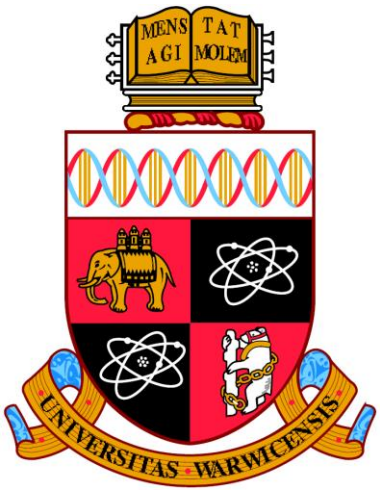

Marine phototroph-heterotroph interactions

By

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A thesis submitted for the degree of Doctor of Philosophy

University of Warwick, School of Life Sciences
September 2018

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List of abbreviations

Å – Ångström unit

AA – Amino acid

ABC – ATP-binding cassette

ANOVA – Analysis of variance

ASW – Artificial Seawater

ATP – Adenosine triphosphate

B1 – Thiamine

B12 – Cobalamin

B7 – Biotin

BMP – Basic membrane protein

Bo – Boron

C – Carbon (when describing nutrients)

C – Concentration (when describing consistency)

Ca – Calcium

Cd – Cadmium

CDS – Coding domain sequences

CFUs – Colony forming units

CH – Carbohydrate

Cl – Chlorine

CO – Carbon monoxide

Co – Cobalt

CO2 – Carbon dioxide

Cox – Carbon monoxide oxidation

Cu – Copper

d – day

DAPI – 4',6-diamidino-2-phenylindole

DF – Degree of freedom (when describing statistics)

DF – Dilution factor (when describing solution)

DHPS – Dihydroxypropanesulfonate

DMA – Dimethylamine

Dmd – DMSP demethylation

DMSP – Dimethylsulfoniopropionate

DNA – Deoxyribose nucleic acid

DOC – Dissolved organic carbon

DOM – Dissolved organic matter

e.g. – *exempli gratia*

EAL – Glutamate-alanine-leucine

EDTA – Ethylendiaminetetraacetic acid

Eh – *E. huxleyi*

ENSP – Ensebl protein ID

ESI – Electron spray ionization

ESW – Enriched seawater

et al. – *et alii*

F – Forward (when describing primers)

FACS – Fluorescence-activated cell sorting

FC – Fold change

FD10 – Log_{10} Fold Change

Fe – Iron

FSC – Forward scatter

g – gram(s) (when describing mass)

g – gravity (when describing centrifugation)

G3P – Glycerol-3-phosphate

Gap – Glyceraldehyde-3-phosphate dehydrogenase

GB – Glycine betaine

GMP – Guanosine monophosphate

Gln – Glutamine

Glu – Glutamate

GTA – Gene transfer agent

GTP – Guanosine triphosphate

h – hour(s)

Het. – Heterotroph

His – Histidine

HPLC – High Performance Liquid Chromatography

i.e. – *id est*

IAA – Indole-3-acetic acid

ID – Identification

Inv2T – Two–tailed inverse

K – Potassium

K_s – Saturation constant of limiting factor s

L – Litre

LB – Lysogeny Broth

LC – Liquid chromatography

LDS – Lithium dodecyl sulfate

LFQ – Label-free quantification

Log – Logarithm

LSD – Least-Significant-Difference

m – meter (when described distance)

m – milli (when described magnitude)

M – Molar

m/z – mass to charge ratio

MB – Marine Broth

Mb – Megabase

MFS – Major facilitator superfamily

Mg – Magnesium

min – minute(s)

ml – millilitre

Mn – Manganese

Mo – Molybdenum

mol – mole

MOPS – 3-(N-morpholino)propanesulfonic acid

Mp – *M. pusilla*

MS – Mass spectrometry (when described in chemical analysis)

MS – mean square error (when described statistics)

Ms – *Micromonas* sp.

n – nano

N – Nitrogen

Na – Sodium

NADH – Nicotinamide adenine dinucleotide (reduced form)

NCBI – National Centre for Biotechnology Information

NF – Not found

NH_4 – Ammonium

Ni – Nickel

NO_3 – Nitrate

NS – Not significant

O – Oxygen

^oC – Degree Celsius

Os – *Ostreococcus tauri*

P – Phosphorus

p – probability value

PAGE – polyacrylamide gel electrophoresis

PCA – Principal component analysis

Ph – *Phaeodactylum triconatum*

pH – potential of hydrogen

PHA – Polyhydroxyalkanoate

Phn – phosphonate utilization

Pho – Alkaline phosphatase

Phot. – Phototroph

Pi – Inorganic phosphorus

PO_3 – Phosphate

POM – Particulate organic matter

ppGpp – phosphatase Ppx/GppA family

Pr – *Prochlorococcus marinus*

Putresc. – Putrescine

q – False discovery rate

R – Reverse (when describing primers)

RA – Relative abundance

RNA – Ribonucleic acid

ROS – Reactive oxygen species

rpm – revolutions per minute

RubisCO – Ribulose-1,5-bisphosphate carboxylase/oxygenase

s – second(s)

S – Sulfur (when describing nutrients)

S7 – *Synechococcu*s sp. WH7803

S8 – *Synechococcu*s sp. WH8102

SD – Significant difference

SDS – Sodium dodecyl sulphate

Se – Selenium

Si – Silicon

SO_4 – Sulfate

Sox – Sulfur oxidation

sp. – species

Spermid. – Spermidine

SSC – Side scatter

SW – Seawater

TCA – Tricarboxylic acid cycle

Th – *Thalassiosira pseudonana*

TMA – Trimethylamine

TMAO – Trimethylamine oxide

TRAP – Tripartite ATP-independent periplasmic transporter

Tris – Tris (hydroxymethyl) aminomethane

V – Volume

WISB – Warwick Integrative Synthetic Biology Centre

Zn – Zinc

μ – Growth rate (when describing growth)

μ – micro (when describing magnitude)

Acknowledgements

I would like to express my sincere gratitude to my primary supervisor, Dr Christie-Oleza for his help and guidance throughout this project, and for putting me forward to apply for the CENTA-NERC doctoral training program scholarship, which funded this project. Many thanks also to my co-supervisors, Prof. Dave Scanlan and Dr Christophe Corre, for their expert guidance and critical review of the project.

I would like to extend my thanks to the group members of Christie-Oleza, Scanlan and Corre labs for their support and advice, especially Mar for preserving the cultures, and helping in experiments always with a smile, Bell for guiding me in my first steps in the lab, Amandeep for sharing difficulties in proteomics, Gabriel and Vinko for the long discussions, Alicja who motivated me for new scientific experiences and Audam for being willing to continue parts of this project. I would also like to acknowledge the members of the Warwick Proteomics facilities, Cleidiane for her technical help and Juan-Ra for his time guiding me through the proteomics analysis.

I thank as well my dear colleagues that I met in the School of Life Sciences at University of Warwick for providing a friendly atmosphere even in the late hours and weekends, such as Giusy, Mark, Anton, Paola, Zelu and especially Chrysa for her support in difficult moments and William for being there for me. Also my friends Antonia and Vassia, along with the new friends that I made during these four years, Elena, Mario and especially Danae for being a great flatmate. I would like to dedicate this thesis to my parents and brother, who always believed on me and supported me even when my world was falling apart.

Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree.

All the work presented (including data generated and data analysis) was carried out by the author, except the experiment described in section 5.3.3 which was performed by Maria Aguilo Ferretjans (School of Life Sciences, University of Warwick). No parts of this thesis have been published by the author.

Abstract

The productivity of marine systems relies on the growth of phytoplankton (phototrophs) which are mainly limited by nutrient availability. Bacteria benefit from the organic matter released by the phototrophs, re-mineralising the scarce nutrients within the system and making them available again to the phytoplankton (Christie-Oleza *et al*., 2017b). Nevertheless, other positive phototroph-heterotroph interactions have been described (*i.e.* the Black Queen Hypothesis) in Morris *et al*. (2012) based on vitamin exchange and alleviation of oxidative stress. Negative interactions such as competition for limited nutrients have also been reported (Thingstad *et al*., 1993) which is represented by the Red Queen Hypothesis as an evolutionary race.

Here, nine phototrophs and 14 heterotrophs were co-cultivated in one-to-one species combinations with the aim of detecting a general behaviour of interactions between marine microbes. The interaction established in each co-culture was characterised based on population abundance and time of survival in both natural oligotrophic (SW) and nutrient-enriched seawater (ESW), compared to mono-culture conditions. Four different types of interactions (*i.e.* positive, semi-positive, neutral and negative) were detected in the 126 co-culture combinations. Many interactions varied between nutrient conditions highlighting nutrient availability as a key player in establishing interactions, as well as the need to test microorganisms in natural SW to reach reliable conclusions.

In order to deepen our understanding of phototroph-heterotroph interactions, the proteomic profile of the co-cultures of the model heterotroph *R. pomeroyi* with each one of the nine phototrophs was performed in SW conditions. In the presence of the phototrophs, *R. pomeroyi* showed generic responses (*e.g.* in amino-acid uptake and N- metabolism, urea metabolism, vitamin biosynthesis and scavenging for aromatic compounds and CO metabolism as energy sources) but also species-specific responses (*e.g.* the relief of P stress, the reduction of specific S compounds and oxidative stress responses). In return, the nine phototrophs reacted differently to the presence of *R. pomeroyi*, with some phototrophs relying on *R. pomeroyi* for key metabolic processes and reaching some level of metabolic coupling with the heterotroph. The magnitude of species-specificity in the interactions between phototrophs and heterotrophs in natural seawater demonstrates the complexity of the system and the usefulness of this data for the interpretation of global biogeochemical cycles.

Chapter 1

Introduction

Microorganisms play an important role in Earth's evolution. Their dominance in our planet in terms of abundance, diversity and metabolic activity make them responsible for the functionality and complexity of natural ecosystems, such as the oceans. Oceans represent 70% of the world's surface and are dominated by microorganisms, with densities of 10^6 planktonic cells per ml of seawater, making up the majority of the oceanic biomass (Azam and Malfatti, 2007). One-half of global primary production is derived from the oceans, specifically from marine phytoplankton (from Greek: φυτον, *phyton*, "plant"; and πλαγκτός, *planktos*, "drifter"). These are prokaryotic or eukaryotic primary producers, inhabiting the photic zone where there is sufficient light and carbon dioxide (CO_2) diffusion from the atmosphere to support photosynthesis. Through oxygenic photosynthesis, these microorganisms generate energy, release O_2 and form organic matter. The remineralisation of the organic matter by bacteria and cycling of nutrients for planktonic organisms is known as the microbial loop (a term coined by Azam *et al*. 1983). A portion of this carbon is respired and returned to the atmosphere; another portion is transferred to higher trophic levels, such as zooplankton and fish, and another is deposited in the deep-sea, known as the carbon pump (Jiao *et al*., 2010), as presented in Figure 1.1. These processes meet increased interest due to the global problems of increasing atmospheric CO_2 levels, climate change, coastal eutrophication and over-fishing (Jackson *et al*., 2001, Azam and Malfatti, 2007).

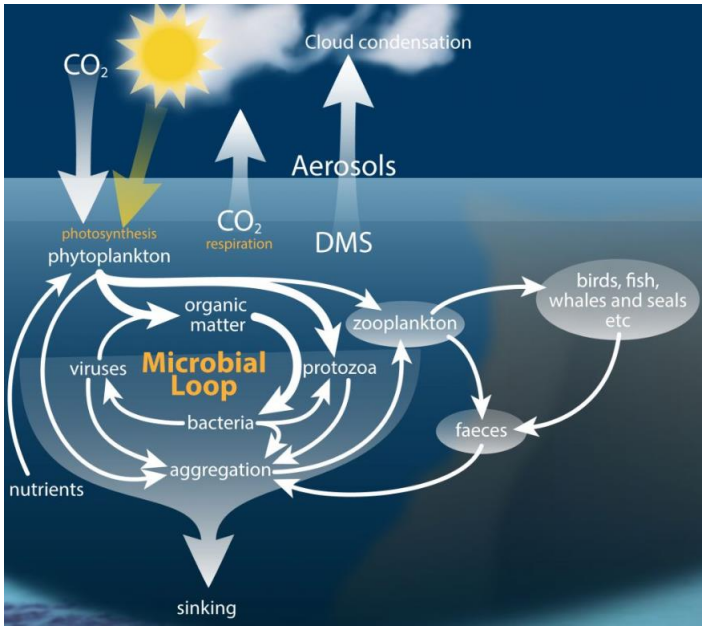


Figure 1.1: Marine microbial loop through grazing and recycle of nutrients play a key role in the transfer of carbon to the higher trophic levels, *i.e*. zooplankton and birds, fish, whales and seals etc. (credits on Dresbach, 2016).

1.1.: The evolution of marine primary producers by their co-existence with heterotrophs

Earth scientists date the beginning of life approximately 3800 million years ago in a hyperthermal environment, where oceans were boiling at about 100 °C (Martin and Tortell, 2008). The initiation of the composition of the atmosphere with oxygen started around 2400-2000 million years ago (Rasmussen *et al*., 2008), by the great oxygenation event when cyanobacteria originated. In this event anoxygenic forms of photosynthesis were replaced by oxygenic processes in cyanobacteria, mainly using water as the electron donor and as a consequence liberating molecular oxygen as a byproduct (Blankenship and Hartman, 1998). Cyanobacteria are algae with bacterial ancestry, proven by molecular phylogeny and polyphasic studies (Ramanan *et al*., 2016). The association of cyanobacteria and bacteria facilitated the evolution of eukaryotic algae through endosymbiotic events, resulting in an astounding phylogenetic diversity of phytoplanktonic eukaryotes. For instance, diatoms derived from a series of secondary endosymbioses approximately 1000 million years ago, when a heterotrophic eukaryotic host domesticated photosynthetic cells (Keeling, 2010, Tirichine *et al*., 2017). In the first endosymbiosis the endosymbiont's nucleus disappeared after the transfer of many genes to the host nucleus. In the second, three membranes were sustained, two new membranes of the plastid of the photosynthetic organism, constructing the periplastid membrane, and the endomembrane system of the host, constructing the chloroplast membrane where ribosomes were attached (Armbrust *et al*., 2004; see Figure 1.1.1).

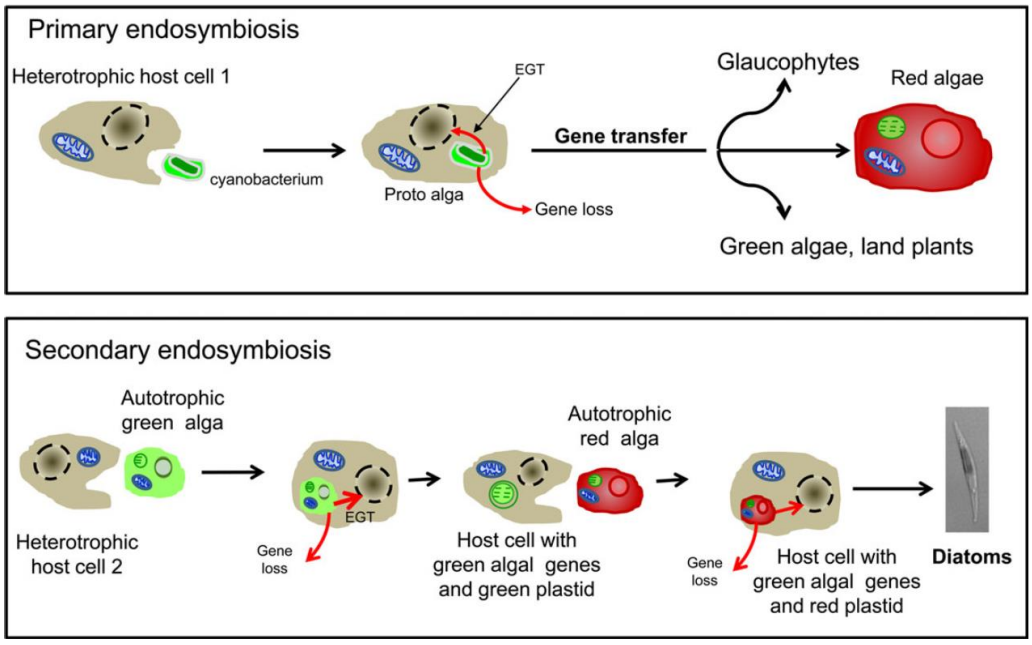


Figure 1.1.1: Representation of the primary and secondary endosymbiont hypothesis during green/red algae (upper panel) and diatom (low panel) evolution, respectively. EGT, endosymbiotic gene transfer (credits on Prihoda et al. 2012).

The association of algae and bacteria in nature can be justified by their co-evolution pattern. For instance, members of the bacterial phyla Proteobacteria and Bacteroidetes are more likely to be associated with phototrophs due to their phylogenetic closeness than others (Ashen and Goff, 2000). Nevertheless, the elements that determine this evolution and, as a consequence, the interaction development between algae and bacteria is yet undefined.

1.2: Ecological hypothesis for the co-existence of organisms

Long-lasting interactions is a major evolutionary process, with some species (beneficiaries) developing strong dependency on functions provided by 'helper' species. This phenomenon has been described as the Black Queen Hypothesis postulating that evolution in stable environments pushes free-living organisms to lose genetically-encoded functions in order to lower their costly energy maintenance (Morris *et al*., 2012). Positive selection for genome reduction is assumed to be the main driver of genome reduction, at least in oligotrophic environments, where retaining a big genome is costly to maintain, replicate and regulate (Dufresne *et al*., 2005, Giovannoni *et al*., 2014). These losses enforce dependency on members of the community that carry out these dispensable, but still vital, functions, as figure 1.2.1 represents.

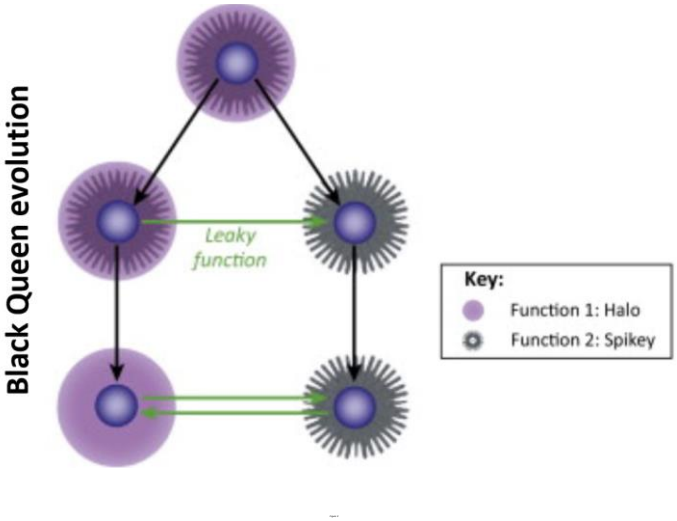


Figure 1.2.1: Schematic representation of Black Queen evolutionary hypothesis of mutualism. Two leaky functions necessary for the survival of all the members of the system are represented as pink Halos and Spikey textures. The ancestor (top) acquires both functions, in the next generation a beneficiary lineage arises, which performs only Spikey function and requires help from a Halo-

performer (leaky function). In the following generation (bottom) the helper loses its Spikey function and depend on the Halo-deficient beneficiary for Spikey production, as a consequence a mutualistic interaction has been developed where both strain depend on the other's presence (credits on Morris, 2015).

The magnitude of dependency relies on the significance of the function and the position of the deletion in the metabolic pathway. For example, the deletion of genes at the end of a bio-synthetic pathway could be more advantageous than the deletion of anterior genes, due to reduction of the anabolism of the pathway (D'Souza *et al*., 2014). The growth advantage of losing a function, may be stronger than the benefit of maintaining it, even for the 'helper' organism. In this way, functions can be lost from the community even if all community members suffer - a phenomenon known as the tragedy of the commons (Hardin, 1998). An example of dependency are the vitamins (Giovannoni, 2012). Humans require the provision of 13 vitamins and nine amino acids all of which are biosynthesised by other species that are consumed as part of our diet. Some of these resources, such as vitamins B1 and B12, are exclusively produced by bacteria, yet they are almost universally required by eukaryotes for core metabolic functions (Morris, 2015). A decrease of vitamin production by bacteria could lead to a decay in exchange of common goods, i.e. vitamins, which will be detrimental for the wellbeing of humans.

The ability to handle essential functions, despite their cost, guarantees some degree of 'security'. Firstly, it allows resistance against environmental changes which can cause fluctuations in the available levels of an element that the other members of the community will compete for. Secondly, in a dependency context, helpers are indispensable, which will partly prevent them from being replaced by competing species (Nadell *et al*., 2008). It is therefore of particular interest to keep, or even to acquire, the status of helper, avoiding the risk of being wiped out as an accessory species whose loss would not have an immediate effect on the community (Mas *et al*., 2016). This was reported by Mas and colleagues (2016), suggesting that helper species may acquire mutations that enhance the production of the common good and approach the commensal. Common goods are described as leaky functions of the helpers, benefiting the beneficiaries (Estrela *et al*., 2012), but also cheaters, that do not contribute but just consume the common resource and threat the cooperative production of the common goods. An example of common goods is the production of siderophores, which are organic compounds secreted to access insoluble inorganic iron. These compounds act as

trace metal buffers that increase the total availability of soluble iron for the entire community, benefiting even the organisms that do not produce siderophores, but can retain them via transporters, acting as cheaters within these systems (Sunda *et al*., 2005, Morris, 2015) as Figure 1.2.2 shows.

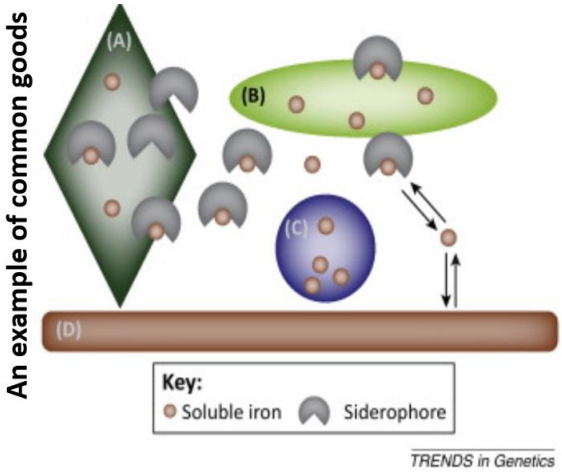


Figure 1.2.2: Schematic representation of siderophores effect on iron availability. Many organisms produce siderophores (grey widgets), which bind extracellularly on iron (orange dots) and then they are uptake by the producers (A). Other organisms are able to bind siderophores produced by others and in this way access iron (B). Another category of organisms cannot even interact with siderophores but benefit from the presence of siderophores that increase the total pool of dissolved iron (C). Free iron exists as insoluble precipitate in oxygenated environments (D). The free iron interacts with the buffer created by the siderophores, and some poorly soluble free iron, which are in rapid dynamic equilibrium (black arrows) are released (credits on Morris, 2015).

The consortium may acquire strategies for eliminating cheating behaviours (Moreno-Fenoll *et al*., 2017), such as the specific structure of siderophore molecules that prevent random uptake of these molecules (Lee *et al*., 2012). In this way, the common goods affects species evolutionary dynamics and their interactions (Morris *et al*., 2012).

The Black queen metaphor was coined as an obvious foil to the well-known Red Queen Hypothesis (Stenseth and Smith, 1984), which is usually applied to the evolutionary 'arms race'. The Red Queen metaphor suggests continuous refinement or improvement of functions or perhaps even the gain of new functions that give players a competitive edge. In this race, the players that are the slowest to lose a function get stuck holding the leaky function and have no choice but to act as helpers for the broader community. In terms of organism and genome complexity, the Black and Red Queen evolutionary strategies can be seen as operating in different directions, with Red Queen races increasing genome size and complexity and Black Queen races leading to smaller, simpler genomes

(Morris *et al*., 2012). It has even been suggested that loss of function is the dominant mode of evolution, not only for microbes but for multicellular life as well, with brief periods of rapid increase in complexity and genome size (driven by brief Red Queen competitions; Wolf and Koonin, 2013). In this way, ecosystems are in two evolutionary modes. The first mode corresponds to the Red Queen condition, a state of continuing evolutionary change, extinction and speciation. The second mode corresponds to an evolutionary stasis of the Black Queen conditions, where organisms are taking advantage of the exchange of goods and establishing a consortium that facilitated their survival. The understanding of the mode that a species is currently encountered can be defined by its fossil records and its position in the current ecosystem (Stenseth and Smith, 1984, Morris *et al*., 2012). In this way, every species is linked to one or several other species, thereby forming an intricate web of direct and indirect interactions, metaphorically described as a tapestry in which the weaving (*i.e*. interactions between species) is as important as the species themselves (Estes *et al*., 2013).

1.3: Phototroph - heterotroph interactions in marine systems

The dual evolutionary mode that all species experience (as discussed above), allow the development of different types of interactions among microorganisms, such as phototrophs and heterotrophs. These interactions have been described in four main ecological categories:

- Mutualism (positive-positive): each individual benefits from the other, it can be considered as co-operation or symbiosis, where two dissimilar organisms living together, involving nutritional cross-feeding, and mechanisms of cross-protection from environmental threats (Zinser, 2018).
- Commensalism (positive-neutral): one individual having a benefit from the activity of the other without the first affecting the second, or when neither of them have any positive or negative effect on each other.
- Parasitism (positive-negative): one individual benefits at the expense of the other.
- Competition (negative-negative): one individual has lower fitness when the other individual is present. The most common case is competition for limiting resources, releasing compounds that cause stress (Amin *et al*., 2012b), such as algicidal compounds (Mayali and Azam, 2004).

Environmental factors and the availability of common goods defines the interactions that take place in nature (Cole, 1982, Carrillo *et al*., 2006, Sher *et al*., 2011, Ramanan *et al*., 2016). The algae - bacteria

system serves as an excellent model to study interactions focusing on the mechanisms and the conditions that results from metabolic complementation or interaction shifts between two organisms (Hom *et al*., 2015). The system of algae and bacteria has been suggested by phycologists to acquire facilitative interactions, since the laboratory algal cultures are often most successful when bacteria are present (Cole, 1982). The onerous task of separating the partners which are naturally bound to each other, such as algae and bacteria, increases the challenge of studying their interactions. This instability of axenic microalgal monocultures (exempt of bacteria) complicates their isolation and maintenance (Amin *et al*., 2015, Ramanan *et al*., 2016) highlighting the importance of microalgal-bacterial interactions for their survival. This is supported in nature due to the presence of specific bacterial communities in algal blooms (Buchan *et al*., 2014).

An interaction that has been thoroughly studied, pointing at the driving forces of the positive connectivity between phototrophs and heterotrophs, is between the model phototroph *Synechococcus* sp. WH7803 and the heterotroph *R. pomeroyi* (Christie-Oleza *et al*., 2017b). In this study the two partners co-existed in one-to-one co-cultures in natural seawater with a large extension in the survival of the phototroph, reaching an equilibrium in the population independent of the initial inoculum (Figure 1.3.1.b).

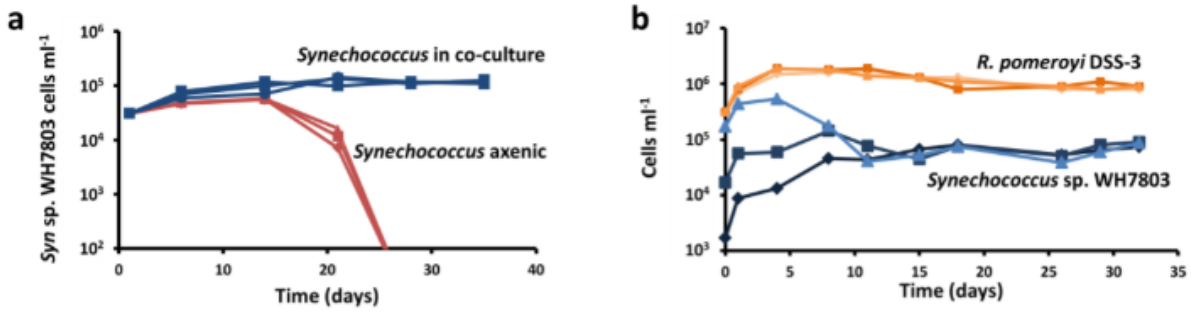


Figure 1.3.1: *Synechococcus* sp. WH7803 and *R. pomeroyi* DSS-3 population levels under nutrient limiting conditions (natural seawater). a) *Synechococcus* sp. WH7803 in the presence and absence of *R. pomeroyi* DSS-3, and b) *Synechococcus* sp. WH7803 and *R. pomeroyi* DSS-3 in co-culture conditions where *Synechococcus* was inoculated at three different concentrations (*i.e*. ~10^3, 10^4 and 10^5 cells/ml). Three culture replicates of each condition are represented in both panels (figure extracted from Christie-Oleza *et al*., 2017b).

This positive correlation between these two model species relies on *R. pomeroyi* scavenging the organic matter produced by *Synechococcus* sp. WH7803 and the recirculation of inorganic nutrients,

such as ammonium, by *R. pomeroyi*, based on nutrient analyses (Christie-Oleza *et al*., 2017b). Nevertheless, in nature the high spatial and temporal environmental heterogeneities increase the possibility of encounter and co-existence of species with diverse interactions. This diversity cannot be detected in natural *in-situ* systems due to the high complexity of the ecosystem.

1.4: Recirculation of macro-nutrients affects the growth of phototrophic and heterotrophic marine microorganisms

Bacteria remineralisation efficiency is depicted as the quantity of organic matter accessible to be consumed at higher trophic levels (as explained in the review of Azam and Malfatti, 2007). This efficiency is affected by membrane-bound transporters and the hydrolytic enzymes that solubilize organic matter (Grossart *et al*., 2003), since dissolved organic matter (DOM) abundance in the ocean is not only derived by primary producers, but also by cell lysis, cell senescence, sloppy feeding by zooplankton, the excretion of waste products by aquatic animals or the dissolution of organic particles from terrestrial inputs (Karel *et al*., 2004). Hence, bacteria have the challenge to position themselves optimally in relation to DOM availability. Alphaproteobacteria such as SAR11 (Morris *et al*., 2002, Giovannoni and Stingl, 2005), Roseobacters (Moran *et al*., 2004, Moran *et al*., 2007) , members of the Bacteroides clades (Kirchman, 2002, Alonso *et al*., 2007), and gammaproteobacteria, such as Alteromonadaceae strains (Williams, 1981, DeLong and Karl, 2005) are good model organisms for defining the adaptive strategies that are used by bacteria in relation to DOM-production regimes in microenvironments (Buchan *et al*., 2014). The metabolic versatility of bacteria facilitates their rapid response to transient nutrient pulses produced by phytoplankton blooms. Our ability to understand the roles that individual bacterial species have in both the formation of blooms and their eventual collapse, will ultimately lead to a better understanding of the forces that control energy flow in the ocean as well as the cycling of compounds (Buchan *et al*., 2014).

The exchanges of macro-nutrients, such as carbon, nitrogen, phosphorus, and sulfur, play a huge role in the development of interactions between phototrophic and heterotrophic communities with an impact on biogeochemical cycling of these nutrients (Cho *et al*., 1996, Grover, 2000, González *et al*., 2000, Grossart *et al*., 2006, Amin *et al*., 2009). Specifically, an interaction can shift from positive to negative if the system is limited by one of these nutrients in regards to their stoichiometric needs (Bratbak and Thingstad, 1985, Vadstein, 2000, Ramanan *et al*., 2016). Stoichiometry was firstly

described by Redfield (1958), who noted that the microscopic plankton of the surface ocean always contained carbon, nitrogen, and phosphorus atoms at ratios of 106:16:1, which was very similar to the ratio found in the deep waters of the ocean, indicating the sinking and decay of the surface microorganisms to the deep ocean. This was the beginning of ecological stoichiometry, a growing field that is providing novel insights into the ecology and elemental cycles of the planet (Sterner and Elser, 2002, Stubbins, 2016). The elemental ratios of carbon, nitrogen, and phosphorus in marine phytoplankton can diverge significantly from the Redfield ratio, but the underlying reasons have been hard to elucidate.

The cellular N:C molar ratio ranges from ~1/5 to 1/10, with low plasticity due to the large dependency for N (Redfield, 1958), since it is the main component of amino acids (Kirchman, 1994). Ammonium is the most common product of any N-containing compound degradation, the main substrate of the anabolic pathways of glutamine and glutamate (Solomon *et al*., 2010) and the preferred N-form of dissolved inorganic nitrogen used by primary producers (Christie-Oleza *et al*., 2017b). Urea is another preferential source of nitrogen for some planktonic species, such as bacteria (Berman and Bronk, 2003, Solomon *et al*., 2010), produced intracellularly via purine catabolism and/or the urea cycle (as presented in Figure 1.4.1), and degraded by two different pathways producing carbon dioxide and ammonium, either with urease or through the allophanate pathway (Hausinger, 2004).

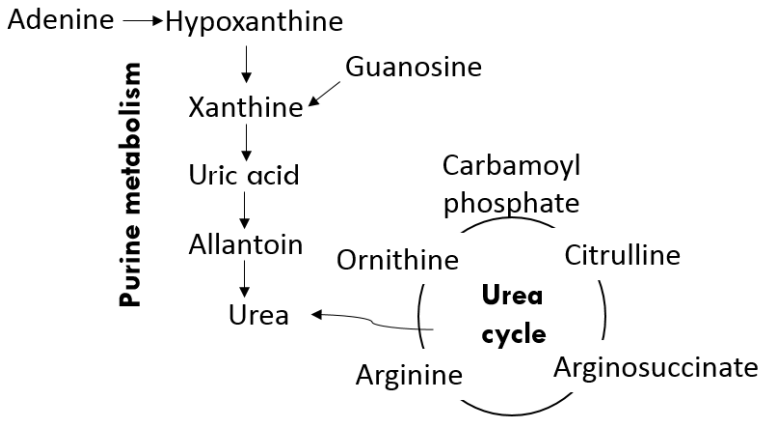


Figure 1.4.1: Urea production through purine metabolism and urea cycle.

The cellular P:C ratio is more variable than the N:C ratio, ranging from ~1/50 to 1/500 (Van Mooy *et al*., 2009). Phosphorus (P) is one of the most limiting nutrients in oligotrophic systems (Lomas *et al*., 2004, Van Mooy *et al*., 2015). The P-pool in nature contains inorganic (Pi, usually phosphate) and

organic phosphorus including C-P (phosphonate) and C-O-P (phosphomonoester) bond classes (Clark *et al*., 2001, Dyhrman and Ruttenberg, 2006). Phosphate is the most commonly used P-form in most bacteria and phototrophs, used in nucleic acids (*e.g*. DNA, RNA), energy (*e.g*. ATP), and phospholipids (Thingstad *et al*., 1993, Dyhrman, 2016). When phosphate availability drops below a critical threshold, the cells induce a P-stress response with an increased production of phosphatases and active membrane transporters for P (Cembella *et al*., 1984, Dyhrman and Ruttenberg, 2006, Sebastian and Ammerman, 2009, Sebastian and Ammerman, 2011). Alkaline phosphatase activity may indicate P-stress but does not necessarily indicate growth limitation (Dyhrman and Palenik, 2003). In addition, many species have lowered their cellular P-demands in order to reduce the high cost of acquiring such a depleted element (Bonachela *et al*., 2011). The reduction in the cellular P quota allows the organism to obtain high growth rates in nutrient-poor regions (Liu *et al*., 1997), such as cyanobacteria and *Pelagibacter* (Dufresne *et al*., 2005, Giovannoni and Stingl, 2005, Van Mooy *et al*., 2006). This can be achieved *via* genome streamlining (Giovannoni and Stingl, 2005, Bertilsson *et al*., 2003, Sun *et al*., 2017), and synthesizing non-phosphorus membrane lipids, such as sulfolipids, when P is not sufficient (Van Mooy *et al*., 2006). Despite the very low P-concentrations, the turnover between organic and inorganic P-forms should be sufficient since it is supporting high primary productivity in these oligotrophic systems (Clark *et al*., 2001) and shaping the species and function composition in a system (Thingstad *et al*., 1993, Sebastian and Ammerman, 2009, Galbraith and Martiny, 2015).

The strategies of nutrients acquisition in microorganisms follow three models, as described by Klausmeier *et al*. (2004b):

1) Michaelis-Menten kinetics (Turpin, 1988): the uptake rate is regulated by the external nutrient concentration.
2) The model of growth: the uptake rate depends on the internal concentration (cell quota) of a single limiting nutrient for the growth rate of each species (Caperon, 1968, Droop, 1968).
3) Liebig's law of the minimum: the growth rate depends on the internal concentration of the most limiting nutrient (Droop, 1974, Rhee, 1978).

The effect of the concentration of nutrients on the growth rate is described by the Monod equation, through a hyperbolic saturation curve for growth rate or nutrient uptake (μ) that is controlled by the

substrate concentration as a limiting factor (Figure 1.4.1; Monod, 1942, De Baar, 1994), using the biological constants of maximal growth rate (μ_{max}) and the half-saturation constant (K_s).

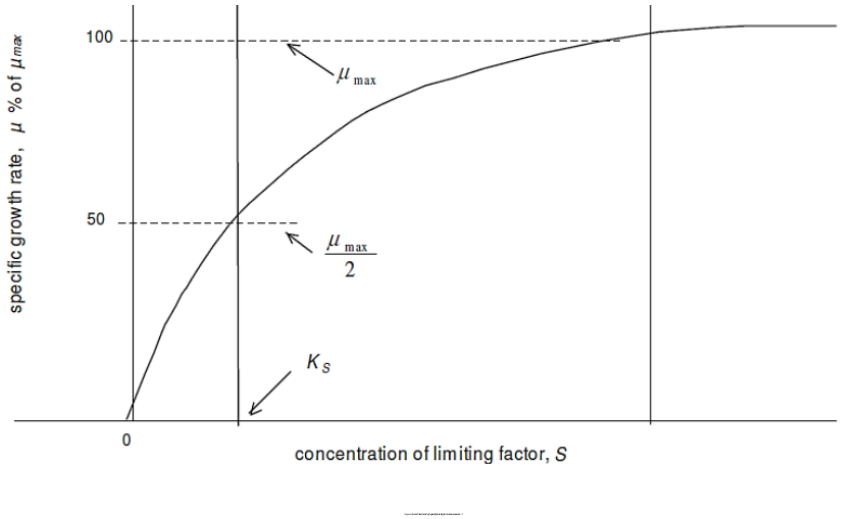


Figure 1.4.2: Monod equation of growth rate (μ) related to concentration of a limiting factor (S) (described by Monod, 1942).

The intracellular nutrients levels needs to be significantly depleted before growth limitation occurs (Droop, 1973, Sterner and Elser, 2002). Nevertheless, in natural environments there are usually more than just one limiting nutrient, often by invoking the term 'co-limitation'. In co-limiting conditions, the uptake of one substrate is dependent on sufficient levels of the second, such as trace metals necessary as co-factors in alkaline phosphatases (Saito *et al*., 2008), where metal starvation will induce phosphorus starvation. Nevertheless, there is a degree of plasticity in the nutrient demands per cell, which varies between species and between clades (Geider and La Roche, 2002).

1.5: Mechanisms for establishing microbial interactions

The balance between intracellular and extracellular nutrients is controlled by membrane transporters that facilitate the release/uptake of essential nutrients, ejection of waste products, and allow the cell to sense the environmental conditions around it. The suite of transporters in one organism also sheds light on its lifestyle and physiology. For prokaryotic species, there is a relatively linear relationship between the genome size and the number of transporter families (Ren and Paulsen, 2005). Having a large array of high-affinity ABC uptake systems may be an advantage for an organism to compete for nutrients. A greater diversity of transporter types presumably allows for a broader range of substrate utilization, described as a strategy of diversification for nutrient acquisition. This is represented in the

high number of ORFs in prokaryotes that encode a large diversity of transporters families (Ren and Paulsen, 2005).

In addition, other functions, such as stress responses, play a great role in the established relationships between two organisms. Osmosis is a problem for all microorganisms living in saline habitats, which is minimized by the production or exchange of osmolytes (compounds which carry water, restoring normal cell volume and fluid balance of the cell), eliminating the detrimental effects of osmosis on protein folding and membrane functions (Galinski, 1995, Kiene *et al*., 1998). More commonly found than osmotic stress is oxidative stress, caused by an increased production of reactive oxygen species (ROS), by products of oxygen-based metabolic pathways such as photosynthesis, photorespiration, and oxidative phosphorylation (Hooper *et al*., 2005), that ultimately lead to cell death. Because ROS are highly reactive forms of oxygen, critical mechanisms for ROS detoxification have evolved consisting of ROS-scavenging enzymes such as glutathione, peroxidases or superoxide dismutases. The detoxification of ROS has been used as a mutualistic interaction example in the Black Queen Hypothesis (Flohé, 2010, Zinser, 2018), since *Prochlorococcus* lacks a catalase and benefits from the catalase activity of surrounding heterotrophic bacteria to decompose hydrogen peroxide (Morris *et al*., 2008, Sher *et al*., 2011, Aharonovich and Sher, 2016, Bernstein *et al*., 2017).

Another example that drives the dependency of phototrophs on bacteria is the exchange of vitamins. Vitamins have been shown to be necessary as cofactors for many enzymes of central and secondary metabolism. As determined through major auxotrophies, three vitamins are known to play a role between phototrophs and heterotrophs: cobalamin (vitamin B12), thiamine (vitamin B1) and biotin (vitamin B7) (Croft *et al*., 2006). Vitamin B12 plays essential roles in amino acid and one-carbon metabolism and vitamin B1 in primary carbohydrate and amino acid metabolism as well as used as an anti-oxidant (Croft *et al*., 2005). Both vitamins have also been found to play a role in polyamine biosynthesis, and in the urea and citric acid cycles, whilst vitamin deficiency may impair the ability of phytoplankton to recover from nitrogen starvation (Bertrand and Allen, 2012). Biotin works as a cofactor for several carboxylase enzymes, including acetyl coenzyme A (CoA) carboxylase, which is involved in fatty acid synthesis (Croft *et al*., 2006). Organisms that require an external source of these essential molecules can be described as vitamin 'auxotrophs'. Over half of all microalgal species surveyed (from fresh, marine and brackish habitats) are auxotrophic for B12, 20% require B1 and 5% require biotin (Croft *et al*., 2006). The synthesis of vitamins can be performed via different pathways

in different organisms (Webb *et al*., 2007). Though the loss of biosynthetic pathways can be partial or complete, such as the lack of one or more genes for the B1 biosynthetic pathway (Croft *et al*., 2006, Worden *et al*., 2009, Bertrand and Allen, 2012). The lack of a particular step could have been specifically induced by selection pressure (*i.e.* via its availability from an external supply) and, as a consequence, the loss of one step could result in a subsequent loss of all the genes in a pathway (Helliwell, 2017). Partial loss of steps in a pathway either can be covered by precursors produced by bacteria or abiotic-degraded vitamin as by UV (Paerl *et al*., 2015). In all (Helliwell *et al*., 2013). An example of substitution is the synthesis of pseudocobalamin in cyanobacteria and a dozen of other forms of B12 found in nature instead of B12 (Helliwell, 2017). The high diversity of B12 forms derive from the complexity of this metabolite, synthesised through more than 20 enzyme-catalysed reactions and found only in bacteria (Warren *et al*., 2002). Algae that are not B12 auxotrophs acquire either pseudogenes, or enzymes that do not demand cobalamin as cofactor, such as the B12 independent form of methionine synthase (Croft *et al*., 2005, Croft *et al*., 2006).

Vitamin auxotrophy is randomly distributed across algal lineages, with no phylogenetic relationship between those organisms that require the nutrient co-factors (Grant *et al*., 2014). The balance between requirements for a vitamin *versus* the metabolic cost of its biosynthesis will determine the selective pressure to maintain biosynthesis of a vitamin. The metabolic expense associated with the biosynthesis of vitamin B12 is therefore likely to be a major contributing factor in the restriction of vitamin B12 biosynthesis to prokaryotes. Since there are just three enzymes that require B12 as a cofactor in eukaryotes, over 20 different cobalamin-dependent enzymes are found in bacteria (Roth *et al*., 1996), making B12 biosynthesis less dispensable in these organisms. It may also mean that bacteria need to synthesise more vitamin B12 to support the growth of algae and indirectly to satisfy their own requirement for nutrients produced by algae. Bacteria within the boundary layer of algae elevated vitamin-assimilation, as supported by the effect that the bacterium *Halomonas* spp. and *D. shibae* had on their B12-dependent dinoflagellate host (Croft *et al*., 2005, Wagner-Dobler *et al*., 2010), or between the green alga *Lobomonas rostrata* and the bacterium *Mesorhizobium loti* (Kazamia *et al*., 2012). The relationships of dependence driven by vitamin exchanges influence marine productivity and species composition (Bertrand and Allen, 2012). Indeed, new evidence suggests several B vitamins lie at the heart of complex marine microbial communities, with 'metabolic outsourcing' for molecules

of value such as vitamins, driving interspecies dynamics and leading to 'vitamin trafficking' between community members (Giovannoni, 2012).

All the processes described above, essential for the establishment of phototroph-heterotroph interactions, take place at a nanoscale distance and specifically in the area surrounding phytoplankton cells, known as "phycosphere" (Bell and Mitchell, 1972). In this area the released molecules are diffused within a boundary layer without mixing with the surrounding (bulk) fluid due to absence of turbulence at such small scales (Amin *et al*., 2012b). In this zone, signalling molecules, such as acylated homoserine lactones, coordinate the activities between individuals of the same species as well as between different species (Schauder and Bassler, 2001, Natrah *et al*., 2014), directing even the mobility of the cells either toward (attractant) or away from (repellent) a chemical gradient. As cell numbers in a population increase, so does the concentration of the secreted molecules. Once the concentration of signalling molecules reaches a threshold required for detection, they bind to specific response regulators, causing a conformational change and triggering a signal transduction cascade that results in the regulation of multiple genes (Ng and Bassler, 2009). For instance, iron complexes siderophores and vibrioferrins, produced by a number of gammaproteobacteria belonging to the Vibrio and Marinobacter genera (Yamamoto *et al*., 1994, Amin *et al*., 2007), can be utilised by phototrophs as a source of iron, excusing the association of these bacterial species with diverse algal cultures (Amin *et al*., 2012a). On the other hand, phototrophs also shape the bacterial community structure by releasing water-soluble molecules, such as by-products of photorespiration, *i.e*. glycolate, or compounds with antibacterial activity, such as fatty acids and esters (Lebeau and Robert, 2003). This ability of environmental sensing provides a fitness advantage over non-sensing or traditional chemotactic cells that depend only on nutrient gradient levels. This ability actually gives opportunities for cooperation or evasion of competitive events (Natrah *et al*., 2014).

1.6: Current approaches to interpret microbial interactions

Interspecies interactions are hard to be observed *in-situ*, since they are indistinguishable under "equilibrium" conditions, and the system must be either disturbed or observed over time. Most of our understanding of interspecies interactions comes from terrestrial environments, primarily from microbe-plant or microbe-mammal interactions, where associations of bacteria with a "stable platform" facilitates observations. In the ocean, stable platforms for studying these interactions exist

in the near-shore/intertidal areas (*e.g*. kelp beds and coral reefs) or on the seafloor (*e.g*., hydrothermal vents and sediments). Few comparable stable structures exist for studying microbial interactions that dominate the vast expanses of the pelagic ocean (Amin *et al*., 2012b).

High-throughput sequencing and other -omics approaches to study microbial ecology has improved our understanding of microbial communities and their environment, with metagenomic and metatranscriptomic studies being performed in complex microbial communities predominated by algae and bacteria (Tringe *et al*., 2005). In addition, studies on the microbiome of algal phycospheres in natural systems and artificial systems, such as photobioreactors using high-throughput sequencing and multi-omics were only recently constituted in natural assemblages (Amin *et al*., 2015, Ramanan *et al*., 2016). Metabolomic analyses have also been performed to decipher exchanged compounds between organisms while metagenomic and transcriptomic approaches were used to gain insights on cluster interactions, genomic machinery and regulatory patterns in natural communities (Cooper and Smith, 2015, Ramanan *et al*., 2016, Rooney-Varga *et al*., 2005, Abby *et al*., 2014). Nevertheless, the 'meta-omics' studies provide an holistic view without revealing the physiological success and the mechanistic factors that lead to the success of individual taxa (Teeling *et al*., 2012, Buchan *et al*., 2014). Recently developed single-species techniques can bridge this gap and offer new insights into the regulation of processes in the environment (Bristow *et al*., 2017).

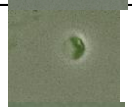
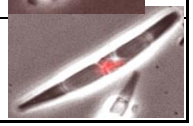
The use of -omics techniques for the interpretation of microbial responses is a new approach for studying interactions under conditions mimicking nature. In the current project an holistic approach was tested, by using a diversity of species (selection presented in Tables 1.7.1 and 1.7.2) in *in-vitro* batch one-to-one species co-cultures, and outlining their vital metabolic coupling through proteomics.

1.7: Model microbes used in this thesis

The microbes used in this study were all representatives of major clades and have been selected on the following basis: i) their proven dominance in worldwide surface ocean waters (Biers *et al*., 2009), ii) they all have an available annotated genome sequence which was essential for the shotgun proteomic analysis, and iii) it was possible to obtain them and culture them.

Phototrophs

Table 1.7.1: The main physiological characteristics of nine phototrophic species tested in the current PhD thesis.

PHOTOTROPHS	Organism	Strain	Reference name in this thesis	Isolation Origine	Isolation Depth	Genome (Mb)	GC(%)	Gene Counts	Average Size	Shape	Microscopic pictures (taken in this project)
Marine Picocyanobacteria	*Prochlorococcus marinus*	MED4/ CCMP1986 / NIES-2087	*Prochlorococcus*	Mediterranean Sea	5m (1989)	1.699	30.8	1847	1.0 µm	Coccoid	
	Synechococcus sp.	WH7803	*Synechococcus* WH7803	Sargasso Sea	25m	2.367	60.2	2591	1.0 µm	Coccoid	
		WH8102	*Synechococcus* WH8102	Carribean Sea		2.434	59.4	2581	1.0 µm	Coccoid	
Green Algae	*Micromonas* sp.	CCMP2709/RC 299/ NOUM17 (*comoda*)	*Micromonas*	Equatorial Pacific	0m (1998)	21.109	63.8	10115	2.0 µm	Coccoid (motile)	
	Micromonas pusilla	CCMP 1545	*M. pusilla*	English Channel		21.964	65.9	10248	1.5 µm	Coccoid (motile)	
	Ostreococcus tauri	RCC745/ OTH95	*Ostreococcus*	Thau lagoon(Mediterran.)	0m	12.92	59.4	7668	0.8 µm	Coccoid	
Haptophyceae	*Emiliania huxleyi*	CCMP 1516 (*Pontosphaera*)	*E. huxleyi*	Equatorial Pacific		155.93	65.7	38549	5µm	Coccoid	
Diatoms	*Thalassiosira pseudonana*	CCMP 1335	*Thalassiosira*	Chile upwelling	0m	32.44	46.9	11771	7µm	Elongated	
	Phaeodactylum tricornutum	CCMP 2561/ CCAP1055/1	*Phaeodactylum*	North Sea	pelagic and benthic habitat	25.05	48.9	9479	9 µm	Elongated	

Prokaryotic phototrophs

Cyanobacteria are a phylum of bacteria with the ability to obtain energy through photosynthesis. Unlike bacteria, cyanobacteria have internal membranes where their photosynthetic machinery is placed, i.e. the thylakoidal membrane (Liberton *et al*., 2008). Oceanic cyanobacteria dominate the photosynthetic biomass in many oligotrophic areas and were first documented just 40 years ago (Waterbury *et al*., 1979, Johnson and Sieburth, 1979). *Synechococcus* has been found at high concentrations in the oceanic euphotic zone and also in regions with low salinity or temperatures. *Synechococcus* species mainly co-exist with *Prochlorococcus*, with the second outnumbering the first in more oligotrophic regions such as in the subtropical gyres (Flombaum *et al*., 2013). Their small cell size and subsequent large surface area/volume ratio allows efficient nutrient uptake (Vaulot and Partensky, 1992, Vaulot *et al*., 1996). Specifically *Prochlorococcus* have lower nutrient requirements (Partensky *et al*., 1999), especially for phosphate due to its adaptation to replace its phospholids to sulfolipids (Van Mooy *et al*., 2006). In addition, *Prochlorococcus* species, despite their absence of motility, have developed a unique combination of chlorophylls that allows them to absorb blue light (Ralf *et al*., 1992*,* Coleman *et al*., 2006), which is the only visible spectrum that penetrates to high depths, i.e. 400m (Zinser *et al.,* 2007). The genomes of many of these picocyanobacteria have currently been sequenced and are available (Scanlan *et al*., 2009, Gonzalez-Esquer *et al*., 2016). The cultivation of marine *Synechococcus* and *Prochlorococcus* species led to rapid advances in understanding the biology of these organisms (Giovannoni and Stingl, 2005, Scanlan *et al*., 2009), such as their large pigment diversity, explaining their successful proliferation in the changing light environments of the surface mixed layers of the ocean (Scanlan and West, 2002).

Eukaryotic phototrophs

Comparing the eukaryotic algal lineages, particular interest arises from their cell wall composition as some have silicate frustules of nanoscale precision (diatoms), others are naked, such as green algae species, and others are covered by ornate calcium carbonate plates (coccoliths) such as haptophytes, increasing their cellular demands for specific nutrients (Worden *et al*., 2015).

Green algae

Green algae from the order Mamiellales have reduced genomes, with the smallest been recorded in *Ostreococcus* (Derelle *et al*., 2006, Palenik *et al*., 2007). *Ostreococcus* contains simple structural

features, such as a single chloroplast (Cardol *et al*., 2008), one mitochondrion, a Golgi body, and a highly reduced cytoplasm compartment (Chrétiennot-Dinet *et al*., 1995, Robbens *et al*., 2007). Isolates from the clonal line of *Ostreococcus tauri*, grew in laboratory conditions with a bacterial microbiome consistently present, with the most prevalent group, being *Flavobacteria* (Abby *et al*., 2014). The motile *Micromonas* genus share only 90% of predicted genes between *Micromonas* sp. and *M. pusilla*, justifying their divergence as distinct species. The shared genes showed more rapid evolutionary rates than core genes, involved in key pathways such as photosynthesis (Worden *et al*., 2009). Their genome divergence raises the question of how this can be depicted in their interactions with bacteria, since only few studies of interactions have included these organisms due to difficulties in maintaining these species in cultures (Abby *et al*., 2014).

E. huxleyi

E. huxleyi is a haptophyte covered with coccoliths, found in almost all marine ecosystems from the equator to sub-polar regions, and from nutrient rich upwelling zones to nutrient poor oligotrophic waters (Okada, 1973, Charalampopoulou, 2011) from the surface to 200m depth (Winter, 1994, Boeckel *et al*., 2008). *E. huxleyi* plays a major role in burying carbon in the bottom of the oceans, especially during dense blooms that cover thousands of square kilometers of the ocean surface (Paasche, 2001, Balch *et al*., 1992, Holligan *et al*., 1993). These blooms also affect the sulfur cycle and, ultimately, climate (Michal *et al*., 2013), since the release of the organic sulphur compound dimethylsulphoniopropionate (DMSP) acts as nucleation sites for cloud formation and enhancing Earth's albedo (Charlson *et al*., 1987, Poulson-Ellestad *et al*., 2016). *E. huxleyi* is one of the few phototrophs previously used to study its interactions with bacteria (Seyedsayamdost *et al*., 2011, Paul *et al*., 2013).

Diatoms

Diatoms are highly diverse and cosmopolitan single celled eukaryotes of the Stramenopile lineage. These organisms form silicon cell walls (Mock *et al*., 2008) that effects their cell division (Huysman *et al*., 2010), susceptibility to grazing, and sinking rates to the deep ocean (Tirichine *et al*., 2017). *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*, representors of centric and pennate diatoms respectively, were the first diatoms with sequenced genome (Bowler *et al*., 2008, Moustafa *et al*., 2009), revealing an unusual gene repertoire and diversity of metabolic potentials (Oudot-Le

Secq *et al*., 2007, Armbrust, 2009, Bowler *et al*., 2010, Shao *et al*., 2018). The genome availability, genetic tractability and ease for cultivation has established *Phaeodactylum tricornutum* as a model diatom (Bowler *et al*., 2008, Yang *et al*., 2014). Bacteria are known to play an important role in the initial phases of diatom blooms and in cell aggregation (Smith *et al*., 1995, Bidle and Azam, 1999, Grossart *et al*., 2006). The most strongly associated genera with diatoms are *Sulfitobacter*, *Roseobacter*, *Alteromonas*, and *Flavobacterium* (Grossart *et al*., 2005, Sapp *et al*., 2007a, Sapp *et al*., 2007b, Tirichine *et al*., 2017).

Heterotrophs

The abundance of heterotrophic bacterial cells is generally positively correlated with the abundance of phytoplankton both in open ocean systems as during the rise and the collapse of blooms, as bacteria continue to use the organic matter that is released from phytoplankton (Cole, 1982, Smriga *et al*., 2016). However, different bacterial species have distinct capacities to consume and remineralize various substrates released by specific phytoplankton species (Alonso-Sáez and Gasol, 2007). For instance, algae and bacteria altered their metabolism to suit each other's needs through the release of compounds, such as the hormone Indole Acetic Acid (IAA) produced by Sulfitobacter in return for organosulfur released by the algae (Amin *et al*., 2015). In this way, it is generally believed that the structure of the bacterial community is coordinated with specific phytoplankton species (Pinhassi *et al*., 2004, Grossart *et al*., 2005). However the study of LeCleir *et al*. (2014) showed that two distinct bloom events, shared similar bacterial composition. Nevertheless, the most commonly associated bacterial lineages with phototrophic blooms are Proteobacteria and Bacteroidetes (Pinhassi *et al*., 2004, Rink *et al*., 2007), with metaproteomic data indicating a high production of rhodopsin (Williams *et al*., 2013), a function that generates energy by light-driven ion-pumping (Gómez-Consarnau *et al*., 2007). This demonstrates the little understanding that we currently have on the role of bacteria in these dynamic events of blooms, with genome and culture-based studies of representative Roseobacter and Flavobacteria strains providing valuable insights into the nature of potential interactions (Buchan *et al*., 2014). The physiological and isolation characteristics of the heterotrophic species that were included in the current project, can be found in Table 1.7.2.

Table 1.7.2: The main physiological characteristics and isolation origin of 14 heterotrophic species used in the current PhD thesis.

HETEROTROPHS	Organism	Strain	Referred in the current study	Origine	Isolation	Characteristics	Genome (Mb)	GC(%)	Gene Counts
Actinobacteria	*Salinispora tropica*	CNB-440/ ATCC BAA-916 / DSM 44818	*Salinispora*	Bahamas	coarse beach sand/ ocean sediments	Gram-positive	5.18	69.5	4639
	Aeromicrobium marinum	T2/ DSM 15272	*Aeromicrobium*	German Wadden Sea	surface waters	Gram-positive	3.08	71.1	3090
Bacteriodetes	*Polaribacter* sp.	MED152	*Polaribacter*	Northwest Mediterranean	0.5 m depth, 1 km off the Blanes Bay	Gram-negative, gliding motility	2.96	30.6	2655
	Algoriphagus machipongonensis	PR1	*Algoriphagus*	Hog Island, Virginia	mud core with a colonial choanoflagellate	Gram-negative, non-motile	4.79	38.7	4026
	Gramella forsetii	KT0803	*Gramella*	North Sea	phytoplankton bloom	Gram-negative, free-living, gliding motility	3.8	36.6	3434
	Formosa agariphila	KMM3901	*Formosa*	Sea of Japan	isolated from alga *Acrosiphonia sonderi*	Gram-negative, gliding motility	4.23	33.6	3573
Alphaproteobacteria Roseobacter clade	*Ruegeria pomeroyi*	DSS-3/ ATCC 700808 / DSM 15171 (*Silicibacter pomeroyi*)	*R. pomeroyi*	Georgia, USA	Coastal sea water Atlantic (salinity = 31; 1999)	Gram-negative, motile with single, complex flagellum	4.11	64.2	3955
	Roseobacter denitrificans	OCh114/ATCC 33942	*R. denitrificans*	Australia	green seaweeds in coastal marine sediments	Gram-negative, with flagella	4.13	59	3895
	Dinoroseobacter shibae	DFL-12/ DSM 16493 / NCIMB 14021	*D. shibae*		*Prorocentrum lima* (benthic dinoflagellate)	Gram-negative, motile by a single flagellum	3.79	66	3638
Gammaproteobacteria	*Alteromonas macleodii*	ATCC 27126 (*Pseudoalteromonas macleodii*)	*Alteromonas*	Hawaii	surface waters (1972)	Gram-negativewith single flagellum	4.65	44.7	3959
	Marinobacter adhaerens	HP15	*Marinobacter*	German Wadden Sea	surface waters aggregates	Gram-negative, motile by a single flagellum	4.42	57.1	4126
	Pseudomonas stutzeri	AN10/CCUG 29243	*Pseudomonas*	West Mediterranean	polluted marine sediments	Gram-negative, motile by a single polar-flagellated	4.71	62.7	4361
	Escherichia coli	S17-1 λpir	*E. coli*		found in liver from a broiler chick with septicemia	Gram-negative			
Verrucomicrobia	*Verrucomicrobiae bacterium*	DG1235 (*Verrucomicrobiales* sp.)	*Verrucomicrobiae*		isolated from a dinoflagellate/deep sea/soil	Gram-negative, non-motile	5.78	54.3	4621

Actinobacteria

This gram-positive phylum is known to produce 70% of the naturally derived antibiotics (Bérdy, 2005), hence, they encode an enormous potential for developing active interactions. Secondary metabolites have been found to be produced in cultures of dinoflagellates (Bai *et al*., 2011).

Salinispora tropica: has a pan-tropical distribution in near-shore marine sediments (Mincer *et al*., 2002), with the production of secondary metabolites playing a role in its ecological diversification (Freel *et al*., 2012).

Aeromicrobium marinum: a gram-positive free living marine bacteria found in surface waters (Bruns *et al*., 2003).

Bacteroidetes

Bacteroidetes are known for high molecular weight organic matter degradation, with a predicted preference for polymeric carbon sources and a distinct capability for surface adhesion (Bauer *et al*., 2006).

Polaribacter sp.: belongs to the family Flavobacteriaceae and grows optimally attached to surfaces where it can move by gliding motility, searching for polymeric substances and using its proteorhodopsin as an energy supplement (Fernandez-Gomez *et al*., 2013).

Gramella forsetii: belongs to the family Flavobacteriaceae and is generally found in phytoplankton blooms (Eilers *et al*., 2001), with adaptations for degradation of polymeric organic matter (Bauer *et al*., 2006).

Formosa agariphila: belongs to the family Flavobacteriaceae and has been found in enrichment cultures of the brown algae *Fucus evanescens* and *Undaria pinnatifida*, the green alga *Acrosiphonia sonderi*, and the marine sponge *Hymeniacidon flavia* (Nedashkovskaya *et al*., 2006, Yoon and Oh, 2011, Park *et al*., 2013).

Algoriphagus machipongonensis: belongs to the family Cyclobacteriaceae and produces lipids that activate and enhance the growth of choanoflagellates (Woznica *et al*., 2016).

Alphaproteobacteria

The Roseobacter clade from the alphaproteobacteria phylum comprises more than 20% of the bacterial cells in coastal waters and 5% in the open ocean (Moran *et al*., 2007, Brinkhoff *et al*., 2008, Wemheuer *et al*., 2015, Sun *et al*., 2017), and is known to associate with phytoplankton (Amin *et al*., 2012b), macroalgae (Egan *et al*., 2014) and marine invertebrates (Thompson *et al*., 2015). An analysis of the collective genetic complement of Roseobacters showed that the group encodes several biogeochemically relevant pathways but only a subset of these pathways are present in any single genome (Newton *et al*., 2010).

Ruegeria pomeroyi: a mixotrophic organism, used as model organism in interaction co-cultures (Christie-Oleza *et al*., 2015, Christie-Oleza *et al*., 2017b, Kaur *et al*., 2018).

Roseobacter denitrificans: a mixotrophic organism found associated with seaweeds, capable of sulfur oxidation and aerobic anoxygenic photosynthesis, without fixing carbon, hence functioning only as an energy supplement (Tang *et al*., 2010).

Dinoroseobacter shibae: a mixotrophic organism known to co-exist and provide vitamins to dinoflagellates (Wagner-Dobler *et al*., 2010, Kazamia *et al*., 2012).

Gammaproteobacteria

The Alteromonadaceae family possesses large genomes that contain many degradation and secondary metabolism genes. Ecologically they are often associated with nutrient-rich environments such as particulate material and marine snow (López-Pérez and Rodriguez-Valera, 2014).

Alteromonas macleodii: it is among the most rapidly dividing bacteria, distributed globally (García-Martínez *et al*., 2002, Ivars-Martinez *et al*., 2008, López-Pérez *et al*., 2012), associated with phytoplankton (Buchan *et al*., 2014, López-Pérez and Rodriguez-Valera, 2014) such as *Prochlorococcus* (Diner *et al*., 2016).

Marinobacter adhaerens: a metabolically flexible genus found in an exceptionally wide range of marine settings (Handley and Lloyd, 2013), associated with dinoflagellates and coccolithophores, producing siderophores (Kazamia *et al*., 2012) and demonstrating attachment and chemotaxis to the diatom *Thalassiosira weissflogii* (Sonnenschein *et al*., 2012).

Pseudomonadaceae: *Pseudomonas stutzeri*: known to reduce nitrate to molecular nitrogen commonly isolated from both soil and marine environments (Grüntzig *et al*., 2001).

Enterobacteriaceae: *Escherichia coli*: not an ordinary marine species, but a model laboratory bacterial organism, and has been found in faecal coliforms in marine systems (Gerba and McLeod, 1976, Chan and Killick, 1995).

Verrucomicrobia

Verrucomicrobia bacterium: found associated with dinoflagellates and has been used as part of the artificial bacterial consortia of the green algae *Chlorella vulgaris* (Ueda *et al*., 2010).

Among the species used in this study, some have been already tested in co-culture experiments as presented in Table 1.7.3. The results in the current study were compared to already published work, although it is worth highlighting that most of the referenced co-culture experiments were performed in nutrient-enriched media in the exponential growth phase.

Table 1.7.3: Recorded co-cultures of the nine phototrophic species in the literature, along with tested details, such as media of incubation and -omics examination.

Co-cultures studies found in the literature	in co-culture with:								
	Prochlorococcus	*Synech*. WH7803	*Synech*. WH8102	*Micromonas* sp.	*M. pusilla*	*Ostreococcus*	*E. huxleyi*	*Thalassiosira*	*Phaeodactylum*
	Bacterial collection in enriched media (Sher et al., 2011)	*Synechococcus* PCC 7002 co-culture with *Shewanella* in enriched media /transcriptome (Beliav et al., 2014)				Flavobacteria in phycosphere (Abby et al., 2014)	*Pseudomonas* / transcriptome 14days (Segev et al., 2016)	*T. weissfloggi* co-culture with *Marinobacter* in enriched media (Kaeppel et al., 2012)	*Alteromonas* in enriched media /N-limitation /transcriptomics (Diner et al., 2016)
	Alteromonas in enriched media /transcriptomic 2nd day (Aharonovich and Sher, 2016)	*R. pomeroyi* /exo-proteome in enriched and natural seawater (Christie-Oleza et al., 2015)				*Marinobacter* in phycosphere (Lupette et al., 2016)	*Phaeobacter* parasitism in enriched media (Wang et al., 2016)	*R. shibae* in enriched media /metabolomics (Paul et al., 2013)	
	Bactrial collection on plate testingoxidative stress (Morris et al., 2008; Zinser, 2018)	*R. pomeroyi* /cellular proteome in natural seawater (Christie-Oleza 2017b)	*Vibrio* in enriched media/transcriptomics (Tai et al., 2009)				*Pseudoaletromonas* in enriched media /metabolomics (Harvey et al., 2016)	*R. pomeroyi* in enriched media /transcriptomics 10th days (Durham et al., 2017)	
		R. pomeroyi /exo-proteome in enriched seawater (Kaur et al., 2018)							

The current co-culture set-up in this study was performed in both nutrient-enriched seawater (*i.e.* the media that was favourable for the growth of each phototrophic species) and in natural oligotrophic seawater derived from the Sargasso Sea, one of the most nutrient limited environments (Menzel and Ryther, 1961). This oligotrophic condition is ideal for studying the nutritional interaction of the organisms and, ultimately, their metabolic coupling, mimicking the natural limiting conditions that the organisms commonly found in the environment.

1.8: Project objectives and hypothesis

Focusing on phototroph-heterotroph interactions, a selection of diverse species from both groups were tested in co-cultivation, extending our understanding from the previously published system described in Christie-Oleza *et al*. (2017b) using the model species *Synechococcus* sp. WH7803 and *R. pomeroyi*. The main findings of the study of Christie-Oleza *et al*. (2017b), summarized by Simon (2017) in Figure 1.8.1, describes the main reasons for the long-term survival of phototroph in co-culture conditions. In the axenic culture, the accumulation of nitrogen-rich dissolved organic matter (DOM) reaches toxic levels detrimental for the survival of *Synechococcus* sp. WH7803. Since the DOM is decomposed and recycled by *R. pomeroyi* as ammonium and phosphorous as phosphate, *Synechococcus* survives in co-culture.

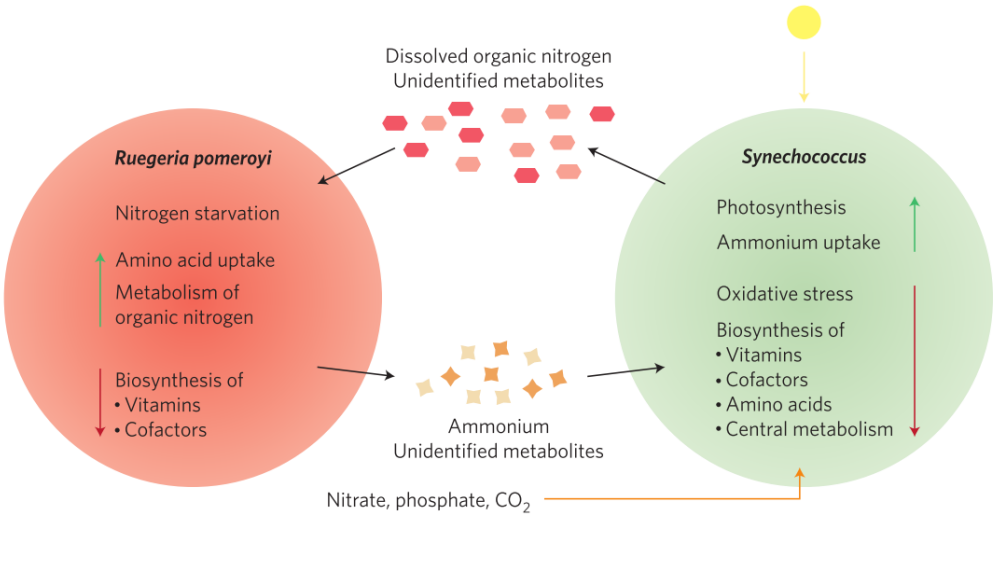


Figure 1.8.1: Mutual interaction between *Synechococcus* sp. WH7803 and *R. pomeroyi* based on nutrient exchange, allows the long-term survival of both in co-culture conditions. *Synechococcus* through photosynthesis releases nitrogen-rich DOM, which is remineralized by *R. pomeroyi* to ammonium releases, which is then used by *Synechococcus*. Both organisms exhibit distinct proteomic features being upregulated (upwards green arrows) and downregulated (downwards red arrows) in the co-culture as compared to monocultures. These features include the metabolism of vitamins,

cofactors and amino acids, and imply that metabolites, not assessed in the study by Christie-Oleza and colleagues, are also being exchanged (credits on Simon, 2017).

In this way, we hypothesised that a generalised mutualistic interaction will occur between most phototrophic and heterotrophic species independent of nutrient availability. The project is divided into two major objectives to address this main working hypothesis:

1) To characterise the interactions that arise from the one-to-one co-cultivation of nine different relevant phototrophs with 14 heterotrophs based on population densities and survival during extended incubation periods in both nutrient-enriched and natural oligotrophic seawater (Chapter 3).
2) To determine the proteomic response of the model heterotroph *R. pomeroyi* to the presence of each one of the nine phototrophs (Chapter 4) and each phototroph to the presence/absence of the heterotroph (Chapter 5) incubated under oligotrophic conditions. We hypothesise that, despite interactions having an overall generic response, species-specific metabolic variations will be detected allowing a deeper understanding of marine phototroph-heterotroph interactions.

Chapter 2

Materials and Methods

2.1 Cell preparation for co-cultures

Nine phototrophic and 14 heterotrophic species (Tables 1.7.1 and 1.7.2) were used in one-to-one phototroph-heterotroph co-culture experiments.

2.1.1: Phototroph cultures

Batch cultures were routinely grown in 20-40 ml final volumes contained in 50 ml tissue culture flasks with vented caps for gas exchange (Sarstedt). Cultures were incubated between 15-22°C under constant light (10 µmol m^{-1} s^{-1}) with shaking (140 rpm, unless stated; Table 2.1.1.1). The optimal conditions and enriched media (ESW) used for each phototrophic species is detailed in Table 2.1.1.1.

Table 2.1.1.1: The main cultivation characteristics of nine phototrophic species used in the current PhD thesis

Cultivation characteristics									
PHOTOTROPHS	Reference name in this thesis	Source	Axenic from source	Medium	Batch culture volume (ml)	T (°C)	Light intensity(µmol photons m-1 s-1)	Shaking (rpm)	Succesful concentration of antibiotic mix
Marine Picocyanobacteria	*Prochlorococcus*	Q.Zeng group	Yes	Pro99	40	20	9	140	
	Synechococcus WH7803	D.Scanlan group	Yes	ASW	20	22	10	140	
	Synechococcus WH8102	D.Scanlan group	Yes	ASW	20	22	10	140	
Green Algae	*Micromonas*	Roscoff culture collection	No*	K	40	20	9	140	5%
	M. pusilla	C.Broussard group	No*	K/ASW	40	15	9	No	**1%
	Ostreococcus	Roscoff culture collection	No*	K	20	20	9	140	10%
Haptophyceae	*E. huxleyi*	Roscoff culture collection	No*	K	20	20	10	140	1%
Diatoms	*Thalassiosira*	T.Mock group	Yes	F/2	20	22	10	140	
	Phaeodactylum	T.Mock group	Yes	F/2	20	22	10	140	

* Axenisation was achieved in the terms of the current project
** only five out of the eight antibitiocs were mixed (details can be found in Table 2.1.1.3)

The four different ESW media were: artificial seawater (ASW), K-media, F/2 media and Pro99 (Table 2.1.1.2). Seawater suitable for plant cell culture (Sigma-Aldrich) was used when required.

Table 2.1.1.2: Enriched media (ESW) nutrient composition, used in different phototrophic cultures (see Table 2.1.1.1). The final concentration is presented for each nutrient in the four media Pro99, ASW, K and F/2.

ASW		**K media**		**F/2 media**		**Pro99**	
in 1L Milli-Q Water		*in 1L Sigma SW*		*in 1L Sigma SW*		*in 1L Sigma SW*	
$NaNO_3$	8.82 10-3 M	$NaNO_3$	8.82x10-4 M	$NaNO_3$	8.82x10-4 M		
K_2HPO_4	1.31 10-4 M	Na2 b-glycerophos	1x10-5 M	$NaH_2PO_4*H_2O$	3.62x10-5 M	$NaH_2PO_4*H_2O$	5 10-5 M
NaCl	4.3 10-1 M	NH_4Cl	5x10-5 M	$Na_2SiO_3*9H_2O$	1.06X10-4 M	NH_4Cl	8 10-4 M
$MgCl_2$	9.83 10-3 M	Tris base (pH7.2)	1X10-3 M				
KCl	3.35 10-3 M						
$CaCl_2$	1.7 10-3 M						
$MgSO_4$	1.42 10-2 M						
Tris (methylamine)	9 10-3 M						
Trace metals		Trace metals		Trace metals		Trace metals	
$FeCl*6H_2O$	1.1 10-2 M	$FeCl_3*6H_2O$	1.17 10-5 M	$FeCl_3*6H_2O$	1.17 10-5 M	$FeCl_3*6H_2O$	1.17 10-6 M
EDTA (Na_2Mg)	1.39 10-3 M	$Na_2EDTA*2H_2O$	1.11 10-4 M	$Na_2EDTA*2H_2O$	1.17 10-5 M	$Na_2EDTA*2H_2O$	1.17 10-6 M
$MnCl_2*4H_20$	9.1 10-3 M	$MnCl_2*4H_20$	9 10-7 M	$MnCl_2*4H_20$	9 10-7 M	$MnCl_2*4H_20$	9 10-8 M
$ZnSO_4*7H_20$	7.7 10-4 M	$ZnSO_4*7H_20$	8 10-8 M	$ZnSO_4*7H_20$	7.65 10-8 M	$ZnSO_4*7H_20$	8 10-9 M
$Co(NO_3)_2*6H_2O$	1.7 10-4 M	$CoCl_2*6H_2O$	5 10-8 M	$CoCl_2*6H_2O$	4.2 10-8 M	$CoCl_2*6H_2O$	5 10-9 M
$Na_2MoO_4*2H_2O$	1.6 10-3 M	$Na_2MoO_4*2H_2O$	2.6 10-8 M	$Na_2MoO_4*2H_2O$	2.6 10-8 M	$Na_2MoO_4*2H_2O$	3 10-9 M
$CuSO_4*5H_2O$	3 10-5 M	$CuSO_4*5H_2O$	1 10-8 M	$CuSO_4*5H_2O$	3.93 10-8 M	$NiSO_4*6H_2O$	1 10-8 M
H_3BO_3	4.6 10-2 M	H_2SeO_3	1 10-2 M			Na_2SeO_3	1 10-8 M
		Vitamins		Vitamins			
		thiamine *HCl	1.48 10-7 M	thiamine *HCl	1.48 10-7 M		
		biotin	1 10-9 M	biotin	1 10-9 M		
		cobalamin	1.85 10-10 M	cobalamin	1.85 10-10 M		

Phototrophs were transferred pouring 30% of an old culture into fresh sterile enriched medium: *Prochlorococcus* every 7 days, green algae every 10 days and the rest every 20 days. Axenic cultures were checked for contamination on contamination and marine broth (MB) plates. Contamination plates were prepared by dissolved 0.8 g of yeast extract and 10 g of agar in 1 L of ASW, and marine broth (MB) plates by dissolving 37.4 g of Marine Broth (Difco) and 10 g of agar in 1 L of boiling water. Both media were autoclaved, poured in sterile 92 x 16 mm transparent polystyrene Petri dishes (Sarstedt) and kept at 4°C when dried. After streaking the phototrophic cultures on the plates they were sealed with parafilm (Parafilm M, Bemis) and kept for at least one month at 22°C (±1°C) after which colony growth was checked, indicating the contaminated cultures.

Prokaryotic phototrophs were checked after partial sequencing of the 16S rRNA sequence, using primer 16S-1492R on a PCR amplification of the gene (*i.e.* using primers 16S-27F (AGA GTT TGA TCC TGG CTC AG) and 16S-1492R (GGT TAC CTT GTT ACG ACT T), melting temperature 58 °C and detailed conditions previously described; Lane, 1991). Sequencing was carried out by GATC, Biotach AG (Konstanz, Germany). Eukaryotic phototrophs were checked after partial sequencing of their 18S rDNA

using primer 18S-1818R on a PCR amplification of the gene (*i.e.* using primers 18S-63F (ACG CTT GTC TCA AAG ATT A) and 18S-1818R (ACG GAA ACC TTG TTA CGA), melting temperature 58 ^{o}C and detailed conditions previously described; Lepere *et al*., 2011).

Each phototrophic species was kept in clonal axenic cultures. Nevertheless, the majority of the phototrophic species were axenised during the current project since they were received in non-axenic cultures from diverse sources (see Table 2.1.1.1). The axenising process was initiated by testing the sensitivity of each phototrophic strain to nine different antibiotics in ratio 1:100 (the list of antibiotics and initial concentration can be found in Table 2.1.1.3). Cultures were kept for one week after which they were examined visually for resistance (no reduction of the pigmentation of the culture). A mix of the antibiotics with no detrimental effects on the phototroph (Table 2.1.1.3) was prepared and added in different concentrations to the non-axenic phototrophic cultures (tested ratios of antibiotic mix to culture; 1:4, 1:10, 5:100; 1:100).

Table 2.1.1.3: List of antibiotics included in the axenisation process of the phototrophic cultures.

Antibiotics	Initial concentration	Antibiotic mix
Nalidix Acid	50 mg/ml	√*
Neomycin	50 mg/ml	√
Kanamycin	50 mg/ml	√*
Streptomycin	50 mg/ml	√
Spectinomycin	50 mg/ml	√
Apramycin	50 mg/ml	√
Ambicillin	100 mg/ml	√
Gentamicin	10 mg/ml	√*

* Excluded by the antibiotic mix used in *M. pusilla*

The cultures that survived after one week (no reduction of the pigmentation of the culture) were checked for contamination (*i.e*. bacterial growth) on LB, marine broth and contamination plates and then were subcultured with fresh sterile ESW containing the same antibiotic concentration. The axenisation process was continued for at least 3 weeks. The axenised cultures with the minimum antibiotic concentration were selected and sub-cultured in antibiotic free media for 4-months prior to their inoculation in the co-culture experiments, ensuring the elimination of antibiotic presence in the

cultures. Absence of bacteria in eukaryotic axenic cultures was checked by 16S rDNA amplification (see conditions above) and routinely monitored by plating on contamination and marine broth plates.

2.1.2: Heterotrophic cultures

The heterotrophic strains were preserved in 25% glycerol stocks in -80°C and grown on the corresponding MB or LB plates (LB: 10 g tryptone, 5 g yeast extract, 10 g NaCl and 15 g agar dissolved in 1L of water) at 28°C for 1 to 6 days, depending on the species (Table 2.1.2.1). Strains were checked after sequencing their partial 16S rDNA as described above. One colony was used to inoculate the corresponding liquid media (*i.e.* 3 ml of media in 15 ml of polypropylene conical falcon tubes) and incubated at 28°C under shaking at 140 rpm for 1 to 6 days (late exponential phase).

Table 2.1.2.1: Cultivation characteristics of 14 heterotrophic strains. Included pictures represent the CFUs diversified by milky colour medium size CFUs (*Salinispora* picture should have been included).

Organism	Medium	Growth on plate/liquid: Slow (S), Medium (M), Fast (F) (days)	Density of liquid cultures (ml of harvested volume)	Colony formations (pictures taken in this project)
Salinispora	MB	S/S (6/5)	Low (8ml)	
Aeromicrobium	MB	S/S (5/5)	Low (8ml)	
Polaribacter	MB	F(but dies fast on plate)/F (2/3)	High (2ml)	
Algoriphagus	MB	F/F (2/2)	Medium (4ml)	
Gramella	MB	M/F (4/2)	Medium (4ml)	
Formosa	MB	F(dies fast on plate/F (2/2)	Medium (4ml)	Yellow CFUs
Ruegeria or *R. pomeroyi*	MB	M/F (3/2)	High (2ml)	
R. denitrificans	MB	M/M (3/3)	High (2ml)	
D. shibae	MB	M/M (3/3)	High (2ml)	
Alteromonas	MB	F/F (1/2)	High (2ml)	
Marinobacter	MB	F/M (2/3)	Medium (4ml)	
Pseudomonas	LB	F/F (1/2)	High (2ml)	
E. coli	LB	F/F (1/2)	High (2ml)	
Verrucomicrobiae	MB	S/S (6/5)	Low (8ml)	

2.2 Co-culture set-up

The one-to-one phototroph-heterotroph co-cultures (126 combinations, Figure 2.2.1) and the corresponding mono-cultures were performed in triplicate in two different media: nutrient-enriched seawater (ESW; Table 2.1.1.2) and the natural oligotrophic seawater (SW originated from the Gulf Stream in the Gulf of Mexico; Sigma-Aldrich).

Phototroph and heterotroph cells were harvested via centrifugation (15 min at 4,000 g using an Eppendorf Falcon Benchtop centrifuge,) and washed twice in sterile SW. Cells were then resuspended in SW and used to inoculate the co-culture experiments, in aimed cell densities in SW cultures; 10^6 cells/ml for bacteria, 10^5 cells/ml for cyanobacteria and 10^4 cells/ml for eukaryotes mimicking natural seawater concentrations (Mühling *et al*., 2005, Azam and Malfatti, 2007, Godhe *et al*., 2008). In ESW co-cultures, cell densities were 2-orders of magnitude higher than those aimed for in SW (Li *et al*., 1992, Grossart, 1999, Aharonovich and Sher, 2016, Diner *et al*., 2016). For the long-term monitoring of interactions (Chapter 3), the resulting 810 cultures (126 combinations plus 9 mono-cultures, in triplicates and in two different media; Figure 2.2.1) were performed in 50 ml tissue culture flasks each containing 30 ml of medium. Those cultures set-up for the proteomic analyses (Chapters 4 and 5) were carried out in 100 ml of SW in sterile 250ml, narrow neck, borosilicate glass Erlenmeyer flasks (Bomex) closed with sterile cotton wool and covered with two layers of aluminium foil. After inoculation, cultures were incubated under optimal conditions for each phototroph (Table 2.1.1.1) for 3 weeks in the case of the proteomics experiment and for 19 weeks in the survival experiments. Details of the different co-culture set-ups can be seen in Table 2.2.1.

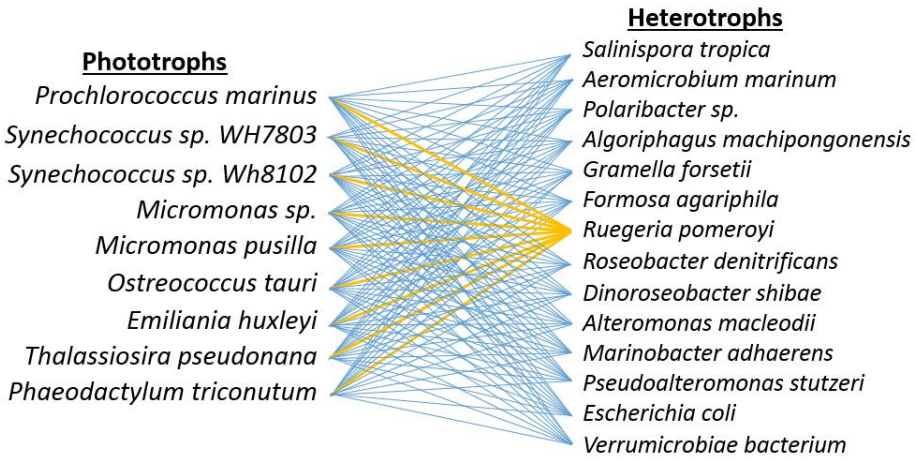


Figure 2.2.1: Co-cultivation depicting the 126 combinations between 9 phototrophs and 14 heterotrophs testing their survival over 19 weeks in both SW and ESW (Chapter 3). Yellow lines represents the co-culture combinations in SW analysed by high-throughput proteomics in Chapters 4 and 5.

Table 2.2.1: Differences among three experimental co-culture setups.

Co-culture experimental differences			
Co-culture experiments:	**Survival in SW**	**Survival in ESW**	**Proteomics experiments**
Results in:	**Chapter 3**		**Chapter 4 and 5**
Seawater type	SW	ESW	SW
Flask type	Falcon tissue culture flasks	Falcon tissue culture flasks	Erlenmeyer flask
Number of phototrophic species tested	9	9	9
Number of heterotrophic species tested	15	15	1
Controls	Phototrophs axenic	Phototrophs axenic	Phototrophs axenic, *R.pomeroyi* mono-culture
Total number of co-cultures	126	126	9
Phototroph inoculated volume (targeted cell concentration)	250 µl (1E+05 cells/ml)	6ml (1E+06 cells/ml)	1ml (1E+05 cells/ml)
Heterotroph inoculated volume (targeted cell concentration)	5µl (1E+07 cells/ml)	100µl (1E+08 cells/ml)	16µl (1E+07 cells/ml)
Total culture volume (ml)	30	30	100
Sampling volume for cell abundance measurements	200µl	200µl	200µl
Sampling volume for proteomics	-	-	100ml
Cell abundance sampling frequence	2-3rd week	2-3rd week	Day 0 and 3rd week
Experimental duration (weeks)	19	19	3

In parallel to the co-cultures, the corresponding control cultures were prepared by ignoring the step of either the heterotrophic species inoculation, describing the axenic phototrophic cultures, or the phototrophic species inoculation, describing the mono-culture for the *R. pomeroyi* in the proteomic experiment in Table 2.2.1.

2.3 Cell abundance measurements

Co-cultures were opened in regular intervals for sampling under strict sterile conditions. The extracted volume for cell abundance measurements was 200 µl. The frequency of sampling was every 2-3 weeks during the whole experimental period (19 weeks) in the survival experiments and only at the beginning (Day 0) and end (3rd week) in the proteomics experiment. An increase in the sampling frequency and volume would have shortened the experimental duration of the survival experiments, since with the current set-up the final volume of the culture in the end of the 19th week has been reduced to half of the initial (30 ml).

2.3.1: Phototrophs

Phototroph cells concentrations were measured twice (technical replicates) by flow cytometry (BD LSRFortessa Cell Analyzer from the WISB facilities). This method determines various physical properties of single particles (e.g. size, shape and fluorescence) as they pass through the flow cell. The specific laser excitation and bandpass emission fluorescence filter was different for each phototroph due to their different cell physiologies, *i.e*. cell size and pigment fluorescence (Table 2.3.1.1).

Table 2.3.1.1: Fortessa flow cytometer laser settings for measuring the cell abundance of four different phototrophic groups.

Flow cytometer laser settings				
Laser (Volt)	*Prochlorococcus*	*Synechococcus*	Green algae	Diatoms
FSC	540	659	540	200
SSC	200	184	200	150
B488-530/30 nm	600	500	600	450
B488-710/50 nm	600	410	600	260
R640-670/14 nm	955	445	955	300
V405-610/20 nm	606	418	606	290
Threshold in 200	FSC	B488-710/50	FSC	
Volt:			B488-710/50	

Cultures were diluted with SW (dilution factor) targeting a flow rate less than 1000 events per second. Phototrophic cells were measured with BD FACSDiva Software, in specific gates in 2-dimension graphs of the lasers presented in Table 2.3.1.1. Phototrophic ell counts were normalised to known bead standards included in the samples (*i.e.* 2,000 particles/ml; 1.7-2.2 μm Polystyrene High Intensity Sphero Nile Red Fluorescent particles, BD Biosciences), measured in the corresponding gate. Bead stock density was measured on a Neubauer's chamber haemocytometer under a fluorescent microscope and maintained in dark cold (4°C) conditions. The phototrophic cell concentration of each sample was calculated based on the measured cell abundance, as follow:

- Every run included known sample volume (V_1 considering the applied dilution factor) derived from the initial cultures with unknown concentration (C_1).
- In each run, beads were added at a final concentration 2.000 beads/ml of sample.
- The final volume of the run was 1 ml (V_2).
- y phototrophic cell events were counted up to 100 beads events (deriving from 2.000 beads/ml concentration of the sample). The concentration of phototrophic cells (C_2) in the sample is:

$$C_2 \text{ (cells ml}^{-1}\text{)} = \text{y phototrophic events} * 2.000 \text{ beads ml}^{-1} / 100 \text{ beads}$$

$$C_2 = y * 20 \text{ cells ml}^{-1}$$

Hence,

$$C_1 * V_1 = C_2 * V_2$$

$$C_1 = \text{y events} * 20 \text{ cells ml}^{-1} * 1\text{ml} / V_1$$

All the samples were measured twice (technical replicates), and the lower limit of detection of the cell abundance was 10^2 cells/ml.

2.3.2: Heterotrophs

Heterotrophic cell abundance was measured as colony forming units (CFUs) in 10 μl droplets from each one of the 1:10 dilution to extinction series (Miles and Misra, 1938). Each dilution was pipetted in triplicate on solid media plates and incubated at 28°C for 2 to 6 days. The heterotrophic cell

concentration of each sample was calculated based on the measured CFUs and its dilution factor (DF), as described below:

$$\text{Heterotrophic cell concentration} = \text{CFUs} / 10^{-2}\text{ml} * \text{DF}$$

The lower limit of detection of the cell abundance was 10^2 cells/ml.

2.4 Proteomics analysis

2.4.1: Sample preparation and nanoLC-MS/MS analysis

Shotgun proteomic analyses of cellular extracts of *R. pomeroyi* - phototroph co-cultures and their corresponding control cultures in SW were carried out, testing the metabolic universality of the model organism *R. pomeroyi* (Christie-Oleza *et al*., 2017b) in a variety of interactions. Cells were collected by centrifugation (4,000 g for 15 min at 4°C; Eppendorf Falcon Benchtop centrifuge) from 100 ml cultures after three weeks incubation. Cell pellets were resuspended in 45 µl of 1x NuPAGE Lithium dodecyl sulfate (LDS buffer; Invitrogen) and incubated for 10 min at 95°C with shaking (650 rpm, dry bath AccuTherm Microtube Shaking Incubator) to lyse and denature the proteins. 30 µl of this sample were loaded on a precast NuPAGE polyacrylamide gel (10% Bis-Tris Gel; Invitrogen, USA) using 3-(N-morpholino)propanesulfonic acid (MOPS; Invitrogen) as the running buffer. SDS-PAGE at 200 V run in an electrophoresis chamber (Mini Vertical Dual Plate Electrophoresis unit) was allowed for a short migration of the entire proteome into the gel (5 mm). The polyacrylamide gel was stained with SimplyBlue SafeStain (Invitrogen; compatible for protein gel analysis through mass spectrometry) and the gel run containing the proteomes were excised and standard in-gel reduction with dithiothreitol (breaking disulfide bonds) and alkylation with iodoacetamide (preventing the reforming of disulphide bonds) were performed prior to trypsin proteolysis (Armengaud, 2010). The resulting tryptic peptide mixture was extracted using 5% formic acid in 25% acetonitrile and concentrated at 40°C in a speed-vac. For mass spectrometry, the samples were resuspended in 2.5% acetonitrile containing 0.05% trifluoroacetic acid (ionizing the peptides) and filtered using a 0.22 µm pore size cellulose acetate spin column 16,000 g for 5 min in order to eliminate undissolved aggregates (Christie-Oleza *et al*., 2017a). 20 µl of sample were analysed by nanoLC-ESI-MS/MS using an Ultimate 3000 LC system (Dionex-LC Packings) coupled to an Thermo Orbitrap Fusion mass spectrometer (Thermo Scientific) using the settings previously described by Christie-Oleza *et al*. (2015). Each sample was analysed over a 2 h LC

separation through the precolumn (Acclaim PepMap µ-precolumn cartridge 300 µm i.d. x 5 mm 5 µm 100 Å), eluted into the analytical column (Acclaim PepMap RSLC 75 µm x 50 cm 2 µm 100 Å (Thermo Scientific) by increasing the mobile phase concentration, where the tryptic peptides were separated in a gradient concentration of mobile phase with acetonitrile from 2% to 40% to 90% and back to 3% over mobile phase buffer by reverse phase chromatography, with settings previously described by Christie-Oleza *et al*. (2017b).

2.4.2: Proteomics data analysis

The mass spectrum files were processed using the software package for shotgun proteomics MaxQuant version 1.6.0.1 for Windows 10 (Tyanova *et al*., 2016a) to identify and quantify protein using the UniProt databases of the nine phototrophic species and *R. pomeroyi* (last updated on 4th of February 2017). Mitochondrial and chloroplast encoded proteins were manually added to the databases of the six eukaryotic phototrophs. Samples were matched between runs (match time window between the spectra was 0.7 min) and the other parameters were set by default in MaxQuant. Spectral mapping for each peptide was performed without normalisation since the number of proteins in the tested samples was unequal. Unique/razor peptide quantification was used, adding the razor peptides in the protein group with the most abundantly detected protein.

The bioinformatic analysis pipeline was completed using the software Perseus version 1.5.5.3 for Windows 10 (Tyanova *et al*., 2016b). This software allows a robust statistical analysis of the proteomic identifications, *i.e*. two-sample and multisample tests, and a calculation of permutation-based false-discovery rates with *q*-value estimation (Tyanova *et al*., 2016b). Relative protein abundance was obtained using a Label free quantification (LFQ) method of the protein intensities from each sample after normalization to protein size (Tyanova *et al*., 2016a). The LFQ method enables an accurate proteome-wide quantification of not labelled proteins by normalizing the intensities according to the algorithms described in Cox *et al*. (2014), through a matrix of the number of samples and number of protein groups as dimensions. The missing values were imputed using the default parameters. Protein quantification and calculation of statistical significance were carried out using two-sample Student's t test using a permutation-based false discovery rate (q = 0.05). The statistical analysis tested the proteomic profile of a co-culture condition with the corresponding control, all in triplicates.

2.4.3: Protein categorisation

The extracted proteins were categorised into metabolic pathways, revealing the magnitude of response of different pathways in the co-culture treatments. This process was initiated by ordering the proteins according to the Ensebl protein ID (ENSP), pointing at proteins derived from the same gene-operons. Then the metabolic functionality of the proteins was defined based on the extracted information of each protein by the Uniprot database, *i.e*. gene name, protein name, Kegg name, Kegg pathway. In many cases, the extracted information and the order of the ENSP number were not descriptive enough. For these proteins a manual curation using Blast in the Conserved Domain search tool from National Centre for Biotechnology Information (NCBI) and Kegg2 platform, were performed. The challenge in the classification process was the diversity of information derived from the different platforms, for which manual literature search was also performed. In addition, some proteins may participate in several different pathways. The uncertainty of the protein functions in parallel to the absence of an internationally accepted method of protein categorisation restricted the current analysis to some targeted metabolic pathways.

Classification of the proteins was followed by calculation of three values for each metabolic pathway: relative abundance, significance and fold change. Firstly, the relative abundance of each protein was calculated based on its LFQ intensity normalised by protein length. The average and the standard deviation of each protein per biological triplicates was calculated. After the protein categorisation in metabolic pathways, a sum of the relative abundances of the proteins involved in each pathway was calculated. The significant regulation of each protein in the co-culture treatment compared to the corresponding control was based on q-values generated during use of a Perseus statistical test. After the categorisation of the proteins into metabolic pathways, the percentage of the included proteins with significant regulation (q-value < 0.05) were confidently considered. Differential regulation of the proteins in the co-culture compared to the mono-culture, known as fold change values, were displayed by Student's T-test Differences in Perseus software. These values were converted to a $\log_{10}$ scale to fit a normal distribution, ranged between negative values (down-regulated proteins) and positive values (up-regulated). This normal distribution of the values allowed the calculation of average values, representing the regulation of a group of proteins categorised in metabolic pathways.

Chapter 3

Evaluation of phototroph - heterotroph interactions based on population behaviour in co-culture

3.1 Introduction

A basic issue in ecology is understanding the underlying principles of how organisms can coexist in severely nutrient-limited ecosystems in comparison to the nutrient-replete conditions of the lab (Simon, 2017). The main nutrients that affect microbial biomass formation in nature are nitrogen, iron and/or phosphorus (Simon, 2017). The general belief is that phototrophic and heterotrophic organisms compete for these elements (Thingstad *et al*., 1993), but also cooperate in order to optimize the use of such limiting nutrients, constructing microbial communities in nutrient-depleted pelagic ecosystems (Seymour *et al*., 2017). The stimulation of microbial activity by extracellular products released by the phytoplankton was first studied by Bell *et al*. (1974), suggesting the 'phycosphere' as a useful concept in discussing bacteria-phytoplankton interactions in aquatic environments (Smriga *et al*., 2016). Sapp *et al*. (2007a) showed that the phycosphere is a niche where bacteria specifically benefit from exudates, and which is targeted by a wide range of bacterial species (Grossart *et al*., 2005, Grossart *et al*., 2006). Each phototrophic species under each physiological state releases different organic compounds, stimulating different bacterial responses (Grossart and Simon, 2007). The demanding growth of phytoplankton (*i.e*. due to the burden of their large and complex photosynthetic apparatus and carbon fixation) is a balanced function of: 1) the availability of macro- and micro-nutrients, 2) physical parameters such as light, temperature, and osmotic pressure, 3) trace organic substances which may be inhibitory or stimulatory, and 4) the genetic potential for growth inherent in the phytoplankton (Frey and Small, 1980). These optimum conditions for growth vary per species, facilitating species succession and distribution in the environment (Rhee, 1982).

Despite the close link between bacteria and phytoplankton, the way that different species interact with each other at the community level is not clear and quite difficult to dissect (Rooney-Varga *et al*., 2005). Simplified communities, *i.e.* one-to-one phototroph-heterotroph co-cultures, are becoming popular to better understand microbial interactions (reviewed in Table 1.7.3). The model study of Christie-Oleza *et al*. (2017b) addressed this question with regard to nutrient availability and recirculation by co-cultivating the cosmopolitan photoautotrophic picocyanobacterium *Synechococcus* sp. WH7803 with the multifunctional heterotrophic bacteria, *R. pomeroyi* DSS-3, known for its significance in marine pelagic ecosystems (Moran *et al*., 2004). In axenic culture, *Synechococcus* sp. WH7803 died after 4-6 weeks due to the absence of bacteria that could re-

mineralise organic nutrients and supply inorganic nutrients to the phototroph. However, the *Synechococcus-R. pomeroyi* co-culture was able to survive over 4 months, through nutrient recycling, depicted as a form of mutualism.

In this chapter, the model co-culture system from Christie-Oleza *et al*. (2017b) was extended to nine phototrophic species co-cultivated in one-to-one combinations with 14 different heterotrophic bacteria. The aim was to examine the generalisation of the recorded positive interaction of the model system, since cooperation, competition, commensalism and parasitism have also been detected among algae and bacteria depending on the physiological status of the organisms (Grossart, 1999). Different interactions were detected in the current project with regards to diverse nutrient availability. The interpretation of individual interactions at the micro-scale (Seymour *et al*., 2010), contributes to understanding of the structure, function and diversity of complex microbial communities in nature (Hibbing *et al*., 2010, Paul *et al*., 2013, Aharonovich and Sher, 2016).

3.2 Materials and methods

The one-to-one species co-culture set-up for testing the survival of the organism under two different seawater types was thoroughly described in Section 2.2 The synopsis of the set-up along with the sampling process which facilitated a regular cell abundance estimation of the two organisms co-existing in co-cultures, is presented in Figure 3.2.1.

Experimental set-up for testing the survival of the phototrophs and heterotrophs in one-to-one species co-cultures:

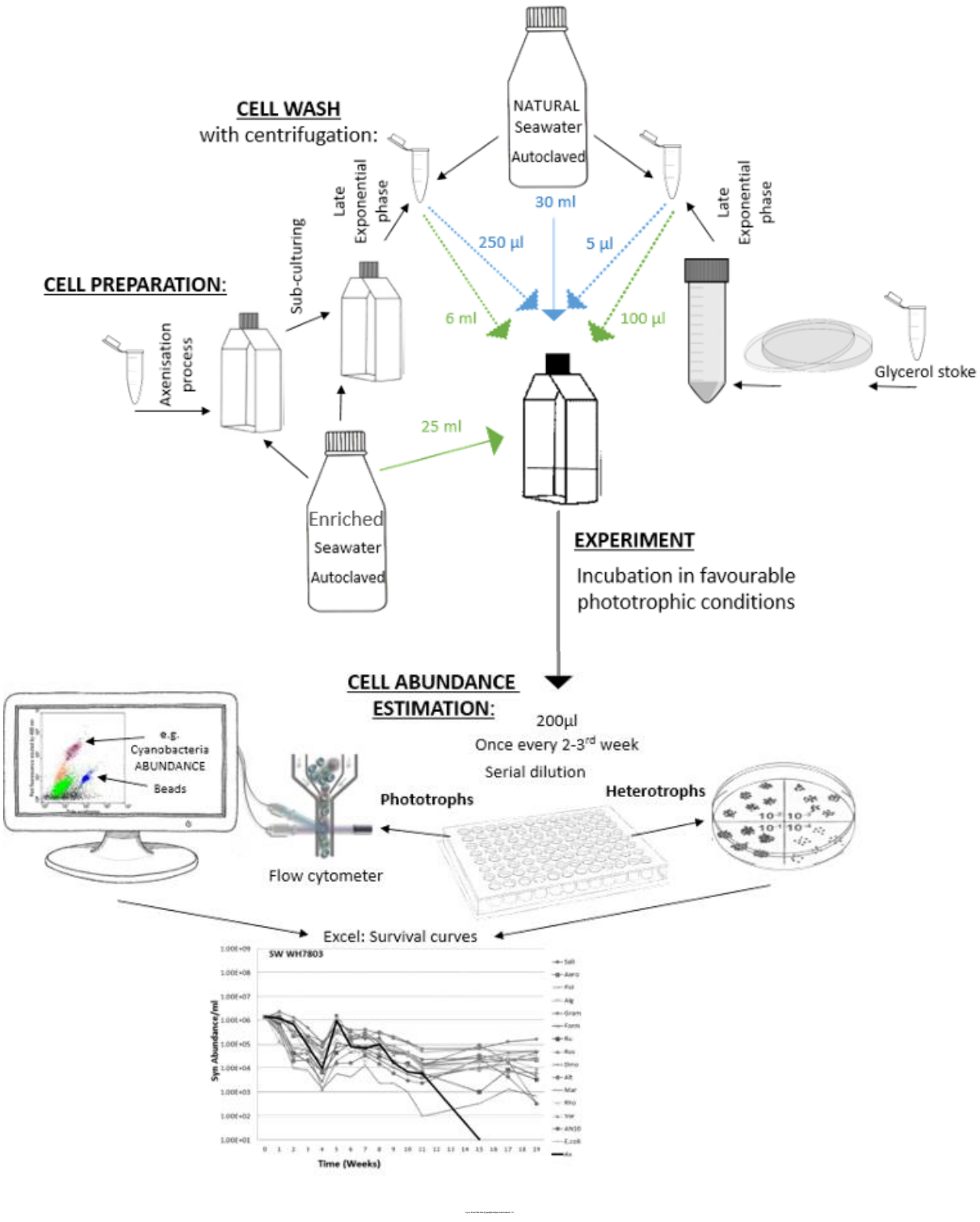


Figure 3.2.1: Experimental representation of the one-to-one co-culture set-up for testing in triplicates the survival of the 14 heterotrophs and 9 phototrophs in every possible combination over 19 weeks in two different seawater types, SW (blue arrows) and ESW (green arrows). Main steps included cell preparation, cell wash, experiment, cell abundance estimation.

3.2.1: Statistical analysis of cell abundance

Population levels were statistically tested, indicating the treatments with significant impact on the performance of each organism. Cell abundance measurements formed two data-categories (see Appendix Table 7.1 to 7.9) regarding the models that they were fitted to:

i. populations survived throughout the whole experimental period were tested with one-way ANOVA, applying the hypothesis that the treatments did not cause any effect on the achieved population levels.
ii. population data where a lethal event occurred, was tested using binary logistic regression, determining the effect of three independent variables, the co-culture treatment, the sampling points and the cell abundance measurements, on predicting the dependent variable, survival.

The applied hypothesis of each statistical test was rejected by a p-value lower than 0.05. Due to the fact that the statistical tests were applied in a set of results and not between two treatments, it was hard to define the treatments that cause the rejection of the tested hypothesis. For the groups tested by one-way ANOVA, the Least-Significant-Difference (LSD) formula was used, determining the treatments with the most signifucant differences compared to the rest.

The LSD formula is:

$$T.Inv2T(0.05,DF) * SQRT(MS(1/n_i + 1/n_{ii})$$

This is based on the information extracted by the one-way ANOVA test, as explained below:

*Two-tailed inverse of a student t-distribution (probability, degree freedom) * square root of (mean square error*(1/counts of group i + 1/counts of group ii)*

The extracted LSD value greater than the Absolute value (Average group i - Average group ii), determined the rejection of the hypothesis for similarity between group i and ii. In the current project, as group i and ii were determined two different treatments. This comparison was repeated for every treatment in a set of results. Finally, a percentage of significant differences achieved by the individual treatment compared to the rest of the treatments was calculated. For instance, a high percentage of difference showed the distinction of this treatment from the rest either due to its very low or very high population level measurements.

The statistical tests were applied on specific set of results (as presented in Figure 3.3.3.1.a, b and c), questioning:

a) Each phototroph cell abundance achieved in 14 heterotrophic treatments, determining the effect of the presence of different heterotrophs on the cell abundance levels of each phototroph,
b) The cell abundance of 14 heterotrophs achieved in the same treatment, determining the effect of the presence of one phototroph on the cell abundance levels of different heterotrophs, since the cultures were set-up per phototroph and the heterotrophic population levels are comparable among the different species. Similar tests could not be applied for the phototrophs, due to the diverse population levels of the participating phototrophic species, such as eukaryotic and prokaryotic population differences (Azam and Malfatti, 2007).
c) Each heterotroph cell abundance achieved in 9 phototrophic treatments, determining the effect of the presence of different phototrophs on the cell abundance levels of each heterotroph.

3.3 Results

The growth of 23 organisms (9 phototrophs and 14 heterotrophs, whose details are summarised in Table 1.7.1 and 1.7.2) as measured in one-to-one co-cultures over 19 weeks in both oligotrophic natural seawater (SW) and the nutrient-enriched seawater (ESW; optimal media routinely used to grow each one of the phototrophic organisms). Based on the detected cell abundances, population curves were constructed, as presented in Figure 3.3.1, facilitating the determination of the impact of the co-existence on population levels and survival of each organism. All raw data of cell abundance measurements for all the cultures, in SW and ESW, can be found in the Appendix (Tables: 7.1 to 7.9), including the population curves of *Synechococcus* WH8102 in SW and ESW (Appendix Figure 7.1).

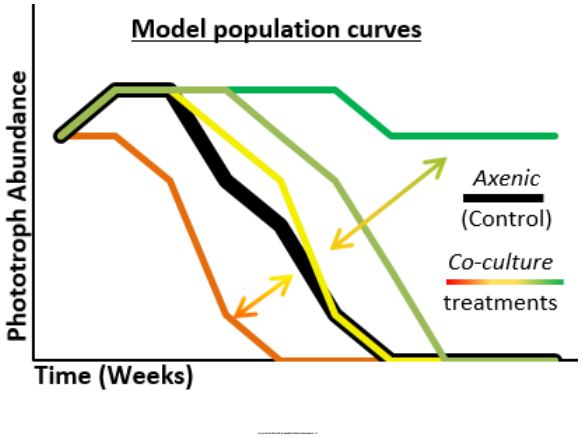


Figure 3.3.1: Model population curves. The four representative patterns of phototrophic survival curves detected when grown in co-cultured with different heterotrophs compared to growth in axenic culture (control, black). Death of the culture was assessed as i) before the control (negative interaction, orange), ii) at the same time as the control (neutral interaction, yellow), or iii) later than the control (semi-positive interaction, light green), and iv) no death (positive interaction, green). Arrows represent the plasticity of the curves, causing an effect on the interaction categorisation.

3.3.1: Interaction categorisation based on the survival of the phototroph

Interactions were categorised based on the extension or shortening of the survival of the phototroph compared to axenic cultures (Figure 3.3.1). The period of survival in co-culture showed four types of interaction: 1) positive interaction, where both the phototroph and heterotroph survived throughout the whole experimental period (*i.e*. 19 weeks); 2) neutral interaction, where the phototroph's death occurred at the same sampling point as the axenic culture, meaning that the presence of the heterotrophic treatment had no effect on the survival of the phototroph; 3) semi-positive interactions, where the phototroph's death occurred later than the axenic culture or, in the case of the heterotroph, when the heterotroph died briefly after the death of the phototroph revealing the high dependence of the heterotroph on the phototroph; and 4) negative interactions, where the death of the phototroph occurred earlier than the axenic culture or, in the case of the heterotroph, when the heterotroph died before the phototroph.

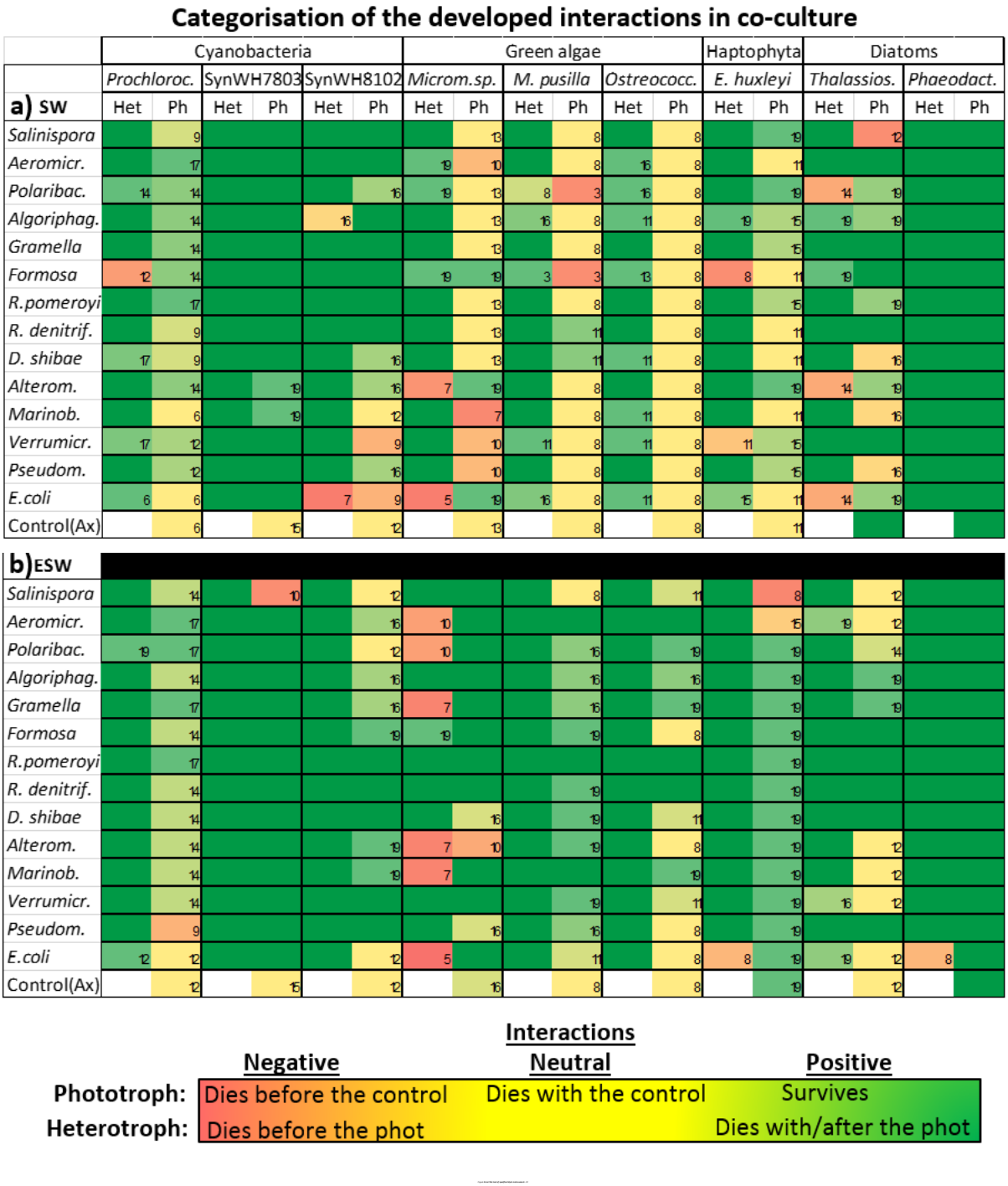


Figure 3.3.1.1: Heat-map categorisation of the one-to-one interactions between 9 phototrophs and 14 heterotrophs incubated in both natural oligotrophic seawater (a; SW) and nutrient-enriched seawater (b; ESW). Each column represents the survival of the heterotroph (Het; left) and phototroph (Ph; right) in each co-culture as compared to the axenic culture (control (Ax); bottom row of a and b panels), as described in Figure 3.3.1. The included numbers represent the number of week in which the death event was detected (no number – no death detected).

Figure 3.1.1.1 summarises the culture survival of each phototroph and heterotroph in all co-culture combinations performed in natural oligotrophic seawater (SW) and in enriched seawater (ESW). In

axenic culture, all phototrophs died at some point during the 19-week experimental period except for the diatoms. Interestingly, both *Thalassiosira* and *Phaeodactylum* survived throughout the 19 weeks in natural oligotrophic SW, although only the former survived the entire period also in ESW (Figure 3.1.1.1.). Overall, the majority of the heterotrophs showed a positive effect in extending the survival of co-cultured phototrophic organisms, especially in ESW. However, in oligotrophic SW, this strong positive effect was less apparent in the green algae, generally presenting neutral effect (Fig 3.1.1.1.a). The most positive effects in SW were achieved by the heterotrophs *Alteromonas* and *Roseobacter* (*i.e*. extending the survival of 5 out of the 9 phototrophs), whereas in ESW most heterotrophs extended the survival of the co-cultured phototroph (*i.e*. Bacteroidetes and Roseobacter species extended the survival of all phototrophs). The most neutral interactions in SW were observed with *Marinobacter* and the most negative ones by *E.coli* and *Formosa*. While *Salinispora* showed general positive interactions in SW, this heterotroph caused strong negative interactions with the phototrophs *Synechococcus* sp. WH7803 and *E. huxleyi* under ESW conditions.

Unexpectedly, *Micromonas* sp. showed a large number of negative effects on the co-cultured heterotroph (*i.e*. six heterotrophs in ESW and two in SW died before the phototroph; Fig 3.1.1.1). In addition, *Micromonas* sp. also experienced a large number of negative effects by the heterotrophs (*i.e*. four combinations in SW and one in ESW died before the axenic culture; Fig 3.1.1.1). More specifically, the combination *Micromonas* - *Alteromonas* is one of the most remarkable negative interactions, with this being the only case of a double negative interaction in ESW. Interestingly, *Micromonas* sp. severely shortened the survival of *Alteromonas* in both SW and ESW conditions which also resulted in the shortening of the survival of *Micromonas* sp. in ESW. This suggests that *Micromonas* sp. shows a competitive behaviour when co-existing with bacteria causing either a losing or winning outcome with respect to accessing nutrients.

The interactions that change from positive in ESW to neutral or negative in SW highlight the importance of nutrients on developing positive interactions among phototrophic and heterotrophic organisms. Green algal species were the most affected by nutrient availability as most interactions with heterotrophs were neutral or negative in SW and became positive in ESW (Fig 3.1.1.1). *Ostreococcus* experienced only neutral interactions in SW, indicating that none of the tested bacterial species could extend the survival of *Ostreococcus* in oligotrophic conditions possibly due to the lack

of mineralisation of some key nutrient or that the supply was insufficient to enhance the survival of the strain.

3.3.2: Cell abundance achieved in each one of the phototroph-heterotroph co-cultures

The cell abundance obtained by each organism in all phototroph-heterotroph co-culture combinations was calculated and represented as the total average of the triplicates during the survival period within the 19 weeks, along with the minimum and maximum measurements (Figure 3.3.2.1 for SW, and Figure 3.3.2.2 for ESW).

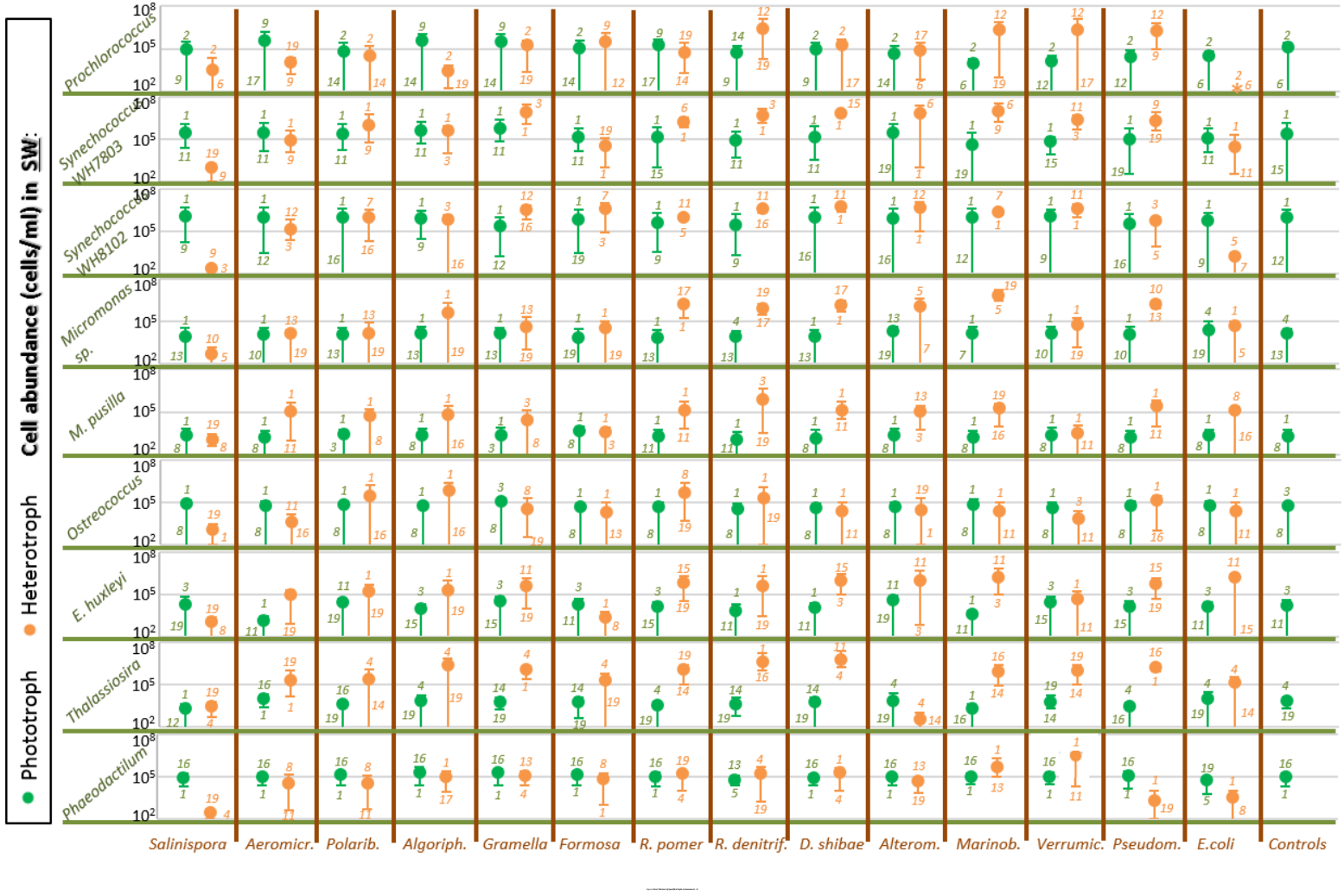


Figure 3.3.2.1: Average cell abundance of 9 phototrophs (green) and 14 heterotrophs (orange) in all co-culture combinations during 19 week incubation in natural seawater (SW). The bars represent the minimum and maximum cell abundance achieved over the experiment, numbers indicate the week that the minimum and maximum cell abundances were measured. The lower limit of detection (10^2 cells/ml) defined the death of the population. The axenic culture of the phototroph is displayed in the last column (controls).

The population abundance obtained at low nutrient availability (in oligotrophic SW) was generally higher for the heterotrophs than for the phototrophs in the majority of the cultures (Figure 3.3.2.1), replicating what is observed in natural marine systems (Azam and Malfatti, 2007). The cell abundance measurements mainly ranged between 10^6 to 10^4 cells/ml for the phototroph and between 10^7 to 10^5 cells/ml for the heterotroph, except for *Salinispora* whose levels were lower than 10^5 cells/ml in all the co-cultures possibly due to cell aggregation (visually observed in the cultures, but not further analysis was carried out). The co-cultures that showed strongest differences between the heterotrophic and phototrophic populations was mainly observed with *Micromonas* sp, *M. pusilla*, *Thalassiosira*, *E. huxleyi* and *R. denitrificans* (up to 2-3 orders of magnitude). Nevertheless, in some co-cultures, *i.e.* with *Ostreococcus* and *Phaeodactylum* and with heterotrophs *Salinispora*, *Aeromicrobium* and *E. coli*, the phototroph cell abundance regularly exceeded the heterotroph (up to 1-2 orders of magnitude). In fact, *Phaeodactylum* kept all heterotrophs at low concentrations ($\leq 10^5$ cells/ml) except for *Verrucomicrobia* which reached 10^6 cells/ml in this co-culture, the highest among all phototrophic treatments.

Interestingly, the heterotrophs *Gramella* and *Alteromonas* contributed to the majority of the highest phototrophic population levels, whereas *R. denitrificans* and *Marinobacter* had the opposite effect on the phototrophs, causing the lowest cell yields. *Marinobacter* though thrived with the majority of the phototrophic species in the currently tested co-culture system, and cyanobacteria and *Micromonas* sp. boosted its population levels to 10^7 cells/ml, although *M. pusilla* and *Ostreococcus* decreased its population to 10^5 cells/ml (Figure 3.3.2.1). The majority of the minimum phototrophic cell abundances was recorded approximately around week 10, coinciding with the maximum abundance of the heterotroph. The alignment of phototroph reduction and heterotroph increase indicated that 1) the inoculated phototroph cell abundance in the beginning of the experiment could not be supported by the available nutrients in the oligotrophic SW with a consequent cell reduction, and 2) that the nutrient released by the dead phototrophic cells boosted the heterotroph cell abundance. The date of the phototroph maximum was aligned with the date of the heterotrophic minimum, or vice-versa, in 38 out the 126 co-culture combinations. From these, 24 cases favoured the heterotroph, mainly in the co-cultures of *E. huxleyi* and *Phaeodactylum*, and with heterotrophs *Pseudomonas* and *Gramella*. The other 14 cases favoured the phototrophs, especially in both *Synechococcus* species and in *D. shibae* co-cultures.

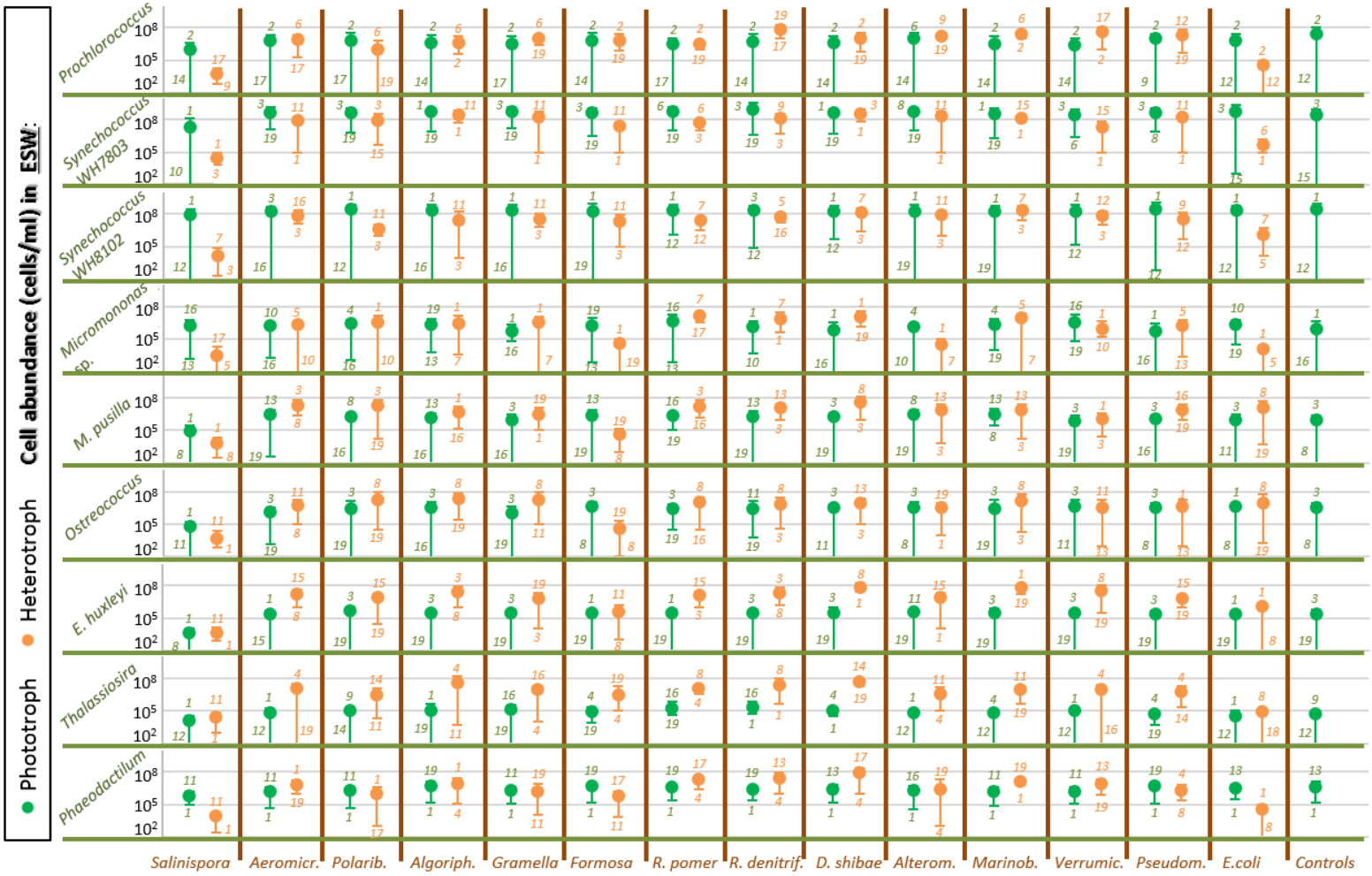


Figure 3.3.2.2: Average cell abundance of 9 phototrophs (green) and 14 heterotrophs (orange) in all co-culture combinations during 19 week incubation in nutrient-enriched seawater (ESW). The bars represent the minimum and maximum cell abundance achieved over the experiment, numbers indicate the week that the minimum and maximum cell abundances were measured. The lower limit of detection (10^2 cells/ml) defined the death of the population. The axenic culture of the phototroph is displayed in the last column (controls).

As expected, the high nutrient levels in ESW supported higher population densities compared to SW, with phototrophs ranging between 10^9 to 10^6 cells/ml and heterotrophs between 10^9 to 10^7 cells/ml (Figure 3.3.2.2). Compared to SW conditions, heterotrophic cell abundances were not consistently higher than phototrophic ones possibly due to the excess of available inorganic nutrients and hence a lower dependence on heterotrophic nutrient mineralisation. Nevertheless, the heterotrophic population did exceed the phototrophic one in *E. huxleyi* and *Thalassiosira* co-cultures (up to 1-2 orders of magnitude). The majority of the phototrophs achieved the highest population levels in week 3 of the experiment, followed by a general decrease in numbers after week 12 and close to the end of the experiment. Only *Phaeodactylum* showed the opposite trend, with the minimum population level recorded at the beginning of the experiment and the maximum at week 11, indicating a population increase until the middle of the experiment.

Noteworthy is the large number of species-specific interactions that were observed in ESW. For example, *Salinispora* was again the heterotroph with the lowest cell numbers due to aggregation, but this strain in ESW also caused the lowest cell abundance in all co-cultured phototrophs, even lower than the control where no bacteria were included. This observation, added to the killing effect observed in *Synechococcus* and *E. huxleyi* (Figure 3.3.2.2), suggests *Salinispora* negatively affected the phototrophic population in rich nutrient environments, possibly through the production of some antimicrobial agent.

The majority of the heterotrophs thrived when co-cultured with both *Synechococcus* species, contrasting with the effect that the green algae (*i.e*. *Micromonas* sp., *M. pusilla* and *Ostreococcus*) had on the heterotrophs. This is possibly due to the fact that the enriched media where *Synechococcus* grows contained larger nutrient concentrations than other media used for other phototrophic strains (see Table 2.1.1.2), since the ASW is completely artificially made compare to the others that have as base natural seawater. Other species-specific observations that are worth highlighting are: i) *Synechococcus* sp. WH8102 and *E. huxleyi* boosted the population of *Marinobacter* to 10^8 cells/ml, whereas it was *Polaribacter* that boosted these phototrophic populations to 10^6 and 10^9 cells/ml respectively; ii) *Synechococcus* sp. WH7803 and *Ostreococcus* boosted the population of *Algoriphagus* to 10^8 cells/ml; iii) *Aeromicrobium*, a gram-positive bacterial species, boosted the population of *Synechococcus* sp. WH7803 and *M. pusilla* to 10^9 and 10^7 cells/ml respectively; and iv) *R. pomeroyi* boosted the population of *Micromonas* sp. and *Thalassiosira* 10^7 and 10^6 cells/ml respectively.

Similar to what was observed in the SW incubations, in ESW 35 out of the 126 combinations showed an alignment between the date of the maximum of one organism and the minimum of the other. In 22 combinations, the maximum cell count of the phototroph was achieved in the date of the minimum of the heterotroph, mainly in co-cultures with *Synechococcus* sp. WH7803 and *Micromonas* sp., as well as with the heterotroph *D. shibae*. This comes as a consequence of the high inorganic nutrient levels present in the ESW that initially supported the growth of the phototrophic organisms. The consequent decrease of the phototroph population levels generated organic matter that boosted the growth of the heterotroph.

3.3.3: Statistical analysis of the phototroph-heterotroph populations within the co-cultures

The cell abundance of some organisms over 19 weeks of incubation was significantly affected by the presence of specific species in the culture, as the statistical analysis revealed (Figure 3.3.3.1). The treatments were tested for their effect either on the achieved population levels, when the organism survived during the whole experimental period (tested with one-way ANOVA) or on the timing of the lethal event that was detected for some organisms (tested in logistic regression model), as described in Section 3.2.1.

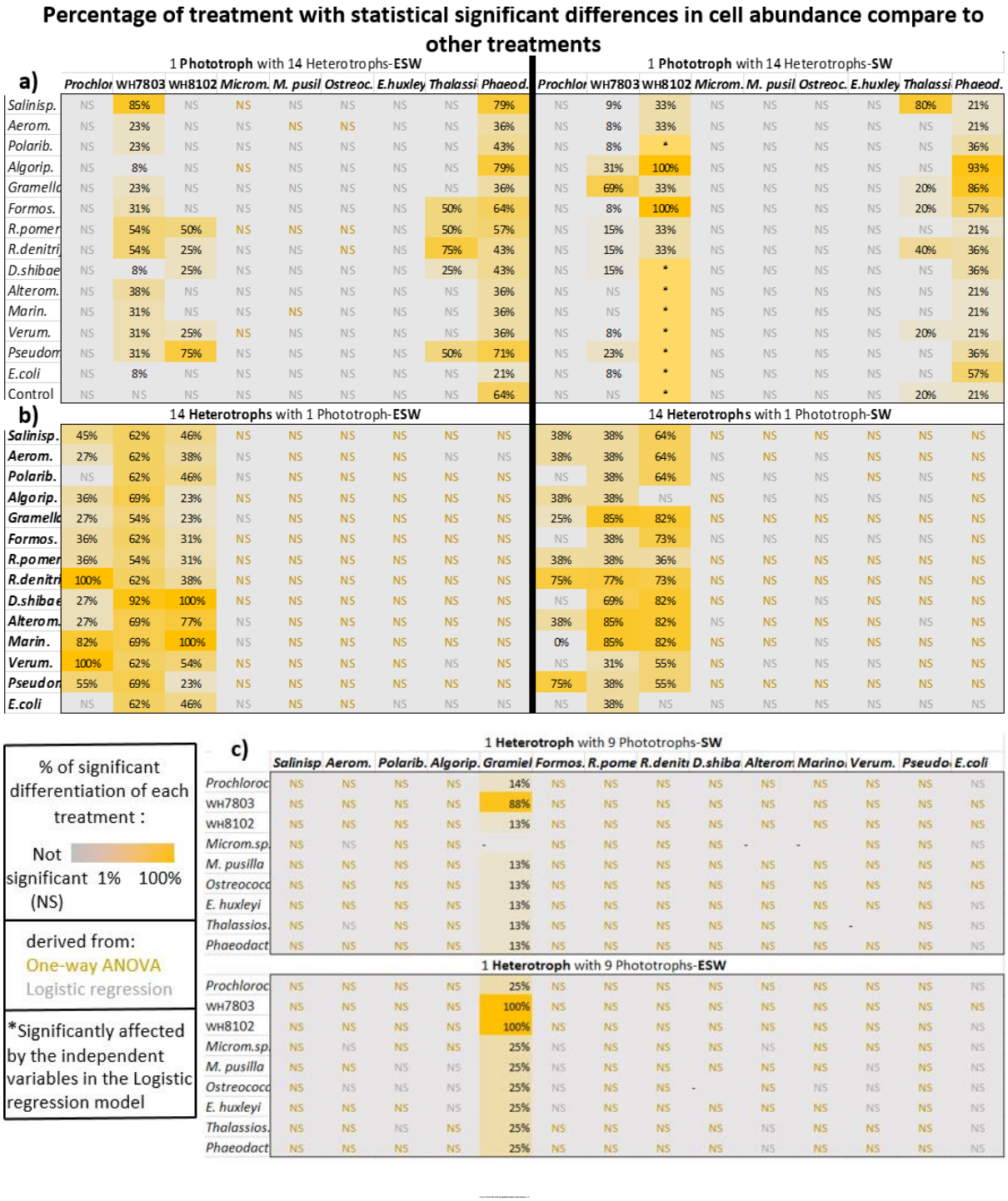


Figure 3.3.3.1: Percentage of significant differences ($p<0.05$) achieved by individual treatments tested against a group of treatments is presented as a heatmap. The cell abundance of each organism measured in ESW and SW, was tested either in one–way ANOVA (when it survived during the whole experiment; yellow values) or in logistic regression (when it died during the experiment; grey values). The tested groups are defined in panel: a) each phototroph under 14 heterotrophic treatments, b) 14 heterotrophs under the same phototrophic treatment and c) each heterotroph under 9 phototrophic treatments.

The statistical test highlights the magnitude of the effect of the co-occurrence of two organisms in co-culture, possibly suggesting a nutritional coupling between the two. Figure 3.3.3.1 depicts the species that were either most affected by or most effective on the co-occurring organism at the population level. In terms of the effect of the heterotrophs on the phototroph population, the two *Synechococcus* species, WH7803 and WH8102, along with the two diatom species, *Thalassiosira* and *Phaeodactylum*, obtained significantly different population levels with regards to the different heterotrophic treatments in both SW and ESW. The phototrophs that had the strongest effect on the heterotrophic populations were the cyanobacteria (*i.e*. *Prochlorococcus*, and *Synechococcus* WH7803 and WH8102). The heterotroph that was most affected by the presence of a phototroph was *Gramella* and especially by the *Synechococcus* species, which caused a significant boost to its population (see Figure 3.3.2.1 and 2).

In 45 out of the 126 combinations a lethal event of at least one of the microorganisms within the co-culture occurred (see Figure 3.3.1.1). The duration of the death phase lasted from just a few weeks to over half of the 19-week experimental period. The patterns of the death phase were tested through a logistic regression model designed on three independent variables for each set of results (see Section 3.2.1). The absence of clear significant effects of the applied variables on the tested model indicated the absence of diversity among the treatments. The only cultures where the variable of the cell abundance and the date of death affected the model was in *Synechococcus* WH8102 incubated in ESW. This suggests that the population abundance achieved during the alive period and the time at which the death event occurred were correlated and significantly different among the treatments, an observation that was not seen for the rest of the organisms. With regard to the growth curves, *Synechococcus* sp. WH8102 incubated in ESW passed through a population bottleneck, similarly to other cultures such as *Synechococcus* WH7803, WH8102 in SW (can be found in Appendix Figure 7.1.a and b) and green algal species in ESW. After the population bottleneck and until the end of the experiment, the recovered populations were tested in a one-way ANOVA test, revealing that only the population levels of *Synechococcus* WH8102 in ESW were significantly different among the treatments. In this way, the population levels, the death event and the recovery from a population bottleneck in *Synechococcus* WH8102 were all significantly affected by the presence of the different heterotrophs in nutrient-enriched SW (ESW).

3.4 Discussion

The goal of this chapter was to outline the population growth curves of 9 phototrophs and 14 heterotrophs co-cultured in 126 combinations in two nutrient conditions, *i.e.* low nutrient (natural oligotrophic seawater; SW) and high nutrient levels (nutrient-enriched seawater; ESW), over 19 weeks. The growth physiology generated a large amount of information and highlighted both generic and species-specific interactions with respect to nutrient availability.

3.4.1: Methodological estimation

Flow cytometry and colony forming units (CFUs) were applied for monitoring phototroph and heterotroph cell abundance in the co-cultures, respectively. Due to the extremely low plating efficiency of the phototroph and the low cell abundance achieved in natural SW incubations, flow cytometry was the most suitable method for counting phototrophic cells by using their natural auto-fluorescence. Nevertheless, the accuracy of flow cytometry in counting live autofluorescent cells is a subject under discussion, debating the fluorescence stability of pigments and the production of chlorotic cells (Seely and Connolly, 1986, Veldhuis *et al*., 2001, Franklin *et al*., 2009). Christie-Oleza *et al*. (2017b) and Dr Daniel Sher (personal communication), have shown that phototroph lose their auto-fluorescence quickly after cell death, despite dead cells still being able to be stained with DNA fluorescing dyes (*e.g*. SYBR green or DAPI). Phototroph cell abundances of all the cultures were measured at regular intervals over the 19 weeks period, and those cultures whose population level was under the detection limit (below 10^2 cell/ml) during the experiment, continued to be measured until the end of the experiment confirming the death of the phototroph.

Heterotroph cell abundance was measured on plates via CFU counts acquiring a reliable measure of active live cells, ranging within 95% confidence (Silliker *et al*., 1979). A detailed comparison of bacterial counts derived from CFUs and flow cytometry was previously done by Christie-Oleza *et al*. (2017b) in the same co-culture set-up as the one used here, detecting no differences in the outcome of the two counting methods. Nevertheless, counting heterotroph cells by flow cytometry after staining with a fluorescent dye (*e.g*. SYBR green or DAPI) did not give reliable population counts as dead cells of both phototrophs and heterotrophs, which still contained DNA, gave false counts and overestimated cell abundance (Nebe-von-Caron *et al*., 2000, Christie-Oleza *et al*., 2017b). Furthermore, the use of plate counts facilitated checking for contamination of the cultures. The population levels of *Salinispora* were

low due to cell aggregation (as confirmed under the microscope), highlighting CFU was inefficient for counting *Salinispora*. Ideally this species should have been measured as biomass (Jensen *et al*., 2015), though variability of the measuring techniques and the presence of the co-cultures phototroph made this impossible.

The use of natural seawater (SW) as well as nutrient-enriched seawater (ESW) represents a unique *in-vitro* approach to replicate natural environmental conditions, such as open ocean oligotrophic surface waters and blooming coastal waters, respectively. All interaction studies (other than Christie-Oleza *et al*., 2017b) have been carried out in enriched media conditions (Table 1.7.3). Among the limiting factors in the currently tested experimental set-up was the increase of salinity due to water evaporation that reduced the culture volume to the half of the initial volume by the end of the experimental period. The accumulation of elements within an enclosed system without media renewal may cause detrimental effects on the performance of the enclosed organisms, especially within natural communities, described as 'Bottle effect' (Massana *et al*., 2001, Ionescu *et al*., 2015). Nevertheless, all microorganisms used in this study are perfectly adapted to *in-vitro* cultivation. Hence, in the current long-term experiment, both co-cultured organisms were forced to co-exist and possibly reach a metabolic coupling over time, which occurred in most of the cases.

3.4.2: Phototroph survival and cell abundance in co-culture

Phototroph-heterotroph interactions are generally affected by nutrient availability (Li *et al*., 1992, Karel *et al*., 2004, Amin *et al*., 2012b, Ramanan *et al*., 2016, Bernstein *et al*., 2017), supporting the present findings where negative or neutral interactions under low nutrient levels (SW) shifted to positive interactions under high nutrient levels (ESW). Nevertheless, the time scale in which this occurred varied with respect to the species. Most studies have used nutrient-rich media and short-term incubations to study marine phototroph-heterotroph interactions reaching, in most cases, misleading conclusions that can only be applied to exceptional blooming events. The co-culture combination of *Synechococcus* sp. WH7803 and *R.pomeroyi* incubated in natural oligotrophic SW is the only experimental set-up carried out in long-term low nutrient experiments (Christie-Oleza *et al*., 2017b, Amandeep *et al*., 2018). The time-course proteomic analysis of Kaur *et al*. (2018) demonstrated the initiation of the metabolic connectivity between the two organisms only after the second week of incubation. This points to the necessity of using long-term incubations like the ones carried out here

to fully understand microbial interactions, *i.e*. phenomenon such as the recovery from a population bottleneck was detected after 10 weeks of incubation in *Synechococcus* species.

The increased performance of the phototroph in the presence of heterotrophic bacteria compared to axenic cultures has been previously recorded in co-culture experiments of *Prochlorococcus* (Sher *et al*., 2011, Aharonovich and Sher, 2016), *Synechococcus* (Palenik, 2012, Beliaev *et al*., 2014, Christie-Oleza *et al*., 2017b, Amandeep *et al*., 2018), *Phaeodactylum* (Lin Wei, 2000, Suleiman *et al*., 2016, Diner *et al*., 2016), *E. huxleyi* (Segev *et al*., 2016) and even in cultures separated with a membrane filter (Paul *et al*., 2013). Nevertheless, the positive effect of the presence of heterotrophs on the phototrophic performance was not a generic phenomenon as observed in the literature (Grossart, 1999) and as shown here (Figure 3.3.1.1), since in several combinations the survival either was not affected or was shortened by the presence of certain bacterial species, described as neutral and negative interactions respectively.

The survival of both diatom species throughout the entire 19 weeks, independent of the presence of the heterotroph, was unique among the tested phototrophic species. *Phaeodactylum* survived independently of the nutrient levels and bacterial species, highlighting its robustness in the tested culture conditions, which confirms its mixotrophic abilities to metabolise organic substrates (Cooksey, 1974, Ukeles and Rose, 1976, Fabregas *et al*., 1996, Cid *et al*., 1992, Cerón García *et al*., 2005, Ceron Garcia *et al*., 2006). Also, continuous light regimes have been reported to enable diatom blooms to persist despite nutrient depletion (Connie *et al*., 2002). Interestingly, *Thalassiosira* survived longer in the absence of bacteria and experienced more positive interactions in low nutrient conditions (SW) than in ESW, displaying the good performance of this species in SW. However, under ESW conditions, *Thalassiosira* decreased its survival and only the presence of certain heterotrophs enhanced it. This demonstrates that low nutrient availability boosts the mixotrophic behaviour of this species, though, under high nutrient availability *Thalassiosira* was able to bloom and switch to a low active state, enhancing sinking processes as a defence against potential grazers (Smetacek, 1985).

As expected, average population levels were higher in ESW than SW for heterotrophs over the phototrophs, explained by the different nutritional levels of the tested seawater medium and the different nutritional requirements of the two microbial groups, respectively. Interestingly, in oligotrophic SW conditions, the average population levels followed the natural range of bacterial and

phytoplankton cell densities found *in situ* (Mühling *et al*., 2005, Azam and Malfatti, 2007, Godhe *et al*., 2008; bacteria 10^6, cyanobacteria 10^5 and eukaryotes up to 10^4 cells/ml). In ESW, cell yields were comparable to those commonly found in *in-vitro* cultivation systems (Li *et al*., 1992, Grossart, 1999, Aharonovich and Sher, 2016, Diner *et al*., 2016), with cell numbers over 2-orders of magnitude compared to natural levels, where the heterotrophic bacteria grows steadily, due to the continuous release of rich organic debris by the phototroph (Grossart, 1999) . Independent of the initial concentration of inoculum, the organisms eventually reached comparable concentrations to those expected, confirming the findings of population balancing with regards to the availability of nutrients by Christie-Oleza *et al*. (2017b).

3.4.3: Population abundance depicts the metabolic performance of the cultures

Phototroph-heterotroph cell abundance data depicts the metabolic status and functioning of the systems, quantifying the coupling of phytoplankton and heterotrophic bacteria (Anderson and Ducklow, 2001), since the primary producer generates organic matter that is consumed by the heterotroph. In the oceanic euphotic zone, the increassed accessibility to light permits high carbon productivity, which eventually supports higher bacterial population levels than the phototrophic ones (Li *et al*., 1992). In the current experiments, the heterotrophic bacterial population was higher than the phototrophic population up to 1-2 orders of magnitude, exposing the connectivity between the producer and the consumer in these cultures which showed close similarities to natural ecosystems. Nevertheless in nature, the heterotrophic population is supported not only by organic substrates derived from primary production, but also by biological leakage from other microbes, cell lysis from viruses or sloppy grazing (Robarts *et al*., 1996, Pinhassi *et al*., 1999, Pedrós-Alió, 2012). The absence of diverse organic substrate sources in the current cultures forced bacteria to depend on the specific organic substrates produced by the co-cultured phototrophic species. For instance, *R. denitrificans* reached high cell abundance in all the cultures in SW. This chemo-lithoheterotroph possibly used its supplementary energy mechanisms (aerobic anoxygenic photosynthesis; Kortlüke *et al*., 1997, Tang *et al*., 2010, and sulfur oxidation; Muthusamy *et al*., 2014) facilitating a more efficient use of the available organic matter *i.e.* more organic matter was used as structural components and, hence, extra cell division, and less was respired in order to generate energy. Ultimately, this allows increasing its own population levels returning less inorganic nutrients back to the co-existing phototroph (Danger *et al*., 2007), justifying the consistently depressed phototrophic population levels in these co-cultures.

Oligotrophic marine environments are highly dominated by *Synechococcus* and *Prochlorococcus* (Scanlan *et al*., 2009, Flombaum *et al*., 2013). The efficiency of the cyanobacteria to grow under low nutrient conditions relies on its level of specialisation, almost entirely relying on the heterotroph to re-mineralise organic matter (Christie-Oleza *et al*., 2017b). This was depicted in the current SW co-cultures as prolonged survival achieved only with the presence of bacteria. The recirculation of nutrients by bacteria plays also a significant role for *Synechococcus* species even in enriched media (ESW), where the continuous remineralisation decreases the toxicity of organic matter accumulation (Christie-Oleza *et al*., 2017b). This points to the importance of the synchronisation of production/consumption rates between phototrophs and heterotrophs in order to reach a sustainable system. Except for detoxification, bacteria were able to produce nutrients that support higher phototrophic population levels than their own in ESW (Figure 3.3.2.2) possibly by metabolising specific nutrients (Campbell *et al*., 2011, Hunt *et al*., 2013) only encountered in ESW.

3.4.4: Negative interactions

The exchange of nutrients between phototrophs and heterotrophs is probably the most common interaction in nature (Christie-Oleza *et al*., 2017b), though both groups of organisms may also compete for limiting nutrients, such as phosphate (Thingstad *et al*., 1993). Unbalanced nutrient levels and organic carbon stocks influence the interactions even in enriched media (Karel *et al*., 2004, Grossart, 1999). However, nutrient imbalance is stronger under SW conditions, inducing a higher variability of interactions compared to co-cultures in ESW due to the diversity of nutrient limitations of each species (Fuszard *et al*., 2012). Bacteria are good competitors in low nutrient environments due to their high chemotactic and uptake rates (Moran *et al*., 2004), along with the release of molecules with detrimental effects on their competitors (Wietz *et al*., 2013, Aguirre-von-Wobeser *et al*., 2015). To some extent, phototrophs can decrease the leakage of organic matter (Bratbak and Thingstad, 1985, Sarmento and Gasol, 2012, Diner *et al*., 2016), and even produce molecules with a variety of effects on uni- and multi-cellular organisms (Gershenzon and Dudareva, 2007, Nunnery *et al*., 2010, Walter and Strack, 2011), such as the detrimental effects observed on bacteria by *Thalassiosira*, *E.huxleyi* and *Micromonas* sp. in this study. The reduction of bacteria below detection limits in the co-culture is, most probably, achieved via the production of antimicrobial metabolites, since the reduction of key nutrients by the phototrophs with detrimental effects (*i.e*. starve to death) would be unlikely, due to

the high resistance of bacteria to prolonged starvation periods (Balaban *et al*., 2004, Keren *et al*., 2004, Justice *et al*., 2008).

Among the tested strains, *Salinispora* is the most well-known species for producing secondary metabolites with antagonistic effects to other organisms, from microbes to multicellular organisms (Challis and Hopwood, 2003, Fenical and Jensen, 2006, Udwary *et al*., 2007, Jensen *et al*., 2007, Fenical *et al*., 2009, Freel *et al*., 2012, Ziemert *et al*., 2014). One possible reason for this behaviour is that *Salinispora* inhabits marine sediments (Ahmed *et al*., 2013), a highly competitive and rich environment. Interestingly, this species caused a strong decrease in the phototrophic population but only under ESW conditions. High nutrient availability and cell densities were shown to enhance the production of molecules in levels accessible for cell-to-cell communication (Davies, 2006). The detrimental effect of *Salinispora* on all phototrophs suggests the production of a secondary metabolite that requires further investigation.

3.4.5: A marine outsider, E. coli

The bacterial species used in this project included the widely studied laboratory species, *E. coli*, as a control. The disability of *E. coli* to grow in standard bacteriological media after a prolonged period with continuous illumination in seawater (Gerba and McLeod, 1976, Xu *et al*., 1982, Barcina *et al*., 1990, Chan and Killick, 1995) justifies the low population levels recorded in the current experimental set-up. The suppression of the restricting effects of salinity and illumination on *E.coli*'s growth could only be achieved by specific inorganics nutrients such as $(NH_4)_2S0_4$ and $(NH_4)_2HPO_4$, and organics, such as glucose, peptone and cysteine (Carlucci and David, 1959, Barcina *et al*., 1990). Here, the presence of certain phototrophs helped *E.coli* to survive the 19-week incubation, pointing at the strong positive effect that some phototrophs have on this species at overcoming stressful conditions.

3.4.6: Marinobacter did not stimulate the phototrophic population

Marinobacter is commonly associated with phytoplankton species (Amin *et al*., 2012a, Singer *et al*., 2011, Sonnenschein *et al*., 2012, Lupette *et al*., 2016) and, hence, this lack of stimulating response on phototrophs was unexpected. Nevertheless, *Marinobacter* thrived in the presence of phototrophs independent of nutrient availability. In most cases this bacterium suggested a parasitic behaviour: i) it did not achieve a metabolic coupling that would benefit the phototrophs in terms of survival, especially in SW where only 2 out of the 9 phototrophs survived longer than their axenic cultures, and

ii) all co-cultured phototrophs reached low population levels compared to the rest of the heterotrophic co-cultures. Interestingly, the positive interaction observed previously between *Thalassiosira weissflogii* and *Marinobacter* (Gärdes *et al*., 2010, Kaeppel *et al*., 2012, Sonnenschein *et al*., 2012) was detected as a neutral interaction here when using *Thalassiosira pseudonana*, pointing at the importance of species-specificity of interactions. *Marinobacter* is known as opportunitroph (Singer *et al*., 2011) with high motility and chemotactic ability (Sonnenschein *et al*., 2012), justifying its survival even in oligotrophic seawater. However, this opportunistic behaviour was absent in ESW cultures of *Prochlorococcus*, *Ostreococcus* and *E. huxleyi* incubated in high nutrient levels, agreeing with the association of members of the *Marinobacter* genus to these species in ESW (Sher *et al*., 2011, Abby *et al*., 2014, Green *et al*., 2015, Lupette *et al*., 2016). This indicates the high impact of nutrient availability on the behaviour of the *Marinobacter*.

3.4.7: The competitive behaviour of Micromonas sp. in co-cultivation with bacteria

The competitive behaviour of *Micromonas* sp. was highlighted by the high number of detrimental interactions that this strain caused on bacteria under high nutrient availability and experienced under low nutrient availability. Nitrogen is the nutrient with the strongest detrimental effects on green algal blooms recorded in the Western English Channel (Not *et al*., 2004) and in lakes (Filzgerald, 1969), suggesting this is a key element for *Micromonas* sp. losing the competition with bacteria in nutrient-limited environments. Interestingly, *Micromonas* co-cultures with *Roseobacter* species showed no negative interactions, agreeing with the co-occurrence of alpha-proteobacterial strains in *Micromonas* sp. cultures (Kawano *et al*., 1997). On the other hand, negative interactions occurred between *Micromonas* sp. and all gamma-proteobacteria in SW. Specifically, *Micromonas* sp. had a striking species-specific detrimental effect on *Alteromonas* which was independent of nutrient availability. *Alteromonas* has been recorded as a competitive organism in previous studies (Long and Azam, 2001, Long *et al*., 2003), but it had never been found to be killed by a phototroph, such as *Micromonas* sp., demonstrating the efficiency of the current experimental setting in uncovering species-specific interactions.

3.4.8: Ostreococcus nutrient requirements are not covered by the presence of bacteria

The sensitivity of *Ostreococcus* to low nutrients, *i.e*. SW incubations, was independent of the presence of bacteria. Nevertheless, some co-culture combinations with specific bacterial species facilitated

prolonged survival in ESW cultures, indicating a possible positive metabolic coupling under high nutrient availability. Bacteroidetes was among these bacterial species, and for which some representatives have been previously detected in *Ostreococcus* cultures (Abby *et al*., 2014). Despite the wide genomic repertoire of *Ostreococcus* (Derelle *et al*., 2006, Palenik *et al*., 2007, Six, 2008, Subirana *et al*., 2013), the absence of some functions, such as motility and chemotaxis (Chrétiennot-Dinet *et al*., 1995), might justify the large differences with the other two green algae species, *Micromonas* sp. and *M. pusilla* . The flagella motility of *Micromonas* may play a key role in the development of bacterial interactions (Omoto and Witman, 1981, Omoto *et al*., 1999).

The effect of nutrient availability on the development of phototroph-heterotroph interactions was analysed in the current chapter, revealing both species-specific and generic interactions. The results obtained help dissect the complex network of marine microbial interactions. Apart from a general nutrient recirculation between the majority of phototrophs and heterotrophs which supports positive interactions, some species are clearly more sensitive to nutritional accessibility than others, such as green algae compared to diatoms. Some interesting and unknown behaviours were also identified such as the competitive behaviour of *Micromonas* sp. and the restricting behaviour of *Salinispora*.

Chapter 4

Proteomic response of *R. pomeroyi* to the presence and absence of nine model phototrophic species in natural seawater

4.1 Introduction

Roseobacters were mostly ignored by microbiologists and ecologists until the early 1990s when culture-independent approaches to assess microbial diversity were applied to marine systems and led to the recognition of this group as one of the most abundant bacterial groups in the oceans (Buchan *et al*. 2014). The characteristics of members of the Roseobacter group is dependent on their life style *i.e.* phytoplankton-associated and planktonic (Luo and Moran, 2014). Phytoplankton-associated Roseobacter isolates have larger genomes (~4.5 Mb) and higher gene content (Newton *et al*., 2010) than other planktonic Roseobacter forms and abundant marine lineages (Swan *et al*., 2013, Billerbeck *et al*., 2016). These generalist microbes encode a large array of auxiliary functions which allow them to take advantage of an extensive array of substrates and adapt to varying environmental conditions (Christie-Oleza *et al*., 2012a). This alteration of behaviour has been observed in the transition from free-living to niche adaptations, which are commonly found associated with phytoplankton (Wagner-Döbler and Biebl, 2006, Luo and Moran, 2014), such as diatoms, corals (Ottesen *et al*., 2011), oysters (Boettcher *et al*., 2000), and dinoflagellates, like *Pfiesteria* (Mohammad *et al*., 2001). The interactions between Roseobacters and phytoplankton are facilitated by chemotaxis towards compounds that are released by the phototroph (*e.g*. DMSP or amino acids; Miller *et al*., 2004). Roseobacters are highly specialised in using the DOM produced by marine photosynthetic organisms as a source of carbon, sulphur, nitrogen and/or phosphorus (for example, DMSP, urea, polyamines, taurine, glycine betaine, methylated amines, phosphoesters and phosphonates; Moran *et al*., 2004, Newton *et al*., 2010, Chen, 2012, Buchan *et al*., 2014). An excellent example of the tight coupling between Roseobacters and phytoplankton comes from the study of the algal osmolyte dimethylsulfoniopropionate (DMSP), which is usually degraded and used as a source of carbon and sulfur by Roseobacters (González *et al*., 2000). This coupling has implications in global climate regulation, since the degradation product DMS is volatile and released into the atmosphere enhancing water condensation and cloud formation (Buchan *et al*., 2014), revealing the importance of these microorganisms in global biogeochemical processes (Wagner-Döbler and Biebl, 2006, Buchan *et al*., 2014).

R. pomeroyi DSS-3 (formerly *Silicibacter pomeroyi*) was the first member of the Roseobacter clade to have its genome completely sequenced and annotated (Moran *et al*., 2004). The genome of *R. pomeroyi* revealed a wide gene pool, where a "mix-and-match" genome arrangement allows

adaptations to a wide diversity of ecological niches (Moran *et al*., 2004, Moran *et al*., 2007, Newton *et al*., 2010) and, most importantly, it is amenable to genetic manipulation. Due to this *R. pomeroyi* has become a model organism for studying the ecological and physiological strategies of heterotrophic marine bacteria from a metabolic point of view (Bürgmann *et al*., 2007, Christie-Oleza *et al*., 2012a, Christie-Oleza *et al*., 2015). Members of the Roseobacter group are also used as model strains to understand alternative energy-conservation strategies in marine microbes known as lithoheterotrophy *e.g.* aerobic anoxygenic photosynthesis, V-type pyrophosphatases, or reduced sulfur and carbon monoxide (CO) oxidation (Luo and Moran, 2014). CO is ubiquitous in surface waters albeit at low concentrations (Moran *et al*., 2007) whereas the energy gain from CO oxidation is sufficient to fulfil the energy demands of the cell (*i.e.* CO dehydrogenase system; Cunliffe, 2013). On the other hand, the oxidation of thiosulfate to sulfate releases 1.4 to 3.0 mol ATP per mol of thiosulfate (Kelly, 1982), with the reduced inorganic sulfur being incorporated into amino acids cysteine and methionine and their derivatives (Giovanelli *et al*., 1980, Matrai and Keller, 1994). These microbes also encode a number of mechanisms for dealing with a heterogeneous and patchy environment, alternating a motile life-style (flagellar and gliding motility) with adhesion strategies (Moran *et al*., 2004, Gifford *et al*., 2012, Kaur *et al*., 2018).

R. pomeroyi is known for the high proportion of genes coding for signal transduction (1.6%) and transport/binding proteins (12.1%) enhancing its ability to sense and respond to environmental conditions outside the cell (Moran *et al*., 2004). The most commonly found transporters in Roseobacter are the TRAP (tripartite ATP-independent periplasmic), MFS (major facilitator superfamily) and ABC (ATP-binding cassette) families (Moran *et al*., 2007, Newton *et al*., 2010, Buchan *et al*., 2014), responsible for cellular uptake and export of a wide range of substrates (Davidson and Chen, 2004, Li *et al*., 2015). The regulation of specific transporters is triggered by signal transductions, accessing information about both their extracellular environment and their intracellular physiological status. Based on this information, they continuously interpret their environment and react rapidly to changes by coordinating gene expression. Recent evidence showed that signalling is not restricted to bacterial cell-to-cell communication, but also allows communication between microorganisms and their hosts (Hughes and Sperandio, 2008).

A solid understanding of the metabolic coupling that takes place during the interactions between bacteria and phototrophs is approached in the current chapter by selecting to examine the proteomic

response of one heterotrophic species, among the 14 that were used in Chapter 3, the model organism *R. pomeroyi* in the absence and presence of each of the nine phototrophs in oligotrophic seawater conditions. In natural oligotrophic seawater the organisms that co-exist require a strong nutritional coupling to support their survival due to the absence of other nutrient inputs (Durham *et al*., 2015, Durham *et al*., 2017). By this way the underlying molecular mechanisms that drive the interactions between microorganisms in nature can be further understood, contributing as well to the understanding of marine biogeochemical processes.

4.2 Materials and methods

R. pomeroyi was co-cultured with nine model phototrophic strains in 100 ml natural oligotrophic seawater (SW), as described in Section 2.2, including the control mono-culture of *R. pomeroyi*. Cells were harvested after three weeks of incubation, measuring cell abundance of the participant organisms (Section 2.3), and then the cell pellet of the cultures were run in proteomics analysis through nanoLC-ESI-MS/MS (Ultimate 3000 LC system coupled to Thermo Orbitrap Fusion mass spectrometer), using the methodology described in Section 2.4.1. Statistical analyses were executed in Maxquant and Perseus (Tyanova *et al*., 2016a, Tyanova *et al*., 2016b), comparing the nine co-cultivation treatments with the mono-culture condition (see Section 2.4). An overall picture of *R. pomeroyi*' s metabolic differences regarding the effect of each treatment on it, was represented in a principal component analysis (PCA) spatial distribution, where the distances between the samples are proportional to their differences (Holland, 2008) in a coordinated system. All the detected proteins including their statistical values were extracted and normalized, as explained in Section 2.4.2. The values of the proteins were not normalized to cell number per culture, since *R. pomeroyi* achieved approximately consistent abundance in all the cultures, see Table 4.3.2.1. After the normalization, the proteins were grouped in metabolic pathways based on the Kegg database. The values of all the included proteins per pathway were summarized in three representative values: i) the summed relative abundance, ii) the average fold change in Log_{10} scale (up or down regulated proteins compared to the mono-culture profile), and iii) the percentage of proteins included in a pathway with significant changes ($p < 0.05$). The steps of the experiment and analysis followed in the current chapter are summarised in Figure 4.2.1.

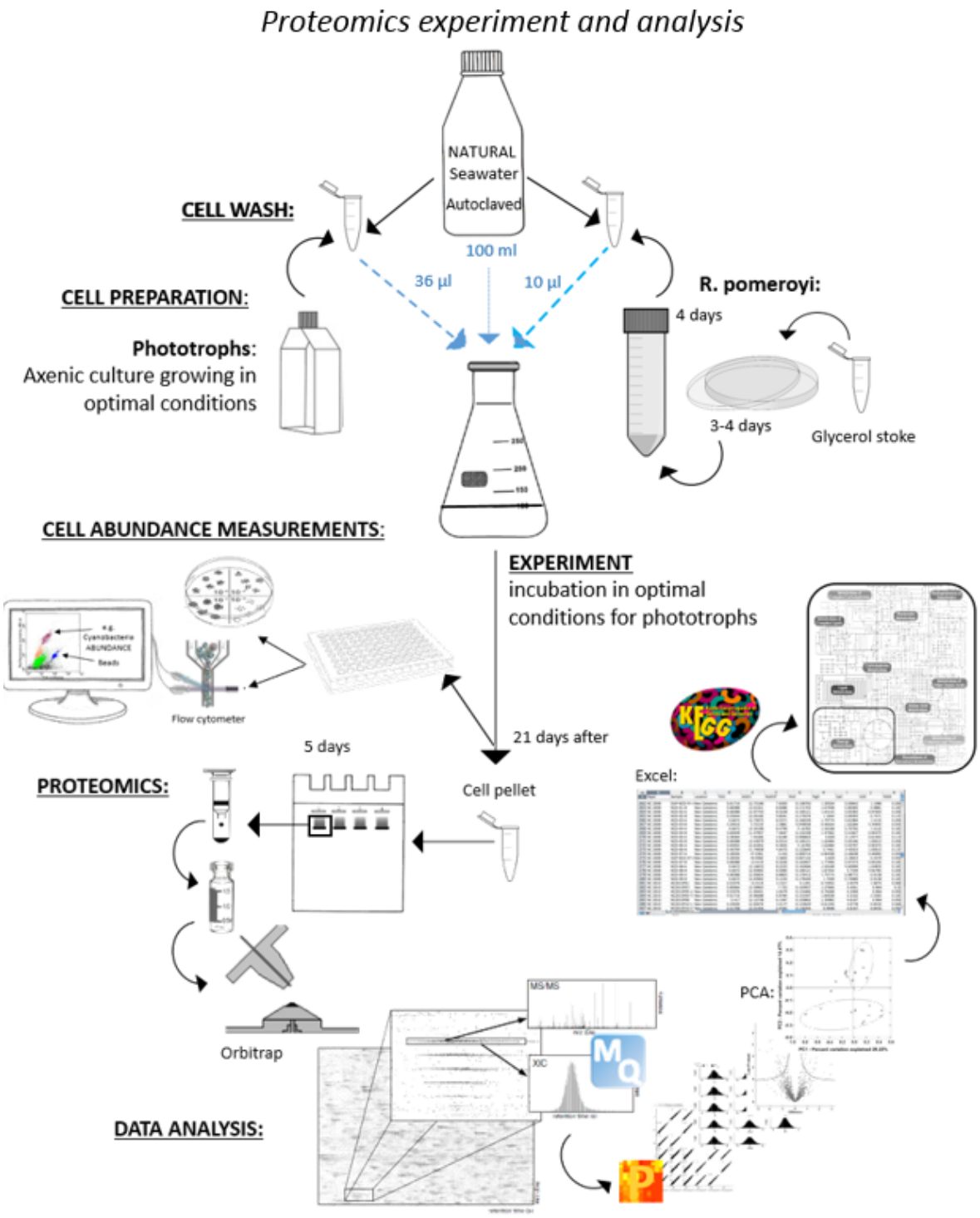


Figure 4.2.1: Workflow of proteomics experiment, including the steps of the co-culture set-up, harvesting and processing of the cultures for proteomic analysis, followed by the extraction and proteomic analysis through MaxQuant (MQ), Perseus (P) software, and the protein categorisation in metabolic pathways.

4.3 Results

4.3.1: Survival of R. pomeroyi in presence/absence of nine different phototrophic organisms

The proteomic response of *R. pomeroyi* to the presence of nine phototrophs in co-culture treatments under low-nutrient availability (natural seawater) were analysed in order to achieve a deeper understanding of the interactions that were observed in the previous chapter. The developed interactions between *R. pomeroyi* and the nine phototrophs were categorised based on their long term survival in co-culture conditions compared to their controls (mono-cultures), and which were presented as population curves in Figure 4.3.1.1 (extracted from the previous chapter, Appendix Tables 7.1 to 7.9).

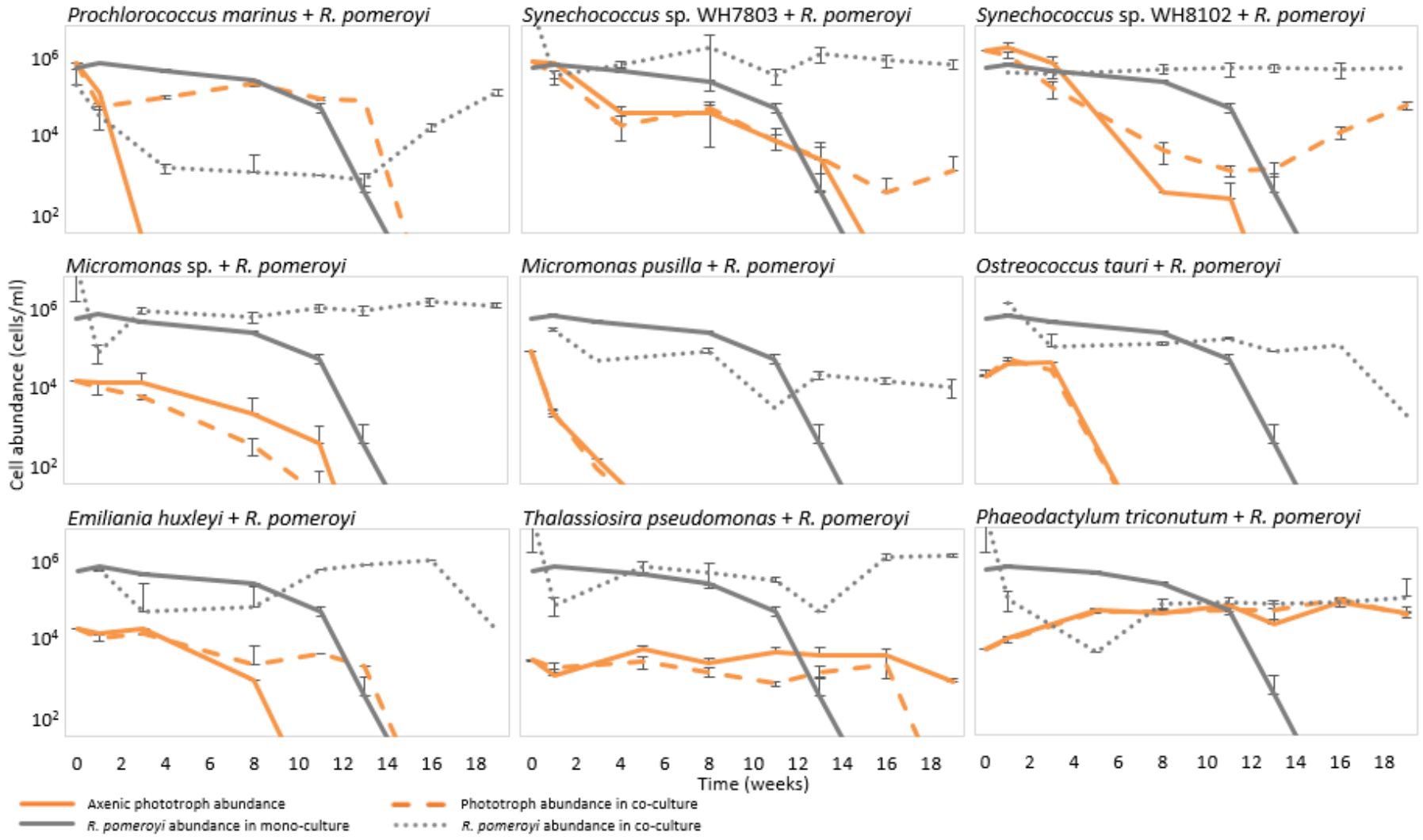


Figure 4.3.1.1: Population curves of *R. pomeroyi* (grey lines) and nine phototrophs (orange lines) in co-culture (dashed line) and control conditions (solid line) in seawater over 19 weeks. The error bars represent the standard deviation of three biological replicates.

The consistent survival of *R. pomeroyi* in all the co-cultures is remarkable, especially compared to its mono-culture condition where it died after 12 weeks incubation (Figure 4.3.1.1). Interestingly, among all the phototrophic treatments, the lowest *R. pomeroyi* cell abundance was measured in the *Prochlorococcus* treatment (10^3 cells/ml) during most of the incubation period, while in the rest of the co-cultures it never went below 10^4 cells/ml. In this co-*culture R. pomeroyi* was outcompeted by *Prochlorococcus*, but its cell density was re-established after the phototroph's death, suggesting that *Prochlorococcus* is more efficient in obtaining essential nutrients in oligotrophic seawater than *R. pomeroyi*. The majority of the phototrophs survived longer during co-cultivation with *R. pomeroyi* compared to their axenic cultures, with the exception of the green algae which died at the same time as their axenic controls. *M. pusilla* experienced the earliest cell death among all the cultures. Interestingly, both *Synechococcus* species survived the whole experimental period only in the presence of *R. pomeroyi*. In these cultures, the phototroph overcame a population bottleneck that their axenic counterparts did not. Both axenic and co-culture incubations of *Phaeodactylum* survived during the whole experimental period, making unclear the effect of co-cultivation with *R. pomeroyi* on its survival.

4.3.2: Collection of the proteomic data

The extended survival of most phototrophs over time due to the presence of *R. pomeroyi* suggests a metabolic coupling between the heterotroph and the phototroph in the co-culture. This was tested by high throughput proteomics. Due to low cell densities achieved in SW, cultures were set-up in 100 ml of seawater in order to have enough material for the proteomics analyses. Cells were harvested after three weeks of incubation for cellular proteomics analysis, when the coupling between the co-cultivated organisms assumed that has been established. The cell abundance at the harvesting point (Table 4.3.2.1) was similar or 10-fold higher than those observed during the long-term co-culture experiment (see Appendix Table 7.1 to 9). This slight difference can be explained by the higher light irradiation that is achieved in larger volume flasks (250 ml Erlenmeyer *versus* 50 ml tissue culture flasks).

The aims of the proteomic analyses were to: i) define the metabolic pattern of *R. pomeroyi* under all the co-culture conditions independently of the phototrophic species, ii) potentially determine the key

metabolites in the coupling between phototrophs and *R. pomeroyi*, and iii) understand the metabolic tendency of *R. pomeroyi* to help increase the survival of some phototrophs.

Table 4.3.2.1: Summary of the main characteristics of the proteome of *R. pomeroyi* extracted by the mono-culture and nine co-cultures.

			Co-culture with:								
		Mono-culture	*Prochlorococcus*	*Synech.* WH7803	*Synech.* WH8102	*Micromonas* sp.	*M. pusilla*	*Ostreococcus*	*E. huxleyi*	*Thalassiosira*	*Phaeodactylum*
R. pomeroyi cell abundance (cells/ml) in 3rd week		1.72E+05	4.90E+05	1.21E+06	3.49E+04	8.95E+05	2.23E+05	3.46E+05	1.17E+06	2.24E+05	2.98E+05
Unknown function proteins (%relative abundance)	302	7.53%	8.76%	10.12%	9.82%	7.27%	7.39%	8.64%	6.51%	7.78%	9.91%
Significant changed proteins (% to the total proteome)			4%	19%	5%	18%	3%	17%	1%	1%	8%
Relative abundance of significant changed preoteins			1.78%	25%	3.23%	18.53%	0.98%	13.52%	0.56%	1.17%	6.48%
Up-regulated proteins (% to the total proteome)			33%	40%	40%	37%	46%	41%	51%	36%	35%
Relative abundance of up-regulated preoteins			43.19%	56.61%	47%	66.54%	59.51%	52.74%	61.45%	54.55%	53.65%

Over 45% of *R. pomeroyi*'s CDS (1936 extracted proteins) were confidently detected in at least one of the culture conditions (Table 4.3.2.1). There was a high abundance of proteins of unknown function, considering the existing functional information in the available protein sequence databases. Specifically this abundance was increased from 7.5% in mono-culture to over 9.8% in the presence of both *Synechococcus* species and *Phaeodactylum*, indicating a selective expression of operons with unknown function in these treatments. In addition, the proteome of *R. pomeroyi* was significantly affected by the phototrophic treatments, especially in co-culture with *Synechococcus* sp. WH7803, *Micromonas* sp. and *Ostreococcus* (19%, 18% and 17% of the total proteome was significantly changed in comparison to the mono-culture, respectively; Table 4.3.2.1). Less than the half of the proteins were up-regulated in co-culture conditions, except in the co-culture with *E. huxleyi* (51%), whereas these proteins dominated the proteome abundance ranging from 43 to 66%.

4.3.3: Spatial distribution of R. pomeroyi's proteomic profile

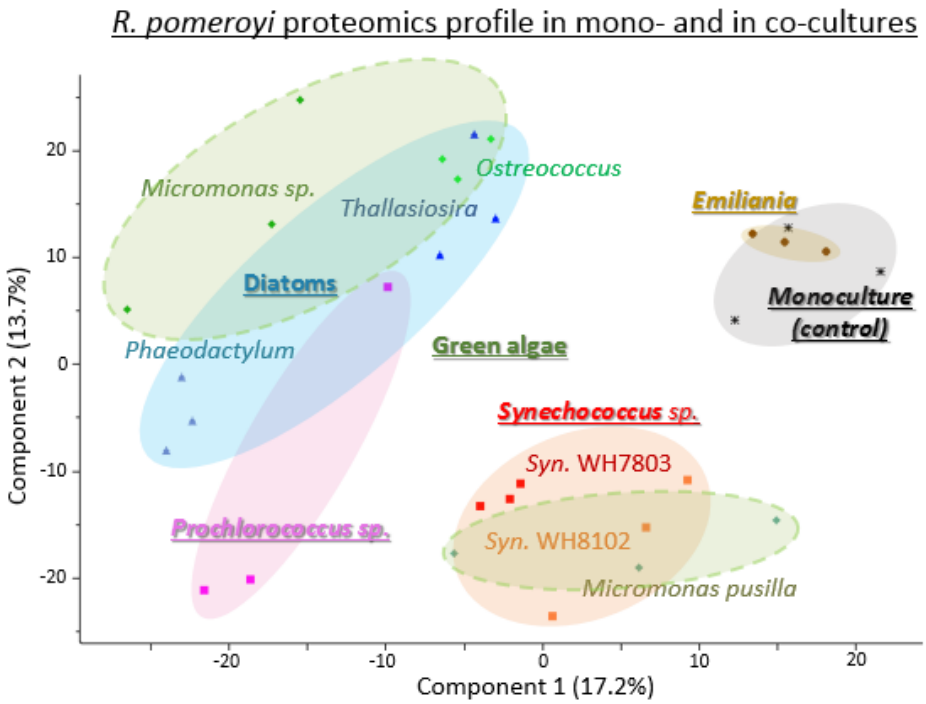


Figure 4.3.3.1: Principal component analysis (PCA) of the proteomics profile of *R. pomeroyi* after three weeks incubation in seawater in monoculture and in the presence of nine different phototrophs.

The proximity of the three biological replicates for each condition highlights the reproducibility of the proteomic response of *R. pomeroyi* to each treatment, with the exception of one of the replicates of *Prochlorococcus* which showed higher variability (Figure 4.3.3.1). Interestingly, the proteome of *R. pomeroyi* in mono-culture is clearly distinct from when it was grown in co-culture with all other phototrophs except for *E. huxleyi*, showing that the majority of the tested phytoplankton species influence of the proteomic profile of *R. pomeroyi*. Noteworthy is the clustering of the heterotroph's proteome when grown in the presence of the two *Synechococcus* species, and how these cluster as well with the *M. pusilla* co-culture. Under the co-culture conditions of *R. pomeroyi* with both *Synechococcus* species, the phototrophs achieved prolonged survival, estimated by the cell abundance measurements during the long-term co-cultivation experiment (Figure 4.3.1.1). Based on this observation, a positive coupling between the two *Synechococcus* species and *R. pomeroyi* is suggested. Under the co-culture conditions of *R. pomeroyi* with *M. pusilla*, where the phototroph experienced an early cell decline regardless of the presence of the heterotroph (Figure 4.3.1.1), *R. pomeroyi* took advantage of the nutrient releases of dying phototrophic cells. Hence, the similar proteomic profiles of *R. pomeroyi* when grown with 'dying' cells of *M. pusilla* and the two

Synechococcus species demonstrates a similar response of *R. pomeroyi* to available photosynthate without any competition involved. On the other hand potential competition was observed between *R. pomeroyi* and *Prochlorococcus*, given the low cell abundance of *R. pomeroyi* throughout the long-term co-cultivation experiment (Figure 4.3.1.1). This competition pressure between *R. pomeroyi* and *Prochlorococcus* is depicted by its proteome and presented as spatial segregation of this treatment in the PCA plot. The presence of other phototrophs *i.e.* the diatoms *Thalassiosira* and *Phaeodactylum* and the green algae *Micromonas* sp. and *Ostreococcus*, whilst showing specific proteomic differences (see Section 4.3.4 below), did not show large differentiated proteomics patterns in *R. pomeroyi* as depicted in the PCA plot.

4.3.4: Categorisation of specific metabolic pathways in R. pomeroyi

From the 1936 proteins detected by proteomics: i) 744 of them were not included in any category due to unclear functions and low abundance values, ii) 302 proteins were sorted as hypothetical due to their uncharacterised function, and iii) 888 proteins were categorised into metabolic pathways (Appendix Tables 7.10 to 7.12). The molecular processes that drive the interaction of *R. pomeroyi* with each one of the phototrophs was dissected and categorised in metabolic pathways, summarised in Figure 4.3.4.1.

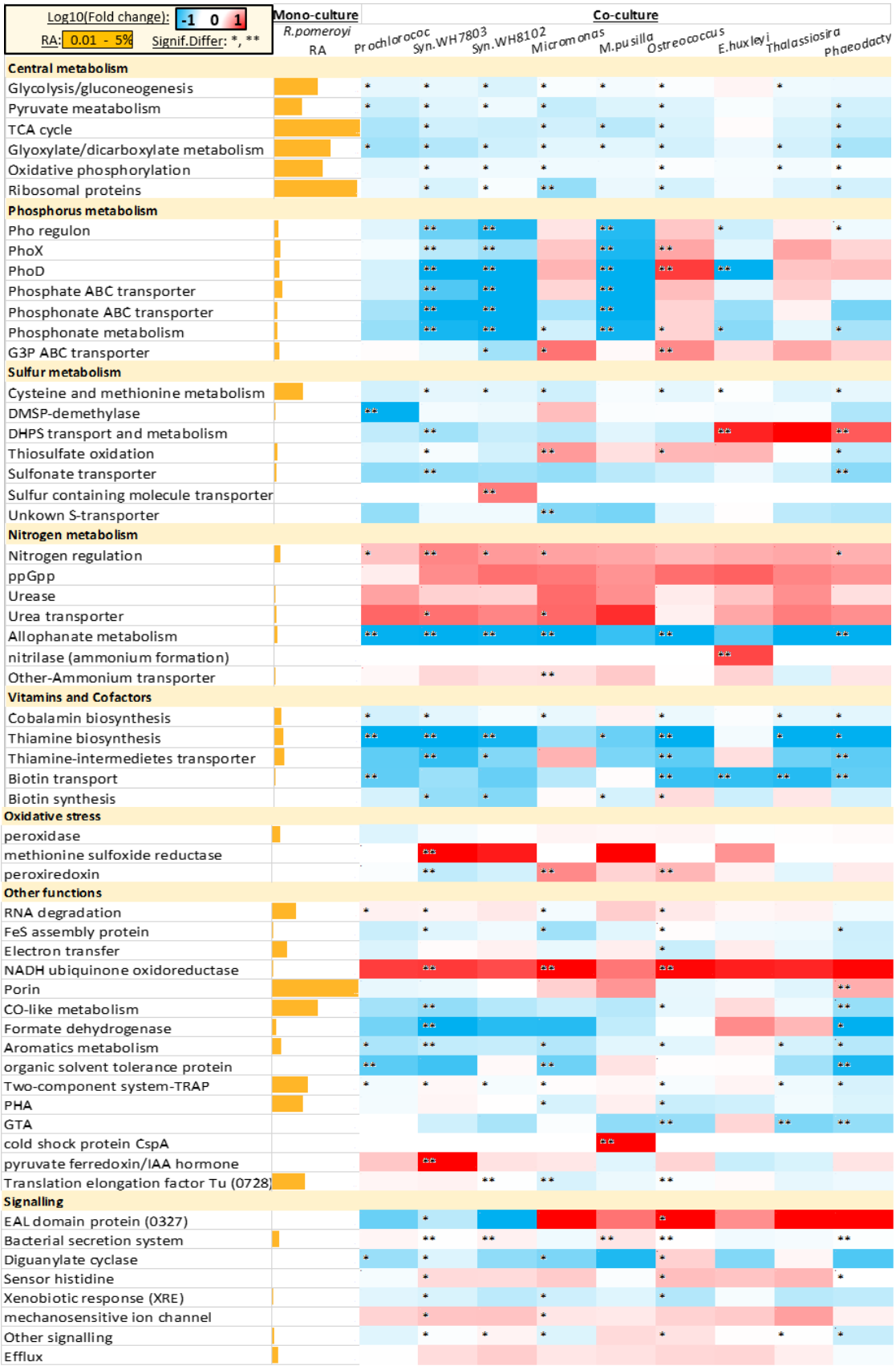


Figure 4.3.4.1: Regulation of different metabolic pathways in *R. pomeroyi* when grown in the presence of nine phototrophic organisms. Orange bars represent the relative abundance (RA) of each pathway in mono-culture. The heatmap represents the down-regulation (blue) and up-regulation (red) of each

pathway when the phototroph was present in relation to the mono-culture. Asterisks represent significant changes (no asterisk: no protein within the pathway was significantly differentially detected; one asterisk: less than 50% of the proteins that made up the pathway were significant; two asterisks: over 50% of the proteins were significant).

The most abundant pathways detected in *R. pomeroyi*, mainly from central metabolic processes (Figure 4.3.4.1), showed an overall down-regulation in all the co-culture treatments when compared to the heterotroph grown in mono-culture, although some specific proteins within this category such as the NADH:ubiquinone oxidoreductase were highly up-regulated (from 3 to 15 fold change in the co-cultures with *M.pusilla* and *Micromonas* sp., respectively; see Appendix Table 7.12). Other highly abundant proteins such as porins (5%), two component systems (2%), carbon storage in the form of polyhydroxyalkanoate (PHA; 1.8%) and RNA degradation (1.5%) also showed a low level of variation amongst conditions. We believe the overall lower relative abundance of these central metabolic functions in the presence of the phototrophs comes as a consequence of the higher investment of the heterotroph for scavenging the higher diversity of scarce compounds in the deplete SW. For example, functions involved in scavenging for alternative sources of energy, *i.e*. formate, aromatic compounds or carbon monoxide, were mainly up-regulated when grown in mono-culture (Figure 4.3.4.1).

The components of pathways involved in the transport and metabolism of key nutrients (*i.e*. phosphorus, sulfur, nitrogen and vitamins) showed the strongest differential expression under each one of the specific treatments:

- The phosphorus (P) response of *R. pomeroyi* was clearly affected by the different phototrophic treatments as shown in Figure 4.3.4.1. A strong and significantly down-regulated P-response was observed in *R. pomeroyi* when it was grown in the presence of both *Synechococcus* species and *M. pusilla* (10 fold change in the Pho regulon, PhoX, PhoD, phosphate transport, and phosphonate transport and metabolism). This response agreed with the closeness of these treatments in the PCA plot (Figure 4.3.3.1). On the other hand, *R. pomeroyi* increased its P-acquisition mechanisms when it was grown in the presence of *Ostreococcus*, *Micromonas* and *Thalassiosira*, which also all grouped in the PCA plot (Figure 4.3.3.1). This suggests that the variability in *R. pomeroyi*'s proteome is mainly driven by its response to P, highlighting the importance of phosphorus in the interaction of *R. pomeroyi* with each phototrophic species. The only function which in the majority of the treatments was downregulated was the

transporters predicted for phosphonate and phosphonate metabolism, except for the *Ostreococcus* treatment, suggesting that phosphonate was not a preferable source of P in the co-cultures as compared to the mono-culture conditions.

- Regarding *R. pomeroyi*'s sulfur (S)-metabolism, a few pathways responded to specific phototrophic treatments, suggesting the production of different reduced sulfur forms by each phototroph. For instance, in co-cultures with *E. huxleyi* and the two diatoms, *R. pomeroyi* showed a strong upregulation of the transport and degradation of dihydroxypropanesulfonate (DHPS; 6, 25, 4 fold change with *E. huxleyi*, *Thalassiosira* and *Phaeodactylum*, respectively). *R. pomeroyi* upregulated the thiosulfate oxidation pathway in the presence of the green algae (up to 2.5 in *Micromonas* sp.), the 'sulfur containing molecule transporter' only in the presence of *Synechococcus* sp. WH8102 (3 fold), whereas the sulfonate and unknown S transporter were down-regulated in most co-culture conditions. Curiously, *R. pomeroyi* did not show a differential expression of enzymes involved in DMSP catabolism (*i.e.* only the DMSP demethylase SPO1913 was detected) even when grown in the presence of *E. huxleyi*.
- A more general response was observed in the nitrogen (N)-metabolism of *R. pomeroyi* in the presence of all phototrophs. Despite the fact that nitrogen metabolism regulatory proteins are mainly regulated by phosphorylation (Zhang *et al*., 2018), an average increase of 2 was observed in these proteins when *R. pomeroyi* was grown in presence of the phototrophs. Among the mechanisms involved in N-regulation was the ppGpp phosphatase (involved in depleting the nitrogen-stress alarmone ppGpp; Magnusson *et al*., 2005, Zhang *et al*., 2018) which was up-regulated in all *R. pomeroyi*-phototroph co-cultures suggesting a decrease in of amino-acid starvation that occured in mono-culture conditions. Apart from the higher availability of N in the form of amino acids, *R. pomeroyi* also showed an increase in urea transport and metabolism independently of the photosynthetic species it co-existed with (*i.e.* the urease and transporter predicted for urea averaged a 2 fold and 3 fold increase in the co-cultures, respectively). The urea degradation by urease in the presence of the phototrophs was replaced by the alternative allophanate pathway (Hausinger, 2004) in the absence of phototrophs (average down-regulation -1.2 fold in co-culture conditions). The preference for specific metabolic pathways for urea degradation by *R. pomeroyi* under different environmental conditions requires further investigation.

- Unexpectedly, an overall decrease in vitamin synthesis and transporters was observed in all co-cultures (*i.e.* cobalamin biosynthesis, -1.2 fold changed; thiamine biosynthesis, -6.5; transport of thiamine-intermediates, -2.5; biotin transporter, -3.3; and biotin synthesis -1.2) suggesting two hypotheses: i) a high production of vitamins takes place under mono-culture conditions, or ii) the additional supply and/or recycling of vitamins by the phototroph. Thiamine was the vitamin that showed the highest down-regulation in the presence of phototrophs (except for *Micromonas* sp. and *E. huxleyi*) and possibly plays a different role, *i.e.* in cell starvation, as discussed below.

The general mechanisms to deal with oxidative stress, *i.e*. hydrogen peroxide (catalase), did not show large variations in the presence of the phototrophs but were generally highly abundant (~0.5%). Nevertheless, when *R. pomeroyi* was growing with both *Synechococcus* species, *M. pusilla*, and *E. huxleyi*, it increased the production of the repair enzyme methionine sulfoxide reductase (average 10 fold increase), whereas with *Micromonas* sp. and *Ostreococcus* it increased the regulating enzyme peroxiredoxin (average 2 fold increase). Nevertheless with *Prochlorococcus* and the two diatom species treatments, *R. pomeroyi* did not adapt a specific to oxidative stress response.

As expected, the signalling response varied between the different phytoplankton species. The most remarkable response was the glutamate-alanine-leucine (EAL) domain protein, identified by Hisert *et al*. (2005) to have a role in bacterial defence processes. This was strongly upregulated only in the presence of eukaryotic phototrophs (10 fold) suggesting a sensing mechanism of *R. pomeroyi* for this group of organisms. The diguanylate cyclase, an enzyme that participates in the formation of the ubiquitous second messenger cyclic-di-GMP, was mainly expressed in mono-culture and in the presence of *Ostreococcus* and *Thalassiosira* (-3.3 fold on average amongst the other phototrophs). Another condition-specific response of *R. pomeroyi* was the significant up-regulation of the cold shock protein A in the *M. pusilla* culture (10 fold up-regulated), which reveals the sensitivity of this species to the currently tested conditions as the co-culture with *M. pusilla* grew at 15^{o}C (instead of the 20-22^{o}C used for all other cultures; see Table 2.1.1.1). Other more generic signalling responses recorded in co-culture conditions were the mechanosensitivity and efflux channels, all up-regulated in the majority of the co-culture treatments (on average 1.6 and 1.2 fold change, respectively).

4.3.5: Active membrane transporters stimulated by the different phototrophic treatments

The current classification of transporter was based on recent publications and on the annotation given by Blast. However automatic prediction of transporters specificity is difficult, hence more targeted experiments are needed to confirm the role of the majority of the currently displayed transporters. Proteins categorised as membrane transport represented 20-30% of *R. pomeroyi*'s proteome, with the minimum encountered in the mono-culture (19.4%) and the maximum found in the presence of *Micromonas* sp. (29.5%). The most dominant group of transporters was for amino acids (AAs), ranging between 10-20%, followed by carbohydrate (CH) and 'other' nutrient transporters (5 and 9%, respectively). The relative abundance of the detected proteins included in specific operons or genetic clusters was summed and displayed in Figure 4.3.5.1 for amino-acid (AA) transporters, Figure 4.3.5.2 for carbohydrate (CH) transporters and Figure 4.3.5.3 for other transporters (raw data can be found in Appendix Table 7.11) .

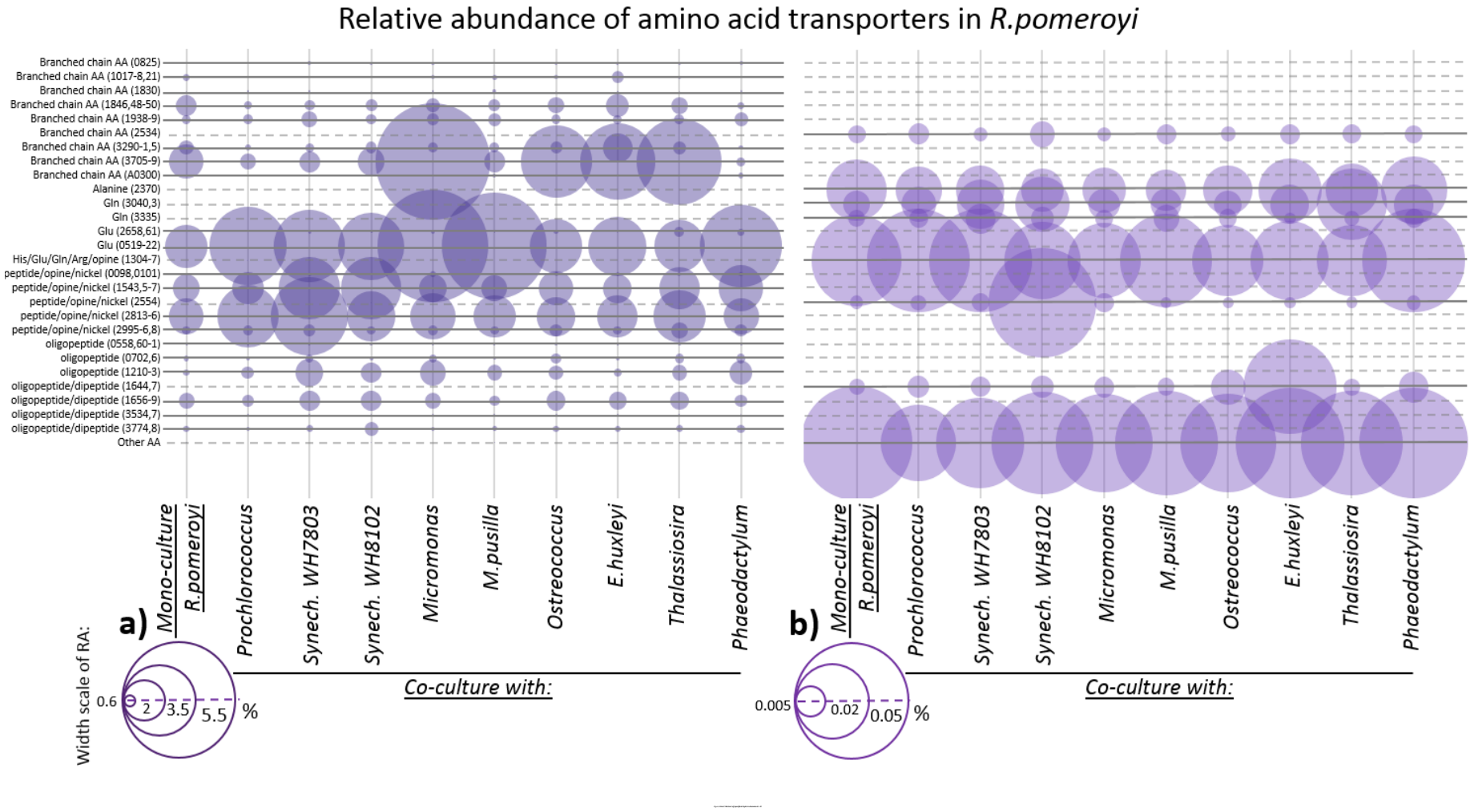


Figure 4.3.5.1: Relative abundance of amino acid (AA) transporter cluster detected from *R. pomeroyi* when grown in mono- and co-culture with nine phototrophic treatments. The annotation number SPO is shown in parenthesis. Cluster abundance is represented by bubble width as displayed in the scale on the bottom left of each chart. Panels a and b illustrate high and low abundant transporters, respectively.

Overall, active membrane transporters for amino acids were mainly upregulated in all co-cultures, and especially in the presence of *Micromonas* sp. (Figure 4.3.5.1). For example, in the presence of *Micromonas* sp., *R. pomeroyi* increased its ABC transporter predicted for glutamate (SPO0519-22; from 2% in mono-culture to 5.25% in co-culture) and branched amino acids (SPO3705-9; from 1.62% to 5.44%), two of the most expressed transporter operons (Figure 4.3.5.1). In addition, two transporters predicted for peptide/opine/nickel were also highly abundant with all cyanobacteria species (SPO2813-6 in the presence of *Prochlorococcus* and *Synechococcus* sp. WH7803, 3% and 3.7%, respectively; and SPO2554 detected only in the *Synechococcus* sp. WH8102 treatment, 0.05%).

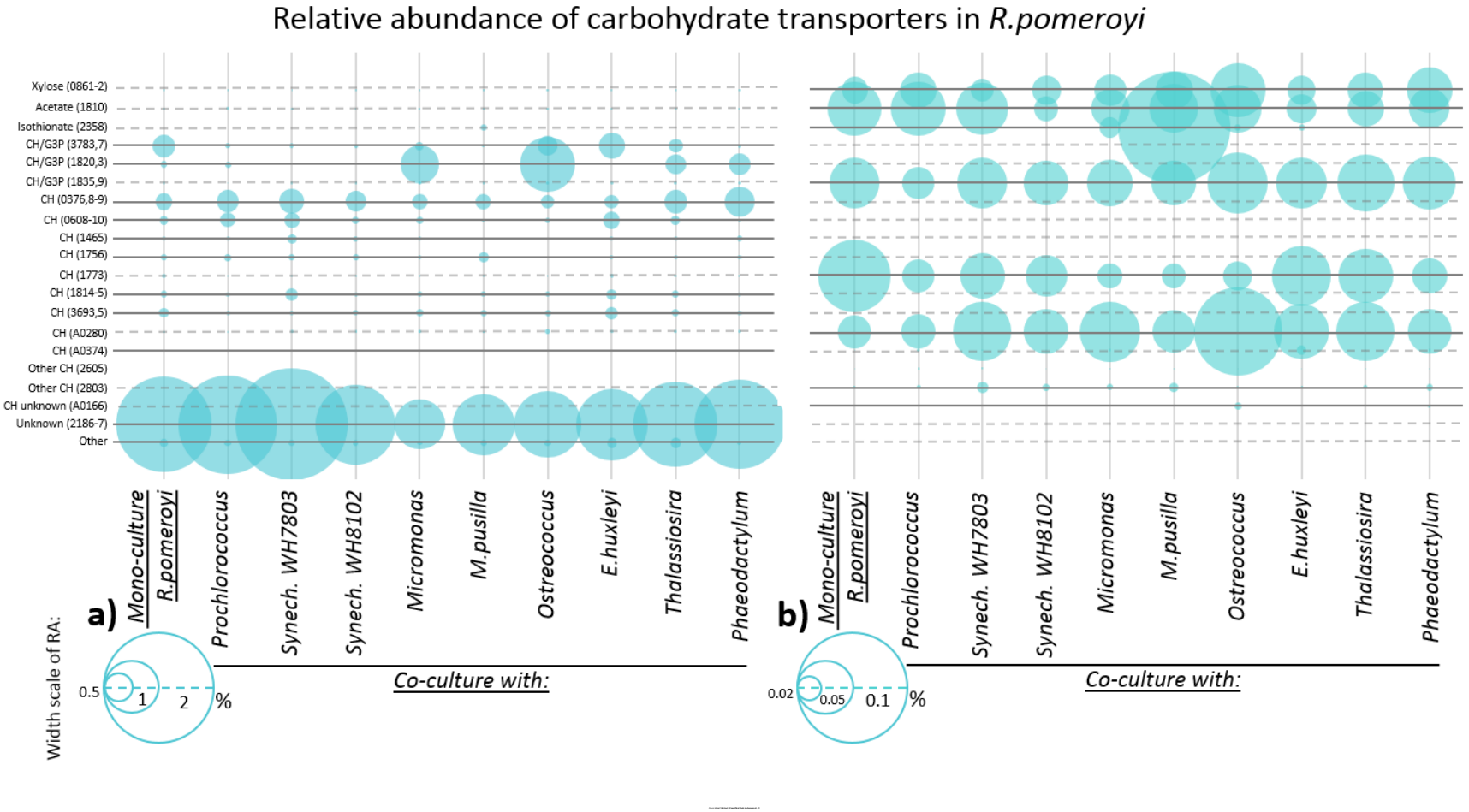


Figure 4.3.5.2: Relative abundance of carbohydrate transporter cluster detected from *R. pomeroyi* when grown in mono- and co-culture with nine phototrophic treatments. The annotation number SPO is shown in parenthesis. Cluster abundance is represented by bubble width as displayed in the scale on the bottom left of each chart. Panels a and b illustrate high and low abundant transporters, respectively.

A large variability within the detected carbohydrate transporters was also observed between the different phototrophic treatments (Figure 4.3.5.2), although the poor functional determination for most carbohydrate transporters poses a limitation in understanding the exact transported substrates. Some sugar transporters were triggered by specific treatments, like the highly expressed sugar transporter operons SPOA0280 (0.09%) and SPO0861-2 for xylose (0.05%) in the presence of *Ostreococcus*. Interestingly, the transporter predicted for xylose also coincided with the upregulation of xylose isomerase (Figure 4.3.6.1) in some phototrophic treatments, and especially in the *Ostreococcus* (3 x upregulated), suggesting increased availability of xylose in this co-culture. In addition, *R. pomeroyi* up-regulated its ABC transporter predicted for isothionate (SPO2358; 0.02% in *Micromonas* sp. and 0.11% in *M. pusilla*) only in the presence both *Micromonas* species, potentially released from taurine metabolism (Krejčík *et al*., 2010).

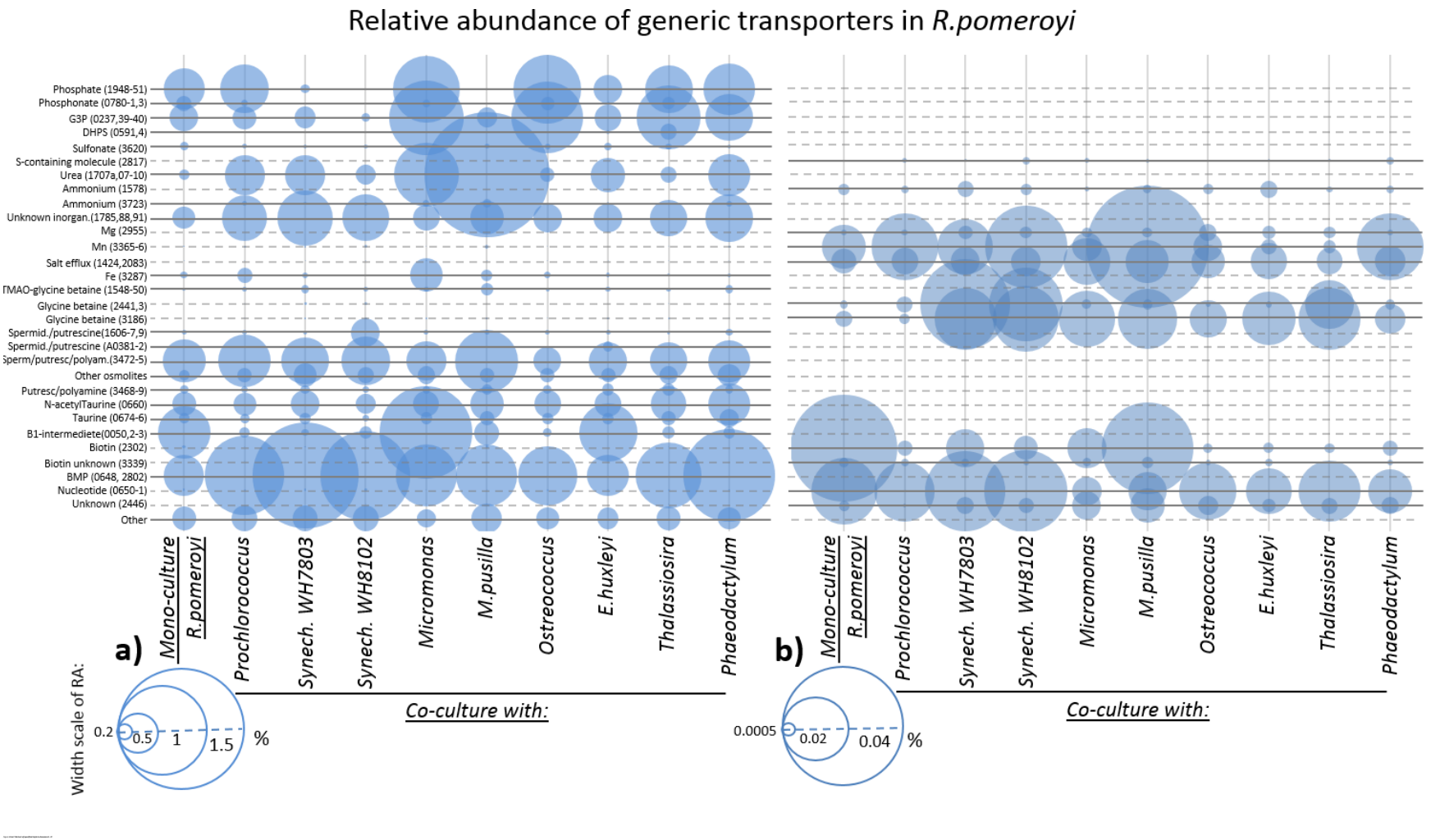


Figure 4.3.5.3*: Relative abundance of 'other'* transporter cluster detected from *R. pomeroyi* when grown in mono- and co-culture with nine phototrophic treatments. The annotation number SPO is shown in parenthesis. Cluster abundance is represented by bubble width as displayed in the scale on the bottom left of each chart. Panels a and b illustrate high and low abundant transporters, respectively.

Transporters for other substrates (other than amino acids and carbohydrates), also showed a large variability among the phototrophic treatments (Figure 4.3.5.3). The most dominant protein in this category was the basic membrane protein (BMP; periplasmic binding-domain, representing 1% of relative abundance), with undefined function. Another highly abundant transporter was for urea, mainly in the presence of *M. pusilla* (1.4%). Interestingly, in the presence of *M. pusilla*, *R. pomeroyi* also increased the abundance of transporters predicted for Mn and biotin (0.04% and 0.03% respectively), transporters that were much less abundant in other treatments (Figure 4.3.5.3). The most abundant expression of generic transporter operons was found in the presence of *Micromonas* sp., where *R. pomeroyi* presented a high abundance of transporters predicted for iron (0.4%), thiamine intermediates (1%) and phosphorus related transporters (1.7%). Specifically for phosphorus, transporters for phosphate and G3P were highly expressed in *Micromonas* sp. (0.8% and 0.9%, respectively) as well as in other treatments were *R. pomeroyi* showed a clear P-response, *i.e. Ostreococcus* (0.8% and 0.8%), *Thalassiosira* (0.5% and 0.7%) and *Phaeodactylum* (0.6% and 0.5%). Another dominant group of transporters among all the conditions were those for osmolytes, showing again a specific regulation depending on the co-cultured phototroph. Hence, while the transporters predicted for glycine betaine SPO3186 was almost generally upregulated in all the co-cultures, SPO2441-3 was only specifically expressed in the presence of both *Synechococcus* species and *Thalassiosira* treatments (0.03% and 0.02%, respectively). In addition, *R. pomeroyi* also up-regulated the osmolyte transporter for spermidine/putrescine (SPO1606-9; 0.33%) specifically in the presence of *Synechococcus* sp. WH8102, when the average abundance for this transporter under other conditions was only ~0.05%. Transporters predicted for organic sulfur compounds such as taurine and DHPS were detected. The first one was highly expressed with eukaryotic phototrophic treatments (N-acetyltaurine average 0.37% and taurine 0.16%), agreeing with the upregulation of taurine-pyruvate aminotransferase in the same treatments (Figure 4.3.6.1). The up-regulation of the transporter for DHPS was also consistent with the increase of DHPS dehydrogenase (Figure 4.3.6.1) in the presence of *E. huxleyi*, *Thalassiosira* and *Phaeodactylum* (DHPS transporter: 0.05%, 0.18%, 0.02% respectively).

4.3.6: Assembly of a metabolic map based on specific protein regulation

The transporter and the metabolic responses supported the distinct nutritional coupling of *R. pomeroyi* with each phototrophic species, and are summarised in specific metabolic pathways as represented in Figure 4.3.6.1 (raw values can be found in Appendix Table 7.11).

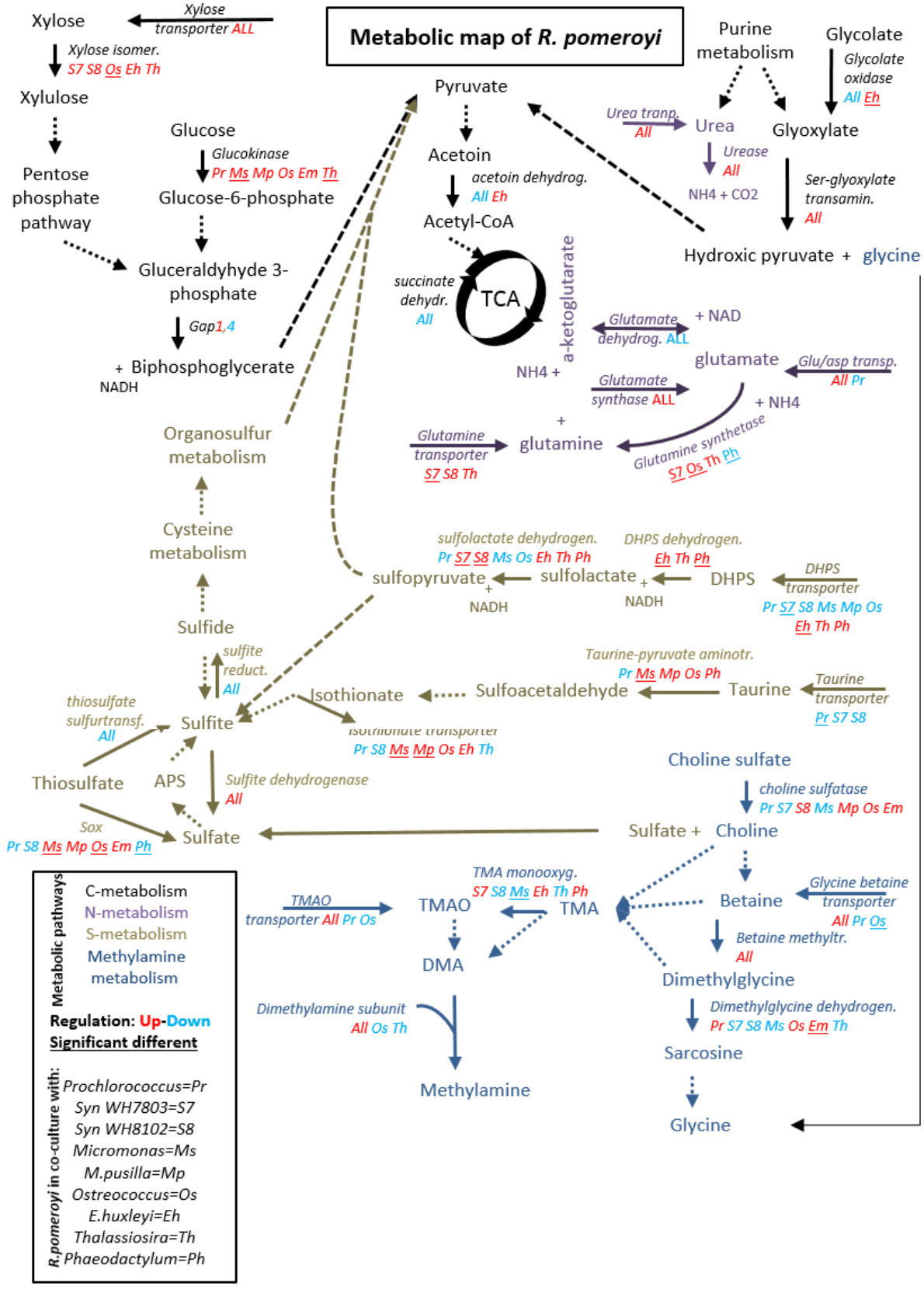


Figure 4.3.6.1: Metabolic map of *R. pomeroyi* based on specific protein detection in the cellular proteomics analysis of mono-culture and co-culture treatments. The specific proteins (in italics) that carry out metabolic steps (solid arrows) involved in four basic metabolic pathways (represented with

different colours) are displayed here along with the regulation (up-regulated in red, down-regulated in blue) and the significance (underlined only if significant) of these specific proteins in the co-culture treatments compared to the mono-culture, all described on the bottom left box. The dashed arrows define potential steps that may be carried out in the cell, but either these proteins were not detected or they were not specifically regulated in the currently extracted proteomics results.

The tricarboxylic acid cycle was fuelled by several forms of carbon when grown with phototrophs (Figure 4.3.6.1). For example, xylose, glucose and glyoxylate metabolism was upregulated with all the phototrophic treatments, along with organosulfur metabolism in the *Synechococcus*, diatoms and *E. huxleyi* treatments, with the latter also upregulating the heterotroph's glycolate metabolism. This highlights the great variety of carbon sources that *R. pomeroyi* had access to under the different phototrophic treatments. N-metabolism was largely up-regulated in all the co-culture conditions (as detailed above) with a potential formation of ammonia from urea, amino acids and osmolytes. However, the release of ammonia requires further investigation. Furthermore, *R. pomeroyi* seems to have a tendency to increase the production of glutamate under all co-culture conditions via the increase of glutamate synthase (up-regulated 2 fold) but not via glutamate dehydrogenase which implies ammonium incorporation (down-regulated -2 fold).

Regarding S metabolism, *R. pomeroyi* showed a diverse response to each phototrophic treatment (Figure 4.3.6.1). Generally, the heterotroph seemed to produce sulfite and sulfate from a variety of reduced sulfur compounds provided by the phototroph. For example in the three green algal species and *E. huxleyi* treatments, *R. pomeroyi*'s source of reduced sulfur was thiosulfate and isothionate, with the latter being derived from taurine. Both diatoms and *E. huxleyi* were clearly providing DHPS.

4.4 Discussion

The response of *R. pomeroyi* to the presence of nine different phototrophs was examined at the population level (see Chapter 3) and at the molecular level via proteomics (current chapter). The overall proteomics response of *R. pomeroyi* highlighted: i) its generic response to co-culture conditions independently of the phototrophic species, and ii) its specific response with respect to each phototroph.

4.4.1: Population response of R. pomeroyi in the co-cultures with nine phototrophs

The cell yields of the organisms in the co-culture compared to when grown alone contributes to the characterisation of the metabolic coupling between two organisms as successful or not. The presence of *R. pomeroyi* extended the survival of all three cyanobacterial species and *E. huxleyi*, but did not have an effect on green algae and diatoms, indicating the selective coupling of this heterotroph to only certain phototrophic species in natural oligotrophic systems (Cavender-Bares *et al*., 2001, Venter *et al*., 2004). *E. huxleyi* experienced survival extension compare to the axenic culture followed by a sudden lethal event in the presence of *R. pomeroyi*, similar to the one recorded during co-cultivation with the Roseobacter strain *Phaeobacter inhibens* (Segev *et al*., 2016), who characterised the interaction as positive that turned harmful over time.

Interestingly, co-culture with *Prochlorococcus* lowered the cell yields of *R. pomeroyi* and only recovered after the death of *Prochlorococcu*s confirming the efficiency of *Prochlorococcu*s in acquiring and retaining nutrients in oligotrophic systems (Lomas *et al*., 2014). *Phaeodactylum*, possibly due to its ability to re-mineralise its own photosynthate, also kept the heterotroph's numbers low. In both cases the low availability of organic matter reduced but did not eliminate the population of *R. pomeroyi*. Generally, the substrates released by phototrophic cell lysis or leakage was the main input of nutrients and kept *R. pomeroyi* alive throughout the 19 weeks. This was not the case when the heterotroph was grown in mono-culture, where *R. pomeroyi* died after 12 weeks. The increased bacterial performance growing with lysed phototrophic cells has observed by Smriga *et al*. (2016) and in natural algal blooms (Straškrabová and Komárková, 1979, Cole, 1982), but never before explored to the detail we presented here due to the lack of previous laboratory experiments carried out in natural oligotrophic seawater.

4.4.2: R. pomeroyi, a model organism for general marine catabolic functions

R. pomeroyi is a model microorganism used for studying a large variety of relevant marine metabolic functions (Christie-Oleza *et al*., 2015). The most commonly studied metabolic functions of *R. pomeroyi* were all detected in this study, such as carbon monoxide oxidation (cox genes), sulfur oxidation (sox genes), DMSP demethylation (dmdA), phosphonate utilization (phn genes), formate dehydrogenase, urea metabolism, glutamine synthetase and taurine metabolism (Moran *et al*., 2007, Gifford *et al*., 2012, Todd *et al*., 2012, Hanson *et al*., 2014, Sun *et al*., 2017). This highlights that this species does, in

fact, use all these metabolic functions to scavenge the wide diversity of scarce compounds within oligotrophic marine systems. In this way, the oligotrophic conditions used here affected the metabolic response of *R. pomeroyi* in a similar way as in nature, facilitating comparison of my findings with the existing literature for the interpretation of the interactions with phototrophs.

4.4.3: A generalist use of carbon and energy sources

R. pomeroyi is known for scavenging a large variety of substrates as sources of carbon and energy in marine oligotrophic environments (Moran *et al*., 2004). When grown in monoculture, where the only source of carbon and energy were the remains of compounds in the seawater, *R. pomeroyi* used aromatic compounds, formate and carbon monoxide (Figure 4.3.4.1). The lithotrophic function of CO metabolism was a supplementary energy source for the maintenance of the cells in oligotrophic conditions (King and Weber, 2007, Hanson *et al*., 2014), as also shown in the currently tested mono-culture conditions. Nevertheless, in the presence of diverse nutrients known to be provided by phototrophic organisms (Osterholz *et al*., 2016), *R. pomeroyi* demonstrated an alteration of its functions in order to metabolise organic compounds produced by the co-occurring primary producers such as glycolate and amino acids (Miller *et al*., 2004). Glycolate is utilised only by bacteria associated with phytoplankton (Lau and Armbrust, 2006) and amino acid are commonly found to be acquired by *R. pomeroyi* (Moran *et al*., 2004, Moran *et al*., 2007) and assimilated into biomass (Bryson *et al*., 2016).

4.4.4: Phosphorus acquisition strategies are driven by the phototrophs

R. pomeroyi obtained a specific response in the absence of the phototrophs by using the phosphonate metabolism, a P-form less preferable compare to phosphate. This response in mono-culture, where there is theoretically higher phosphate availability due to the lack of a competing phototroph, indicates a signalling role in interactions and requires further examination. The low phosphate availability in the cultures facilitated competition or cooperation between phytoplankton and bacteria as previously reported (Cembella *et al*., 1984, Thingstad *et al*., 1993). *R. pomeroyi* has been well-studied for its P-acquisition mechanisms (PhoD; Zaheer *et al*., 2009, Sebastian and Ammerman, 2011 and PhoX; Moran *et al*., 2007) highly expressed in oligotrophic environments, such as Sargasso Sea (Sebastian and Ammerman, 2009, Sebastian and Ammerman, 2011). It is interesting to note the down-regulation of P-stress occurred specifically with cyanobacteria and *E. huxleyi*, phototrophs known to dominate P-depleted environments and have higher phosphatase activity (Van Mooy *et al*., 2006).

This demonstrates that these phototrophic organisms complemented the extracellular phosphatase activity of the heterotroph and were able to make more P accessible to the system, establishing a P-metabolic coupling. The reduction of P-stress in co-culture with *M. pusilla* can be due to the increased lysis of phototrophic cells releasing P for the bacteria. *Prochlorococcus* did not induce a strong down-regulation, probably due to the low cellular P-stoichiometry (Baer *et al*., 2017), since this is one of the lowest P-demanding species (Scanlan and Wilson, 1999, Fuszard *et al*., 2012).

The rest of the co-cultures increased *R. pomeroyi*'s P-acquisition mechanisms, prompting the 'phytoplankton-bacteria paradox' (Bratbak and Thingstad, 1985). According to this paradox, in P-limited conditions, phytoplankton increases extracellular organic releases, such as glucose, encouraging the proliferation of its P-competitor, bacteria (Birgit *et al*., 2005). High glucose availability is supported by the up-regulation of glycokinase in all the treatments except of *Synechococcus* which, as suggested above, was a non-P-limited culture. Despite the increase of the P-acquisition mechanisms of *R. pomeroyi* in these co-cultures, the survival of the phototrophs was not shortened (see Figure 4.3.1.1) indicating that the P-competition did not have detrimental effects on the co-existed organism due to the supplement of other essential nutrients, such as nitrogen (Danger *et al*., 2007).

4.4.5: Nitrogen metabolism compensate the carbon stability of primary production

The recycling of nitrogen supports approximately 80% of primary productivity in surface waters (Bristow *et al*., 2017), with urea and ammonium being the preferable sources for bacteria and phytoplankton (Dortch, 1990, Solomon *et al*., 2010, Allen *et al*., 2011, Bertrand and Allen, 2012). The proteomic profile of *R. pomeroyi* showed the massive degradation of urea to ammonium by two distinct pathways, whose activation was affected by the presence or absence of phototrophs in the culture. In the presence of phototrophs, *R. pomeroyi* used urease through ATP-independent hydrolysis (Kanamori *et al*., 2004) and in the absence of phototrophs, it was catabolised using the three steps degradation pathway via allophanate (Hausinger, 2004). The question is why *R. pomeroyi* switches so strongly between both pathways. On the one hand, the urease pathway carries out degradation in one step, requiring CO_2/bicarbonate, GTP hydrolysis, and nickel ions (Mulrooney and Hausinger, 2003). On the other hand, for the degradation process via allophanate, the cells require biotin and bicarbonate, plus ATP as substrates for the reaction (Hausinger, 2004). Biotin biosynthesis and oxidative phosphorylation were both up-regulated in the absence of phototrophs, proposing them as

candidate functions for the regulation of degradation via allophanate. Although the reasons for encoding two different pathways is unknown, Hausinger (2004) suggested a few possible explanations: i) the available concentrations of nickel or bicarbonate in the cellular environment affect the expression of the two pathways, ii) the utilisation of one pathway at high substrate concentrations and the other at low concentrations, iii) one of the pathways having potentially more roles than urea hydrolysis. To these explanations I would like to add that these two pathways may have different kinetics and inhibition levels by the final product, ammonium. Due to the clear regulation of these two pathways regarding the mono-culture and the co-culture conditions, the currently tested experimental set-up is highly recommended for examining the alteration between the two pathways in *R. pomeroyi*.

Urea can be produced along with glyoxylate by purine metabolism (Solomon *et al*., 2010), both up-regulated in the co-cultures, indicating the increased requirement for urea metabolism for fulfilling the ammonium demands of phototrophs. The increased N-demands of phototrophs has been recorded as limited stoichiometric flexibility compared to C and P (Chan *et al*., 2012). In this way, the demands of the phototrophs for ammonium was depicted as nitrogen pressure on *R. pomeroyi*, which along with the urea metabolism, nitrogen regulation proteins, glutamine synthetase, urea and ammonium transporters were up-regulated in co-cultures, a pattern previously recorded in N-limited conditions (Chan *et al*., 2012).

4.4.6: Phototrophs triggered reduced sulfur utilisation in R. pomeroyi

Sulfur (S) is another essential nutrient for the growth of all living organisms, assimilated in the photosynthetic machinery (Ratti *et al*., 2011), amino acids and lipid metabolism (Michal *et al*., 2013). In these experiments *R. pomeroyi* seems to switch between a sulfate/sulfite metabolism in mono-culture to a larger use of reduced S-compounds in the presence of phytoplankton (Chan *et al*., 2012). The main sources of sulfur in the co-cultures with diatoms or *E. huxleyi*, was 2,3-dihydroxypropane-1-sulfonate (DHPS), instead of DSMP commonly produced by these species (González *et al*., 2000, Todd *et al*., 2012). DHPS is an abundant metabolite in diatom blooms, located in the *Thalassiosira* cytosol (Durham *et al*., 2017), and currently recoded to be produced by *Thalassiosira*, *E. huxleyi* and *Phaeodactylum*, and acquired by *R. pomeroyi* in the oligotrophic tested system. Furthermore, in the presence of *Micromonas*, *R. pomeroyi* presumably synthesized and exported an organic S-compound

isothionate (Moran *et al*., 2004, Durham *et al*., 2017) via taurine metabolism as described by Krejčík *et al*. (2010). This suggests increased taurine availability in the co-cultures with *Micromonas*, agreeing with the findings obtained during an amine analysis of *Micromonas* cultures by Dr Robert Haas in 2016 (unpublished data). In the co-cultures with green algae, sulfate was produced through thiosulfate oxidation (Sox), a lithotrophic system to utilise sulfur as an alternative method for energy supply (Kelly, 1982) when carbon is limited (Muthusamy *et al*., 2014, Luo and Moran, 2014). The stimulation of these functions demonstrates the behaviour of *R. pomeroyi* as a specialist for metabolising specific compounds when they become available from the primary producers.

4.4.7: Transporters nicely correlate with the metabolic processes

The regulation of transporters gives the most subtle clue of the organic and inorganic compounds that are used by microbes in the ocean. The high recorded abundance of transporters in the *R. pomeroyi* proteome was expected by the high dominance of transporter operons encoded in its genome (Moran *et al*., 2004) and high abundance observed in previous proteomic analyses (Christie-Oleza *et al*., 2012b, Kaur *et al*., 2018). Often, transporter's abundance is inversely proportional to substrate concentrations (Bergauer *et al*., 2017), regulated by the saturation levels of the cell. For this reason transporters have been mainly studied under nutrient-limited conditions (Moran *et al*., 2004, Moran *et al*., 2007, Vetting *et al*., 2015). However, this only applies to transporters that target essential nutrients, *e.g.* when P or N are limiting in the cell. The increased abundance of transporters for amino acids, substrates highly produced in the current co-culture by the phototrophs, is a response previously recorded in other co-culture experiments (Durham *et al*., 2017) and in natural samples (Kiene *et al*., 1998, Bergauer *et al*., 2017). The fact that *R. pomeroyi* was not amino-acid starved in the co-cultures, but only in the mono-culture conditions can be supported by i) the degradation of the signalling molecule, ppGpp (guanosine tetra- or penta-phosphate; Zhang *et al*., 2018) by the corresponding phosphatase, and ii) the reduction of diguanylate cyclase that synthesises the extracellular sensor related to amino-acid depletion (Camilli and Bassler, 2006, Zyskind and Smith, 1992, Magnusson *et al*., 2005) in co-culture conditions. This indicates that the abundance of transporters can also be increased under nutrient-repletion, as suggested by the Michaelis-like approximation by Armstrong (2008) supporting the replacement of the untargeted diffusion mechanism by targeted nutrient uptake. In addition, the absence of coordinated regulation of a series of transporters similar to the ones recorded in nutrient-limited experiments from the literature

(Moran *et al*., 2007, Vetting *et al*., 2015), such as the transporters TRAP (SPO1463), oligopeptides (SPO1645), alanine (SPO2370), branched amino-acids (SP03291 and SPOA0299) and carbohydrates (SPO37830), amongst others, indicates that individual transporters were up-regulated in the presence of specific organic compounds and not of due to starvation.

Also, the increase of osmolyte transporters in *R. pomeroyi* highlights the release of these compounds by the phototrophs, *e.g.* glycine betaine, putrescine/spermidine (Moran *et al*., 2004) and transporters predicted for trimethylamine N-oxide (TMAO) were up-regulated. TMAO is commonly found in natural seawater (Sowell *et al*., 2009, Lidbury *et al*., 2014, Li *et al*., 2015), with its transporter being regulated in response to trimethylamine (TMA) cellular levels (Ticak *et al*., 2014). The fluctuation of the intracellular TMA concentration is regulated by the parallel metabolism of glycine betaine by glycine betaine methyltransferase (Ticak *et al*., 2014), which was highly activated ensuring the continuous import of osmolytes in the cells in the current co-cultures (see Figure 4.3.6.1). In addition the stimulation of osmolyte transport systems along with sodium-based respiratory pumps (sodium symporters; SPO1810 and SPO2370 and a component of a multigene NADH:ubiquinone oxidoreductase; SPOA0030; Moran *et al*. 2007) regulated the ionic homeostasis of the cells in seawater, generating energy (Tringe *et al*. 2005) in co-culture conditions.

4.4.8: Vitamin exchange and oxidative stress alleviation

Vitamins are considered to play an important role in the chemical signalling between the majority of the auxotrophic algae species and bacteria, which are capable of synthesizing vitamins (Croft *et al*., 2005). The proteomic profile of *R. pomeroyi* during the co-cultures was not up-regulated as anticipated (Gonzalez *et al*., 1992, Bertrand and Allen, 2012, Bertrand *et al*., 2013, Helliwell *et al*., 2013), agreeing with a few studies of co-cultivation of *R. pomeroyi* with vitamin auxotrophs (Durham *et al*., 2015, Durham *et al*., 2017). The cultures were incubated in natural seawater obtained from the Sargasso Sea, a system known to contain extremely low vitamin levels (Gutowska *et al*., 2017). Despite the prototrophy of cyanobacteria and diatoms and the auxotrophy of green algae and *E. huxleyi*, *R. pomeroyi* displayed almost the same down-regulated behaviour with all co-cultured phototrophs, supporting the idea that vitamin exchange may not be actively regulated as part of the phototroph-heterotroph interaction, as was previously suggested (Durham *et al*., 2017). The high vitamin synthesis and release under mono-culture conditions proposes a targeting behaviour of *R. pomeroyi* to attract

phototrophs and after encountering them, the vitamin production being reduced. Only in the presence of *Micromonas* sp. and *E. huxleyi*, does *R. pomeroyi* up-regulate transport of thiamine intermediates indicating an attempt by *R. pomeroyi* to cover their needs releasing thiamine intermediates (see Figure 4.3.4.1; Bertrand and Allen, 2012, Gutowska *et al*., 2017). Another explanation for the strong reduction of thiamine biosynthesis in the presence of the phototrophs is that the phosphorylated product of thiamine was part of the cell starvation response (Lakaye *et al*., 2004, Gigliobianco *et al*., 2010). Hence, in mono-culture, *R. pomeroyi* was starved and produces thiamine triphosphate due to metabolic stress, a situation that was decreased by the presence of the phototroph.

The general belief is that bacteria alleviate primary producers from oxidative stress (Flohé, 2010, Poole *et al*., 2011), such as *Prochlorococcus* and some *Synechococcus* strains, *e.g*. WH8102, which lack a catalase function (Zinser, 2018). The absence of a peroxidase response in *R. pomeroyi*'s proteome along with no oxidative stress response with *Prochlorococcus*, indicates that the accumulation of reactive oxygen species in these co-cultures is not an issue, as already suggested by Christie-Oleza and colleagues (2017b). Nevertheless, peroxiredoxin (thiol-dependent peroxidase), was up-regulated with *Micromonas* and *Ostreococcus* treatments, and methionine sulfoxide reductase mainly with the *Synechococcus* treatments, demonstrating the selection of *R. pomeroyi* to use enzymes for specific oxygen species detoxification (Cabiscol Català *et al*., 2000, Dickschat *et al*., 2010) or for their dual role, for instance in cell redox signalling processes (Flohé, 2010, Poole *et al*., 2011).

To sum up, *R. pomeroyi* has a clear response to the presence or absence of phototrophs in the culture independent of the species, especially with regard to nitrogen, vitamins metabolism and energy responses. More specific responses were observed in *R. pomeroyi* in terms of membrane transporters, phosphorus and sulfur metabolism. The addition of metabolomics analysis would confirm the currently described predictions. Nevertheless, the wide range of metabolic activity of *R. pomeroyi* supports the generalist lifestyle of this organism along with its specialised responses in trophic interactions (Mou *et al*., 2008). In order to understand the effect *R. pomeroyi* had on the phototrophs, the proteomic response of the phototrophs was also examined in the next chapter, interpreting the metabolic coupling between the phototrophs and *R. pomeroyi*.

Chapter 5

Proteomic response of nine relevant phototrophic species to the presence of *R. pomeroyi* in natural seawater

5.1 Introduction

Marine phytoplankton are the primary producers of the oceans and contribute approximately half of the global net primary productivity (Field *et al*., 1998), comprising both prokaryotic cyanobacteria and eukaryotic photosynthetic microorganisms. Phytoplankton along with bacterioplankton numerically dominate the ocean planktonic community (Sarmento and Gasol, 2012) driving the microbial loop which, at a global scale, controls the carbon cycle and, ultimately, climate (Landa *et al*., 2016). Several studies has proven that heterotrophic bacteria influence algal growth and survival (Amin *et al*., 2015, Ramanan *et al*., 2016) by regulating nutrient availability (Christie-Oleza *et al*., 2017b) and reducing stressors, such as oxidative stress (Zinser, 2018). Among all nutrients, nitrogen has been found as the crucial macronutrient for phytoplankton growth (Christie-Oleza *et al*., 2017b). Despite the variety of inorganic (*e.g.* nitrate, ammonium) and organic nitrogen forms (*e.g.* urea) that phytoplankton can use (Glibert *et al*., 1986, Paerl, 1991, Allen, 2005, Read *et al*., 2013), the fastest growth rates are achieved with ammonium or urea (Dortch, 1990, Dortch *et al*., 1991, Peers *et al*., 2000), due to the low energetic costs associated with the assimilation of these forms into amino acids (Hildebrand, 2005). An example of the importance of nitrogen in algae is the diatoms that acquire cellular nitrogen homeostasis system, regarding the intracellular and the extracellular N-levels (Rosenwasser *et al*., 2014) and the complete recirculated urea metabolism. This is the only urea metabolic system where urea is not excreted as waste but intracellularly degraded by an urease to retain the C and N redistribution via the tricarboxylic acid cycle (TCA) and the glutamine synthetase/glutamate synthase pathway (Armbrust *et al*., 2004, Allen *et al*., 2011, Bender *et al*., 2012). Comparing the genome of two cyanobacteria for nitrogen related genes, *i.e. Prochlorococcus* MED4 and *Synechococcus* WH8102, highlighted that *Prochlorococcus* contains the common cyanobacterial genes necessary for the use of urea, cyanate and oligopeptides, but no nitrate reductase which was genetically lost along with its carbonic anhydrase, affecting the inorganic carbon concentration in the *Prochlorococcus* cells (Rocap *et al*., 2003). Despite these gene losses, cyanate and urea are the key players in the N cycle for cyanobacteria in marine oligotrophic environments (Kamennaya and Post, 2011). Cyanate can be found intracellularly as a by-product of the urea cycle or via the degradation of carbamoyl phosphate (Scanlan *et al*., 2009), but can also be imported by a specific membrane transporter (Espie *et al*., 2007).

Cyanobacteria, along with *E. huxleyi*, thrive in P-limited environments. Specifically, *E. huxleyi* encodes for six inorganic phosphate transporters and a high-efficiency alkaline phosphatase, placing this organism as a strong competitor for phosphorus (Read *et al*., 2013). Cyanobacteria have very low intracellular P-requirements, which is depicted in their stoichiometry and genomic content and organisation, allowing cells to establish a low P, relative to C and N, stoichiometric balance (Bertilsson *et al*., 2003). Nevertheless the outcome of occupying oligotrophic environments is not the same for *Prochlorococcus* and *Synechococcus* as different strategies are used by both *i.e. Prochlorococcus* ecotypes adapt to light intensity while *Synechococcus* to nutrient gradients (Zwirglmaier *et al*., 2008). Despite marine picocyanobacteria being known as strict photoautotrophs, they have also been suggested as mixotrophs through the use of an array of organic compounds (Rocap *et al*., 2003, Zubkov *et al*., 2003, Zubkov *et al*., 2004, del Carmen Muñoz-Marín *et al*., 2013).

Like *Prochlorococcus* and *Synechococcus*, many other marine phytoplankton species that belong to the same phylum have evolved completely different metabolic pathways, adopting diverging life strategies. For instance, the two genome-sequenced diatom genera *Thalassiosira* and *Phaeodactylum* are genomically distant as human and fish (Bowler *et al*., 2010), and species within the genus *Micromonas* shows divergence equivalent to the separation between corn and rice (Worden *et al*., 2009, Worden *et al*., 2015). *Ostreococcus* and *Micromonas*, despite both belonging to the green algal order *Mamiellales*, the first is one of the smallest in genome and cellular size among eukaryotic organisms, achieved by metabolic fusion, increased dependence on extracellular supplements and loss of motility (Derelle *et al*., 2006). Oppositely, both *Micromonas* species, sp. and *pusilla*, have larger genome than *Ostreococcus* and retain flagella (Worden *et al*., 2009).

The complication of the comparison of different species is derived by the genomic potential of each species aligned with their stoichiometric needs. The performance of each species in the environment is influenced by the relative abundance of both macronutrients and trace elements, in accordance to their stoichiometric needs. For instance, limitation for certain trace metals will affect species that encode enzymes that require them as a cofactor, as occurs in specific alkaline phosphatases (Bruland *et al*., 1991, Sunda and Huntsman, 1995b, Sunda and Huntsman, 1995a, Saito *et al*., 2002, Yeala *et al*., 2006). Vitamins are other micronutrients that play a key role in the physiology of phototrophs, with only a fraction of the eukaryotic species being able to synthesize them or use a vitamin-independent metabolism. Those that are vitamin auxotrophs need to develop relationships of dependency with

vitamin producing bacteria (Croft *et al*., 2005, Helliwell *et al*., 2011, Bertrand and Allen, 2012, Bertrand *et al*., 2012). Incomplete biosynthetic pathways in the evolutionary process depend on the import of a precursor that can be more abundant in seawater than the vitamin itself (McRose *et al*., 2014).

Extracellular and intracellular concentrations of macro and micro-nutrients drive the response of gene expression in all organisms. The presence of bacteria surrounding phytoplankton influences nutrient availability and as a consequence their gene expression. In order to determine the effect of bacteria on phytoplankton, nine relevant marine phototrophs were cultivated in the presence and absence of the model bacteria, *R. pomeroyi*, in oligotrophic seawater. The task of this chapter was to dissect the metabolic demands of the phototroph that were covered by the co-cultured heterotroph and contributing to their survival. The combination of the results of *R. pomeroyi* (Chapter 4) with the phototrophs (current Chapter) reveals the metabolic coupling that depicted as interactions in the co-cultures (Chapter 3).

5.2 Materials and methods

The results of the current chapter were extracted by the same experiment as in Chapter 4, co-cultivating nine phototrophs with *R. pomeroyi* in natural oligotrophic seawater (SW) for three weeks using the process described in Figure 4.2.1. For the requirements of this chapter, axenic cultures of the nine phototrophic species were also included, allowing the detection of the effect of the presence of *R. pomeroyi* on the phototrophic metabolism. The differences and similarities between the two results chapters (4 and 5) are exposed in Table 5.2.1.

Table 5.2.1: Experimental differences between the proteomic analysis of Chapter 4 and current chapter.

Differences in the experimental concistency of Chapter 4 and 5		
Co-culture experiments:	*R. pomeroyi* proteomics (Chapter 4)	Phototrophs proteomics (Chapter 5)
Control conditions	*1: R. pomeroyi* mono-culture	9: axenic phototrophic cultures
Co-culture treatments	9: *R. pomeroyi* in nine co-cultures *	9: nine phototrophs with *R. pomeroyi* *
Number of analysed conditions	10	18
Number samples: co-culture+controls	27*+3	27*+27

* The same co-culture samples were used for extracting two different data sets

27 samples were run twice through MaxQuant, applying the database of each of the two co-existing organisms. The protein databases that were used, were derived from Uniprot, which based on translations of genome sequence submissions to the International Nucleotide Sequence Database Consortium. The currently used databases were updated on the 4th February 2017, with the analysis taking place in November 2017. In the current chapter the analysis of the co-culture samples was done by extracting the proteins from the phototrophic species in each sample, and comparing them with the corresponding axenic culture proteome through Perseus. The absence of a universal control for all the phototrophs did not allow the statistical comparison of the proteins among the species, but just within the species for treatment condition (co-culture) to the control (axenic). The process of proteomic data analysis is described in Sections 2.4.2 and 2.4.3. Categorisation of the phototrophic proteomes used the same basic metabolic processes as the ones described in the previous chapter, permitted correlation of the metabolism of each phototroph with *R. pomeroyi*, and predicting the efficiency of their coupling. The categories among the different phototrophs were not consisted by the same proteins, since different proteins were extracted from each phototroph, as it is described in the column "Detected proteome" in Table 5.3.1.1. The synopsis of each phototrophic profile was constructed separately, summing up the following values: i) the relative abundance, ii) the fold change in Log_{10} scale (up or down regulated proteins compared to the axenic culture) and iii) the percentage of proteins with significant change ($p < 0.05$), raw values can be found in Appendix Table 7.13.

5.2.1: Assessment of vitamin requirements of green algae species and E. huxleyi

Growth of the three green algae species (*Micromonas*, *M. pusilla* and *Ostreococcus*) and the one haptophyte species *E. huxleyi* was tested in the absence of each one of the three vitamins (thiamine, biotin and cobalamin) routinely added to their growth media. Cells from axenic cultures were harvested by centrifugation (15 min at 4,000 g) and resuspended in 10 ml of the corresponding media (*Micromonas*, *Ostreococcus*, *E. huxleyi* in K media, *M. pusilla* in K/ASW media) containing all vitamins according to the consistency described in Table 2.1.1.2 (positive control), no vitamins (negative control) and media lacking each one of the three vitamins: i) Thiamine exclusion, ii) Cobalamin exclusion, iii) Biotin exclusion and iv) only Thiamine inclusion. Cultures in all the above media were incubated in 50 ml tissue flasks under the specific optimal conditions for each phototroph as described in Table 2.1.1.1. Half of the cultures were sub-cultured weekly into fresh corresponding media at least 4 times, and the other half were sustained over three weeks without sub-culturing. Pictures of all the

cultures were taken weekly and used as evidence for the survival of the cultures in the corresponding condition.

5.3 Results

The proteomic profile of the phototrophs was determined after three weeks of incubation. The cell abundance of all the co-culture and axenic conditions was similar between the long-term survival experiment (Chapter 3, see Appendix Tables 7.1 to 9) and the culture performed to generate the biological material for proteomics (see Table 5.3.1.1).

5.3.1: Protein detection from the nine phototrophs

The genomic details and protein detection for each one of the nine tested phototrophs is detailed in Table 5.3.1.1.

Table 5.3.1.1: Summary of the genomic and proteomic characteristics of each phototrophic species

Organism	Protein annotation file name in Uniprot	Number of chromosomes	Coding sequence domain (updated on the 4/2/2017)	Mitochondrial proteins	Chloroplastic proteins	Cells/ ml achieved in co-culture conditions on the 3rd week	Number of detected proteins (%of the total predicted proteins)	Number of detected common proteins between the phototroph and *R. pomeroyi*	Detected mitochondrial (% of total detected proteome)	Detected chloroplastic (%of total detected proteome)	Significantly changed proteins (%of total detected proteome)	Upregulated preoteins in co-culture (%of total detected proteome)	% of categorised proteins (Up:Down-regulated in co-culture conditions)	% categorised as hypothetical
Prochlorococcus	Prochlorococcus MED4-1.942-4fEBR2017uniprot-proteome%3AUP000001026		1942			2.47E+06	1447 (75%)	0			8%	67%	15% (99:123)	16%
***Synech* WH7803**	SynWH7803_uniprotAUP000001566_AND_28proteomecomponent_3A-Chromosome_29		2530			4.62E+05	1271 (50%)	0			0.2%	43%	20% (135:127)	21%
***Synech* WH8102**	Synechococcus_WH8102_uniprot-proteome_UP000001422_March2017		2512			3.20E+05	1126 (45%)	0			1.5%	19%	22% (84:162)	24%
***Micromonas* sp.**	Micromonas commodaRCC299-10.115-4Febr2017uniprot-proteome%3AUP000002009	17 (polyploid?)	10116	85	124	3.98E+05	1454 (14%)	8	19 (1.3%)	53 (3.6%)	19%	40%	18% (126:130)	52%
M. pusilla	Micromonas pusilla-CCMP1545-10.250-4fEBR2017uniprot-proteome%3AUP000001876	19 (polyploid?)	10250	78	111	3.26E+04	735 (7.2%)	8	12 (1.7%)	41 (5.5%)	3.7%	31%	24% (82:94)	18%
Ostreococcus	OstreococcusTauri-OTH595-7745-4Feb2017-uniprot-proteome%3AUP000009170	20 (haploid)	7782	94	64	4.42E+04	1124 (14%)	4	22 (2%)	26 (2%)	15.5%	40%	18% (148:38)	65%
E. huxleyi	Emiliania huxleyi-CCMP1516-35707-22Aug2017-uniprot-proteome%3AUP000013827	?(diploid)	35728	176	44	2.07E+04	1196 (3%)	15	25 (2%)	7 (0.5%)	2.5%	42%	22% (36:222)	52%
Thalassiosira	Thalassiosira pseudonana CCMP1335-11718-4fEBR2017-uniprot-proteome%3AUP000001449	24 (diploid)	11752	51	65	1.53E+04	2026 (17%)	4	23 (1%)	17 (0.8%)	2%	42%	17% (109:235)	41%
Phaeodactylum	Phaeodactylum tricornutum-CCAP1055-10465-4Febr2017-uniprot-proteome%3AUP000000759	33 (diploid)	10498	28	63	1.45E+05	1482 (14%)	8	14 (1%)	13 (0.9%)	1.4%	39%	21% (144:162)	47%

The nine model marine phototrophs used for this study have a large range of genome sizes and, consequently, encode for very different genetic potential (*i.e.* from 1,942 CDS in *Prochlorococcus* to 35,728 in *E. huxleyi*; Table 5.3.1.1). Nevertheless, the range of detected proteins for each organism was much smaller *i.e.* between 2026 proteins detected in *Thalassiosira* to 736 in *M. pusilla*, with 5.5% of the latter encoded by the chloroplast. The percentage of proteins mapped to chloroplast proteins using the Uniprot database was high in *M. pusilla* along with *Micromonas* sp. compared to the rest of the eukaryotes (Table 5.3.1.1), revealing the dominance of these compartments in the metabolism of the *Micromonas* species. Despite the fact that cyanobacteria have the smallest genomes, their detected proteome size was comparable to the rest of the phototrophs (over 1100 proteins). As a consequence, the cyanobacteria achieved the highest proteome coverage *i.e.* 75% of the CDS of *Prochlorococcus* were detected. By contrast, the lowest coverage of proteins was achieved for *E. huxleyi*, which had the largest genome among all the tested species (*i.e*. 35,728 CDS of which only 3% were detected).

The presence of *R. pomeroyi* induced a significant change in less than 4% of the detected proteins in two out of the nine phototrophs (Table 5.3.1.1). S*ynechococcus* WH7803 was the strain that showed the smallest proteomic variation among the extracted proteomes with only 0.2% of the proteins being significantly regulated. A higher percentage of significantly changed proteins were observed in *Prochlorococcus*, *Ostreococcus* and *Micromonas*, with the latter obtaining the highest percentage (19%). This suggests that some organisms have been more affected by the presence of *R. pomeroyi* than others after the three-week incubation. This response is mainly driven by a down-regulation of protein expression (60-70% of the differentially detected proteins were down-regulated whereas 30-40% were up-regulated). *Synechococcus* sp. WH7803 shows a stronger tendency to down-regulate proteins in the presence of the heterotroph (80% down-regulated and 20% up-regulated) and *Prochlorococcus* is the only strain that shows an opposite trend with the majority of its proteins being up-regulated (33% down-regulated and 67% up-regulated; Table 5.3.1.1).

Nine PCA plots of the proteomic data obtained from axenic *versus* co-cultured phototrophs show, in most cases, a strong separation between the two conditions (*i.e*. mostly separated within the PC1 which represented the highest variability between the samples; Figure 5.3.1.1). On the one hand the majority of the axenic replicates grouped nicely in the plots, except of the *Synechococcus* sp. WH7803 and *Ostreococcus*. On the other hand the majority of the co-culture replicates did not grouped nicely,

either with one of the replicates being distant than the other two, such as in *Prochlorococcus*, *Synechococcus* sp. WH8102 and *Micromonas*, or all the three replicates showed high variability, such as in *E. huxleyi* and *Thalassiosira*.

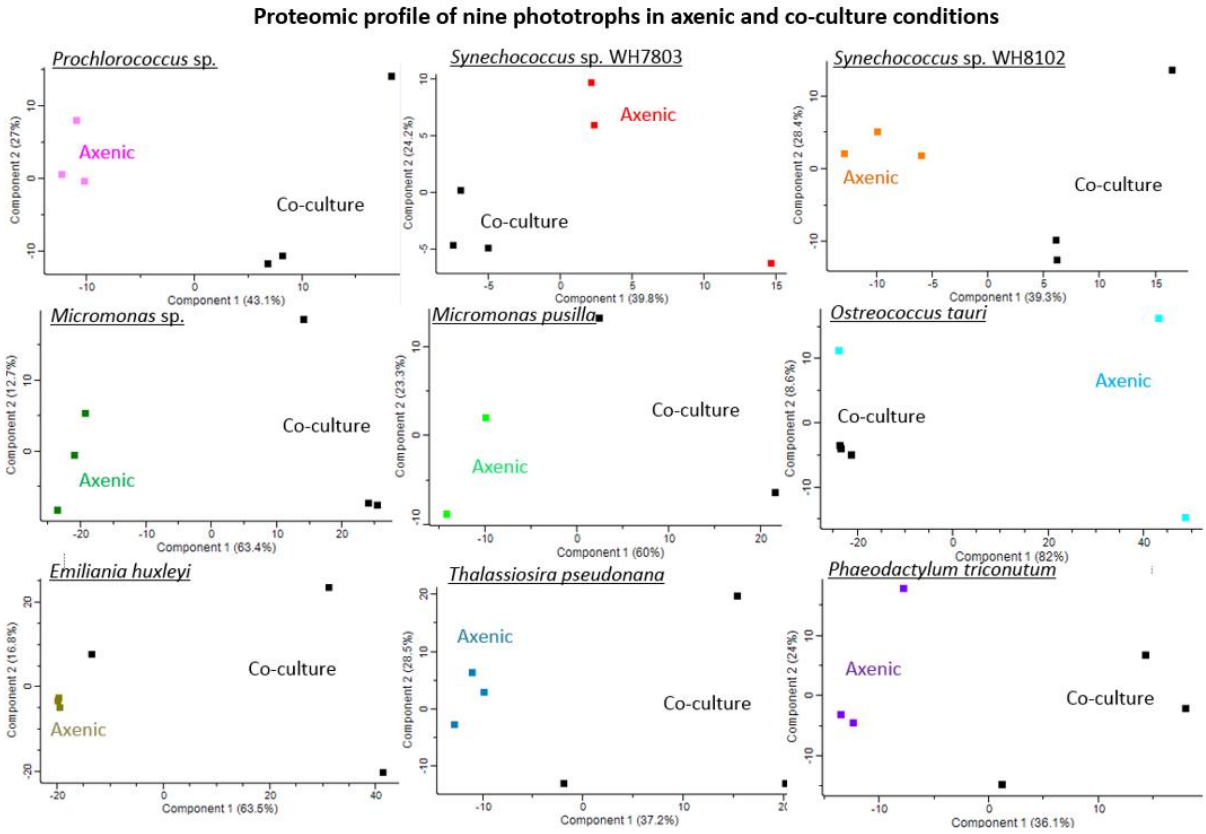


Figure 5.3.1.1: Principal component analysis (PCA) of the proteomic profile of nine phototrophs after three weeks incubation in seawater in axenic and co-cultivation with *R. pomeroyi*.

5.3.2: Proteomic categorisation of the phototroph response to the presence/absence of R. pomeroyi

The proteome of each phototroph was further examined by categorising the proteins into metabolic pathways (Figure 5.3.2.1). In this analysis almost 25% of the total detected proteins were categorised while almost 40% of *R. pomeroyi*'s proteome was covered in Chapter 4. This is mainly due to the lack of knowledge of most functions encoded in phototrophic microorganisms. The majority of the proteins with known function were mapped onto basic cellular metabolic pathways such as nucleotide and amino acid synthesis, though most proteins were poorly characterised and categorised as hypothetical, especially in eukaryotes (with the exception of *M. pusilla* with only 18% of its proteome

being uncharacterised proteins; Table 5.3.1.1). *Ostreococcus* and *Micromonas* were the two species with the highest percentage of proteins with unknown function (65% and 52%, respectively), along with the most significantly changed among all the phototrophs (Table 5.3.1.1). The high abundance of uncharacterised proteins in the eukaryotic cells was also apparent in Figure 5.3.2.1. For instance, in both *Micromonas* species the relative abundance of the uncharacterised proteins were 28% and 23%, respectively.

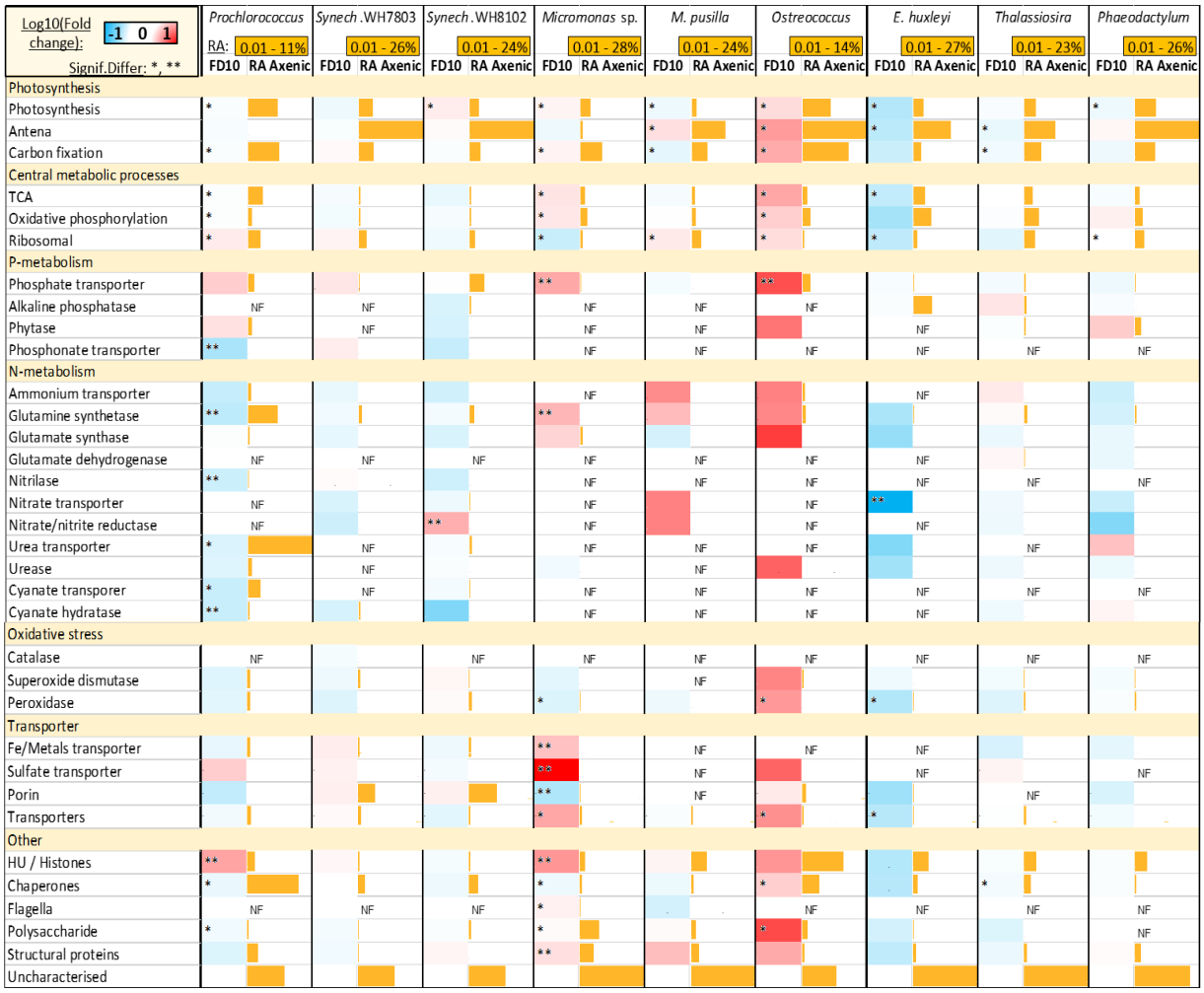


Figure 5.3.2.1: Regulation of different metabolic pathways in nine phototrophs in the presence of *R. pomeroyi*, considering the absence of pathways (not found-NF) from some organisms. Orange bars represent the relative abundance (RA) of each pathway in axenic culture, with the range specified for each phototroph in the top row. The heatmap represents the down-regulation (blue) and up-regulation (red) of each pathway when *R. pomeroyi* was present in relation to the corresponding axenic culture. Asterisks represent significant changes (no asterisk: no protein within the pathway was significantly differentially detected; one asterisk: less than 50% of the proteins that made up the pathway were significant; two asterisks: over 50% of the proteins were significant).

The categorisation of proteins revealed details of the interaction of each phototrophic organism with *R. pomeroyi* (Figure 5.3.2.1). As expected, proteins involved in photosynthesis and carbon fixation were among the most abundant functions in all phototrophs, averaging up to 30% of the total proteome. Specifically the proteins of the photosynthetic antenna were the most abundant of all, with a relative abundance of 26% in *Synechococcus* WH7803 and *Phaeodactylum*. Conversely, *Micromonas* sp. and *Prochlorococcus* whose antenna pigment proteins was almost undetectable with less than 1% abundance, including only phycoerythrobilin in *Prochlorococcus*. Nonetheless the photosystems I and II, and carbon fixation proteins were highly detected in both *Micromonas* sp. and *Prochlorococcus* (15% and 10%, respectively). Other proteins that dominated the proteomes were DNA packaging proteins (*i.e*. histones ranging from 5 to 9% in eukaryotes and 2% histone-like proteins in cyanobacteria) and chaperones (averaging 2%, with *Prochlorococcus*' proteome reaching up to 9%).

As mentioned above, the majority of phototrophs showed a stronger down-regulation of proteins in the presence of *R. pomeroyi* (Table 5.3.1.1) and this may come as a consequence of reducing the need for scavenging nutrients from different sources when the heterotroph is present. Nitrogen metabolism is a clear example that depicts this (Figure 5.3.2.1). Apart from the green algae, all phototrophs downregulated most of the pathways involved in acquiring and assimilating nitrogen. *Prochlorococcus* lowered its urea acquisition by downregulating the urea ABC transporter (from 11% in the axenic cultures to 6% in co-culture with *R. pomeroyi*; p-value 0.02) and urease (from 0.67% to 0.61%) as well as its cyanate metabolism (transporter predicted for cyanate, 2% to 1.5%, p-value 0.04%; cyanate hydratase, 0.03% to 0.01%, p-value 0.02). Similar to *Prochlorococcus*, *E. huxleyi* down-regulated the use of urea, as well as nitrate (transporter predicted for nitrate, 0.03% to 0%, p-value 0). The reduction in the use of nitrate was a generic trend except for *Synechococcus* sp. WH8102, which did show an upregulation of its nitrate oxidase despite the presence of *R. pomeroyi* (Figure 5.3.2.1). As shown in Chapter 4, *R. pomeroyi* showed a strong up-regulation of its urea metabolism, suggesting the production of ammonium (agreeing with the findings of Christie-Oleza *et al*. 2017b), the main source of nitrogen for the phototrophs. The co-regulation of specific pathways between the phototroph and heterotroph (*e.g*. urea metabolism, up-regulated in *R. pomeroyi* and downregulated in *Prochlorococcus* during co-cultivation) exhibits the correlation of the metabolic demands by the presence of each other. Nevertheless, *R. pomeroyi* triggered a different response in the green algae, which up-regulated their transporters predicted for ammonium and glutamine/ glutamate synthesis,

as well as the acquisition of other nitrogen sources in co-culture conditions (*e.g*. nitrate and urea, Figure 5.3.2.1).

While the strongest response of *R. pomeroyi* to the presence of phototrophic species was in phosphorus acquisition (Chapter 4), this was not so obvious in the case of the phototrophs (Figure 5.3.2.1). The phosphate transporter was highly abundant in some phototroph proteomes independent of the presence of the heterotroph, *i.e.* the transporter predicted for phosphate in *Synechococcus* WH8102 (5% relative abundance), *Prochlorococcus* and *Ostreococcus* (1%). Although *Ostreococcus* and *Micromonas* did show a significant up-regulation of their transporter predicted for phosphate in the presence of *R. pomeroyi* (2 and 5 fold change, respectively). This upregulation coincided with the proteomic profile that *R. pomeroyi* showed in co-culture with *Ostreococcus* and *Micromonas*, where the heterotroph was stimulated as well its P-acquisition mechanisms.

It is also worth noting the extremely high abundance of alkaline phosphatase in *E. huxleyi* (8%). It is uncertain if other phototrophs also produce such large amounts of secreted phosphatases to remineralise organic forms of P as these enzymes can be secreted to the periplasm or to the extracellular milieu (Kaur *et al*., 2018) and, hence, an exoproteomic analysis would be required to have a full picture of this function. Phytase was upregulated in *Prochlorococcus*, *Phaeodactylum* and *Ostreococcus* cells (1.2, 3 and 1.6 fold, respectively), suggesting an increase in degradation of their phosphorus storage components as a response to low phosphorus availability (Tetu *et al*., 2009). *Prochlorococcus*, as well as *Synechococcus* sp. WH8102, down-regulated its transporter predicted for phosphonate in the presence of *R. pomeroyi* (-2 and -1.6 fold change, respectively), demonstrating this as the least preferable P source under co-culture conditions.

The proteome of *R. pomeroyi* was mainly dominated by transporters (Chapter 4), something that was not the case in the proteome of the phototrophs, except for the porin proteins which were highly abundant in the two *Synechococcus* species (10% in *Synechococcus* WH8102 and 7% in WH7803). The most significantly up-regulated transporters were observed in *Micromonas* sp. in the presence of the heterotroph (*i.e*. predicted as ABC-transporters predicted for metals, calcium and magnesium, 8, 4 and 1.6 fold change respectively) which, curiously, was the co-culture treatment that also caused the highest abundance of transporters in *R. pomeroyi* (25% *versus* the average 20% when co-cultured with other phototrophs, Chapter 4), revealing a potential competitive response for acquisition of nutrients

in this co-culture. Among the transporters detected in *Micromonas* sp. was the one predicted for sulfate which, similar to the *Ostreococcus* culture, was significantly upregulated in the co-culture conditions (fold change: 25 in *Micromonas* sp. and 4 in *Ostreococcus*). This supports the idea that there is S-coupling between theses strains and *R. pomeroyi*, with the heterotroph releasing sulfate (see section 4.4.7) and the phototroph acquiring it.

Many publications have suggested that marine phototroph-heterotroph interactions are based on the quenching of reactive oxygen species (ROS) by the heterotroph due to the loss of catalase function in *Prochlorococcus* and some *Synechococcus* strains (*e.g*. WH8102; Zinser 2018). The presence of *R. pomeroyi* showed a subtle decrease in the phototroph's response to oxidative stress, mainly in terms of the production of superoxide dismutase, peroxidase and catalase (Figure 5.3.2.1). Nevertheless, *Ostreococcus* was the only strain that increased the abundance of proteins involved in ROS defence (superoxide dismutase 3 and peroxidase 2.5 fold change in the presence of *R. pomeroyi*).

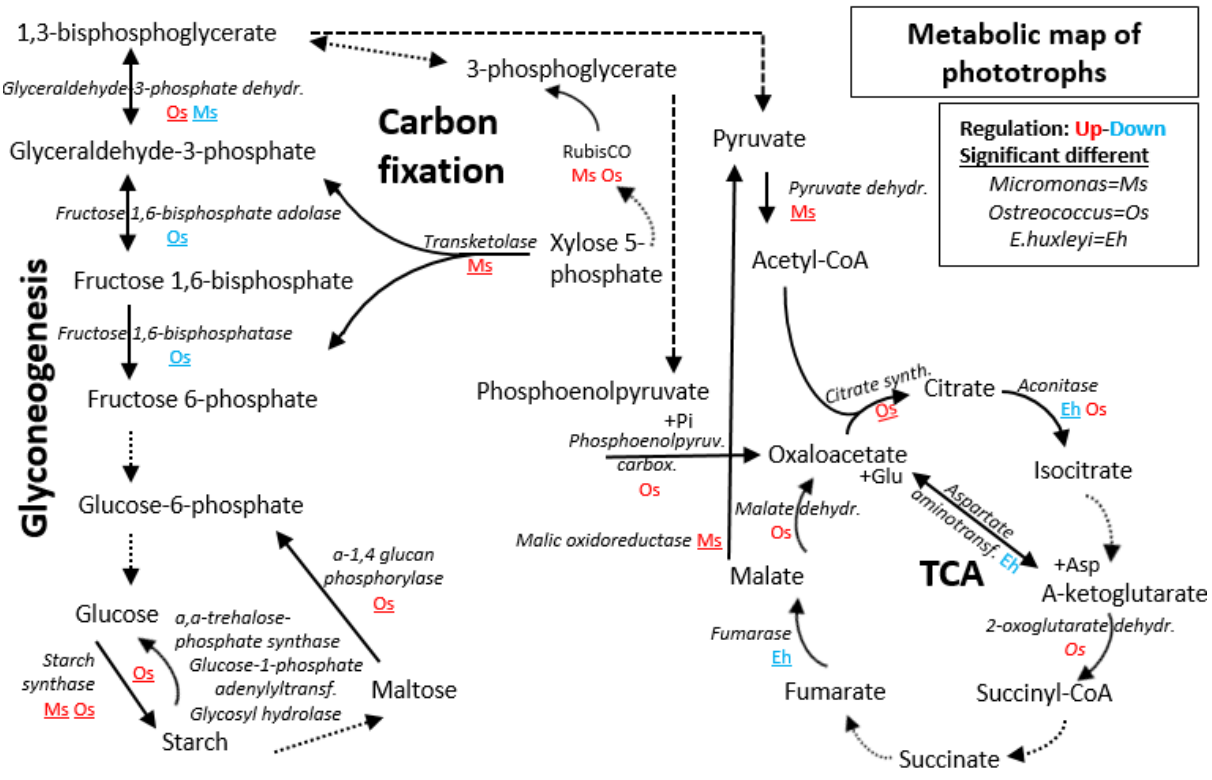


Figure 5.3.2.2: Central metabolic map of the three phototrophic species *Micromonas* sp. (Ms), *Ostreococcus* (Os) and *E. huxleyi* (Eh), including specific proteins (in italics) that carry out metabolic steps (solid arrows) involved in the Calvin cycle, glyconeogenesis and tricarboxylic acid cycle (TCA) are displayed here along with the regulation (up-regulated in red, down-regulated in blue) and the

significance (underlined only if p-value < 0.05) of these specific proteins in the co-culture treatments compared to the axenic conditions. The dashed arrows define potential steps that may be carried out in the cell, but either these proteins were not detected or they were not specifically regulated in the currently extracted proteomics results.

Micromonas sp., *Ostreococcus* and *E. huxleyi* were the species that showed a series of differential regulation of their central carbon metabolism in the presence of *R. pomeroyi* (Figure 5.3.2.2). While carbon fixation in *Ostreococcus* and *Micromonas* was mainly increased (*e.g.* RuBisCO varied from 2 and 3% in axenic cultures to 2.5 and 3.5% in co-culture, respectively), the glycogenesis in *Ostreococcus* was decreased and specifically the conversion of glyceraldehyde 3-phosphate to fructose 6-phosphate. Both *Ostreococcus* and *Micromonas* upregulated their starch synthase suggesting an increase in the storage of glucose in the form of starch, although *Ostreococcus* also up-regulated its degradation pathway back into glucose or to glucose 6-phosphate (Figure 5.3.2.2). Enzymes from the TCA cycle were mainly up-regulated in both green algal strains and down-regulated in *E. huxleyi* in the presence of the heterotroph. The TCA cycle requires equimolar oxaloacetate and acetyl-coA in order to complete the cycle and while *Ostreococcus* shows a large input of pyruvate/acetyl-coA into the cycle requiring a repletion of oxaloacetate (via phosphenolpyruvate carboxylase) in the presence of *R. pomeroyi*, *Micromonas* showed a trend requiring the conversion of malate into pyruvate when the heterotroph was present (Figure 5.3.2.2). On the other hand, *E. huxleyi* generally reduced its TCA enzymes in the presence of *R. pomeroyi*. Interestingly, the second step of the TCA cycle (*i.e*. citrate to isocitrate catalysed by the aconitase) was strongly down-regulated in *E. huxleyi* in co-culture (-8.3 fold change, p-value 0.02). Further work is required in order to determine if there is a metabolic coupling of TCA cycle intermediate compounds between *R. pomeroyi* and the phototrophs, in which case it would require the exchange of metabolites as previously suggested by (Braakman *et al*., 2017).

5.3.3: Testing the vitamin auxotrophy of eukaryotic phototrophs

The vitamin response of the phototrophs was not analysed through the extracted proteome due to the reduced detection of relative proteins, but through the growth of the eukaryotic auxotrophic species in vitamin depleted enriched media. Regarding the literature, the three green algal species, *Micromonas* sp., *M.pusilla* and *Ostreococcus*, and the haptophyte *E. huxleyi* have been recorded to lack many steps of the B1 and B12 biosynthetic pathway and are unable to grow in vitamin-deplete media.

Table 5.3.3.1: Literature and experimental evidence for vitamin auxotrophies in the six tested eukaryotic strains. On the left, genomic and culture evidence for B12 and B1 auxotrophy derived from literature reviews. On the right, experimental growth of the four phototrophs (*i.e*. three green algae and *E. huxleyi*) in the absence of all vitamins (No vitamins), in the absence of each one of the three vitamins (No B1, No B12, No B7), in the presence of only B1 (Only B1) and in the presence of all the vitamins (All vitamins). The displayed pictures of the four species were taken after sub-culturing four time in each media.

	Evidences of vitamin auxotrophy									
	Literature				Current experimental growth after 4-subcultivations in vitamin limited media					
	B12 auxotrophy by genome	B12 auxotrophy by cultures	B1 auxotrophy by genome	B1 auxotrophy by cultures	No vitamins	No B1	Only B1	No B12	No B7	All vitamins
Micromonas sp.	**Yes** (Helliwell, 2017)	**Yes** (Helliwell, 2017)	**Yes** (Bertrand 2012, Helliwell 2017)	**Yes** (Bertrand 2012, Helliwell 2017)						
M. pusilla	**Yes** (Bertrand 2012, Helliwell 2017)	**Yes** (Bertrand 2012, Helliwell 2017)	**Yes** (Bertrand 2012, Helliwell 2017)	**Yes** (Bertrand 2012, Helliwell 2017)						
Ostreococcus	**Yes** (Bertrand 2012, Helliwell 2017)	**Yes** (Bertrand 2012, Helliwell 2017)	**Yes** (Bertrand 2012, Helliwell 2017)	**Yes** (Bertrand 2012, Helliwell 2017)						
E. huxleyi	**Yes** (Bertrand 2012, Helliwell 2017)	-	**Yes** (Bertrand 2012, Helliwell 2017)	**Yes** (Bertrand 2012, Helliwell 2017)						

All green algae and *E. huxleyi* were clearly auxotrophic for thiamine (vitamin B1) but, unexpectedly, they all grew in the absence of cobalamin (vitamin B12), contradicting previous work (Table 5.3.3.1; Bertrand and Allen, 2012, Helliwell, 2017). Hence, while the four axenic strains showed a strong dependency for thiamine (*i.e*. cultures did not even grow after the first sub-cultivation), these seemed to grow nicely in the absence of cobalamin (vitamin B12) and biotin (vitamin B7) even after four sub-cultivations in the depleted media (Table 5.3.3.1, pictures). This surprising result was repeated in three different experiments and cultures were checked to discard heterotrophic contamination. Therefore, thiamine seems to be a key element for the survival of these four strains under the applied conditions, suggesting that their demands could have been covered by *R. pomeroyi*'s release of B1 or B1 intermediates (Table 5.3.3.1).

5.4 Discussion

The proteomic response of the nine phototrophs to the presence and absence of *R. pomeroyi* after three weeks of incubation in oligotrophic seawater reveals the effect of the presence of a heterotrophic species on their metabolism.

5.4.2: Common proteomic response of all phototrophs to the presence of R. pomeroyi

The higher number of down-regulated proteins in co-culture conditions with *R. pomeroyi* suggests an increased metabolic reliance of the phototrophs on the bacteria, as previously suggested by Aharonovich and Sher (2016). The proteome of the phototrophs was dominated by proteins of unknown function (whose abundance reached up to 28% of the detected proteins), complicating the interpretation of the extracted results, with the highest percentages observed within the eukaryotic species. Genomic analysis of these strains depicts the complexity of the organisms, such as *E. huxleyi*'s genome, with only 36% of its CDS being assigned to a known function due to the dominance of high density unclassified tandem repeats and low-complexity regions (Read *et al*., 2013). This justifies the high percentage of 52% unclassified proteins detected in the current *E. huxleyi*'s proteome (Table 5.3.1.1). Oppositely, the better-studied species *Phaeodactylum* had 62% of its proteoeme identified (Read *et al*., 2013) demonstrating the high scientific interest on organisms with industrial applications. Based on proteomics records, uncharacterised proteins in *Ostreococcus* were 13% of the total extracted proteome (Jancek *et al*., 2008) and 19% in *Thalassiosira* (Armbrust *et al*., 2004), percentages lower than the ones detected in the current study. This highlights the problem of missing information or even may lead to misinterpretations of results, increasing the necessity of the common task to assign functions to the large number of unknown proteins.

The next most abundant protein category, after the uncharacterised, were those involved in photosynthesis (*i.e*. photosystems and antennae), pointing to their importance as the main input of energy and the enormous burden this process means to the cell. The energy generated by photosynthesis is distributed among different functions (Beardall *et al*., 2001). The generic metabolic stimulation increases cellular energy demands, lowering the turnover rates of ATP and NADPH, forms commonly used as electron acceptors in photosystem I, which eventually leads to accumulation of intracellular reactive oxygen species (ROS) (Kana, 1992). The metabolic up-regulation of *Ostreococcus* in the presence of *R. pomeroyi*, probably increased its energy demands causing an oxidative stress response in *R. pomeroyi* producing peroxiredoxin (see Figure 4.3.4.1). However, the response of *R. pomeroyi* seemed not enough to alleviate the oxidative stress of *Ostreococcus*, which possibly was the reason for the up-regulated expression of superoxide dismutase and peroxidase in co-culture conditions (see Figure 5.3.2.1). A similar response of *R. pomeroyi* in other co-cultures, such as *Micromonas* sp. and *E. huxleyi*, could have been more effective on alleviating their oxidative stress,

since their peroxidase expression was down-regulated in co-culture conditions. In addition, the absence of an oxidative stress response and photosynthetic response in the presence of *R. pomeroyi*, contradicts previous findings of *Prochlorococcus'* co-cultures with bacterial strains (Morris *et al*., 2008, Zinser, 2018), suggesting that *Prochlorococcus* likely did not produce ROS in toxic levels in the current experimental set-up.

5.4.3: Proteomic response of Prochlorococcus

The large up-regulation of *Prochlorococcus* proteins (see Table 5.3.1.1) is in disagreement with the massive down-regulation of the transcriptome of the same organism when co-cultivated with *Alteromonas* by Aharonovich and Sher (2016), nevertheless this co-cultivation took place in enriched media, which is the crucial difference with the current study. While functions such as phosphonate and urea transporters, and cyanate metabolism were down-regulated in both studies, supporting the nutrient coupling with the bacteria, proteins involved in photosynthesis, protein synthesis, stress responses, biosynthesis of amino acids, purines, pyrimidines, fatty acids, phospholipids and cell cycle were differentially regulated in both studies (down-regulated in the co-cultures done by Aharonovich and Sher (2016) and up-regulated in the current co-cultures). We attribute such differences to the fact the transcriptomic analysis of the *Prochlorococcus-Alteromonas* co-cultures where performed on exponentially growing cultures in enriched media. Despite the differences between the two studies, they both suggest synergistic interactions. The dominance of the *Prochlorococcus* proteome by urea uptake and degradation (11%), a function that is missing in other phototrophs, such as *Synechococcus* WH7803 and *M. pusilla* (Solomon *et al*., 2010), along with its ability to metabolise ammonium (Moore *et al*., 2002), cyanate (García-Fernández and Diez, 2004), but not nitrate (DuRand *et al*., 2001), demonstrates the importance of nitrogen in the performance of *Prochlorococcus* cells (El Alaoui *et al*., 2001). Hence, a relationship based on the exchange of N was possibly developed between *Prochlorococcus* and *R. pomeroyi*, but not for other functions as observed previously in the co-cultures of Aharonovich and Sher (2016). This indicates the plasticity of *Prochlorococcus* with regards to nutrient availability, which derived from the highly sensitive two-component regulatory systems, such as histidine kinases and response regulators, and its high affinity ABC transporters for nutrients, despite their low number compared to other cyanobacteria (Scanlan and West, 2002). This plasticity can be considered more efficient than the one acquired by *R. pomeroyi* in this co-culture, since it was outcompeted at the population levels (see Figure 4.3.1.1).

All the *Prochlorococcus* species have evolved very low P-requirements even in P-rich media (Heldal *et al*., 2003), with common adaptation to P-limited conditions, such as high affinity phosphate transporter (Sabah *et al*., 2003, Martiny *et al*., 2006, Fuszard *et al*., 2012). The down-regulation of these functions in the presence of *R. pomeroyi* suggests that it covers its cellular P-needs, allowing a P-coupling with *R. pomeroyi* (slight down-regulation of *R. pomeroyi*'s P-acquisition mechanism; Figure 4.3.4.1) in the current experimental set-up.

5.4.4: Absence of a strong proteomic response of Synechococcus and diatoms in the presence of R. pomeroyi

Both *Synechococcus* did not show a strong proteomic response to co-cultivation with the heterotroph, a behaviour previously described in the literature (Palenik, 2012, Christie-Oleza *et al*., 2017b). It was suggested that *Synechococcus* is highly specialised in carrying out photosynthesis and carbon fixation and is not able to re-mineralise its own leaked photosynthate (Christie-Oleza *et al*., 2017b). This finding supports the requirement of *Synechococcus* to co-exist with heterotrophs that can recycle its organic matter and can this be the reason for the sustained survival of this strain in oligotrophic seawater (Cavender-Bares *et al*., 2001, Venter *et al*., 2004). This scenario has been described by Hagstr *et al*. (1988), who pointed at the balanced consumption/production rates of *Synechococcus* and bacteria in oligotrophic environments. The metabolism that *Synechococcus* adopts in oligotrophic conditions, and especially WH8102, involves the metabolism of urea, cyanate (Hanson *et al*., 2014), and reduction of phycoerythrin (the dominant N-rich pigment; Lourenço *et al*., 1998, Scanlan *et al*., 2009, Garcia *et al*., 2016). The absence of protein regulation in these pathways, similarly to previous studies of N-limited cultivation conditions (Six *et al*., 2004, Su *et al*. 2006), indicates that none of the current conditions limited N-availability. Nevertheless, the coordinated up-regulation of nitrate reductase (SYNW2464) and a nitrogen regulator (SYNW2496) in *Synechococcus* sp. WH8102 in the presence of the heterotroph, reveals the increased nitrate availability in the system, a N-form that cannot be utilised by *R. pomeroyi* (Christie-Oleza *et al*., 2017b), but is a significant N-source for *Synechococcus* sp. WH8102 (Su *et al*., 2006). This response was not observed in *Synechococcus* sp. WH7803.

In terms of P-limitation, the *Synechococcus* cells do not show a determined P-response due to the overlap of the protein functions facilitating a multi-coordinated response (Tetu *et al*., 2009). Among the mechanisms that *Synechococcus* uses to acquire P, we detected by proteomics the alkaline

phosphatase (Moore *et al*., 2005), high phosphate uptake transporters (Donald *et al*., 1997) and phosphonate metabolism, absent in eukaryotes (Palenik *et al*., 2003). The dominant presence of the P-related porin in both *Synechococcus* species (7% WH7803; SynWH7803_1779, 2236, and 11% WH8102; SYNW2224,7), indicates the importance of P in cell survival to P-limitation (Tetu *et al*., 2009, Palenik, 2012, Bernstein *et al*., 2017). The increased abundance of the P-related porin in *Synechococcus* WH7803 under SW incubation compare to ESW, previously recorded by Christie-Oleza *et al*. 2017b, suggests that cells were strongly affected by the oligotrophic seawater conditions independently of the presence of *R. pomeroyi*.

Diatoms are well-studied organisms not only for their genome and proteome, but also in cultivation and interaction studies (Geider *et al*., 1993, Brown *et al*., 1996, Fabregas *et al*., 1996, Armbrust *et al*., 2004, Urbani *et al*., 2005, Grossart and Simon, 2007, Amin *et al*., 2012b, Du *et al*., 2013, Paul *et al*., 2013, Poulson-Ellestad *et al*., 2014, Suleiman *et al*., 2016). In the current experiments, both diatom species *Thalassiosira* and *Phaeodactylum* were the least affected by the presence of *R. pomeroyi*, since they survived over time even in axenic cultures and their proteome showed only small variations. The unaffected metabolism of diatoms during their co-culture with bacteria was previously recorded in *Phaeodactylum* with *Alteromonas* by Diner *et al*. (2016). Nevertheless, the response of the diatoms was mainly to the low nutrient availability of the currently tested oligotrophic seawater since their response to nutrient starvation, and especially to N-limited conditions, has been recorded in *Phaeodactylum* (Allen *et al*., 2011, Feng *et al*., 2015, Longworth *et al*., 2016) and in *Thalassiosira* (Van Mooy *et al*., 2009, Dyhrman *et al*., 2012). Among the previously recorded starvation responses of *Phaeodactylum*, histone and DNA-methylation regulation was observed, revealing the importance of the chromatin state for expression or repression of genes and transposable elements in nutrient-limited conditions (Tirichine *et al*., 2017). Histones were highly abundant in the majority of the phototrophs but only up-regulated in *Prochlorococcus*, *Micromonas* and *Ostreococcus*, suggesting higher control of gene expression in the presence of *R. pomeroyi* in these strains but not in the case of the diatoms.

5.4.5: E. huxleyi's nutrient coupling with R. pomeroyi in co-cultures

Our current study agrees with the findings of McKew *et al*. (2013) regarding the abundance of proteins of unknown function and involved in energy production in the detected proteome. Interestingly, the

proteomic response detected here was similar to the one achieved when *E. huxleyi* was incubated under high irradiation (McKew *et al*., 2013), with a high detection of proteins involved in oxidative stress, chaperones, glutamate synthase, urea metabolism and membrane transporters pointing to the higher light received per cell when grown in highly dilute oligotrophic environments compared to when grown in ESW conditions, where cells shade each other.

Among the detected proteins, the alkaline phosphatase (EOD07255) showed a high relative abundance (8%), confirming the reputation of *E. huxleyi* as one of the most effective competitors for phosphorus among phytoplankton species (Riegman *et al*., 2000), blooming in phosphorus-deplete environments (Lessard *et al*., 2005). The alkaline phosphatase is regulated under P stress (Dyhrman and Palenik, 2003), but *E. huxleyi* kept the levels of alkaline phosphatase almost constant in the presence and absence of *R. pomeroyi.* On the other hand, the nitrogen metabolism of *E. huxleyi* did respond to the presence of *R. pomeroyi* probably due to the increased availability of ammonium released by *R. pomeroyi* (Christie-Oleza *et al*., 2017b), which down-regulated nitrate assimilation, as previously shown by Page *et al*. (1999). The uptake of ammonium by *E. huxleyi* has been recorded by the N-isotopic study of Nathalie *et al*. (1998), releasing ammonia in return, suggesting a nitrogen balance in the current *E. huxleyi* co-cultures.

5.4.6: Green algae metabolic stimulation in co-culture

Among the green algae, *Micromonas* sp. is the most commonly found species in oligotrophic water with a repertoire of high affinity transporters to scavenge and compete for nutrient (Worden *et al*., 2009), explaining the significant up-regulation of transporters in the co-culture conditions such as those for metals. The increased transporter detection could justify a competitive behaviour for nutrients with detrimental effects on the survival of *Micromonas* sp. and its bacterial partners (Figure 3.3.1.1). *R. pomeroyi* responded to this competitive behaviour also by increasing the expression of transporters. The other *Micromonas* strain, *M. pusilla*, is possibly less reliant on the extracellular levels of nutrients for its survival, since its genome encodes fewer transporter genes, as far as it is known, for instance it lacks some nitrogen-related uptake transporters such as for urea-uptake (Worden *et al*., 2009). Nevertheless, *M. pusilla* is able to acquire nitrate and ammonium (Conway, 1977, Collos, 1989), with the presence of ammonium suppressing nitrate uptake (Cochlan and Harrison, 1991). The small amount of ammonium made available by *R. pomeroyi* did not seem enough to suppress the uptake of nitrate in this study as shown by the upregulated use of nitrate in the co-culture.

Both Micromonas were the only motile phototrophs via flagella in this study. Proteins related to flagella and photosynthesis in *Micromonas* species were stimulated by the presence of *R. pomeroyi* (1.1 average fold change). Motility can be stimulated by a higher nutrient availability in co-culture conditions allowing also the increase of photosynthesis since the pigment synthesis in *Micromonas* species is nutrient dependent (Raven and Richardson, 1984, Halsey *et al*., 2014).

The absence of flagella in *Ostreococcus* (Halsey *et al*., 2014) has been correlated with less targeted nutrient uptake mechanisms, such as nutrient diffusion (Taylor and Stocker, 2012). *Ostreococcus* encodes a high number of transporters predicted for ammonium (two currently detected in this study with 0.5% relative abundance) compared to other eukaryotes such as *Thalassiosira* (Derelle *et al*., 2006) and *E. huxleyi* (Riegman *et al*., 2000), pointing at the importance of this element in the metabolism of *Ostreococcus*. In this case, the increased availability of ammonium in the co-cultures, stimulated a specific metabolic response similar to those previously recorded in nitrogen replete conditions (Martin *et al*., 2011b, Le Bihan *et al*., 2011).

The large effect *R. pomeroyi* had on the metabolism of the green algae did not explain the neutral interaction these organisms had in terms of their survival. A possible reason could have been the detrimental effects of the cultivation set-up, such as the continuous light regime in combination with the low-nutrient availability, since the circadian cycle of the currently tested green algal species is crucial for their survival (Kana and Glibert, 1987, Ottesen *et al*., 2013, Waltman *et al*., 2016). Having said this, a few bacterial species were able to prolong the survival of the two *Micromonas* species (Figure 3.3.1.1), demonstrating that it may be that *R. pomeroyi* could not alleviate green algal stress. In addition, the general lethal event of *Ostreococcus* with all heterotrophs in SW agrees with the natural findings of this strain: 1) it rarely co-occurs with bacteria and it is mainly stimulated by bottom-up nutrient injections (Sophie *et al*., 2017), and 2) it is not commonly found in highly oligotrophic seawater (Demir-Hilton *et al*., 2011). However, these strains thrived in enriched media pointing at the importance of the abundance of specific compounds, such as trace metals and vitamins.

5.4.7: Survival of the eukaryotic auxotrophs in the absence of vitamins

The survival of the eukaryotes in the absence of the vitamin B12 was not expected according to the auxotrophies reported in the literature (Bertrand and Allen, 2012, Helliwell, 2017). A possible explanation to reverting this auxotrophy is a substitution of the vitamin requirements by other

pathways. For instance, N-deficiency induces the up-regulation of pathways that demand B12 (Bertrand *et al*., 2012), and potentially N-repletion may allow recirculation of B12 and maintenance of population levels. Another explanation is that the natural SW used in these experiments contained high levels of B12 supporting the survival of the auxotrophic cultures, despite previous studies stated that B12 levels in natural filtered oligotrophic seawater are extremely low and not sufficient to support the growth of auxotrophs (Helliwell *et al*., 2016). Nevertheless, the auxotroph *Ostreococcus* has been detected in environments with extremely low ambient concentrations of vitamins (Sañudo-Wilhelmy *et al*., 2012), indicated that the mechanisms for acquiring vitamins are extremely efficient via the use of high affinity transporters (Croft *et al*., 2005).

B1 exchange between phototrophs and heterotrophs was less puzzling compared to B12, since the survival results of the auxotrophic species fit with literature reports (Table 5.3.3.1). *E. huxleyi* lacks the pathway for just one precursor for B1 biosynthesis (McRose *et al*., 2014), while *Micromonas* and *Ostreococcus* lack larger portions of the vitamin's biosynthetic pathway and encode a plant-like enzyme necessary for the condensation of precursors, whose addition is enough for the phototroph's survival (Bertrand and Allen, 2012, Paerl *et al*., 2015).

In summary the overall prediction of the phototrophic responses suggests that *Prochlorococcus* and *E. huxleyi* metabolically responded to the presence of *R. pomeroyi* after three weeks of incubation, though the *Synechococcus* and diatom species did not acquire such a strong response at this time point. Though most of these co-cultures had a positive outcome in terms of their survival extension compared to axenic cultures, this was not observed in the case of the green algae, whose metabolism was strongly affected by the presence of *R. pomeroyi,* without any beneficial effect on prolonging the survival of the phototrophs. This suggests that possibly the coupling between green algae and *R. pomeroyi* was not sufficient to alleviate the stress caused by the currently tested oligotrophic set-up of the cultures. These findings combined with the *R. pomeroyi* metabolic profiles (Chapter 4) and the long-term survival results (Chapter 3), contribute to the interpretation of the establishment of interactions between phototrophs and heterotrophs.

Chapter 6

Summary and Future perspectives

The interactions between phototrophs and heterotrophs was examined based on their survival and cell abundance in one-to-one co-cultures over time (Chapter 3). To deepen our understanding of these interactions, the proteomic profile of a series of cultures was analysed, revealing the proteomic variations of the heterotroph *R. pomeroyi* in the presence of nine different phototroph (Chapter 4), and each of phototrophs in the presence/absence of the heterotroph (Chapter 5) in natural oligotrophic SW. The species-specificity of interactions was demonstrated in this thesis.

6.1: Hypotheses on varying Phototrophs - Heterotrophs interactions due to different nutritional regimes

The long-term growth experiments illustrated the metabolic coupling between phototrophs and heterotrophs as the majority of combinations resulted in positive interactions suggesting a robust nutrient exchange between phototrophs and heterotrophs. Nevertheless, among the 126 one-to-one co-culture combinations, 52 varied between nutrient conditions, with the majority (*i.e.* 41) changing from a negative or neutral interactions in SW into a positive interaction in ESW, described in Figure 6.1.1. This illustrates the fragility of specific relationships in nutrient-deplete systems and highlights the need of testing interactions in natural oligotrophic seawater, contrary to most marine phototroph-heterotroph reports to date (Sher *et al*., 2011, Paul *et al*., 2013, Beliaev *et al*., 2014, Aharonovich and Sher, 2016, Lupette *et al*., 2016, Harvey *et al*., 2016, Wang *et al*., 2016, Durham *et al*., 2017, Tai *et al*., 2009, Zinser, 2018).

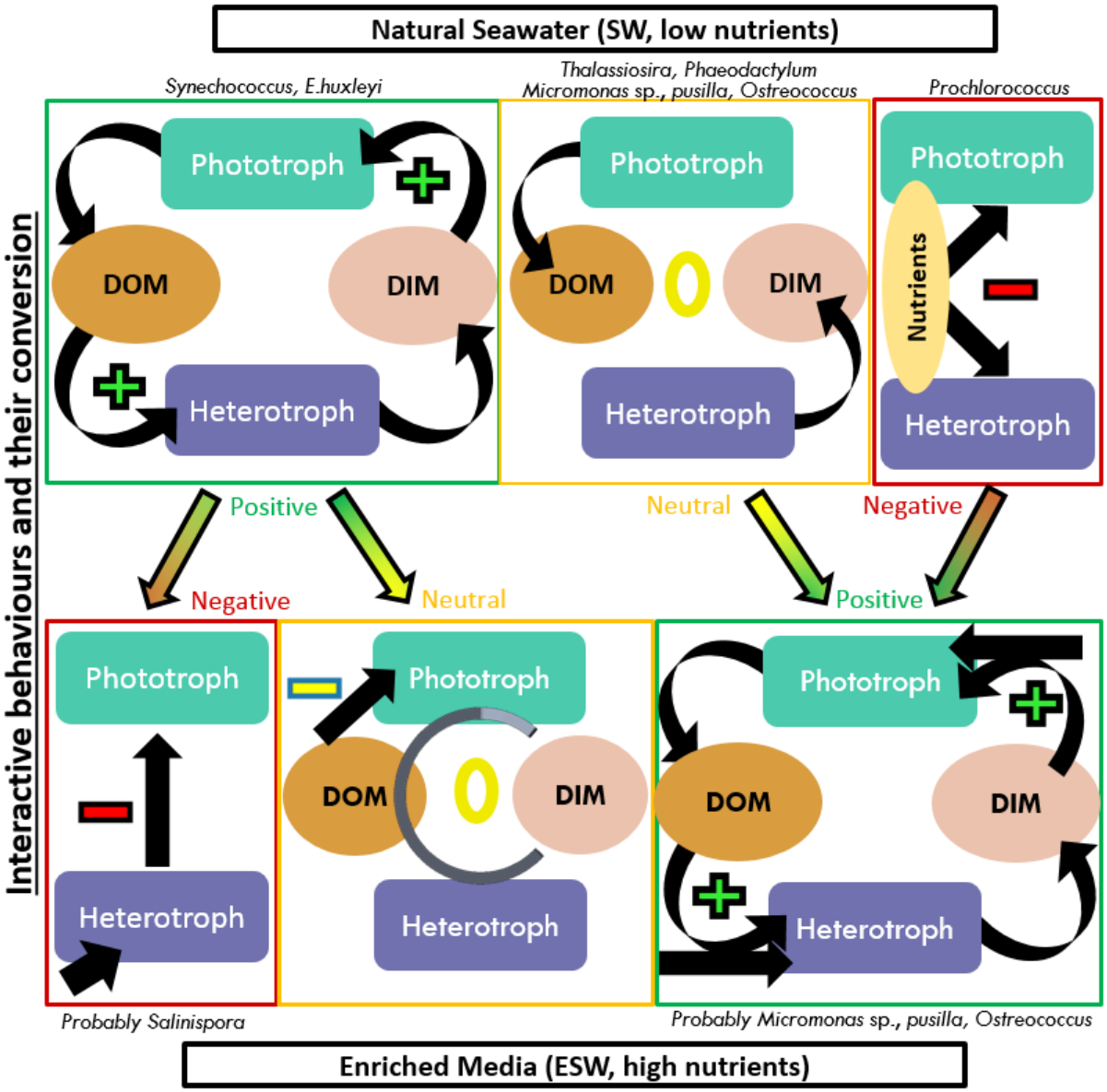


Figure 6.1.1: Phototroph-heterotroph interactions (positive; green, neutral; yellow, and negative; red) changed (represented with arrows) regarding the nutrient availability: low (SW; top panel) and high (ESW; bottom panel). The production and consumption of dissolved organic matter (DOM) and dissolved inorganic matter (DIM) are presented as the key elements of metabolic connectivity. The phototrophic species that behave according to each model when grown with *R. pomeroyi* are indicated.

The hypotheses behind the variations that occur between different nutrient conditions is schematically described in Figure 6.1.1. The consistency of positive interactions regardless of nutrient availability demonstrates that in most cases the recycling of nutrients is achieved in most phototroph-heterotroph combinations, especially with both *Synechococcus* and *E. huxleyi* incubated with *R.*

pomeroyi, categorising their metabolic connectivity as positive. Positive interactions under low nutrient availability suggests a strong metabolic connectivity or, at least, that the scarce nutrients in the system are all being recycled without the need for external supplements. Those interactions that are positive only under high nutrient availability (ESW) suggests there is not the genetic potential to recycle all organic matter generated in the system and, possibly, a key nutrient is becoming inaccessible in SW and the co-culture requires higher doses of nutrients to overcome this loss. Hence, the negative interactions in SW were likely driven by nutrient deficiency, stimulating a competitive behaviour between the phototroph and the heterotroph such as in the co-culture between *Prochlorococcus* with *R. pomeroyi*. Despite the absence of any detrimental effect on their survival, *Prochlorococcus* sustained *R. pomeroyi*'s population levels low, highlighting *Prochlorococcus*'s known potential for efficient nutrient uptake in oligotrophic environments (Lomas *et al*., 2014). A more detrimental competitive behaviour was observed in *Micromonas* sp. co-cultures, which worked in its favour only under high nutrient availability (ESW), and not under low nutrient availability (SW). The proteome of the co-culture of *Micromonas* sp. with *R. pomeroyi* in SW showed an increased abundance of active membrane transporters in both strains (up to 1% and 25%, respectively) pointing at this competitive behaviour and their high reliance on nutrient import. The survival of all three green algae was not affected by the presence of *R. pomeroyi* under SW conditions, while under ESW conditions survival was prolonged, indicating the importance of nutrients for the establishment of beneficial metabolic connection with bacteria.

The uncommon conversion of a positive interaction in SW to neutral or negative in ESW suggests nutrient excess can have a negative effect, possibly due to i) the production of substrates at rates that the heterotroph is not able to re-mineralise, with a consequent accumulation of DOM to toxic levels for the phototroph as described previously (Christie-Oleza *et al*., 2017b), or ii) the production of molecules by the heterotroph with detrimental effects for the phototroph, such as in *Salinispora*, species known to produce secondary metabolites with antagonistic effects to other organisms (Challis and Hopwood, 2003, Fenical and Jensen, 2006, Udwary *et al*., 2007, Jensen *et al*., 2007, Fenical *et al*., 2009, Freel *et al*., 2012, Ziemert *et al*., 2014).

6.2: Trace metals, the candidate elements for the survival of green algae

Among the elements added in ESW were trace metals. The sensitivity of green algae to low levels of trace metals compared to other clades has been reported by Tung-Yuan *et al*. (2003). Despite the high variability of trace metal levels in the Sargasso Sea (Jickells *et al*., 1984), the use of seawater derived from this environment has been used as a trace metals depleted condition in previous experiments (Brand *et al*., 1983), confirming the assumption that their addition in ESW facilitated the growth of green algae. The added elements in ESW of each phototroph are displayed in Table 6.2.1 (final concentrations can be found in Table 2.1.1.2).

Table 6.2.1: Summary of the added compounds in the corresponding enriched media for each phototrophic species. The final concentration of each element in the media can be found in Table 2.1.1.2.

Compounds added in enriched media									
	Prochlorococcus	*Syn* WH7803	*Syn* WH8102	*Micromonas sp.*	*M. pusilla*	*Ostreococcus*	*E. huxleyi*	*Thalassiosira*	*Phaeodactylum*
NO_3		√	√	√	√	√	√	√	√
NH_4	√			√	√	√	√		
PO_3	√	√	√	√	√	√	√	√	√
Mg		√	√		√				
K		√	√		√				
Ca		√	√		√				
Si								√	√
Fe	√	√	√	√	√	√	√	√	√
Mn	√	√	√	√	√	√	√	√	√
Zn	√	√	√	√	√	√	√	√	√
SO_4	√	√	√	√	√	√	√	√	√
Ni	√								
Bo		√	√		√				
Se				√	√	√	√		
Cu		√	√	√	√	√	√	√	√
Vitamins				√	√	√	√	√	√

The cellular concentrations of metals for the majority of the phototrophic species follow this order: Fe>Mn>Zn>Cu, with Fe, Zn and Cu highly affecting green algae performance (Tung-Yuan *et al*., 2003). The substitution of metals allows the continuous enzymatic functionality of the cells (Sunda and Huntsman, 2000), since many of them have low recycling rates (Schoemann *et al*., 1998). Estimation of the levels of trace metals with potential effects on green algal performance is initiated with Fe, one of the most efficiently reused metals (Hutchins and Bruland, 1995), with crucial effects on chlorophyll

synthesis (Martin and Fitzwater, 1988, Latifi *et al*., 2009) and nitrate reductase performance (Muggli and Harrison, 1996, Peers and Price, 2004, Schoffman *et al*., 2016). The up-regulation of both, chlorophyll and nitrate reductase in the green algae co-culture conditions (see Figure 5.3.2.1), indicates sufficient Fe levels for the green algal needs.

Secondly, Zn affects the carbon and phosphorus response, through its use as a co-factor in carbonic anhydrase and alkaline phosphatase (Morel *et al*., 1994). Green algae were not Zn-limited, shown by the presence of carbonic anhydrase in their proteome. Nevertheless, Zn levels were limiting for *Synechococcus* WH8102 and substituted by Cd, since hypothetical proteins related to Cd handling (SYNW0908, SYNW0670, SYNW0827, SYNW0406) were highly abundant. These proteins have been previously found in Zn-limited conditions correlated with alkaline phosphatase activity (Cox and Saito, 2013). In addition, Cox and Saito (2013) recorded down-regulation of alkaline phosphatase in *Synechococcus* WH8102 under Zn-limitation, as we do, causing high P-availability in this co-culture and as a consequence down-regulation of the Ca-dependent alkaline phosphatase of *R. pomeroyi* (Yamane and Maruo, 1978, Majumdar *et al*., 2005), as Figure 4.3.4.1 showed.

The continuous growth of phototrophs in a metal enriched environment prior to the initiation of the co-culture experiments (see Section 2.1), may have caused susceptibility of the internal metal pool control of the phototrophs to a sudden metal deficiency (Brand *et al*., 1986). In addition, phosphorus-limited cells are not efficient at metal efflux, causing intracellular accumulation, with Cu having one of the most detrimental effects on the cell (Reed and Gadd, 1989). *Thalassiosira* has been reported as highly Cu-sensitive when under P-stress (Rijstenbil *et al*., 1998), though this species was not affected by the current conditions. Nevertheless, the green algae may have been affected by the Cu-levels of the tested SW, and aligned with the strong response of P-acquisition mechanisms in these co-cultures (for *R. pomeroyi* and *Micromonas*/*Ostreococcus*, see Figure 4.3.4.1 and 5.3.2.1 respectively), it is suggested that the green algae and especially *Ostreococcus* reduced the metal-releases from their cells with detrimental effects for their survival that the presence of *R. pomeroyi* could not prevent. Further evidence is required to support this assumption.

6.3: The proteome coupling of nine phototrophs and R. pomeroyi in SW

Proteome analysis of different co-culture combinations allowed the characterisation of phototroph - *R. pomeroyi* interactions based on the supply and use of substrates via the up/down-regulation of metabolic pathways, described in Chapter 4 and 5 and summarised in Figure 6.3.1.

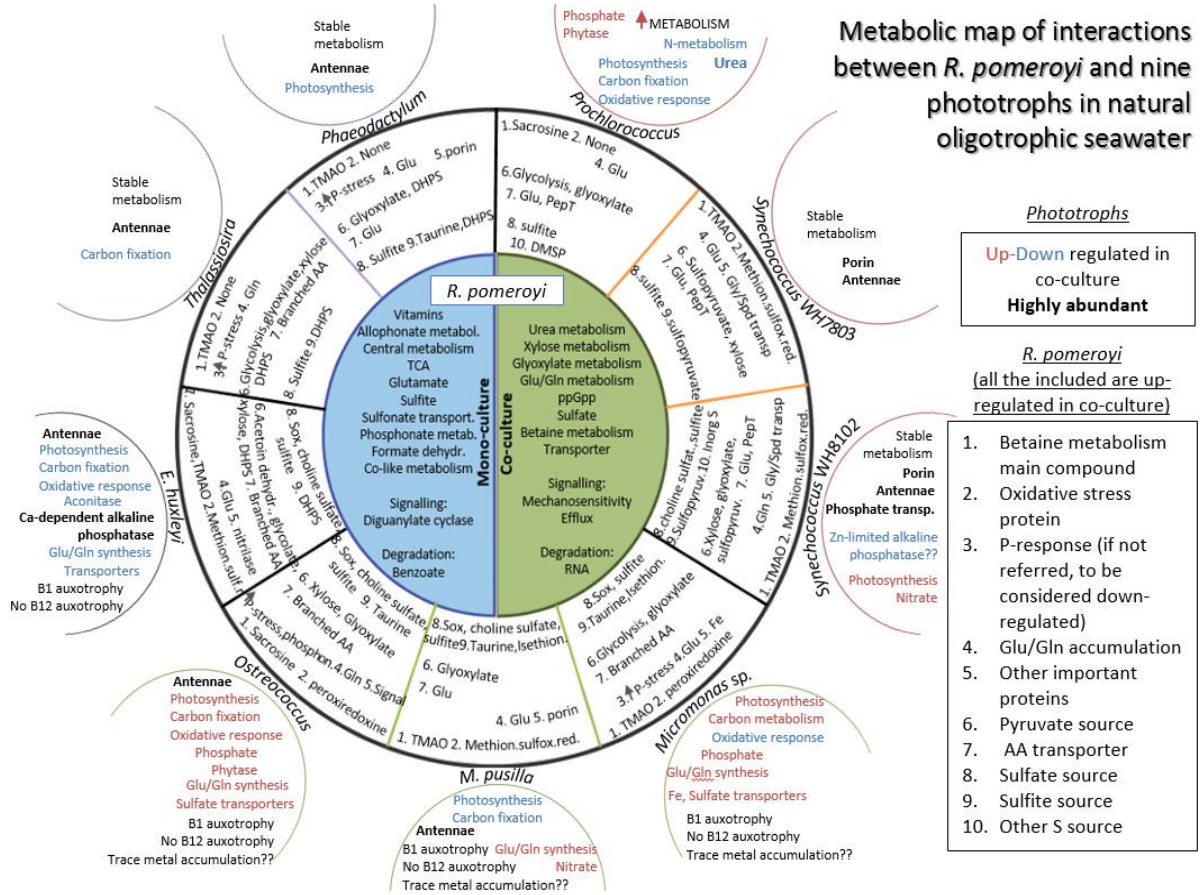


Figure 6.3.1: Map of the metabolic coupling between nine phototrophs (external semi-bubbles) and *R. pomeroyi* (central circle), based on their cellular proteomic profile after three weeks of incubation in natural oligotrophic seawater. The inner circle of *R. pomeroyi* describes the generic profile of *R. pomeroyi* up-regulated in mono-culture (blue-semi cycle) and in co-culture (green-semi cycle). The external circle describes the specific metabolic responses adopted by *R. pomeroyi* to the co-cultivation with each phototrophic species. The functions have been enumerated based on the categories described in the bottom right box, all the included functions were up-regulated in the corresponding condition. The external semi-bubbles present the pattern adapted by the nine phototroph species, with the colour and font coding described in the right middle box.

R. pomeroyi encodes a well-studied functional repertoire which allows it to degrade a large array of typically-produced substrates by marine phototrophic organisms (Christie-Oleza *et al.*, 2012a) and

possibly this may be the reason why this heterotroph showed the best performance in producing positive interactions. In oligotrophic systems, bacteria are highly dependent on primary production (Cole *et al*., 1988), explaining why *R. pomeroyi* survived in the presence of all phototrophs but died after 12 weeks in monoculture (see Figure 4.3.1.1). Though even in this environment, bacteria remineralise organic matter based on its stoichiometric ratios (Caron, 1994, Kirchman, 1994), which vary dependent on the phototrophic species (Obernosterer and Herndl, 1995, Grossart and Simon, 2007, Sarmento *et al*., 2016). An example is the metabolism of different organosulfur forms such as DHPS, isothionate and sulfopyruvate, produced by different phototrophic species. Among these detected sulfur forms, the absence of the corresponding metabolic response in *R. pomeroyi* for the well-studied DMSP, known to be released by *E. huxleyi* and diatoms (Geng and Belas, 2010, Paul *et al*., 2013, Segev *et al*., 2016) indicated that instead of DMSP, *R. pomeroyi* acquires glycine betaine in these co-cultures. Both elements are structurally and functionally similar (Anthoni *et al*., 1991, González *et al*., 2000), and are transported by the same system, nevertheless only the most abundant will be transported, inhibiting the other's uptake (Kiene *et al*., 1998). In microalgae the replacement of DMSP production by glycine betaine has been previously observed in N-rich environments (Keller *et al*., 1999, Bertrand and Allen, 2012) and sulfate-limited conditions (Michal *et al*., 2013). The current conditions of co-cultures can be considered N-rich based on the nutrient analysis of Christie-Oleza *et al*. (2017), where the presence of *R. pomeroyi* in SW caused high ammonium levels. This suggests that the N-levels can be increased by the presence of *R. pomeroyi*, and as a consequence this could affects the algal DMSP production.

Despite the different organic forms produced by the phototrophic species, the metabolism of *R. pomeroyi* suggested that it released sulfate and ammonium in all co-cultures (see Figure 4.3.6.1), elements required to fulfil phototrophic demands (Ratti *et al*., 2011, Amin *et al*., 2012b, Bender *et al*., 2012, Michal *et al*., 2013, Suleiman *et al*., 2016). Specifically, the presence of *R. pomeroyi* and its releases of ammonium possibly replaced the production of some proteins related to N-metabolism in Prochlorococcus and *E. huxleyi*, while its releases of S-related elements up-regulated the production of sulfate transporters in *Micromonas* sp. and *Ostroeococcus* (see Figure 5.3.2.1). Though others, such as *Synechococcus* and diatom proteomes did not respond in the presence of *R. pomeroyi*. This absence of response suggests that either the species *R. pomeroyi*, or its achieved low population levels, were not efficient at stimulating any metabolic response, before the third week when their proteome was

extracted. These species-species metabolic coupling between phototrophs and bacteria support the black queen hypothesis, although this requires further work.

R. pomeroyi can be considered as a good candidate to test the Black Queen hypothesis, due to its high plasticity as demonstrated in its P response regarding the different phototrophs. *R. pomeroyi* decreased its P-acquisition mechanisms with all the phototrophic species known from the literature to have efficient P-uptake systems, *i.e*. cyanobacteria and *E. huxleyi* (Donald *et al*., 1997, Riegman *et al*., 2000), but not with the rest (Figure 4.3.4.1). This suggests that the P-demands of organisms which encode highly efficient P-acquisition mechanisms, are easily fulfilled by P-metabolic coupling with bacteria, based on the stoichiometry detected by Klausmeier *et al*. (2004a) in oligotrophic environments. In contrast to this, *R. pomeroyi* failed to fulfil its P-demands by the material released by other phototrophs, such as *Micromonas* sp., *Ostreococcus* and diatoms, and up-regulated its P-acquisition mechanisms. Diatoms were not affected by the presence of *R. pomeroyi*, but strongly adapted to the oligotrophic properties of seawater, with a possible adaptation being the replacement of P containing lipids with sulfolipids and betaine lipids (Van Mooy *et al*., 2009, Martin *et al*., 2011a, Dyhrman *et al*., 2012). *Micromonas* sp. and *Ostreococcus* responded in the presence of *R. pomeroyi* by up-regulating their P-acquisition mechanisms, a failed attempt at P-coupling with *R. pomeroyi* depicted in the unaffected population survival of the green algae over time, which could have been affected by the absence of other essential elements, such as vitamins. This suggests that the relationships of dependence described by the Black Queen hypothesis can be not only species-specific but also specific to each metabolic cycle.

6.4: Reduced vitamin role in the currently developed interactions

In the current project, a decreased role of vitamins was detected for the survival of phototrophs, known from the literature as being vitamin auxotrophs (see Table 5.3.3.1). This was supported by the reduction of vitamin production by *R. pomeroyi* in the presence of all the phototrophs (see Figure 4.3.4.1) and by the survival of the auxotrophic species in vitamin limited conditions (see Table 5.3.3.1), such as B12. The absence of any effect of the phototrophs' population levels by the limitation of B12, indicates recirculation of vitamins by the phototrophs or vitamin passage to their offspring, theories that have not been tested or proved. Another explanation can be the substitution of vitamin demand by other pathways or use of precursors, indicating an effect of the numerous generations of tested

phototrophic cultures incubated in non-axenic conditions and in vitamin rich media, before the initiation of this project (see Section 2.1). Theoretically the regular exogenous supply of a vitamin can relax the selective pressure on its biosynthetic pathway (Helliwell *et al*., 2013), as Helliwell *et al*. (2015) managed to evolve a B12-dependency through transposition after continuous cultivation in an exogenous supply of vitamin B12. The evolution of a B12-independency could explain the survival of the auxotrophic strains in our experiment, but it is hard to be hypothesized, since it requires gene insertion. However, such a hypothesis is not supported by any literature, since the expression of B12-independent enzymes in non-auxotrophs auxotrophic was suppressed under B12-rich conditions (Bertrand *et al*., 2012, Bertrand *et al*., 2013), and the use of the highly efficient B12-dependent enzymes was stimulated (Gonzalez *et al*., 1992, Xie *et al*., 2013).

The exchange of vitamin B1 between phototrophs and heterotrophs is more clear, with the survival of the auxotrophic species agreeing with published literature (see Table 5.3.3.1). B1 auxotrophy is based on the lack of portions of the B1 biosynthesis pathway, which potentially can be covered by precursors (Bertrand and Allen, 2012, Paerl *et al*., 2015). *Micromonas* and *Ostreococcus* miss a bigger portion of this pathway than *E. huxleyi* which lacks one step, and the addition of this precursor allows *E. huxleyi* growth, but not of *Micromonas* and *Ostreococcus* (McRose *et al*., 2014). Thiamine precursors are produced by proteobacteria (McRose *et al*., 2014), and *R. pomeroyi* responded partially to the demands of *Micromonas* sp. and *E. huxleyi* by up-regulating its transporters predicted for thiamine-intermediate (see Figure 4.3.4.1), facilitating the prolonged survival of *E. huxleyi* (McRose *et al*., 2014), but not of *Micromonas* sp. (Paerl *et al*. 2017; see Figure 4.3.1.1), in agreement with the corresponding literature. The fact that *R. pomeroyi* responded only through thiamine-intermediates to the presence of *Micromonas* sp. and *E. huxleyi*, but not to the other two auxotrophic species, *M. pusilla* and *Ostreococcus*, despite their demands, suggests that it preferably expressed the intermediates and not the final product due to inefficient metabolic coupling. This supports the idea of the use of vitamins as signalling molecules, and not only for fulfilling the phototrophs nutrient demands. Increased vitamin production has been previously recorded during amino acid limitation (Lakaye *et al*., 2004) and organic matter starvation (Gigliobianco *et al*., 2010) by bacteria, conditions that *R. pomeroyi* experienced in mono-culture conditions.

The opposition of the currently presented results of vitamin metabolism with the anticipated results based on the existing literature, suggests the necessity for using a combination of methods in order

to define the role of challenging elements, such as vitamins, in interactions. Current evidence supports that the vitamin metabolism in bacteria was affected more by the nutrient availability than by the prototrophic or auxotrophic demands of the organism that it co-existed with. In this way, the relationships of dependence between organisms cannot be considered as a generic interaction between two organisms, but is probably specific to metabolic cycles and condition-dependent. Hence, the variety of metabolic responses detected in *R. pomeroyi* and the nine phototrophs in the current study, revealed the complexity of the metabolic interaction between two organisms and their fragile equilibrium between competition and cooperation, with both being possible depending on the conditions. As a consequence, it can be considered inaccurate to describe an interaction based on one single hypothesis, such as Black or Red Queen, since the profile of an interaction between is the mosaic of both theories.

6.5: Limitations of the current study and future study directions

Some of the most important the limitations of this study, are displayed below, along with suggested experiments that could complete the currently presented project:

1) The *in-vitro* experimental set-up of co-cultures, even though it was close to a natural system due to the use of SW, was missing the realism of natural conditions, such as the light-dark cycles, the seasonal temperature changes, the water column mix and the involvement in a complex system. Other conditions, such as *in-situ* incubations of each organism or of co-cultures, for instance in dialysis bags, could be tested. Similarly, more complex *in-vitro* co-culture experiments could be carried out by incorporating more than two organisms, such as two bacteria and one phototroph. In this case, the controls will not be considered only the mono-cultures, but also the co-cultures of all the double combinations among the three organisms.
2) The absence of repetition of experiments was counterweighted by the high number of treatments. Nevertheless the repetition of the co-cultures in the same set-up or in higher volume could increase the reliability of the results.
3) The absence of further work in the negative interactions, such as in the *Salinispora* co-cultures in ESW, raises questions regarding the components that shape the development of a negative interaction. For this reason, these cultures can be used in studies of metabolomics targeting

the exchange molecules, since secondary metabolites are known to have detrimental effects on the microbial survival (Freel *et al*., 2012).

4) The usage of *R. pomeroyi* as the only bacteria to investigate the interactions in the co-culture conditions, by studying its proteomic profile, could be considered insufficient for drawing generic conclusions. One should keep in mind that the current project was used to generalise the positive interaction between *R. pomeroyi* and *Synechococcus* WH7803, already described by Christie-Oleza *et al*. (2017), by replacing *Synechococcus* WH7803 with eight more phototrophic species. Nevetheless, it would be interesting to replace *R. pomeroyi* with other bacterial species and study their proteomic profile. In this way more generic conclusions could be outlined for the interactions between heterotrophs and phototrophs and for who is driving these interactions. Hence, the current results can be considered promising since there are clear metabolic patterns in the interactions, though more work need to be done to determine whether these are specific to *R. pomeroyi* or not.
5) The time of the third week, when the proteomic profile of the co-culture organisms was described, could be also considered insufficient for the organisms to develop an interaction. This time was decided based on the time-course exo-proteomic analysis of the co-culture *R. pomeroyi* and *Synechococcus* WH7803, made by Kaur *et al*. (2018), where the change of metabolism started in the third week. However the time required for developing an interaction could vary between species. A similar analysis as the one done by Kaur *et al*. (2018), with several timepoints, could be carried out for each phototrophic species, considering the survival curves presented in the current project. For instance, the co-cultures of *E. huxleyi* and *Prochlorococcus*, where their survival pattern changed over the 19 weeks, it will be interested to observe the change in metabolism between the third week and the time before they die.
6) Last but not least, the methodological limitations of the current study is one of the most critical points of this project. In the first part of the project, the sampling for measuring the cell abundance of the co-cultures can be considered as not high throughput method, since there was continuously high risk for contamination of the cultures and it was quite time-consuming. Eventually, low-cost small culture flasks connected with a sampling system for flow cytometer and nutrient analysis, could efficiently replace the manual manipulation of a

large scale experimental set-up like the currently described. In this way, many parameters will be measured providing a holistic view of the physiology of the cultures, allowing a complete view of the survival of the culture.

7) In terms of proteomics analysis, the economical limitations of the method and the unreliable protein annotation, leads to a reduced amount of valid information. And this raises two points, firstly the generated conclusions of the proteomics results in this study has to be confirmed with other methods, such as metabolomics analysis and transcriptomic analysis or even with independent experiments, using mutants and nutrient analysis. Secondly, I would recommend the proteomic results to be re-analysed in a few years when the annotation system of the proteins will have increased its reliability in function identification.

6.6: Applications of the current findings

A deeper understanding of the interactions between phototrophs and heterotrophs contributes to the prediction of ecosystem changes along with new insights in the industrial applications of this system. Firstly, an outline of realistic trophic modes with true complexities, indicates key elements in interactions that are involved in global elemental cycle models (Worden *et al*., 2015). Then the discovery of the functional plasticity of the organisms points towards their position in the complex natural metabolic network (Beier *et al*., 2014), allowing the prediction of their risk of extinction or how their distribution may be modified, under changes in ocean temperature which can cause large-scale changes in phytoplankton community structure with a large impact on ocean ecosystems (Flombaum *et al*., 2013). Secondly, discovery of the plasticity of bacteria and phytoplankton has industrial relevance, enabling the discovery of novel products and exploitable functions that will contribute in solving contemporary problems, such as antibiotic-resistance. Studies like the current one provide knowledge of the mechanisms that favour mutualism (Geng and Belas, 2010), indicating ways to increase the efficiency of mass algal culture in closed growth systems, such as photobioreactors (Grima *et al*., 1999, Green *et al*., 2015). An improved growth system with high cell density benefits the large-scale production of algal products with high-value products such as pharmaceuticals and cosmetics, low value food products for aquaculture and animal feed, medium value chemicals such as fuels and lipids, as well as environmental technologies, such as wastewater treatment (Ramanan *et al*., 2016).

This was the first approach to understand the demands of microorganisms in nature, based on an *in-vitro* cultivation system, which can be expanded upon in the future by the addition of more organisms producing an *in-vitro* 'version' of nature. The addition of each organism in this system should be based on a complete understanding of its utility in the system, such as making a puzzle. This way, not only we will understand the potential of the organisms, but possibly we will be able to cultivate species that are currently unculturable, since each species can be considered part of a chain.

6.7: Conclusions

The conclusions from this thesis, along with their potential use in future studies, are summarised as follows:

1. The initial hypothesis that a generalised positive interaction will occur between phototrophs and heterotrophs independent of nutrient availability, based on the previously described model system of *Synechococcus* sp. WH7803 and *R. pomeroyi* (Christie-Oleza *et al*., 2017b), was rejected. Whilst positive was the dominant form of interaction determined by the long term survival of both phototroph and heterotroph over the 19 weeks of experimental set-up, other interactions (*i.e.* negative, neutral and semi-positive interactions) were also recorded in specific combinations.
2. Nutrient availability strongly influenced the development of phototroph-heterotroph interactions, since 80% of the co-cultures in ESW developed positive interactions, whereas only 50% were positive in oligotrophic SW. Hence, high nutrient availability facilitates the coupling between most phototrophs with almost any heterotroph, and highlights a flaw in most interaction studies published to date in which ESW has been used.
3. Nutrient cycling plays a major role in phototroph-heterotroph interactions in oligotrophic SW, where the heterotroph relies on the phototroph as the source of carbon and energy, whereas the phototroph requires the mineralisation of its leaked photosynthate to continue photosynthesising and fixing carbon. A gap in this nutrient cycling causes nutrient starvation and potentially competition. The best heterotroph 'helpers' were *Alteromonas* and the *Roseobacter* stains, possibly because of their higher recycling potential.

4. Some phototrophs, *i.e.* the diatoms, have the potential to recycle all of their photosynthate liberating them from the need to coexist with heterotrophic organisms to carry out this function.
5. High nutrient availability (*i.e.* ESW conditions in this study or marine blooms in nature) induces the production of secondary metabolites that actively influence microbe-microbe interactions, *e.g.* the restricting behaviour of *Salinispora*, an active interaction that was not observed in nutrient-deplete conditions.
6. The presence of bacteria stimulates a competitive behaviour in *Micromonas* sp., up-regulating high number of transporters, with the potential of winning or losing the contest depending on the co-cultured bacterial species and the nutrient availability.
7. *Ostreococcus* was not influenced by the presence of heterotrophs under SW conditions, suggesting a lack of recycling of specific nutrients, such as trace metals, or an inability to adapt to such oligotrophic conditions. The proteomic response of both *R. pomeroyi* and green algae in co-culture indicates a metabolic response (*e.g*. both organisms up-regulate P and N acquisition), but not a successful nutrient exchange coupling that could prolong the survival of the green algae.
8. The picocyanobacteria show clear positive interactions with most heterotrophs, though their proteomic profile did not respond to the presence of the heterotroph on the third week, when their proteome was extracted. Nevertheless, the *Synechococcus* species seemed to be leaky organisms ('generously feeding' the heterotroph with photosynthate as shown by the proteome of *R. pomeroyi*), this was not the case with *Prochlorococcus*, which showed a more parasitic behaviour, reducing *R. pomeroyi* cell abundance and relying on it for essential functions, such as N-production (as shown by the *Prochlorococcus* data).
9. *R. pomeroyi* relies on the DOM produced by the phototrophs as a source of carbon and energy in order to survive and, as expected, *R. pomeroyi* showed strong variations in its proteome when grown in the presence of the different phototrophs: i) decreasing the scavenging of diverse sources of energy *i.e.* CO and aromatic hydrocarbon metabolism, ii) lowering its N stress due to the availability of N-rich amino acids and osmolytes, iii) strongly decreasing its response to P-stress in the presence of certain phototrophs establishing P-coupling, iv) upregulating catabolic pathways for specific compounds produced by certain phototrophs *e.g.*

the reduced sulphur compound DHPS produced by *E. huxleyi* and diatoms, v) switching the urea degradation from an allophanate pathway when in mono-culture to a urease degradation in the presence of the different phototrophs, and vi) tuning its active membrane transporters to the nutrients produced by the different phototrophs.

10. Vitamin exchange between the phototrophs and heterotroph needs re-evaluation. While vitamin B12 did not show such a strong role in the development of interactions as expected (in fact, phototrophs reported as auxotrophs for vitamin B12 in the literature did not require this vitamin in this study, and the biosynthetic pathway in *R. pomeroyi* was only slightly downregulated in the presence of most phototrophs), vitamin B1 did potentially have a strong influence on the phototroph-heterotroph interaction as shown by the down-regulation of the biosynthetic pathway in the presence of the phototrophs, despite the strong dependency of some phototrophs on this vitamin (*e.g*. the three green algae species and *E. huxleyi*). The partially coverage of the phototrophic B1-needs indicates that *R. pomeroyi* B1-metabolism was more affected by amino-acid starvation than by the phototrophic demands.

This study has allowed to dissect generic and species-specific elements involved in marine phototroph-heterotroph interactions, mainly involving nutrient exchange and cycling at the base of the trophic pyramid. This is the first step towards understanding the demands of microorganisms in nature, based on an *in-vitro* cultivation system with environmentally-relevant nutrient levels and, in doing so, allows the testing of specific hypotheses generated from fieldwork studies.

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Appendixes

Table 7.1: Raw data of the abundance of Prochlorococcus (up-part) in co-cultures with 14 heterotrophs (down-part) over 19 weeks of incubation (number of week on the top raw), tested with logistic regression (white cells) or two-way ANOVA (grey-cells).

Prochlorococcus

SW	0			2			6			9			12			14			17		
Sali	1E+06	3E+05	3E+05	4E+05	2E+03	2E+03	9E+03	1E+02	1E+02	1E+02											
Aero	1E+06	3E+05	4E+05	3E+05	1E+05	1E+05	1E+05	1E+06	8E+05	3E+06	2E+05	2E+05	2E+05	1E+04	1E+04	2E+04	1E+02	1E+02	1E+02		
Pol	1E+06	3E+05	5E+05	4E+05	4E+04	1E+05	4E+03	9E+02	9E+02	3E+02	1E+04	6E+03	6E+03	1E+02	1E+02	1E+02					
Alg	1E+06	4E+05	3E+05	4E+05	2E+05	2E+05	1E+05	1E+06	8E+05	3E+05	2E+05	3E+04	1E+05	1E+02	1E+02	1E+02					
Gram	1E+06	4E+05	4E+05	4E+05	2E+05	1E+05	2E+05	1E+06	1E+06	3E+05	1E+05	1E+05	7E+04	1E+02	1E+02	1E+02					
Form	1E+06	4E+05	3E+05	3E+05	1E+05	1E+05	1E+05	4E+02	6E+02	5E+02	7E+03	6E+03	7E+03	1E+02	1E+02	1E+02					
Ru	1E+06	1E+05	1E+05	8E+04	2E+05	2E+05	2E+05	4E+05	4E+05	5E+05	2E+05	2E+05	2E+05	2E+05	2E+05	2E+04	1E+02	1E+02	1E+02		
Ros	1E+06	2E+05	2E+05	3E+05	8E+03	1E+04	8E+03	1E+02	1E+02	1E+02											
Dino	1E+06	3E+05	2E+05	3E+05	4E+03	4E+03	4E+03	1E+02	1E+02	1E+02											
Alt	1E+06	1E+05	2E+05	2E+05	4E+04	3E+04	5E+04	4E+04	1E+04	3E+04	2E+03	9E+02	3E+03	1E+02	1E+02	1E+02					
Mar	1E+06	2E+04	2E+04	1E+04	1E+02	1E+02	1E+02														
Ver	1E+06	3E+04	2E+05	2E+04	2E+04	2E+03	1E+03	3E+04	1E+03	8E+02	1E+02	1E+02	1E+02								
AN10	1E+06	8E+04	1E+05	2E+05	3E+04	2E+04	5E+04	8E+02	1E+03	5E+03	1E+02	1E+02	1E+02								
E.coli	1E+06	7E+04	4E+04	4E+04	1E+02	1E+02	1E+02														
Ax	1E+06	2E+05	2E+05	4E+05	1E+02	1E+02	1E+02														
ESW																					
Sali	4E+07	4E+06	3E+06	3E+06	7E+04	1E+05	1E+05	4E+04	3E+04	3E+04	3E+04	1E+05	5E+04	1E+00	1E+00	1E+00					
Aero	4E+07	2E+07	2E+07	1E+07	8E+06	5E+06	7E+06	2E+06	3E+06	4E+06	7E+05	4E+07	9E+05	2E+05	2E+05	2E+05	1E+00	1E+00	1E+00		
Pol	4E+07	4E+07	3E+07	5E+07	7E+05	1E+06	3E+05	2E+06	2E+06	3E+06	7E+04	5E+04	9E+04	1E+05	1E+05	2E+05	1E+00	1E+00	1E+00		
Alg	4E+07	2E+07	2E+07	2E+07	8E+05	7E+05	1E+06	2E+05	2E+05	2E+05	6E+04	5E+04	5E+04	1E+00	1E+00	1E+00					
Gram	4E+07	2E+07	1E+07	2E+07	7E+05	3E+06	8E+03	4E+05	4E+05	4E+05	1E+05	1E+05	1E+05	1E+05	1E+05	2E+05	1E+00	1E+00	1E+00		
Form	4E+07	3E+07	3E+07	3E+07	5E+05	5E+05	7E+05	3E+05	2E+05	3E+05	6E+04	7E+04	8E+04	1E+00	1E+00	1E+00					
Ru	4E+07	1E+07	9E+06	1E+07	4E+06	2E+06	2E+06	2E+06	2E+06	2E+06	1E+06	1E+06	2E+06	8E+05	8E+05	7E+05	1E+00	1E+00	1E+00		
Ros	4E+07	2E+07	2E+07	2E+07	2E+05	2E+05	2E+05	1E+05	1E+05	2E+05	6E+04	5E+04	4E+04	1E+00	1E+00	1E+00					
Dino	4E+07	2E+07	2E+07	2E+07	2E+05	4E+05	4E+05	2E+05	2E+05	2E+05	7E+04	8E+04	9E+04	1E+00	1E+00	1E+00					
Alt	4E+07	2E+07	1E+07	2E+07	5E+07	2E+07	2E+07	8E+05	8E+05	7E+05	8E+04	3E+04	7E+04	1E+00	1E+00	1E+00					
Mar	4E+07	2E+07	1E+07	2E+07	3E+06	8E+05	5E+03	7E+05	4E+05	7E+05	6E+04	2E+04	5E+04	1E+00	1E+00	1E+00					
Ver	4E+07	1E+07	1E+07	2E+07	3E+05	3E+06	4E+06	8E+04	1E+05	2E+04	7E+05	8E+05	4E+04	1E+00	1E+00	1E+00					
AN10	4E+07	3E+07	3E+07	3E+07	3E+06	2E+06	2E+06	1E+00	1E+00	1E+00											
E.coli	4E+07	3E+07	3E+07	2E+07	9E+04	1E+05	2E+05	1E+05	7E+04	7E+04	1E+00	1E+00	1E+00								
Ax	4E+07	2E+08	7E+07	6E+07	1E+05	2E+05	5E+05	2E+05	1E+05	1E+05	1E+00	1E+00	1E+00								
SW																					
Sali	6E+02	1E+03	1E+03	3E+04	1E+02	1E+02	1E+02	1E+02	1E+02	1E+02	1E+02	1E+02	1E+02	1E+02	1E+02	1E+02	2E+02	4E+02	1E+02	3E+02	2E+02	2E+02
Aero	3E+05	2E+03	2E+03	2E+03	4E+03	3E+03	3E+03	2E+04	1E+04	1E+04	1E+03	2E+03	2E+03	1E+04	1E+04	2E+04	2E+04	3E+04	3E+03	2E+04	2E+04	3E+04
Pol	2E+03	2E+05	1E+05	2E+05	2E+02	8E+02	1E+03	6E+02	8E+02	3E+03	2E+02	1E+02										
Alg	1E+04	1E+02	1E+02	1E+02	2E+03	2E+03	1E+03	6E+02	2E+03	1E+03	3E+02	4E+03	1E+02	3E+02	3E+03	3E+02	3E+02	1E+04	1E+02	2E+02	1E+02	1E+02
Gram	2E+04	4E+05	4E+05	4E+05	2E+05	2E+05	3E+05	2E+05	2E+05	2E+05	7E+04	8E+04	8E+04	3E+05	2E+05	3E+05	2E+04	2E+05	2E+05	2E+03	2E+03	2E+03
Form	2E+05	2E+04	2E+04	1E+04	3E+03	1E+04	2E+04	2E+06	2E+06	3E+05												
Ru	4E+05	3E+04	5E+04	7E+04	3E+03	4E+03	5E+03	3E+03	3E+03	4E+03	2E+03	2E+03	3E+03	2E+03	2E+03	2E+03	4E+04	4E+04	3E+04	3E+05	3E+05	2E+05
Ros	2E+05	3E+05	3E+05	3E+05	5E+05	4E+05	5E+05	1E+05	1E+05	1E+05	1E+07	1E+07	1E+07	4E+06	3E+06	4E+06	5E+05	5E+05	5E+05	1E+02	2E+04	2E+04
Dino	2E+05	4E+05	4E+05	5E+05	4E+05	3E+05	3E+05	3E+05	3E+05	3E+05	3E+04	3E+04	3E+04	3E+04	1E+04	2E+04						
Alt	2E+05	2E+04	1E+04	2E+04	4E+02	1E+03	5E+02	3E+03	2E+03	2E+03	3E+04	2E+04	2E+04	4E+04	8E+04	7E+04	3E+05	3E+05	3E+05	2E+05	2E+05	1E+05
Mar	3E+04	1E+06	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+07	2E+03	7E+06	1E+06	7E+06	1E+05	1E+02	1E+02	1E+03	1E+02	1E+02
Ver	5E+03	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+02	1E+07	1E+07	1E+07									
AN10	3E+05	8E+05	4E+05	4E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	6E+06	6E+06	6E+06	5E+06	5E+06	4E+06	1E+05	8E+05	4E+05	2E+05	4E+05	1E+05
E.coli	2E+04	1E+02	1E+02	1E+02																		
ESW																						
Sali	1E+04	5E+02	9E+02	3E+03	4E+03	1E+03	7E+02	8E+02	7E+02	4E+02	3E+03	5E+03	3E+03	4E+03	5E+03	2E+03	1E+04	1E+04	3E+04	3E+04	1E+04	3E+03
Aero	6E+06	5E+06	2E+07	1E+07	2E+07	2E+07	2E+07	3E+06	4E+06	6E+06	1E+04	1E+06	2E+06	1E+07	2E+07	2E+07	2E+05	2E+05	2E+05	3E+06	4E+06	4E+06
Pol	4E+04	1E+05	1E+05	1E+05	7E+06	7E+06	6E+06	5E+05	6E+04	7E+04	1E+03	1E+03	1E+03	1E+03	8E+02	1E+03	2E+05	1E+05	2E+05	1E+00	1E+00	1E+00
Alg	2E+05	3E+05	3E+05	4E+05	2E+07	2E+07	2E+07	2E+06	2E+06	2E+06	4E+06	3E+06	3E+06	3E+06	3E+06	3E+06	2E+06	2E+06	2E+06	1E+06	1E+06	1E+06
Gram	3E+05	2E+07	1E+07	5E+06	3E+07	2E+07	2E+07	1E+07	2E+07	2E+07	5E+06	4E+06	7E+06	8E+06	2E+07	1E+07	3E+06	4E+06	4E+06	3E+06	3E+06	2E+06
Form	3E+06	3E+07	3E+07	2E+07	1E+07	3E+06	8E+06	7E+05	8E+05	3E+05	3E+06	2E+06	2E+06	3E+06	2E+06	3E+06	1E+06	1E+06	2E+06	1E+06	7E+05	8E+05
Ru	8E+06	4E+06	6E+06	1E+07	4E+06	2E+06	3E+05	3E+06	3E+06	3E+06	3E+06	3E+06	2E+06	3E+06	3E+06	6E+06	2E+06	2E+06	2E+06	5E+05	7E+05	2E+06
Ros	5E+06	5E+07	5E+07	4E+07	3E+07	3E+07	4E+07	4E+07	4E+07	4E+07	8E+07	7E+07	6E+07	5E+07	6E+07	6E+07	1E+07	1E+07	1E+07	2E+08	2E+08	2E+08
Dino	5E+06	4E+07	3E+07	3E+07	6E+06	3E+06	5E+06	3E+06	4E+06	3E+06	3E+06	3E+06	4E+06	2E+07	2E+07	2E+07	3E+06	3E+06	2E+06	6E+05	5E+05	5E+05
Alt	3E+06	2E+07	2E+07	1E+07	1E+07	2E+07	2E+07	3E+07	2E+07	3E+07	2E+07	2E+07	1E+07	1E+07	2E+07	1E+07	1E+07	2E+07	2E+07	3E+06	2E+07	1E+07
Mar	2E+06	1E+07	1E+06	1E+07	5E+07	4E+07	4E+07	3E+07	3E+07	3E+07	4E+07	4E+07	3E+07	4E+07	4E+07	2E+07	3E+07	4E+07	3E+07	2E+07	2E+07	2E+07
Ver	1E+05	1E+06	1E+06	1E+05	4E+07	4E+07	4E+07	2E+07	2E+07	2E+07	7E+07	7E+07	7E+07	4E+07	3E+07	4E+07	1E+08	2E+08	3E+07	2E+07	3E+07	2E+07
AN10	6E+06	1E+07	7E+06	1E+07	2E+07	2E+07	2E+07	3E+07	2E+07	2E+07	7E+07	7E+07	7E+07	9E+06	7E+06	3E+06	2E+07	2E+07	3E+07	5E+05	5E+05	6E+05
E.coli	3E+05	1E+05	8E+04	6E+04	3E+04	2E+04	1E+04	4E+03	4E+03	4E+03	1E+00	1E+00	1E+00									

Table 7.2: Raw data of the abundance of Synechococcus WH7803 *(up-part) in co-cultures with 14 heterotrophs (down-part) over 19 weeks of incubation (number of week on the top raw), tested with logistic regression (white cells) or two-way ANOVA (grey-cells).*

	Syn WH7803																								
SW	0	1	3	6			7			3			11			15			17			13		
Sali	1E+06	1E+06	2E+05	3E+05	3E+05	5E+05	2E+05	1E+05	2E+05	5E+04	3E+04	4E+04	2E+04	3E+04	1E+04	3E+04	2E+04	3E+04	2E+04	2E+04	3E+04	6E+04	4E+04	4E+04
Aero	1E+06	1E+06	2E+05	1E+05	2E+05	2E+05	1E+05	3E+04	5E+05	5E+04	6E+04	5E+04	2E+04	3E+03	1E+04	5E+04	3E+04	3E+04	3E+04	2E+04	2E+04	3E+04	1E+04	2E+04
Pol	1E+06	1E+06	1E+05	3E+05	1E+05	2E+05	2E+05	2E+05	3E+05	7E+04	6E+04	1E+05	2E+04	2E+04	2E+04	3E+04	2E+04	3E+04	4E+04	5E+04	4E+04	6E+04	4E+04	5E+04
Alg	1E+06	2E+06	2E+05	2E+05	2E+05	1E+05	2E+05	2E+05	2E+05	1E+05	2E+05	2E+05	5E+04	5E+04	4E+04	6E+04	7E+04	7E+04	4E+04	6E+04	5E+04	6E+04	5E+04	6E+04
Gram	1E+06	2E+06	5E+05	3E+05	5E+05	4E+05	8E+05	2E+05	3E+05	1E+05	3E+05	3E+05	5E+04	8E+04	7E+04	1E+05	3E+04	1E+04	1E+05	1E+05	1E+05	2E+05	1E+05	1E+05
Form	1E+06	6E+05	6E+04	7E+04	1E+05	6E+04	7E+04	7E+04	1E+05	5E+04	6E+04	5E+04	2E+04	1E+04	1E+04	2E+04	2E+04	2E+04	3E+04	2E+04	1E+04	3E+04	2E+04	3E+04
Ru	1E+06	7E+05	4E+04	3E+04	1E+05	1E+05	7E+04	5E+04	8E+04	2E+04	1E+04	2E+04	8E+03	5E+03	6E+03	1E+03		2E+03	8E+03	1E+04	7E+03	1E+03	8E+03	1E+03
Ros	1E+06	4E+05	5E+04	4E+04	2E+04	9E+04	4E+04	5E+04	4E+04	2E+04	2E+04	2E+04	5E+03	3E+03	7E+03	1E+04	1E+04	9E+03	8E+03	5E+03	8E+03	2E+03	2E+03	2E+04
Dino	1E+06	8E+05	2E+04	2E+05	4E+04	3E+04	6E+04	5E+04	4E+04	2E+04	8E+03	3E+03	2E+03	4E+03	5E+03	5E+03	1E+04	1E+04	1E+04	2E+04	2E+04	8E+03	4E+03	2E+03
Alt	1E+06	1E+06	1E+05	2E+05	3E+05	2E+05	3E+05	3E+05	3E+05	4E+04	5E+04	5E+04	2E+04	3E+03	1E+04	1E+05	8E+04	3E+04	2E+04	2E+04	2E+04	1E+00	1E+00	1E+00
Mar	1E+06	3E+05	8E+03	7E+03	2E+03	4E+03	1E+04	1E+04	2E+04	5E+03	4E+03	2E+03	1E+03	2E+03	2E+03	1E+03	1E+03	2E+03	1E+03	3E+03	2E+03	1E+00	1E+00	1E+00
Ver	1E+06	1E+05	3E+04	1E+05	1E+05	1E+05	4E+04	5E+04	7E+04	3E+04	4E+04	4E+04	5E+03	1E+04	1E+04	1E+04	3E+03	6E+03	1E+04	2E+04	4E+04	5E+03	1E+04	1E+04
AN10	1E+06	5E+05	2E+04	2E+04	2E+04	1E+04	4E+04	4E+04	3E+04	7E+03	5E+03	6E+03	3E+03	2E+03	2E+03	4E+04	2E+04	1E+04	7E+03	2E+03	4E+03			1E+03
E.coli	1E+06	5E+05	1E+05	5E+04	5E+04	6E+04	6E+04	1E+05	3E+04	6E+04	5E+04	6E+04	1E+04	1E+04	1E+04	2E+04	2E+04	1E+04	3E+04	2E+04	3E+04	2E+04	3E+04	3E+04
Ax	1E+06	1E+06	8E+04	2E+03	2E+05	9E+04	4E+03	8E+04	1E+05	6E+03	6E+03	4E+04	1E+04	2E+04	2E+04	1E+00	1E+00	1E+00						
ESW																								
Sali	2E+08	1E+08	2E+07	7E+04	1E+05	9E+04	2E+04	3E+04	3E+04	6E+04	6E+04	8E+04	1E+04	2E+04	1E+04	1E+04	1E+04	1E+04	1E+04	1E+04	1E+04	1E+04	1E+04	3E+04
Aero	2E+08	8E+08	1E+09	3E+08	5E+08	4E+08	7E+08	6E+08	5E+08	2E+08	1E+08	1E+08	1E+08	3E+07	8E+07	1E+08	1E+08	1E+08	1E+08	1E+08	3E+07	1E+07	1E+07	1E+07
Pol	2E+08	6E+08	1E+09	6E+08	5E+08	5E+08	7E+08	5E+08	4E+08	3E+08	2E+08	3E+08	2E+08	2E+08	1E+08	7E+07	7E+07	8E+07	8E+06	1E+07	2E+07	2E+06	6E+06	1E+07
Alg	2E+08	1E+09	3E+08	8E+08	8E+08	1E+09	5E+08	6E+08	7E+08	3E+08	2E+08	2E+08	3E+07	1E+08	1E+08	1E+08	7E+07	1E+08	3E+07	7E+07	7E+07	8E+06	7E+06	1E+07
Gram	2E+08	1E+09	1E+09	6E+08	7E+08	9E+08	6E+08	8E+08	1E+09	2E+08	2E+08	3E+08	2E+08	1E+08	1E+08	1E+08	1E+08	2E+08	1E+08	8E+07	1E+08	3E+07	1E+07	1E+07
Form	2E+08	7E+08	1E+09	4E+08	6E+08	7E+08	3E+08	2E+08	3E+08	2E+08	3E+08	5E+06	3E+07	3E+08	8E+07	8E+07	4E+06	2E+08	3E+07	3E+07	2E+07	5E+06	3E+06	2E+06
Ru	2E+08	1E+09	1E+09	1E+09	2E+09	1E+09	8E+08	1E+09	2E+09	1E+08	9E+07	8E+07	5E+07	5E+07	7E+07	2E+08	2E+08	3E+08	2E+08	2E+08	3E+08	2E+07	1E+07	9E+05
Ros	2E+08	2E+09	4E+09	1E+09	1E+09		2E+09	3E+08		4E+08	5E+08	5E+08	2E+08	2E+08	2E+08	1E+08	3E+07	1E+08	3E+07	5E+07	4E+07	1E+06	1E+07	6E+06
Dino	2E+08	3E+08	4E+08	2E+09	4E+08	3E+08	6E+08	8E+08	2E+09	2E+08	1E+08	1E+08	1E+08	6E+07	3E+07	8E+07	3E+07	1E+08	5E+07	7E+07	9E+07	1E+06	5E+06	7E+06
Alt	2E+08	6E+08	3E+08	1E+09	8E+08	7E+08	1E+09	1E+09	2E+09	2E+08	1E+08	4E+08	2E+08	6E+07	5E+07	2E+08	1E+08	1E+08	1E+08	1E+08	1E+08	1E+07	1E+07	1E+07
Mar	2E+08	1E+09	6E+08	2E+08	3E+08	4E+08	3E+08	7E+08	2E+08	3E+07	7E+07	6E+07	2E+07	4E+07	6E+07	9E+07	1E+08	1E+08	7E+07	4E+07	3E+07	3E+06	2E+06	1E+06
Ver	2E+08	6E+08	1E+09	3E+08	2E+08	3E+08	3E+08	2E+08	2E+08	6E+07	7E+06	4E+07	2E+06	8E+06	3E+07	3E+06	2E+08	8E+05	1E+04	8E+07	4E+07	8E+06		
AN10	2E+08	1E+09	1E+09	7E+08	1E+09	5E+08	2E+08	3E+07	5E+07	8E+06	4E+07	5E+07	3E+07	3E+07	4E+07	3E+07	4E+07	5E+07	5E+07	3E+07	7E+07	3E+06	7E+06	1E+07
E.coli	2E+08	1E+09	3E+09	3E+08	5E+08	6E+07	6E+08	1E+09	8E+08	2E+07	1E+07	7E+06	3E+07	3E+07	5E+07	2E+07	5E+07	2E+07	1E+05	2E+04	7E+04	1E+07	4E+05	3E+04
Ax	2E+08	6E+08	3E+08	2E+08	2E+08	4E+08	5E+05	1E+08	1E+08	1E+05	1E+05	3E+06	1E+06	6E+05	5E+04	1E+00	1E+00	1E+00						
SW																								
Sali	2E+05	1E+03	5E+02	6E+02	5E+02	4E+02	2E+02	2E+02	2E+02	1E+02	1E+02	1E+02	1E+03	2E+03	2E+03	2E+03	1E+03	1E+03	7E+02	7E+02	2E+03	2E+03	1E+03	2E+03
Aero	5E+07	4E+05	6E+04	7E+04	5E+04	5E+04	5E+04	4E+04	4E+04	6E+03	1E+04	2E+04	2E+04	1E+04	1E+04	4E+04	1E+04	2E+04	3E+02	5E+02	4E+02	1E+04	1E+04	1E+04
Pol	7E+06	5E+06	2E+05	2E+05	2E+05	2E+05	2E+05	1E+05	1E+05	8E+04	8E+04	2E+04	2E+05	2E+05	2E+05	4E+04	1E+05	7E+04	6E+03	2E+04	1E+04	2E+05	2E+05	2E+05
Alg	6E+07	3E+05	1E+04	6E+05	3E+05	4E+05	3E+05	2E+05	3E+05	5E+05	1E+05	3E+05	1E+05	3E+05	3E+05	1E+04	3E+04	2E+04	1E+03	3E+03	2E+03	1E+05	4E+05	4E+05
Gram	1E+07	1E+06	3E+07	6E+06	7E+06	7E+06	8E+06	4E+06	5E+06	4E+06	5E+06	7E+06	8E+06	6E+06	6E+06	5E+06	4E+06	3E+06	6E+06	7E+06	2E+06	3E+06	3E+06	3E+06
Form	1E+05	1E+03	2E+04	8E+03	8E+03	7E+03	2E+03	2E+03	2E+03	4E+03	4E+03	6E+04	8E+04	8E+04	8E+04	7E+03	7E+03	7E+03	2E+03	1E+02	1E+03	1E+05	6E+04	1E+05
Ru	3E+07	6E+05	1E+06	2E+06	5E+06	3E+06	7E+05	7E+05	1E+06	7E+05	6E+05	8E+05	2E+06	3E+06	2E+06	2E+06	1E+06	2E+06	1E+06	1E+06	2E+06	1E+06	1E+06	1E+06
Ros	3E+07	1E+06	1E+07	4E+06	6E+06	8E+06	2E+06	5E+06	2E+06	3E+06	3E+06	8E+06	1E+06	3E+06	3E+06	2E+06	4E+06	8E+06	3E+06	4E+06	5E+06	2E+06	2E+06	2E+06
Dino	8E+07	4E+06	8E+06	4E+06	5E+06	4E+06	2E+06	2E+06	4E+06	7E+06	4E+06	5E+06	8E+06	3E+06	8E+06	7E+06	1E+07	3E+06	1E+07	2E+07	2E+07	8E+06	8E+06	1E+07
Alt	3E+07	1E+03	3E+05	1E+07	3E+07	2E+07	6E+07	6E+07	1E+08	4E+06	4E+06	3E+06	8E+06	5E+06	4E+06	7E+06	7E+06	8E+06	5E+06	6E+06	6E+06	5E+06	4E+06	8E+06
Mar	7E+07	3E+06	2E+06	4E+07	2E+07	3E+07	2E+07	1E+07	2E+07	2E+06	2E+06	2E+06	5E+06	5E+06	3E+06	4E+06	3E+06	6E+06	2E+07	5E+07	6E+07	2E+07	2E+07	2E+07
Ver	1E+07	7E+05	5E+05	5E+06	1E+06	3E+06	3E+06	1E+06	2E+06	2E+06	1E+06	2E+06	7E+06	4E+06	4E+06	5E+06	2E+06	3E+06	1E+06	1E+06	1E+06	1E+06	1E+06	1E+06
AN10	2E+07	7E+05	5E+05	1E+06	8E+05	2E+06	6E+05	7E+05	1E+06	3E+06	6E+06	1E+07	7E+05	6E+05	3E+05	4E+05	8E+05	1E+06	6E+05	6E+05	7E+05	3E+05	4E+05	5E+05
E.coli	3E+07	2E+05	3E+03	3E+03	2E+03	1E+03	2E+03	3E+02	7E+02	1E+03	8E+03	6E+02	1E+02	5E+02	3E+02	4E+02	4E+02	3E+02	1E+03	5E+02	2E+03	1E+03	1E+03	1E+03
ESW																								
Sali	3E+06	1E+05	7E+03	4E+03	4E+04	1E+04	4E+03	2E+04	6E+03	7E+03	5E+04	5E+04	3E+04	3E+04	4E+04	2E+04	2E+04	2E+04	1E+04	4E+04	5E+03	2E+04	1E+04	2E+04
Aero	1E+09	1E+05	2E+07	1E+08	1E+08	1E+08	3E+08	3E+08	5E+08	4E+07	2E+07	6E+07	2E+08	1E+08	1E+08	1E+08	1E+08	7E+07	2E+08	2E+08	2E+08	1E+08	1E+08	2E+08
Pol	1E+08	8E+07	4E+08	6E+07	3E+07	1E+08	6E+07	2E+08	2E+08	3E+07	2E+07	3E+07	3E+07	3E+07	3E+07	6E+05	3E+05	4E+05	6E+07	3E+07	6E+07	1E+07	1E+07	2E+07
Alg	1E+09	5E+07	7E+07	2E+08	1E+08	1E+08	2E+08	2E+08	1E+08	6E+08	1E+08	1E+08	3E+08	7E+08	6E+08	4E+08	6E+08	4E+08	3E+08	3E+08	3E+08	1E+08	1E+08	3E+07
Gram	2E+08	1E+05	3E+06	2E+08	1E+08	1E+08	2E+08	2E+08	2E+08	3E+08	3E+08	3E+08	1E+08	1E+09	6E+08	1E+08	1E+08	2E+08	7E+07	2E+08	2E+08	1E+08	2E+08	2E+08
Form	2E+06	1E+05	2E+07	2E+07	2E+07	1E+07	3E+07	6E+07	1E+08	3E+07	5E+07	4E+07	4E+07	4E+07	7E+07	6E+07	3E+07	7E+07	2E+06	2E+06	5E+06	4E+06	4E+06	1E+07
Ru	5E+08	2E+07	1E+07	7E+07	1E+08	1E+08	1E+08	1E+08	8E+07	4E+07	4E+07	4E+07	5E+07	7E+07	6E+07	5E+07	2E+07	3E+07	1E+08	6E+07	1E+08	9E+07	8E+07	8E+07
Ros	2E+09	2E+07	5E+06	3E+07	2E+07	3E+07	2E+08	2E+08	2E+08	2E+08	4E+08	3E+08	2E+08	3E+08	3E+08	1E+08	2E+08	2E+08	2E+08	2E+08	2E+08	2E+08	2E+08	2E+08
Dino	2E+09	6E+07	3E+08	4E+08	2E+08	2E+08	3E+08	5E+08	4E+08	2E+08	4E+08	3E+08	6E+08	3E+08	3E+08	4E+08	4E+08	2E+08	1E+08	2E+08	2E+08	2E+08	1E+08	2E+08
Alt	6E+08	1E+05	5E+07	1E+08	2E+08	2E+08	6E+07	6E+07	1E+08	2E+08	2E+08	7E+08	1E+08	1E+09	6E+08	1E+08	3E+08	4E+08	4E+08	5E+08	6E+08	2E+08	2E+08	2E+08
Mar	1E+09	6E+07	8E+07	2E+08	8E+07	2E+08	6E+07	7E+07	9E+07	9E+07	1E+08	9E+07	1E+08	1E+08	1E+08	3E+08	3E+08	2E+08	2E+08	2E+08	2E+08	1E+08	1E+08	1E+08
Ver	2E+08	1E+05	2E+06	7E+07	3E+07	3E+07	5E+07	4E+07	3E+07	1E+07	1E+07	7E+06	1E+07	1E+07	1E+08	1E+07	1E+08	1E+07	8E+05	1E+07	3E+07	1E+07	3E+07	2E+07
AN10	3E+08	1E+05	8E+05	1E+08	3E+07	8E+07	1E+08	3E+08	2E+08	1E+08	1E+08	1E+08	5E+08	2E+08	3E+08	3E+08	#DIV/0!	3E+08	3E+08	2E+08	2E+08	3E+08	2E+08	2E+08
E.coli	6E+08	1E+05	2E+05	1E+06	2E+06	2E+06	3E+05	7E+05	1E+05	6E+05	3E+05	8E+05	3E+05	4E+05	4E+05	2E+05	2E+05	2E+05	5E+05	7E+05	1E+06	3E+05	4E+05	4E+05

Table 7.3: Raw data of the abundance of Synechococcus WH8102 *(up-part) in co-cultures with 14 heterotrophs (down-part) over 19 weeks of incubation (number of week on the top raw), tested with logistic regression (white cells) or two-way ANOVA (grey-cells).*

	Syn WH8102																								
SW	0	1			3			5			7			9			12			16			19		
Sali	3E+06	4E+06	6E+06	6E+06	2E+06	2E+06	2E+06	8E+04	3E+04	8E+04	5E+03	7E+03	7E+03	1E+04	2E+04	2E+04	1E+05	4E+04	6E+04	5E+04	4E+04	5E+04	7E+04	7E+04	7E+04
Aero	3E+06	4E+06	4E+06	7E+06	3E+06	2E+06	2E+06	2E+05	1E+05	2E+05	2E+03	5E+03	3E+03	2E+04	2E+04	3E+03	3E+03	3E+03	3E+03	2E+03	3E+02	8E+04	1E+02	3E+02	2E+05
Pol	3E+06	4E+06	3E+06	3E+06	2E+06	2E+06	2E+06	2E+05	2E+05	1E+05	9E+04	3E+04	5E+04	4E+04	4E+04	3E+03	6E+04	2E+02	8E+02	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00
Alg	3E+06	3E+06	3E+06	3E+06	2E+06	2E+06	1E+06	2E+05	1E+05	1E+05	9E+04	8E+04	9E+04	3E+04	3E+04	3E+04	4E+05	1E+04	3E+05	2E+05	1E+04	2E+05	4E+05	2E+05	3E+05
Gram	3E+06	2E+06	3E+03	1E+05	1E+06	1E+03	2E+03	1E+05	7E+04	1E+03	2E+04	2E+04	1E+04	8E+03	6E+03	4E+03	5E+03	1E+02	1E+02	2E+03	1E+02	1E+02	1E+03	1E+02	1E+02
Form	3E+06	2E+06	4E+06	4E+06	3E+05	1E+06	1E+06	5E+04	6E+04	3E+04	1E+04	3E+04	2E+04	1E+03	5E+03	3E+03	3E+05	3E+05	3E+05	2E+05	2E+05	2E+05	5E+05	5E+05	5E+05
Ru	3E+06	2E+06	2E+06	2E+06	5E+05	3E+05	2E+05	2E+04	2E+04	1E+04	1E+04	7E+03	1E+04	3E+03	3E+03	4E+03	3E+03	4E+03	4E+03	3E+04	2E+04	3E+04	2E+05	1E+05	1E+05
Ros	3E+06	2E+06	1E+06	2E+06	1E+05	1E+05	7E+04	5E+03	2E+03	6E+03	3E+03	6E+03	6E+03	1E+03	3E+03	2E+03	7E+03	4E+03	4E+03	2E+04	1E+04	2E+04	1E+05	3E+04	2E+04
Dino	3E+06	6E+06	4E+06	4E+06	6E+05	7E+05	8E+05	2E+04	3E+04	4E+04	6E+03	7E+03	9E+03	6E+03	5E+03	3E+03	3E+03	8E+02	6E+02	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00
Alt	3E+06	5E+06	3E+06	4E+06	3E+05	7E+05	8E+05	7E+04	8E+04	1E+05	5E+04	1E+05	6E+04	3E+04	3E+04	4E+04	1E+04	1E+04	2E+04	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00
Mar	3E+06	2E+06	6E+06	4E+06	2E+05	5E+05	5E+05	2E+03	1E+04	2E+04	2E+03	1E+03	1E+03	2E+03	1E+03	1E+03	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00
Ver	3E+06	3E+06	3E+06	4E+06	1E+06	8E+05	8E+05	2E+04	2E+04	2E+04	1E+03	1E+02	1E+03	1E+03	1E+03	1E+03	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00
AN10	3E+06	1E+06	2E+06	2E+06	1E+05	2E+05	2E+05	3E+03	3E+03	8E+03	1E+03	5E+03	3E+03	1E+02	1E+03	1E+02	5E+02	6E+02	4E+02	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00
E.coli	3E+06	1E+06	9E+05	5E+05	2E+05	3E+05	2E+05	8E+04	4E+04	1E+03	2E+03	1E+02	1E+02	1E+02	1E+02	1E+02	4E+02	2E+02	1E+02	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00
Ax	3E+06	2E+06	4E+06	3E+06	6E+05	2E+06	2E+06	1E+05	1E+05	1E+05	6E+04	3E+04	2E+04	1E+03	1E+03	1E+03	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00
ESW																									
Sali	3E+08	3E+08	3E+08	3E+08	3E+07	3E+07	1E+08	4E+07	5E+07	4E+07	8E+05	2E+06	1E+06	2E+04	1E+04	1E+04	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00
Aero	3E+08	4E+08	3E+08	4E+08	6E+08	6E+08	4E+08	2E+08	2E+08	2E+08	1E+08	7E+07	9E+07	4E+06	3E+06	6E+06	1E+03	1E+02	2E+04	1E+02	1E+02	1E+02	0E+00	0E+00	0E+00
Pol	3E+08	4E+08	1E+09	7E+08	5E+08	7E+08	7E+08	2E+08	2E+08	2E+08	8E+07	7E+07	9E+07	8E+05	8E+05	6E+05	1E+00	1E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00
Alg	3E+08	4E+08	9E+08	8E+08	3E+08	4E+08	4E+08	1E+08	2E+08	2E+08	1E+08	1E+08	1E+08	1E+07	7E+06	6E+06	2E+05	8E+04	8E+04	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00
Gram	3E+08	6E+08	5E+08	1E+09	5E+08	5E+08	4E+08	2E+08	2E+08	2E+08	1E+08	1E+08	1E+08	7E+06	7E+06	6E+06	2E+04	1E+04	2E+03	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00
Form	3E+08	8E+08	1E+09	8E+08	3E+08	3E+08	4E+08	1E+08	1E+08	2E+08	1E+08	1E+08	7E+07	2E+07	2E+07	2E+07	1E+06	8E+05	8E+05	2E+04	1E+00	1E+00	1E+00	0E+00	0E+00
Ru	3E+08	6E+08	9E+08	6E+08	6E+08	5E+08	5E+08	2E+08	3E+08	2E+08	1E+08	1E+08	1E+08	2E+07	2E+07	2E+07	9E+05	1E+06	2E+06	1E+07	1E+07	1E+07	2E+07	2E+07	2E+07
Ros	3E+08	5E+08	5E+08	6E+08	5E+08	7E+08	7E+08	3E+08	2E+08	2E+08	1E+08	1E+08	1E+08	9E+06	1E+07	9E+06	7E+04	2E+05	1E+05	5E+06	1E+07	8E+06	1E+07	1E+07	1E+07
Dino	3E+08	9E+08	4E+08	5E+08	4E+08	4E+08	5E+08	2E+08	2E+08	2E+08	6E+07	8E+07	6E+07	3E+06	5E+06	5E+06	1E+05	6E+05	7E+05	7E+06	5E+06	4E+06	6E+06	4E+06	3E+06
Alt	3E+08	1E+09	5E+08	6E+08	4E+08	4E+08	5E+08	2E+08	2E+08	2E+08	6E+07	7E+07	7E+07	1E+07	3E+06	1E+07	1E+06	6E+05	1E+06	1E+02	1E+02	5E+03	1E+00	1E+00	1E+00
Mar	3E+08	7E+08	5E+08	5E+08	4E+08	3E+08	4E+08	1E+08	1E+08	2E+08	5E+07	5E+07	5E+07	2E+06	2E+06	7E+06	4E+05	1E+06	2E+06	8E+03	1E+02	1E+02	0E+00	0E+00	0E+00
Ver	3E+08	9E+08	3E+08	6E+08	4E+08	4E+08	4E+08	2E+08	1E+08	2E+08	4E+07	4E+07	6E+07	2E+06	3E+06	4E+06	5E+04	2E+05	2E+05	7E+06	7E+06	2E+07	8E+06	1E+07	1E+07
AN10	3E+08	7E+08	1E+09	2E+09	6E+08	5E+08	6E+08	2E+08	2E+08	2E+08	6E+07	3E+07	2E+07	3E+06	8E+05	3E+04	1E+03	2E+03	1E+02	3E+04	2E+05	7E+04	1E+05	3E+05	2E+05
E.coli	3E+08	6E+08	5E+08	6E+08	5E+08	4E+08	5E+08	2E+08	2E+08	2E+08	4E+07	7E+06	3E+06	1E+04	2E+04	1E+04	1E+03	1E+00	1E+00	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00
Ax	3E+08	6E+08	6E+08	1E+09	6E+08	4E+08	5E+08	2E+08	1E+08	2E+08	1E+08	1E+08	1E+08	2E+05	1E+07	7E+06	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00
SW																									
Sali		1E+02	1E+02	1E+02	1E+02	1E+02	1E+02	1E+02	1E+02	2E+02	3E+02	3E+02	1E+02	4E+02	3E+02	3E+02	2E+02	2E+02	2E+02	3E+02	3E+02	4E+02			
Aero		3E+04	3E+04	3E+04	3E+04	3E+04	2E+04	4E+04	2E+04	4E+04	3E+05	2E+05	3E+05	3E+04	6E+04	7E+04	5E+05	6E+05	7E+05	3E+04	3E+04	2E+04			
Pol		6E+05	5E+05	6E+05	5E+05	6E+05	4E+05	2E+06	2E+06	1E+06	4E+06	3E+06	3E+06	7E+04	1E+05	1E+05	2E+05	3E+05	3E+05	2E+04	1E+04	2E+04			
Alg		2E+04	3E+05	1E+06	3E+05	2E+06	2E+06	3E+05	4E+05	1E+06	2E+05	2E+05	3E+05	1E+06	8E+05	1E+06	1E+06	8E+05	1E+06	1E+00	1E+00	1E+00			
Gram		2E+06	2E+06	6E+06	2E+06	2E+06	2E+06	6E+06	3E+06	3E+06	2E+06	3E+06	5E+06	3E+05	2E+06	6E+06	5E+06	6E+06	3E+06	2E+05	2E+05	2E+06			
Form		2E+04	1E+04	2E+04	8E+04	1E+05	5E+04	1E+06	6E+06	6E+06	1E+07	7E+06	5E+06	4E+06	4E+06	4E+06	5E+06	6E+06	1E+06	2E+06	3E+06	2E+06			
Ru		8E+05	8E+05	7E+05	7E+05	7E+05	8E+05	7E+05	8E+05	5E+05	6E+05	1E+06	1E+06	1E+06	1E+06	3E+05	1E+06	8E+05	1E+06	3E+05	3E+05	1E+06			
Ros		3E+06	3E+06	5E+06	4E+06	2E+06	3E+06	1E+06	4E+06	7E+06	5E+06	5E+06	4E+06	5E+06	3E+06	4E+06	3E+06	7E+06	5E+06	2E+06	2E+06	2E+06			
Dino		1E+06	1E+06	3E+05	2E+06	3E+06	2E+06	5E+06	7E+06	4E+06	7E+06	8E+06	4E+06	5E+06	3E+06	7E+06	1E+07	6E+06	6E+06	2E+06	4E+06	5E+06			
Alt		2E+03	3E+03	2E+03	1E+05	1E+06	2E+06	2E+06	4E+06	1E+06	3E+06	2E+06	5E+06	4E+06	5E+06	3E+06	1E+07	6E+06	2E+07	2E+06	2E+06	2E+06			
Mar		4E+05	6E+05	7E+05	1E+06	1E+06	1E+06	5E+06	3E+06	3E+06	4E+06	4E+06	3E+06	4E+06	2E+06	2E+06	4E+06	3E+05	1E+06	3E+06	3E+06	2E+06			
Ver		7E+05	5E+05	4E+05	1E+06	1E+06	5E+05	5E+06	2E+06	4E+06	5E+06	5E+06	3E+06	2E+06	2E+06	2E+06	1E+07	6E+06	5E+06	3E+06	3E+06	3E+06			
AN10		1E+06	7E+05	1E+06	1E+06	8E+05	1E+06	8E+03	1E+04	6E+03	5E+05	1E+06	1E+06	5E+05	1E+06	8E+05	3E+05	5E+05	8E+05	3E+05	3E+05	4E+05			
E.coli		1E+05	1E+05	2E+05	2E+03	2E+03	2E+03	2E+03	3E+03	3E+03	1E+00	1E+00	1E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00	0E+00			
ESW																									
Sali		1E+02	2E+02	2E+02	1E+02	2E+02	3E+02	7E+03	1E+03	4E+03	3E+04	8E+04	6E+04	1E+04	5E+03	6E+03	5E+02	2E+03	3E+02	3E+03	2E+03	2E+03			
Aero		2E+06	2E+06	2E+06	2E+07	1E+07	1E+07	3E+06	2E+07	2E+07	2E+07	4E+07	6E+07	2E+07	2E+07	2E+07	5E+07	1E+08	1E+08	8E+06	1E+08	5E+08			
Pol		2E+06	2E+06	3E+06	1E+06	3E+06	7E+06	5E+06	1E+07	7E+06	6E+06	5E+06	3E+06	2E+06	1E+06	2E+06	4E+05	1E+06	7E+06	8E+05	1E+06	2E+06			
Alg		3E+05	3E+05	4E+05	1E+04	5E+05	2E+06	1E+06	3E+06	4E+06	4E+06	6E+06	1E+07	1E+07	8E+07	1E+08	6E+06	2E+07	2E+07	1E+06	1E+06	2E+06			
Gram		1E+07	3E+06	6E+06	6E+06	8E+06	6E+06	8E+06	1E+07	1E+07	5E+07	6E+07	2E+07	5E+07	7E+07	3E+07	1E+07	5E+07	1E+07	3E+06	8E+06	3E+06			
Form		3E+05	5E+04	2E+05	1E+05	3E+04	5E+04	1E+06	4E+06	5E+06	7E+06	6E+06	5E+06	5E+07	4E+07	3E+07	1E+06	1E+06	3E+06	2E+06	2E+07	6E+05			
Ru		1E+07	1E+07	1E+07	2E+07	2E+07	2E+07	3E+07	6E+07	2E+07	7E+07	4E+07	4E+07	4E+07	2E+07	3E+07	3E+06	3E+06	6E+06	1E+07	2E+07	2E+07			
Ros		7E+06	1E+07	1E+07	3E+07	2E+07	4E+07	2E+08	7E+07	7E+07	6E+07	1E+08	3E+07	5E+07	4E+07	5E+07	2E+07	2E+07	3E+07	2E+07	2E+07	1E+07			
Dino		2E+08	1E+08	8E+07	3E+06	2E+06	3E+06	1E+08	1E+08	2E+08	3E+08	2E+08	5E+08	8E+07	1E+08	8E+07	1E+08	2E+08	2E+08	1E+08	8E+07	1E+08			
Alt		1E+06	1E+06	1E+07	1E+06	3E+07	4E+07	5E+07	5E+07	8E+07	1E+08	3E+07	7E+07	3E+07	5E+07	8E+07	1E+08	2E+08	1E+08	8E+07	8E+07	3E+07			
Mar		7E+06	4E+06	3E+06	3E+07	2E+07	3E+07	3E+08	3E+08	2E+08	5E+08	7E+08	5E+08	1E+08	2E+08	1E+08	2E+08	4E+07	3E+08	2E+08	3E+07	1E+08			
Ver		2E+07	1E+07	6E+06	1E+07	8E+06	4E+07	7E+07	4E+07	3E+07	6E+07	1E+08	1E+08	3E+07	1E+08	6E+07	2E+08	2E+08	2E+08	5E+07	5E+07	7E+07			
AN10		4E+07	3E+07	5E+07	1E+07	3E+06	1E+07	5E+07	4E+07	2E+07	4E+07	7E+07	8E+07	2E+08	1E+08	1E+08	5E+05	6E+05	3E+05	1E+06	2E+06	3E+06			
E.coli		3E+06	5E+06	6E+06	4E+05	3E+05	2E+05	3E+04	3E+03	3E+03	6E+06	5E+06	5E+06	2E+06	8E+05	1E+06	8E+05	2E+05	6E+05	2E+05	2E+05	2E+05			

Table 7.4: Raw data of the abundance of Micromonas sp. (up-part) in co-cultures with 14 heterotrophs (down-part) over 19 weeks of incubation (number of week on the top raw), tested with logistic regression (white cells) or two-way ANOVA (grey-cells).

	Micromonas sp.																				
SW	0	1	4			7			10			13			16			19			
Sali	3E+04	3E+04	7E+03	1E+04	5E+03	2E+02	2E+02	2E+02	2E+02	4E+02	3E+02	1E+00	1E+00	1E+00							
Aero	3E+04	3E+04	2E+04	2E+04	3E+03	5E+02	2E+02	3E+02	1E+00	1E+00	1E+00										
Pol	3E+04	3E+04	2E+04	2E+04	2E+04	2E+02	8E+02	2E+03	2E+02	3E+02	2E+02	1E+00	1E+00	1E+00							
Alg	3E+04	4E+04	4E+04	3E+04	2E+04	2E+03	6E+02	1E+03	3E+02	3E+02	3E+02	1E+00	1E+00	1E+00							
Gram	3E+04	3E+04	3E+04	3E+04	3E+04	3E+03	2E+03	2E+03	2E+02			1E+00	1E+00	1E+00							
Form	3E+04	3E+04	2E+04	2E+04	2E+04	2E+02	5E+02	2E+02	6E+02			1E+00	1E+00	1E+00	3E+02	2E+03	1E+03	1E+00	1E+00	1E+00	
Ru	3E+04	2E+04	1E+04	1E+04	1E+04	6E+02	1E+03	8E+02	2E+02			1E+00	1E+00	1E+00							
Ros	3E+04	1E+04	2E+04	2E+04	2E+04	3E+03	3E+03	4E+03	6E+02	3E+02	4E+02	1E+00	1E+00	1E+00							
Dino	3E+04	2E+04	2E+04	2E+04	1E+04	3E+03	2E+03	2E+03	1E+02	2E+02	3E+01	1E+00	1E+00	1E+00							
Alt	3E+04	2E+03	2E+04	2E+04	3E+04	3E+04	3E+04	3E+04	4E+04	7E+04	4E+04	3E+04	1E+04	3E+03	3E+03	1E+03	1E+03	1E+00	1E+00	1E+00	
Mar	3E+04	4E+04	2E+02	3E+01	1E+01	1E+00	1E+00	1E+00													
Ver	3E+04	4E+04	2E+04	2E+04	2E+04	3E+02	2E+02		1E+00	1E+00	1E+00										
AN10	3E+04	4E+04	1E+04	1E+04	1E+04	8E+02	3E+02	1E+03	1E+00	1E+00	1E+00										
E.coli	3E+04	2E+04	3E+04	6E+04	4E+04	3E+04	2E+04	1E+04	3E+03	3E+03	1E+04	4E+04	4E+04	2E+04	2E+04	2E+04	1E+04	1E+00	1E+00	1E+00	
Ax	3E+04	3E+04	5E+04	2E+04	2E+04	1E+04	2E+02	3E+02	3E+03	1E+03	7E+02	1E+00	1E+00	1E+00							
ESW																					
Sali	3E+05	3E+06	8E+04	1E+05	7E+04	4E+04	2E+04	1E+04	3E+03	3E+03	4E+03	4E+03	3E+02	5E+02	1E+07	5E+06	7E+05	3E+06	4E+06	4E+06	
Aero	3E+05	2E+06	2E+06	3E+06	3E+06	3E+06	3E+06	3E+06	3E+06	3E+06	4E+06	1E+05	1E+05	1E+05	2E+04	6E+03	1E+04	4E+04	8E+04	4E+04	
Pol	3E+05	5E+06	6E+06	5E+06	3E+06	4E+06	2E+06	2E+06	4E+05	2E+06	6E+06	2E+06	3E+06	3E+05	4E+03	6E+03	3E+03	8E+03	3E+01	1E+04	
Alg	3E+05	3E+06	3E+05	1E+06	1E+06	4E+05	4E+05	6E+05	8E+05	6E+05	8E+05	2E+03	3E+03	1E+04	6E+04	4E+05	4E+06	3E+05	4E+06	2E+07	
Gram	3E+05	2E+06	5E+05	7E+05	6E+05	3E+05	3E+05	3E+05	3E+05	2E+05	2E+05	3E+05	6E+05	4E+05	4E+04	6E+04	8E+04	5E+04	1E+05	8E+04	
Form	3E+05	3E+06	5E+05	4E+05	3E+05	4E+04	3E+04	3E+04	5E+03	5E+03	5E+03	5E+02	1E+03	3E+01	2E+06	5E+06	2E+05	3E+06	1E+07	2E+06	
Ru	3E+05	4E+06	2E+06	3E+05	2E+06	3E+04	8E+04	8E+04	3E+04	3E+04	2E+04	1E+04	3E+05	2E+03	6E+05	1E+06	5E+07	4E+06	8E+06	2E+07	
Ros	3E+05	4E+06	5E+05	1E+06	3E+05	2E+04	2E+04	8E+03	8E+03	5E+03	2E+03	8E+04	3E+05	2E+05	2E+05	7E+05	4E+05	3E+05	1E+07	2E+04	
Dino	3E+05	3E+06	1E+06	6E+05	1E+06	5E+04	8E+04	3E+04	5E+04	5E+04	8E+04	3E+04	2E+04	5E+03	1E+00	1E+00	1E+00				
Alt	3E+05	2E+06	3E+06	2E+06	1E+06	7E+05	3E+05	2E+05	1E+00	1E+00	1E+00										
Mar	3E+05	3E+06	8E+06	5E+06	3E+06	2E+06	1E+06	5E+05	5E+04	4E+04	6E+04	1E+06	1E+06	3E+05	1E+05	3E+04		3E+03	3E+03	2E+04	
Ver	3E+05	3E+06	3E+05	1E+06	7E+05	4E+05	5E+05	3E+05	6E+04	5E+04	7E+04	1E+06	1E+05	1E+06	2E+07	3E+07	3E+06	4E+04	5E+06	2E+05	
AN10	3E+05	3E+06	6E+05	4E+05	3E+05	8E+04	6E+04	6E+04	4E+04	3E+04	4E+04	5E+02	3E+02	3E+01	1E+00	1E+00	1E+00				
E.coli	3E+05	2E+06	3E+06	4E+06	5E+06	5E+06	7E+06	6E+06	8E+06	5E+06	5E+06	1E+05	2E+04	3E+04	1E+05	1E+04	4E+04	1E+05	7E+02	5E+04	
Ax	3E+05	4E+06	3E+06	2E+05	4E+05	1E+05	3E+04	1E+05	5E+03	5E+03	1E+04	1E+00	1E+00	1E+00							
SW																					
Sali	1E+05	1E+02	1E+02	6E+02	8E+02	1E+02	1E+03	1E+03	2E+03	2E+03	1E+03	2E+03	4E+02	8E+02	2E+02	1E+02	2E+02	6E+02	1E+02	1E+02	
Aero	6E+03	1E+04	1E+04	1E+04	2E+04	1E+04	2E+04	2E+04	1E+04	2E+04	2E+04	4E+04	2E+04	1E+04	1E+04	9E+03	3E+03	1E+00	1E+00	1E+00	
Pol	4E+03	4E+03	8E+03	8E+03	6E+03	7E+03	6E+03	5E+03	6E+03	3E+03	5E+03	1E+05	6E+04	7E+04	6E+03	5E+03	5E+03	1E+00	1E+00	1E+00	
Alg	1E+10	2E+06	1E+03	5E+03	1E+06	1E+03	2E+03	1E+03	1E+04	1E+04	7E+03	8E+04	4E+04	9E+04	3E+03	2E+03	3E+03	1E+02	1E+02	1E+02	
Gram	1E+04	4E+04	2E+03	9E+03	2E+04	2E+03	9E+03	1E+04	2E+04	1E+04	1E+04	2E+05	2E+05	2E+05	3E+03	3E+03	4E+03	1E+03	1E+02	1E+02	
Form	1E+03	1E+05	6E+04	5E+04	4E+04	2E+04	3E+04	3E+04	7E+03	4E+04	3E+04	4E+04	4E+04	1E+05	1E+02	1E+02	1E+02	1E+00	1E+00	1E+00	
Ru	2E+08	2E+05	1E+06	2E+06	2E+06	9E+05	1E+06	1E+06	2E+06	2E+06	2E+06	1E+06	2E+06	1E+06	2E+06	2E+06	3E+06	2E+06	1E+05	1E+05	
Ros	4E+08	5E+05	6E+05	5E+05	7E+05	9E+05	9E+05	1E+06	1E+06	1E+06	1E+06	1E+06	1E+06	1E+06	3E+05	3E+05	3E+05	2E+06	1E+05	1E+05	
Dino	1E+10	5E+05	1E+06	1E+06	3E+05	1E+06	3E+05	8E+05	1E+06	1E+06	1E+06	1E+06	2E+06	1E+06	2E+06	2E+06	5E+06	2E+06	1E+05	1E+05	
Alt	9E+09	1E+03	4E+06	2E+06	3E+05	1E+00	1E+00	1E+00													
Mar	6E+09	5E+06	2E+06	3E+06	2E+06	2E+06	3E+06	3E+06	6E+06	7E+06	8E+06	1E+07	8E+06	1E+07	4E+06	4E+06	5E+06	2E+07	1E+07	2E+07	
Ver	8E+09	2E+05	2E+03	2E+03	2E+03	1E+05	1E+05	2E+05	8E+04	6E+04	6E+04	1E+04	3E+04	3E+04	2E+03	3E+03	3E+03	1E+03	1E+02	1E+02	
AN10	1E+08	1E+06	2E+06	2E+06	2E+06	2E+06	2E+06	2E+06	2E+06	3E+06	3E+06	9E+05	7E+05	9E+05	2E+06	2E+06	3E+06	9E+05	8E+04	8E+04	
E.coli	1E+08	1E+05	1E+00	1E+00	1E+00																
ESW																					
Sali	1E+04	2E+03	1E+02	1E+02	1E+02	4E+02	7E+02	1E+03	5E+02	3E+02	3E+02	3E+03	2E+03	1E+03	2E+04	2E+04	2E+04	5E+02	7E+02	6E+02	
Aero	6E+08	3E+06	5E+06	3E+06	1E+06	1E+05	1E+05	2E+05	1E+00	1E+00	1E+00										
Pol	4E+08	1E+07	4E+03	8E+03	7E+03	1E+04	5E+03	8E+03	1E+00	1E+00	1E+00										
Alg	1E+09	1E+07	1E+05	9E+05	2E+06	2E+03	6E+03	6E+03	1E+04	5E+05	3E+05	1E+06	1E+05	1E+05	3E+05	3E+05	3E+05	1E+06	2E+06	2E+06	
Gram	1E+03	1E+07	1E+04	5E+03	3E+03	1E+00	1E+00	1E+00													
Form	1E+08	1E+05	4E+04	3E+04	7E+04	1E+05	5E+04	3E+04	1E+03	5E+03	1E+04	2E+04	1E+04	1E+04	1E+04	6E+03	2E+04	1E+02	1E+02	1E+02	
Ru	2E+07	2E+07	3E+06	4E+06	4E+06	2E+07	2E+07	3E+07	1E+07	2E+07	2E+07	1E+07	1E+07	2E+07	4E+06	3E+06	4E+06	2E+07	1E+07	1E+07	
Ros	4E+07	5E+05	1E+06	1E+07	2E+07	2E+07	3E+07	3E+07	7E+06	9E+06	1E+07	6E+06	1E+07	1E+07	2E+06	3E+06	3E+06	2E+06	3E+06	3E+06	
Dino	1E+09	4E+07	1E+05	7E+06	5E+05	2E+07	1E+07	2E+07	9E+06	5E+06	4E+06	1E+07	1E+07	6E+06	2E+06	2E+06	1E+06	1E+06	1E+05	1E+05	
Alt	9E+08	1E+05	1E+03	1E+02	1E+02	1E+00	1E+00	1E+00													
Mar	6E+08	3E+06	2E+07	1E+05	1E+05	1E+00	1E+00	1E+00													
Ver	8E+08	5E+06	3E+05	2E+05	3E+05	3E+05	3E+05	2E+05	1E+05	3E+05	2E+05	2E+05	3E+05	3E+05	3E+05	2E+05	2E+05	2E+05	2E+05	2E+05	
AN10	1E+08	1E+06	6E+06	6E+06	5E+06	1E+06	1E+06	2E+05	2E+06	1E+06	1E+05	3E+05	1E+06	9E+05	1E+06	1E+06	1E+06	3E+03	2E+02	1E+02	
E.coli	1E+08	3E+04	1E+00	1E+00	1E+00																

Table 7.5: Raw data of the abundance of M. pusilla (up-part) in co-cultures with 14 heterotrophs (down-part) over 19 weeks of incubation (number of week on the top raw), tested with logistic regression (white cells) or two-way ANOVA (grey-cells).

M. pusilla

SW	0	1			3	8	11			13			16			19		
Sali	1.7E+05	7.3E+03	5.5E+03	7.0E+03	8.5E+01	1.0E+00												
Aero	1.7E+05	4.2E+03	5.0E+03	4.5E+03	3.4E+02	1.0E+00												
Pol	1.7E+05	5.7E+03	5.3E+03	4.8E+03	1.0E+00	1.0E+00												
Alg	1.7E+05	7.0E+03	7.7E+03	6.3E+03	8.5E+01	1.0E+00												
Gram	1.7E+05	7.3E+03	9.5E+03	5.4E+03	1.7E+02	1.0E+00												
Form	1.7E+05	1.2E+04	8.5E+03	6.1E+03	1.0E+00	1.0E+00												
Ru	1.7E+05	6.5E+03	5.3E+03	4.3E+03	2.6E+02	1.0E+00												
Ros	1.7E+05	5.2E+03	3.1E+03	3.2E+03	2.6E+02	1.7E+02	1.0E+00	1.0E+00	1.0E+00									
Dino	1.7E+05	5.5E+03	5.0E+03	5.0E+03	2.6E+02	1.7E+02	1.0E+00	1.0E+00	1.0E+00									
Alt	1.7E+05	7.2E+03	5.3E+03	6.2E+03	1.7E+02	1.0E+00												
Mar	1.7E+05	3.4E+03	4.8E+03	4.8E+03	2.6E+02	1.0E+00												
Ver	1.7E+05	6.7E+03	8.2E+03	7.1E+03	1.7E+02	1.0E+00												
AN10	1.7E+05	5.5E+03	5.4E+03	3.6E+03	4.3E+02	1.0E+00												
E.coli	1.7E+05	6.5E+03	4.3E+03	6.8E+03	3.4E+02	1.0E+00												
Ax	1.7E+05	5.0E+03	6.3E+03	4.4E+03	4.3E+02	1.0E+00												
ESW																		
Sali	2.1E+06	2.6E+05	2.1E+05	2.8E+05	1.3E+04	1.0E+00												
Aero	2.1E+06	7.0E+05	7.8E+05	7.8E+05	4.3E+06	1.5E+04	1.1E+07	8.6E+06	1.7E+06	2.0E+06	2.9E+06	1.8E+07	1.4E+06	3.2E+06	4.1E+04	2.1E+04	2.1E+04	1.2E+03
Pol	2.1E+06	4.4E+05	5.4E+05	3.6E+05	2.3E+06	3.3E+06	8.9E+06	4.8E+06	7.2E+05	3.2E+06	1.6E+06	6.0E+03	1.0E+00	1.0E+00	1.0E+00			
Alg	2.1E+06	4.4E+05	6.0E+05	4.8E+05	3.6E+06	1.0E+04	8.7E+06	4.5E+06	6.4E+06	8.2E+06	1.1E+07	5.5E+06	1.0E+00	1.0E+00	1.0E+00			
Gram	2.1E+06	4.6E+05	5.1E+05	4.3E+05	2.9E+06	1.1E+06	5.2E+05	1.8E+06	1.2E+06	7.5E+03	8.4E+04	4.6E+04	1.0E+00	1.0E+00	1.0E+00			
Form	2.1E+06	3.6E+05	3.5E+05	3.5E+05	1.9E+06	1.3E+06	2.0E+06	4.9E+06	2.7E+06	1.3E+07	3.0E+06	5.3E+06	1.2E+06	3.8E+05	4.1E+05	1.0E+00	1.0E+00	1.0E+00
Ru	2.1E+06	3.9E+05	3.7E+05	3.4E+05	3.1E+06	1.1E+06	2.1E+06	4.2E+06	7.5E+05	4.6E+05	8.0E+06	2.3E+06	6.0E+06	4.1E+06	7.9E+06	1.5E+05	4.9E+04	2.4E+05
Ros	2.1E+06	3.4E+05	3.1E+05	5.0E+05	2.5E+06	7.0E+05	4.1E+06	6.5E+06	7.4E+05	7.7E+06	8.4E+06	2.1E+06	1.1E+04	5.5E+03	2.8E+03	1.0E+00	1.0E+00	1.0E+00
Dino	2.1E+06	5.0E+05	2.9E+05	1.6E+05	3.8E+06	1.4E+06	4.7E+06	2.5E+06	3.4E+06	2.8E+06	1.9E+06	5.4E+06	7.2E+03	1.0E+04	3.3E+06	1.0E+00	1.0E+00	1.0E+00
Alt	2.1E+06	1.4E+05	2.0E+05	2.9E+05	2.9E+06	6.7E+06	3.9E+06	3.8E+06	6.1E+06	5.9E+06	3.4E+06	7.9E+06	2.3E+06	1.1E+06	1.1E+04	1.0E+00	1.0E+00	1.0E+00
Mar	2.1E+06	3.2E+05	8.6E+05	6.0E+05	2.1E+06	2.5E+05	5.6E+06	7.9E+06	5.6E+05	5.2E+06	1.5E+07	8.8E+06	1.0E+06	4.6E+05	2.7E+06	8.0E+03	8.8E+03	8.5E+05
Ver	2.1E+06	3.8E+05	3.6E+05	3.5E+05	2.5E+06	4.8E+05	2.2E+05	4.4E+05	6.6E+05	8.9E+05	7.5E+03	1.8E+06	7.4E+05	8.8E+05	1.5E+06	1.0E+00	1.0E+00	1.0E+00
AN10	2.1E+06	4.4E+05	1.8E+05	5.3E+05	2.4E+06	5.1E+05	1.2E+06	2.0E+06	5.6E+05	2.0E+06	4.5E+06	3.3E+06	1.0E+00	1.0E+00	1.0E+00			
E.coli	2.1E+06	5.2E+05	3.8E+05	6.6E+05	2.8E+06	7.1E+04	1.0E+00	1.0E+00	1.0E+00									
Ax	2.1E+06	7.6E+05	8.9E+05	8.4E+05	2.1E+06	1.5E+06	1.0E+00	1.0E+00	1.0E+00									
SW																		
Sali	3.5E+03	7.0E+02	1.3E+03	9.0E+02	5.3E+02	3.7E+02	6.0E+02	9.5E+02	7.8E+02	2.0E+03	1.6E+03	2.1E+03	6.0E+02	6.5E+02	6.3E+02	3.1E+03	3.0E+03	2.0E+03
Aero	4.3E+06	4.5E+05	3.9E+05	4.2E+05	2.9E+05	3.8E+03	1.0E+03	5.7E+03	1.1E+04	1.0E+04	1.5E+04	1.2E+04	1.0E+03	1.9E+04	2.8E+04	3.6E+04	3.7E+04	5.2E+04
Pol	2.3E+06	1.8E+05	2.0E+05	1.1E+05	1.0E+03	1.0E+00												
Alg	2.5E+06	3.0E+05	2.6E+05	2.5E+05	1.3E+05	1.0E+02	1.0E+03	5.8E+02	3.4E+02	1.5E+02	1.0E+02	1.0E+02	1.0E+00	1.0E+00	1.0E+00			
Gram	1.0E+05	2.6E+04	2.1E+04	2.6E+04	1.3E+05	1.0E+02	8.5E+03	9.3E+03	1.0E+04	1.6E+04	1.3E+04	1.1E+04	3.4E+03	2.0E+03	2.7E+03	7.0E+02	1.0E+02	1.0E+02
Form	5.0E+06	1.5E+04	1.1E+03	6.7E+03	1.0E+00													
Ru	4.1E+07	5.8E+05	5.4E+05	5.6E+05	1.0E+05	1.7E+05	7.0E+03	1.9E+06	3.1E+06	4.8E+04	3.4E+04	5.3E+04	3.2E+04	2.3E+04	3.7E+04	2.4E+04	1.2E+04	3.2E+04
Ros	1.6E+07	3.5E+05	3.2E+05	3.8E+05	4.7E+06	2.0E+05	5.0E+04	4.0E+04	2.7E+04	3.5E+04	4.4E+04	2.8E+04	3.3E+04	3.5E+04	3.9E+04	3.5E+03	3.7E+03	3.0E+03
Dino	4.1E+07	4.9E+05	5.9E+05	5.2E+05	1.0E+05	1.6E+05	4.2E+04	3.9E+04	2.9E+04	4.1E+04	3.8E+04	4.8E+04	3.8E+04	4.9E+04	4.7E+04	4.8E+04	3.7E+04	3.0E+04
Alt	1.3E+07	1.0E+05	5.3E+04	8.3E+04	6.0E+03	1.1E+05	1.0E+05	1.5E+05	8.7E+04	2.1E+05	2.2E+04	1.5E+05	1.0E+04	1.5E+05	1.9E+05	2.9E+05	2.3E+05	3.1E+05
Mar	2.7E+07	3.1E+05	4.6E+05	3.1E+05	2.0E+04	1.4E+05	1.0E+05	1.8E+05	2.7E+05	2.6E+05	3.6E+05	2.2E+05	1.0E+04	1.4E+05	2.5E+05	2.8E+05	3.6E+05	4.2E+05
Ver	4.0E+06	9.7E+03	9.7E+03	1.5E+04	2.3E+03	1.0E+02	1.0E+00	1.0E+00	1.0E+00									
AN10	1.9E+07	7.5E+05	6.9E+05	5.7E+05	2.5E+05	1.3E+05	1.0E+04	8.3E+04	1.1E+05	1.6E+05	1.4E+05	1.8E+05	3.0E+05	2.7E+05	3.5E+05	3.4E+05	2.8E+05	2.1E+05
E.coli	4.9E+07	3.4E+04	2.9E+04	4.1E+04	1.4E+05	2.4E+05	2.3E+05	2.2E+05	2.2E+05	2.0E+05	2.1E+05	2.0E+05	1.0E+00	1.0E+00	1.0E+00			
ESW																		
Sali	3.5E+05	2.6E+04	2.1E+04	2.1E+04	1.2E+04	3.0E+02	1.5E+03	1.2E+03	8.0E+02	1.7E+03	1.9E+03	1.2E+03	2.0E+03	1.1E+03	1.0E+03	3.6E+03	4.4E+03	3.5E+03
Aero	4.3E+08	1.0E+07	1.3E+07	1.7E+07	6.7E+07	2.1E+06	1.1E+07	2.6E+07	2.6E+07	4.0E+07	2.6E+07	3.2E+07	1.1E+07	1.6E+07	1.6E+07	4.5E+06	1.2E+07	6.5E+06
Pol	2.3E+08	7.0E+06	6.3E+06	7.3E+06	6.0E+07	1.8E+06	1.7E+07	1.1E+07	1.4E+07	1.5E+07	3.0E+06	1.4E+08	2.0E+04	1.6E+04	1.6E+04	1.5E+04	1.3E+04	1.9E+04
Alg	2.5E+08	1.6E+07	1.6E+07	1.6E+07	1.0E+06	1.8E+06	1.4E+06	1.5E+07	1.5E+06	1.1E+06	2.1E+07	1.7E+06	1.6E+05	1.0E+05	1.6E+05	3.9E+05	3.8E+05	3.0E+05
Gram	1.0E+07	1.0E+05	6.2E+05	1.3E+06	1.1E+06	2.0E+06	1.5E+06	1.5E+06	1.5E+06	1.2E+06	2.1E+06	3.1E+06	1.6E+06	2.0E+06	2.4E+06	7.7E+06	2.0E+07	1.2E+07
Form	5.0E+08	1.0E+05	5.7E+04	2.9E+04	1.4E+04	1.0E+03	1.0E+03	1.0E+03	3.3E+03	1.0E+03	5.5E+03	4.4E+04	1.0E+04	8.3E+04	1.1E+05	1.6E+05	1.3E+05	1.8E+05
Ru	4.1E+03	9.7E+06	1.3E+07	1.3E+07	6.3E+07	2.4E+06	2.8E+06	2.3E+06	1.9E+06	3.7E+06	4.2E+06	3.2E+06	1.7E+06	1.5E+06	1.1E+06	1.7E+07	5.3E+06	9.7E+06
Ros	1.6E+03	1.4E+07	1.2E+07	1.7E+07	1.0E+06	3.0E+06	2.0E+06	2.0E+06	1.9E+06	4.3E+06	3.6E+07	2.6E+07	2.3E+06	3.2E+07	2.9E+07	2.4E+07	1.5E+07	1.4E+07
Dino	4.1E+03	3.0E+07	3.3E+07	3.1E+07	1.0E+06	1.2E+08	1.3E+06	1.7E+06	1.8E+06	4.4E+07	3.5E+07	4.1E+07	3.1E+07	2.5E+07	3.4E+07	2.3E+07	2.6E+07	2.5E+07
Alt	1.3E+03	1.0E+05	5.3E+04	3.2E+06	6.0E+03	6.3E+06	8.5E+06	1.4E+07	2.0E+07	2.0E+07	2.5E+07	2.0E+07	1.0E+05	7.4E+06	9.0E+06	1.5E+07	1.1E+07	1.3E+07
Mar	2.7E+03	5.7E+06	6.0E+06	5.0E+06	1.5E+04	1.0E+06	1.0E+06	1.6E+07	1.9E+07	3.1E+07	2.2E+07	1.9E+07	1.0E+05	8.4E+06	1.5E+07	1.7E+07	2.2E+07	1.3E+07
Ver	4.0E+08	3.7E+06	3.7E+06	4.7E+06	2.8E+04	1.0E+06	1.0E+06	8.4E+05	8.4E+05	8.4E+05	7.0E+05	7.1E+05	6.7E+05	5.6E+05	5.3E+05	3.5E+05	1.2E+05	1.8E+05
AN10	1.9E+03	1.1E+07	9.7E+06	1.3E+07	1.0E+06	1.1E+06	1.0E+06	5.0E+05	7.6E+06	1.0E+03	1.5E+07	2.0E+07	2.3E+07	2.5E+07	2.1E+07	7.0E+05	1.2E+06	9.4E+05
E.coli	4.9E+03	4.0E+05	4.5E+05	4.4E+05	2.5E+07	5.0E+07	1.3E+04	2.0E+04	2.5E+04	5.3E+06	5.3E+06	5.1E+06	7.5E+04	1.8E+05	1.0E+05	5.3E+03	5.6E+03	5.2E+03

Table 7.6: Raw data of the abundance of Ostreococcus (up-part) in co-cultures with 14 heterotrophs (down-part) over 19 weeks of incubation (number of week on the top raw), tested with logistic regression (white cells) or two-way ANOVA (grey-cells).

Ostreococcus

SW	0	1	3	8	11			13			16			19		
Sali	4E+04	1E+05	1E+05	1E+00												
Aero	4E+04	1E+05	7E+04	1E+00												
Pol	4E+04	1E+05	3E+04	1E+00												
Alg	4E+04	1E+05	6E+04	1E+00												
Gram	4E+04	2E+05	2E+05	1E+00												
Form	4E+04	8E+04	7E+04	1E+00												
Ru	4E+04	1E+05	6E+04	1E+00												
Ros	4E+04	8E+04	3E+04	1E+00												
Dino	4E+04	8E+04	5E+04	1E+00												
Alt	4E+04	1E+05	4E+04	1E+00												
Mar	4E+04	2E+05	4E+04	1E+00												
Ver	4E+04	1E+05	3E+04	1E+00												
AN10	4E+04	1E+05	5E+04	1E+00												
E.coli	4E+04	1E+05	7E+04	1E+00												
Ax	4E+04	8E+04	3E+04	1E+00												
ESW																
Sali	7E+05	2E+05	3E+04	1E+03	1E+00	1E+00	1E+00									
Aero	7E+05	1E+06	4E+06	1E+06	5E+05	9E+05	1E+06	4E+05	6E+05	4E+06	2E+05	2E+05	8E+04	2E+03	2E+03	2E+03
Pol	7E+05	2E+06	1E+07	1E+04	1E+04	1E+05	2E+06	4E+05	5E+05	5E+06	2E+04	1E+04	5E+03	1E+00	1E+00	1E+00
Alg	7E+05	2E+06	1E+07	4E+06	1E+06	2E+06	5E+05	4E+03	1E+04	3E+03	1E+00	1E+00	1E+00			
Gram	7E+05	3E+05	5E+06	1E+06	6E+05	1E+06	1E+06	3E+04	6E+04		4E+05	2E+05	3E+05	1E+00	1E+00	1E+00
Form	7E+05	1E+06	1E+07	1E+00												
Ru	7E+05	2E+06	7E+06	5E+06	2E+06	2E+06	4E+06	3E+05	5E+05	1E+06	4E+05	2E+05	6E+05	4E+04	3E+03	4E+04
Ros	7E+05	2E+06	2E+06	8E+04	4E+07	2E+06	2E+06	1E+06	8E+05	1E+06	4E+05	3E+05	4E+05	7E+03	3E+03	5E+03
Dino	7E+05	2E+06	6E+06	5E+06	1E+00	1E+00	1E+00									
Alt	7E+05	1E+06	1E+07	1E+00												
Mar	7E+05	2E+06	2E+07	8E+02	1E+04	3E+04	4E+04	6E+04	4E+05	2E+05	3E+05	3E+04	6E+05	1E+00	1E+00	1E+00
Ver	7E+05	8E+05	2E+07	2E+02	1E+00	1E+00	1E+00									
AN10	7E+05	1E+06	8E+06	1E+00												
E.coli	7E+05	7E+06	6E+06	1E+00												
Ax	7E+05	2E+06	3E+06	1E+00												
SW																
Sali	3E+03	1E+02	1E+03	1E+03	3E+02	7E+02	3E+02	2E+02	1E+02	1E+02	3E+03	3E+03	3E+03	3E+03	3E+03	2E+03
Aero	4E+06	5E+03	2E+03	2E+03	1E+04	2E+04	1E+04	5E+02	1E+03	2E+03	1E+02	1E+00	1E+00			
Pol	2E+06	2E+06	1E+05	1E+03	2E+03	4E+03	1E+03	1E+02	7E+02	4E+02	1E+02	1E+00	1E+00			
Alg	3E+06	3E+06	2E+05	3E+02	1E+00	1E+00	1E+00									
Gram	1E+05	1E+04	1E+03	2E+05	2E+04	2E+03	2E+03	1E+04	2E+03	1E+03	2E+03	1E+03	6E+02	4E+02	1E+02	1E+02
Form	5E+06	1E+05	1E+03	1E+02	1E+02	1E+02	1E+02	1E+00	1E+00	1E+00						
Ru	4E+07	2E+06	2E+05	2E+05	3E+05	3E+05	4E+05	1E+05	2E+05	2E+05	3E+05	2E+05	2E+05	5E+03	5E+03	4E+03
Ros	2E+07	1E+06	8E+04	7E+04	1E+04	1E+04	1E+04	1E+04	3E+03	6E+03	1E+04	1E+04	1E+04	1E+02	1E+02	1E+02
Dino	4E+07	1E+05	1E+02	1E+02	1E+02	1E+02	1E+02	1E+00	1E+00	1E+00						
Alt	1E+07	1E+02	1E+02	1E+03	8E+03	7E+03	1E+04	1E+03	7E+04	4E+04	1E+05	2E+05	1E+04	1E+05	3E+05	2E+05
Mar	3E+07	1E+05	1E+02	1E+02	1E+02	1E+02	1E+02	1E+00	1E+00	1E+00						
Ver	4E+06	1E+03	3E+04	5E+02	1E+00	1E+00	1E+00									
AN10	2E+07	3E+05	2E+05	3E+05	5E+04	5E+04	4E+04	3E+04	3E+04	2E+04	1E+03	2E+04	3E+04	3E+04	4E+04	3E+04
E.coli	5E+07	1E+05	1E+02	1E+02	1E+00	1E+00	1E+00									
ESW																
Sali	3E+05	8E+02	1E+03	2E+03	3E+04	8E+03	3E+04	9E+02	1E+03	2E+03	3E+03	4E+03	3E+03	4E+03	3E+03	3E+03
Aero	4E+08	1E+07	1E+06	1E+05	2E+07	1E+07	2E+07	1E+07	6E+06	3E+06	1E+05	4E+05	5E+05	7E+05	7E+05	5E+05
Pol	2E+08	1E+07	1E+06	1E+08	3E+06	2E+06	3E+06	1E+07	1E+06	7E+05	1E+04	1E+05	1E+04	3E+04	3E+04	2E+04
Alg	3E+08	8E+06	3E+07	7E+07	2E+07	2E+07	3E+07	1E+07	2E+07	2E+07	5E+05	4E+05	5E+05	3E+05	3E+05	3E+05
Gram	1E+07	1E+06	1E+06	1E+08	7E+06	8E+06	3E+06	1E+05	2E+06	5E+06	1E+05	4E+06	7E+06	7E+06	8E+06	1E+07
Form	5E+08	1E+04	5E+03	1E+02	1E+04	3E+04	7E+04	1E+03	5E+04	1E+05	1E+04	3E+04	2E+05	2E+05	2E+05	3E+05
Ru	4E+09	6E+06	1E+06	3E+07	1E+07	1E+07	2E+07	7E+06	1E+07	1E+07	3E+04	3E+04	2E+04	2E+07	2E+07	2E+07
Ros	2E+03	1E+06	4E+04	3E+07	3E+06	9E+06	1E+07	4E+06	4E+06	4E+06	1E+05	3E+06	7E+06	5E+05	6E+06	1E+07
Dino	4E+03	1E+05	3E+04	9E+06	2E+07	9E+06	8E+06	3E+07	2E+07	2E+07	1E+05	6E+06	7E+06	1E+07	1E+07	7E+06
Alt	1E+03	1E+04	1E+04	1E+06	6E+06	9E+06	6E+06	1E+06	1E+06	2E+06	6E+06	1E+06	4E+06	1E+07	7E+06	6E+06
Mar	3E+03	3E+06	2E+04	5E+07	2E+07	2E+07	2E+07	2E+06	2E+06	3E+06	2E+06	2E+07	5E+06	1E+07	2E+07	1E+07
Ver	4E+08	3E+06	2E+04	1E+06	2E+07	2E+07	2E+07	1E+03	7E+04	1E+05	2E+05	1E+05	2E+05	3E+05	3E+05	3E+05
AN10	2E+03	2E+07	1E+06	1E+05	1E+07	7E+06	6E+06	1E+03	1E+06	2E+06	3E+06	2E+06	3E+06	8E+05	8E+05	6E+05
E.coli	5E+09	2E+04	1E+06	5E+07	1E+04	1E+06	2E+06	2E+06	3E+06	4E+06	3E+04	1E+05	1E+05	2E+03	9E+02	1E+03

Table 7.6: Raw data of the abundance of E. huxleyi (up-part) in co-cultures with 14 heterotrophs (down-part) over 19 weeks of incubation (number of week on the top raw), tested with logistic regression (white cells) or two-way ANOVA (grey-cells).

	E. huxleyi														
SW	0	1			3	8	11			15			19		
Sali	4E+04	3E+04	3E+04	3E+04	6E+04	5E+03	3E+04	2E+04	4E+04	3E+03	2E+03	2E+03	1E+00	1E+00	1E+00
Aero	4E+04	2E+03	2E+03	4E+03	1E+03	2E+03	1E+00	1E+00	1E+00						
Pol	4E+04	4E+04	3E+04	3E+04	5E+04	4E+04	6E+04	6E+04	2E+04	4E+03	1E+04	3E+03	1E+00	1E+00	1E+00
Alg	4E+04	2E+04	2E+04	1E+04	2E+04	3E+03	3E+03	1E+04	2E+04	1E+00	1E+00	1E+00			
Gram	4E+04	6E+04	5E+04	5E+04	7E+04	2E+04	2E+04	1E+04	2E+04	1E+00	1E+00	1E+00			
Form	4E+04	3E+04	2E+04	3E+04	5E+04	3E+03	1E+00	1E+00	1E+00						
Ru	4E+04	2E+04	3E+04	2E+04	3E+04	6E+03	2E+03	2E+04	3E+03	1E+00	1E+00	1E+00			
Ros	4E+04	2E+04	2E+04	3E+04	8E+03	7E+02	1E+00	1E+00	1E+00						
Dino	4E+04	2E+04	2E+04	2E+04	2E+04	2E+03	1E+00	1E+00	1E+00						
Alt	4E+04	4E+04	3E+04	3E+04	6E+04	4E+04	1E+05	6E+04	5E+04	2E+04	1E+04	1E+04	1E+00	1E+00	1E+00
Mar	4E+04	5E+03	1E+04	6E+03	5E+03	5E+03	1E+00	1E+00	1E+00						
Ver	4E+04	4E+04	5E+04	4E+04	7E+04	6E+03	5E+04	2E+04	1E+03						
AN10	4E+04	2E+04	3E+04	3E+04	3E+04	2E+03	1E+04	3E+03	2E+04						
E.coli	4E+04	2E+04	3E+04	2E+04	3E+04	4E+03	1E+00	1E+00	1E+00						
Ax	4E+04	3E+04	3E+04	3E+04	4E+04	2E+03	1E+00	1E+00	1E+00						
ESW															
Sali	7E+05	1E+04	6E+03	3E+03	3E+03	1E+00									
Aero	7E+05	1E+05	2E+05	5E+05	3E+05	2E+05	2E+05	3E+05	2E+05	1E+00	1E+00	1E+00			
Pol	7E+05	3E+05	2E+05	3E+05	1E+06	6E+05	5E+05	6E+05	7E+05	2E+05	1E+05	2E+05	1E+00	1E+00	1E+00
Alg	7E+05	3E+05	5E+05	4E+05	5E+05	4E+05	5E+05	4E+05	4E+05	6E+04	1E+05	1E+05	1E+00	1E+00	1E+00
Gram	7E+05	2E+05	4E+05	1E+05	6E+05	5E+05	3E+05	4E+05	3E+05	3E+04	5E+04	2E+04	1E+00	1E+00	1E+00
Form	7E+05	3E+05	5E+05	6E+05	5E+05	3E+05	4E+05	4E+05	3E+05	8E+04	1E+05	1E+05	1E+00	1E+00	1E+00
Ru	7E+05	4E+05	5E+05	6E+05	5E+05	3E+05	3E+05	3E+05	4E+05	3E+04	3E+03	2E+04	1E+00	1E+00	1E+00
Ros	7E+05	6E+05	8E+05	6E+05	6E+05	2E+05	3E+05	3E+05	2E+05	2E+04	1E+04	6E+03	1E+00	1E+00	1E+00
Dino	7E+05	6E+05	8E+05	4E+05	3E+05	4E+05	2E+05	2E+05	2E+05	2E+03	2E+03	2E+03	1E+00	1E+00	1E+00
Alt	7E+05	2E+05	2E+05	4E+05	6E+05	3E+05	6E+05	6E+05	7E+05	1E+05	1E+05	3E+05	1E+00	1E+00	1E+00
Mar	7E+05	5E+05	5E+05	5E+05	8E+05	2E+05	2E+05	3E+05	2E+05	2E+03	3E+04	2E+04	1E+00	1E+00	1E+00
Ver	7E+05	2E+05	3E+05	3E+05	6E+05	3E+05	4E+05	3E+05	4E+05	8E+04	1E+05	1E+05	1E+00	1E+00	1E+00
AN10	7E+05	4E+05	5E+05	4E+05	5E+05	6E+04	3E+05	3E+05	2E+05	5E+04	6E+04	6E+04	1E+00	1E+00	1E+00
E.coli	7E+05	5E+05	2E+05	4E+05	4E+05	1E+05	2E+05	3E+05	2E+05	2E+03	2E+04	7E+04	1E+00	1E+00	1E+00
Ax	7E+05	2E+05	4E+05	6E+05	6E+05	1E+05	2E+05	2E+05	2E+05	2E+04	2E+04	8E+03	1E+00	1E+00	1E+00
SW															
Sali	3E+03	3E+02	4E+02	5E+02	3E+02	1E+02	1E+03	2E+03	2E+03	2E+03	2E+03	2E+03	3E+03	2E+03	2E+03
Aero	4E+06	1E+04	3E+04	2E+04	2E+05	2E+05	2E+05	6E+04	6E+04	1E+02	1E+02	#DIV/0!	8E+02	1E+02	1E+02
Pol	2E+06	7E+05	4E+05	3E+05	9E+04	1E+05	1E+05	1E+05	1E+05	8E+04	4E+04	2E+04	1E+02	1E+02	1E+02
Alg	3E+06	7E+05	1E+06	5E+05	1E+05	3E+04	6E+04	5E+04	4E+04	4E+04	2E+04	3E+03	1E+00	1E+00	1E+00
Gram	1E+05	1E+05	2E+05	3E+05	2E+05	2E+05	2E+06	1E+06	3E+05	3E+05	4E+05	3E+05	1E+04	6E+02	2E+04
Form	5E+06	5E+03	6E+03	6E+03	1E+03	1E+00									
Ru	4E+07	1E+06	4E+05	1E+06	1E+05	1E+05	1E+06	7E+05	1E+06	2E+06	2E+06	2E+06	4E+04	4E+04	3E+04
Ros	2E+07	2E+06	2E+06	2E+06	1E+05	2E+05	2E+04	2E+04	2E+04	2E+04	2E+04	2E+04	2E+03	3E+03	3E+03
Dino	4E+07	1E+06	1E+06	1E+06	1E+05	3E+05	1E+06	2E+06	1E+06	2E+06	2E+06	3E+06	5E+05	4E+05	5E+05
Alt	1E+07	3E+03	4E+03	1E+04	7E+02	1E+03	1E+05	3E+06	7E+06	7E+05	1E+06	1E+06	3E+05	3E+05	3E+05
Mar	3E+07	2E+05	3E+05	6E+05	1E+05	1E+05	8E+06	4E+06	1E+07	2E+06	2E+06	2E+06	1E+05	1E+03	1E+03
Ver	4E+06	2E+05	2E+05	1E+05	2E+04	1E+03	1E+00	1E+00	1E+00						
AN10	2E+07	6E+05	1E+06	6E+05	2E+05	4E+05	6E+05	5E+05	4E+05	2E+06	1E+06	1E+06	6E+04	4E+04	4E+04
E.coli	5E+07	1E+06	1E+06	1E+06	2E+06	3E+06	1E+00	1E+00	1E+00						
ESW															
Sali	3E+05	1E+03	7E+02	3E+02	6E+03	2E+03	1E+04	2E+04	1E+04	4E+03	2E+03	3E+03	2E+03	4E+03	2E+03
Aero	4E+08	1E+07	2E+07	2E+07	5E+06	1E+06	2E+07	3E+07	2E+07	5E+07	4E+07	4E+07	3E+06	1E+07	2E+07
Pol	2E+08	1E+07	1E+07	1E+07	3E+06	2E+06	2E+07	1E+07	1E+07	2E+07	2E+07	1E+07	3E+04	3E+04	2E+04
Alg	3E+08	2E+07	2E+07	2E+07	8E+07	1E+06	6E+07	2E+07	2E+07	1E+07	3E+06	6E+06	7E+06	4E+06	3E+06
Gram	1E+07	5E+06	3E+06	5E+06	1E+04	1E+06	7E+06	5E+06	5E+06	1E+06	1E+06	2E+06	2E+07	3E+07	2E+07
Form	5E+08	1E+04	6E+03	8E+05	2E+04	1E+03	2E+06	2E+06	1E+06	5E+05	6E+05	7E+05	3E+05	1E+05	2E+05
Ru	4E+09	1E+07	1E+07	2E+07	1E+06	3E+06	1E+07	2E+07	2E+07	3E+07	2E+07	2E+07	7E+06	1E+07	1E+07
Ros	2E+09	1E+07	1E+07	2E+07	6E+07	2E+06	7E+06	6E+06	6E+06	1E+07	2E+07	2E+07	1E+07	1E+07	2E+07
Dino	4E+09	3E+07	2E+07	2E+07	7E+07	1E+08	6E+07	1E+08	8E+07	4E+07	3E+07	3E+07	4E+07	2E+07	3E+07
Alt	1E+09	1E+04	5E+05	7E+06	3E+05	1E+06	1E+07	3E+06	3E+06	1E+07	2E+07	2E+07	1E+07	1E+07	1E+07
Mar	3E+09	7E+07	3E+07	1E+08	1E+03	8E+07	2E+07	2E+07	1E+08	3E+07	2E+07	3E+07	1E+07	2E+07	2E+07
Ver	4E+08	#DIV/0!	7E+06	1E+07	4E+07	3E+07	2E+07	2E+07	2E+07	2E+07	2E+07	1E+07	5E+05	2E+05	1E+05
AN10	2E+09	5E+06	3E+06	1E+07	8E+06	5E+06	1E+06	4E+06	4E+06	1E+07	1E+07	1E+07	7E+05	3E+05	1E+06
E.coli	5E+09	2E+06	2E+06	2E+06	1E+06	1E+06	1E+00	1E+00	1E+00						

Table 7.8: Raw data of the abundance of Thalassiosira (up-part) in co-cultures with 14 heterotrophs (down-part) over 19 weeks of incubation (number of week on the top raw), tested with logistic regression (white cells) or two-way ANOVA (grey-cells).

	Thalassiosira																		
SW	0	1	4		8		12		14			16			19				
Sali	7E+03	4E+03	2E+03	4E+03	2E+03	3E+02	9E+02	1E+03	1E+00	1E+00	1E+00								
Aero	7E+03	3E+03	1E+04	1E+04	1E+04	6E+03	8E+03	3E+03	1E+04	1E+04	8E+03	1E+04	1E+04	6E+03	3E+04	3E+03	2E+04	3E+03	5E+03
Pol	7E+03	2E+03	7E+03	6E+03	7E+03	5E+03	5E+03	8E+03	5E+03	2E+03	5E+03	4E+03	3E+03	3E+03	4E+03	5E+03	1E+04	1E+00	1E+00
Alg	7E+03	3E+02	2E+04	1E+04	2E+04	1E+04	1E+04	1E+04	6E+03	2E+03	1E+03	3E+03	6E+03	8E+03	1E+04	1E+04	1E+04	1E+00	1E+00
Gram	7E+03	3E+03	7E+03	1E+04	4E+03	4E+03	3E+03	3E+03	1E+04	6E+03	8E+03	2E+04	6E+03	1E+04	1E+04	6E+03	6E+03	1E+02	2E+03
Form	7E+03	4E+03	6E+03	7E+03	8E+03	3E+03	5E+03	3E+03	8E+03	7E+03	1E+04	2E+04	3E+03	1E+04	7E+03	3E+03	8E+03	4E+02	1E+02
Ru	7E+03	5E+03	3E+03	5E+03	6E+03	3E+03	4E+03	3E+03	2E+03	2E+03	2E+03	4E+03	4E+03	2E+03	3E+03	3E+03	4E+03	1E+00	1E+00
Ros	7E+03	6E+03	2E+03	2E+03	2E+03	7E+02	9E+02	8E+02	5E+03	6E+03	7E+03	9E+03	1E+04	1E+04	9E+02	1E+03	3E+01	1E+02	6E+02
Dino	7E+03	4E+03	5E+03	3E+03	1E+04	6E+03	4E+03	2E+03	3E+03	3E+03	4E+03	1E+04	1E+04	6E+03	1E+00	1E+00	1E+00		
Alt	7E+03	1E+03	3E+04	2E+04	2E+04	1E+04	7E+03	3E+03	2E+03	3E+03	3E+03	4E+03	4E+03	6E+03	6E+03	4E+03	3E+03	1E+00	1E+00
Mar	7E+03	4E+03	3E+03	3E+03	3E+03	1E+03	1E+03	2E+03	2E+02	9E+01	2E+02	3E+02	3E+02	6E+03	1E+00	1E+00	1E+00		
Ver	7E+03	4E+03	8E+03	6E+03	6E+03	5E+03	5E+03	8E+03	4E+03	7E+03	6E+03	1E+03	2E+03	2E+03	4E+03	5E+03	3E+03	2E+04	1E+04
AN10	7E+03	3E+03	6E+03	4E+03	5E+03	3E+03	3E+03	4E+03	5E+03	3E+03	3E+03	3E+02	2E+03	3E+03	1E+00	1E+00	1E+00		
E.coli	7E+03	2E+04	3E+04	2E+04	2E+04	1E+04	1E+04	1E+04	2E+03	2E+03	3E+03	3E+03	2E+03	3E+03	3E+03	1E+03	4E+03	1E+00	1E+00
Ax	7E+03	4E+03	1E+04	1E+04	1E+04	8E+03	5E+03	6E+03	1E+04	9E+03	1E+04	2E+04	6E+03	6E+03	1E+04	4E+03	1E+04	2E+03	1E+02
ESW																			
Sali	3E+04	3E+04	5E+03	1E+04	6E+03	3E+03	3E+03	6E+03	5E+03	6E+03	4E+03	8E+02	3E+03	2E+03	1E+00	1E+00	1E+00		
Aero	3E+04	2E+05	3E+04	6E+04	7E+04	2E+04	1E+04	1E+04	1E+00	1E+00	1E+00								
Pol	3E+04	8E+04	1E+05	2E+05	2E+05	2E+05	2E+05	2E+05	1E+05	3E+04	6E+04	1E+00	1E+00	1E+00					
Alg	3E+04	4E+05	3E+04	7E+04	1E+05	4E+04	6E+04	1E+05	1E+04	2E+04	3E+04	2E+04	3E+04	2E+04	3E+04	4E+04	2E+04	1E+00	1E+00
Gram	3E+04	6E+04	8E+04	2E+05	1E+05	2E+05	2E+05	2E+05	7E+04	4E+04	4E+04	2E+05	2E+05	8E+04	3E+05	4E+05	1E+05	1E+00	1E+00
Form	3E+04	5E+04	2E+05	2E+05	3E+05	1E+05	1E+05	1E+05	1E+05	8E+04	1E+05	6E+04	5E+04	3E+04	3E+04	6E+04	7E+04	8E+03	4E+04
Ru	3E+04	5E+04	2E+05	8E+04	2E+05	3E+04	7E+04	7E+04	2E+05	2E+05	2E+05	1E+05	1E+05	1E+05	8E+05	1E+06	2E+05	4E+04	1E+05
Ros	3E+04	6E+04	2E+05	2E+05	1E+05	1E+05	8E+04	7E+04	7E+04	6E+04	2E+05	8E+04	1E+05	1E+05	7E+05	3E+05	5E+05	2E+05	3E+05
Dino	3E+04	3E+04	6E+04	4E+05	7E+04	2E+05	1E+05	1E+05	8E+04	5E+04	3E+04	1E+05	1E+05	6E+04	3E+05	1E+05	1E+05	3E+04	7E+04
Alt	3E+04	1E+05	1E+05	6E+04	4E+04	3E+04	1E+04	7E+03	1E+00	1E+00	1E+00								
Mar	3E+04	1E+05	1E+05	7E+04	4E+04	1E+04	7E+03	4E+03	1E+00	1E+00	1E+00								
Ver	3E+04	2E+05	1E+05	1E+05	3E+04	3E+04	1E+05	1E+05	1E+00	1E+00	1E+00								
AN10	3E+04	6E+04	5E+04	6E+04	1E+05	4E+04	5E+04	6E+04	8E+04	1E+05	1E+05	3E+04	8E+04	7E+04	1E+05	1E+05	1E+05	5E+04	5E+03
E.coli	3E+04	1E+05	2E+04	3E+04	4E+04	1E+04	6E+03	2E+04	1E+00	1E+00	1E+00								
Ax	3E+04	6E+04	8E+04	6E+04	8E+04	1E+05	8E+04	8E+04	1E+00	1E+00	1E+00								
SW																			
Sali	1E+04	5E+03	3E+02	8E+02	5E+02	8E+02	1E+03	8E+02	1E+03	2E+03	1E+03	1E+03	1E+03	1E+03	3E+03	3E+03	4E+03	6E+03	5E+03
Aero	6E+08	1E+04	4E+04	8E+04	1E+05	3E+04	3E+04	3E+04	2E+05	2E+05	2E+05	2E+03	2E+04	3E+04	7E+04	4E+04	4E+04	6E+05	1E+06
Pol	4E+08	6E+03	1E+06	1E+06	1E+06	1E+04	1E+04	3E+04	2E+04	3E+03	5E+03	1E+00	1E+00	1E+00					
Alg	1E+03	2E+06	7E+06	5E+08	1E+06	2E+05	3E+05	4E+05	2E+04	3E+04	1E+05	3E+03	2E+03	3E+03	1E+03	1E+03	2E+03	1E+00	1E+00
Gram	1E+03	3E+05	2E+06	2E+06	2E+06	2E+06	1E+06	1E+06	5E+05	1E+06	8E+05	1E+06	1E+06	2E+06	1E+06	8E+05	1E+06	1E+06	1E+06
Form	1E+08	1E+05	6E+05	5E+07	2E+05	2E+05	2E+05	2E+05	2E+05	2E+05	2E+05	1E+05	1E+05	2E+05	3E+04	1E+05	1E+05	1E+00	1E+00
Ru	2E+07	2E+05	1E+06	1E+06	2E+06	1E+06	5E+05	3E+05	6E+05	7E+05	6E+05	1E+05	1E+06	1E+06	2E+06	2E+06	2E+06	2E+06	2E+06
Ros	4E+07	2E+07	3E+06	3E+06	3E+06	2E+06	1E+06	2E+06	1E+06	1E+06	1E+06	2E+06	2E+06	2E+06	1E+06	8E+05	1E+06	1E+06	1E+06
Dino	1E+03	4E+06	1E+06	1E+06	2E+06	2E+06	3E+06	3E+06	2E+07	2E+07	3E+07	3E+06	3E+06	3E+06	2E+06	2E+06	2E+06	2E+06	2E+06
Alt	3E+08	1E+02	1E+03	6E+02	8E+02	6E+02	3E+02	4E+02	1E+02	5E+01	3E+01	1E+00	1E+00	1E+00					
Mar	6E+08	4E+05	1E+06	3E+08	2E+06	3E+06	3E+06	3E+06	3E+06	4E+06	2E+06	1E+05	1E+04	1E+05	2E+06	3E+06	2E+06	2E+05	2E+05
Ver	8E+08	5E+05	2E+06	1E+06	8E+05	2E+05	8E+05	1E+06	6E+05	6E+05	5E+05	1E+05	6E+05	3E+05	1E+06	3E+04	1E+06	2E+06	2E+06
AN10	1E+06	8E+05	3E+05	2E+06	2E+06	2E+06	2E+06	2E+06	2E+06	2E+06	2E+06	2E+06	2E+06	2E+06	7E+06	7E+05	7E+05	3E+05	8E+05
E.coli	1E+06	1E+05	3E+05	3E+05	3E+05	2E+05	2E+05	2E+05	1E+02	5E+01	3E+01	1E+00	1E+00	1E+00					
ESW																			
Sali	1E+05	1E+03	7E+03	5E+03	2E+04	3E+04	5E+04	4E+04	4E+04	3E+04	7E+04	2E+04	2E+04	2E+04	3E+04	1E+04	2E+04	3E+03	2E+04
Aero	6E+09	2E+07	3E+07	2E+07	2E+07	1E+07	2E+07	2E+07	1E+04	2E+07	2E+07	4E+06	4E+06	4E+06	1E+06	5E+05	3E+05	1E+00	1E+00
Pol	4E+09	7E+06	2E+04	3E+06	2E+06	2E+04	2E+06	3E+05	2E+04	2E+04	2E+04	1E+07	1E+07	6E+06	1E+04	3E+04	2E+04	3E+05	3E+05
Alg	1E+10	2E+07	1E+08	8E+07	1E+08	7E+07	4E+07	6E+07	7E+03	2E+03	5E+03	1E+06	6E+05	3E+05	1E+05	2E+05	2E+05	2E+05	3E+05
Gram	1E+04	7E+06	1E+04	4E+06	2E+06	3E+06	4E+06	8E+06	2E+07	5E+06	1E+07	2E+07	2E+07	2E+07	2E+07	2E+07	2E+07	3E+05	5E+05
Form	1E+03	1E+05	1E+05	1E+05	1E+05	2E+05	1E+06	2E+06	2E+05	2E+05	7E+05	8E+05	5E+05	3E+05	7E+05	7E+05	6E+05	2E+07	3E+06
Ru	2E+08	2E+07	1E+06	1E+07	1E+06	2E+07	2E+07	3E+07	2E+07	2E+07	2E+07	1E+07	3E+06	8E+06	1E+07	6E+06	8E+06	7E+06	8E+06
Ros	4E+08	4E+05	1E+06	7E+05	8E+05	1E+08	7E+07	3E+07	3E+07	3E+07	3E+07	2E+07	2E+07	2E+07	2E+07	2E+07	1E+07	1E+07	1E+07
Dino	1E+10	2E+07	5E+07	5E+07	4E+07	5E+07	5E+07	5E+07	7E+07	8E+07	8E+07	1E+08	3E+07	8E+07	6E+07	6E+07	6E+07	2E+07	4E+07
Alt	3E+03	6E+05	1E+05	4E+05	2E+05	6E+06	8E+06	6E+06	1E+07	2E+07	1E+07	2E+06	2E+06	2E+06	1E+06	2E+06	2E+06	4E+05	3E+05
Mar	6E+09	7E+06	2E+07	1E+07	2E+07	2E+07	2E+07	2E+07	2E+07	2E+07	2E+07	3E+06	3E+06	2E+06	2E+06	3E+06	2E+06	5E+05	4E+05
Ver	8E+09	1E+07	2E+07	2E+07	2E+07	1E+07	1E+07	1E+07	1E+06	1E+06	1E+06	2E+06	2E+06	1E+06	1E+00	1E+00	1E+00		
AN10	1E+07	1E+06	2E+07	2E+07	2E+07	1E+06	1E+05	1E+06	1E+07	2E+07	2E+07	2E+05	3E+05	2E+05	5E+05	5E+05	7E+05	6E+05	6E+05
E.coli	1E+07	1E+05	2E+05	6E+04	6E+04	3E+05	2E+04	3E+04	2E+04	1E+04	1E+04	3E+04	1E+05	7E+04	1E+05	1E+05	1E+05	1E+00	1E+00

Table 7.9: Raw data of the abundance of Phaeodactylum (up-part) in co-cultures with 14 heterotrophs (down-part) over 19 weeks of incubation (number of week on the top raw), tested with logistic regression (white cells) or two-way ANOVA (grey-cells).

	Phaeodactylum																				
SW	0	1	3	6			8			11			13			16			19		
Sali	1E+04	2E+04	6E+04	1E+05	1E+05	1E+05	3E+04	3E+04	3E+04	1E+05	1E+05	2E+05	3E+04	7E+04	4E+04	2E+05	2E+05	2E+05	7E+04	4E+04	5E+04
Aero	1E+04	2E+04	7E+04	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	3E+04	3E+04	1E+04	2E+05	1E+05	2E+05	9E+04	1E+05	3E+04
Pol	1E+04	2E+04	8E+04	1E+05	2E+05	1E+05	1E+05	1E+05	1E+05	2E+05	2E+05	1E+05	5E+04	4E+04	3E+04	2E+05	3E+05	2E+05	2E+05	1E+05	2E+05
Alg	1E+04	2E+04	7E+04	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	2E+05	2E+05	2E+05	1E+05	3E+04	7E+04	5E+05	5E+05	5E+05	5E+05	3E+05	4E+05
Gram	1E+04	3E+04	7E+04	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	2E+05	2E+05	2E+05	1E+05	1E+05	3E+05	4E+05	3E+05	4E+05	3E+05	3E+05	4E+05
Form	1E+04	2E+04	7E+04	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	2E+05	1E+05	1E+05	1E+05	5E+05	1E+05	2E+05	3E+05	2E+05	2E+05
Ru	1E+04	2E+04	6E+04	1E+05	1E+05	1E+05	1E+05	3E+04	1E+05	1E+05	1E+05	1E+05	5E+04	2E+05	1E+05	2E+05	2E+05	2E+05	8E+04	8E+04	3E+04
Ros	1E+04	3E+04	7E+04	1E+05	1E+05	1E+05	3E+04	3E+04	1E+05	6E+04	5E+04	7E+04	2E+04	2E+04	3E+04	4E+04	8E+04	7E+04	6E+04	6E+04	5E+04
Dino	1E+04	3E+04	7E+04	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	7E+04	6E+04	5E+04	4E+04	2E+04	5E+04	2E+05	1E+05	1E+05	5E+04	5E+04	4E+04
Alt	1E+04	3E+04	6E+04	3E+04	1E+05	3E+04	8E+04	3E+04	3E+04	1E+05	1E+05	1E+05	5E+04	6E+04	6E+04	2E+05	1E+05	2E+05	2E+05	1E+05	1E+05
Mar	1E+04	3E+04	6E+04	3E+04	1E+05	1E+05	1E+05	3E+04	3E+04	1E+05	1E+05	1E+05	3E+04	5E+04	3E+04	2E+05	2E+05	1E+05	1E+05	3E+04	2E+05
Ver	1E+04	3E+04	6E+04	1E+05	1E+05	3E+04	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	7E+04	3E+04	2E+04	2E+05	2E+05	2E+05	1E+05	1E+05	1E+05
AN10	1E+04	2E+04	7E+04	1E+05	1E+05	1E+05	1E+05	1E+05	1E+05	8E+04	8E+04	8E+04	8E+04	3E+04	1E+05	2E+05	2E+05	3E+05	1E+05	2E+05	2E+05
E.coli	1E+04	6E+04	8E+04	7E+04	7E+04	7E+04	6E+04	4E+04	4E+04	6E+04	7E+04	8E+04	4E+04	2E+04	3E+04	1E+05	1E+05	1E+05	7E+03	5E+03	6E+03
Ax	1E+04	2E+04	3E+04	1E+05	1E+05	1E+05	1E+05	8E+04	3E+04	1E+05	1E+05	1E+05	6E+04	3E+04	5E+04	2E+05	1E+05	2E+05	7E+04	1E+05	1E+05
ESW																					
Sali	8E+04	3E+04	4E+05	7E+05	8E+05	4E+05	4E+05	4E+05	5E+05	1E+06	2E+06	1E+06	7E+05	7E+05	1E+06	6E+05	8E+05	8E+05	3E+05	2E+05	3E+05
Aero	8E+04	5E+04	2E+05	2E+06	3E+06	3E+06	2E+06	2E+06	2E+06	4E+06	4E+06	4E+06	2E+06	2E+06	1E+06	2E+06	3E+06	1E+06	7E+04	8E+04	8E+04
Pol	8E+04	5E+04	4E+05	2E+06	3E+06	3E+06	2E+06	2E+06	2E+06	3E+06	5E+06	3E+06	2E+06	2E+06	2E+06	2E+06	3E+06	3E+06	1E+06	2E+06	2E+06
Alg	8E+04	1E+05	1E+06	2E+06	3E+06	1E+06	2E+06	2E+06	2E+06	5E+06	7E+06	6E+06	4E+06	2E+06	2E+06	1E+07	3E+06	1E+07	2E+07	2E+07	7E+06
Gram	8E+04	1E+05	4E+05	2E+06	3E+06	4E+06	3E+06	3E+06	3E+06	5E+06	3E+06	5E+06	2E+06	3E+06	2E+06	4E+06	4E+06	4E+06	1E+06	5E+05	4E+06
Form	8E+04	2E+05	1E+06	3E+06	3E+06	2E+06	3E+06	3E+06	3E+06	6E+06	1E+07	5E+06	2E+06	2E+06	3E+06	6E+06	7E+06	6E+06	9E+06	9E+06	7E+06
Ru	8E+04	2E+05	2E+06	3E+06	3E+06	3E+06	2E+06	2E+06	3E+06	6E+06	5E+06	5E+06	4E+06	3E+06	4E+06	4E+06	5E+06	6E+06	6E+06	7E+06	7E+06
Ros	8E+04	3E+05	2E+06	3E+06	3E+06	3E+06	3E+06	4E+06	3E+06	3E+06	3E+06	2E+06	1E+06	1E+06	6E+05	2E+06	3E+06	3E+06	3E+06	4E+06	4E+06
Dino	8E+04	1E+05	8E+05	2E+06	2E+06	2E+06	2E+06	2E+06	2E+06	5E+06	5E+06	6E+06	5E+06	6E+06	6E+06	1E+06	3E+05	1E+06	6E+05	3E+05	3E+05
Alt	8E+04	4E+04	3E+05	2E+06	3E+06	4E+06	3E+06	2E+06	2E+06	4E+06	4E+06	4E+06	2E+06	2E+06	2E+06	5E+06	4E+06	5E+06	1E+06	1E+06	1E+06
Mar	8E+04	7E+04	4E+05	2E+06	3E+06	3E+06	2E+06	2E+06	1E+06	3E+06	4E+06	4E+06	1E+06	1E+06	1E+06	3E+06	4E+06	3E+06	6E+05	2E+06	6E+05
Ver	8E+04	1E+05	3E+05	2E+06	3E+06	5E+06	3E+06	2E+06	2E+06	3E+06	3E+06	7E+06	2E+06	7E+05	2E+06	2E+06	2E+06	2E+06	7E+05	3E+05	4E+05
AN10	8E+04	1E+05	1E+06	2E+06	4E+06	2E+06	3E+06	3E+06	3E+06	5E+06	5E+06	5E+06	5E+06	4E+06	5E+06	6E+06	5E+06	7E+06	1E+07	3E+06	1E+07
E.coli	8E+04	3E+05	3E+05	4E+06	4E+06	3E+06	3E+06	3E+06	2E+06	5E+06	6E+06	5E+06	7E+06	7E+06	4E+06	2E+06	2E+06	2E+06	1E+06	8E+05	7E+05
Ax	8E+04	1E+05	1E+05	2E+06	3E+06	3E+06	2E+06	2E+06	2E+06	5E+06	5E+06	5E+06	3E+06	1E+07	2E+07	3E+06	5E+06	4E+06	2E+06	3E+06	3E+06
SW																					
Sali	1E+04	2E+02		1E+02	1E+02	1E+02	2E+02	1E+02	1E+02	1E+02	1E+02	1E+02	2E+02	7E+02	2E+02	4E+02	5E+02	5E+02	4E+02	4E+02	6E+02
Aero	6E+08	1E+05		2E+03	3E+03	2E+03	6E+04	2E+05	2E+05	5E+02	5E+02	3E+02	3E+03	2E+04	2E+04	4E+02	6E+02	8E+02	2E+03	3E+03	2E+03
Pol	4E+08	8E+04		2E+03	1E+03	2E+03	1E+05	1E+05	1E+05	1E+03	3E+02	1E+02	1E+04	2E+04	1E+04	2E+04	2E+04	2E+04	2E+04	2E+04	2E+04
Alg	1E+09	2E+05		1E+04	1E+04	5E+04	2E+05	2E+05	2E+05	1E+05	2E+05	1E+05	5E+03	9E+03	2E+04	6E+03	6E+03	1E+04	2E+04	2E+04	2E+04
Gram	1E+03	2E+05		2E+04	3E+04	3E+04	2E+04	4E+04	2E+04	7E+02	2E+04	1E+05	2E+05	3E+05	3E+05	7E+04	1E+05	1E+05	2E+05	1E+05	2E+05
Form	1E+08	1E+03		3E+04	2E+04	2E+04	2E+05	2E+05	2E+05	1E+05	9E+04	1E+05	2E+04	2E+04	1E+05	5E+03	8E+03	1E+03	1E+05	1E+05	1E+03
Ru	2E+07	2E+05		1E+04	7E+04	1E+05	1E+05	2E+05	1E+05	2E+05	2E+05	1E+05	1E+05	2E+05	2E+05	1E+05	2E+05	2E+05	5E+05	1E+05	8E+04
Ros	4E+07	4E+05		5E+05	6E+05	4E+05	7E+04	3E+04	1E+05	3E+04	5E+04	5E+04	1E+05	1E+05	1E+05	2E+03	1E+03	2E+03	2E+03	1E+03	2E+03
Dino	1E+09	4E+05		1E+04	$DIV/0!	$DIV/0!	6E+04	6E+04	1E+05	1E+05	1E+05	2E+05	2E+05	2E+05	3E+05	2E+05	2E+05	2E+05	2E+05	2E+05	2E+05
Alt	3E+08	3E+04		6E+04	7E+04	7E+04	7E+04	8E+04	7E+04	8E+04	8E+04	7E+04	3E+04	3E+04	7E+04	8E+03	1E+04	1E+04	5E+03	6E+03	3E+03
Mar	6E+08	2E+06		8E+04	8E+04	1E+05	1E+05	3E+04	1E+05	2E+05	1E+05	1E+05	8E+05	8E+05	1E+06	1E+05	1E+05	2E+05	1E+05	1E+05	1E+05
Ver	8E+08	4E+08		3E+04	1E+04	3E+04	4E+05	4E+05	3E+05	1E+04	2E+04	3E+04	1E+05	2E+05	2E+05	1E+06	1E+06	1E+06	1E+06	8E+05	1E+06
AN10	1E+06	1E+04		1E+03	7E+02	9E+02	6E+02	5E+02	8E+02	4E+02	7E+02	3E+02	2E+03	7E+02	8E+02	1E+02	1E+02	1E+02	1E+02	1E+02	1E+02
E.coli	1E+06	1E+04		1E+03	1E+02	1E+02	1E+00	1E+00	1E+00												
ESW																					
Sali	1E+05	3E+02		1E+04	4E+03	3E+04	1E+04	1E+04	1E+04	2E+04	1E+04	2E+04	3E+03	1E+04	7E+03	2E+03	3E+03	3E+03	6E+03	4E+03	3E+03
Aero	6E+09	2E+07		7E+06	5E+06	7E+06	5E+06	7E+06	3E+06	3E+06	3E+06	1E+07	3E+06	4E+06	3E+06	2E+06	2E+06	1E+06	1E+06	1E+04	1E+04
Pol	4E+09	4E+06		3E+04	3E+05	7E+05	2E+04	8E+05	5E+05	2E+04	7E+05	4E+05	2E+05	2E+05	3E+05	1E+03	3E+04	2E+05	2E+05	3E+05	2E+05
Alg	1E+10	3E+07		2E+05	1E+05	1E+05	4E+06	4E+06	4E+06	1E+06	2E+06	4E+06	2E+06	1E+06	1E+06	1E+07	1E+07	2E+07	1E+04	7E+03	3E+03
Gram	1E+04	1E+04		2E+04	1E+04	5E+04	1E+04	5E+04	1E+05	1E+04	9E+04	1E+05	2E+05	2E+05	2E+05	3E+06	3E+06	4E+06	1E+07	9E+06	4E+06
Form	1E+09	5E+04		7E+04	1E+05	5E+04	1E+04	2E+04	1E+05	7E+03	3E+03	1E+04	6E+05	7E+05	1E+06	2E+06	2E+06	2E+06	9E+05	1E+06	2E+06
Ru	2E+08	2E+07		2E+06	2E+06	3E+06	2E+07	2E+07	2E+07	1E+07	1E+07	2E+07	3E+07	3E+07	3E+07	3E+07	3E+07	3E+07	2E+07	3E+07	3E+07
Ros	4E+08	2E+07		1E+06	1E+06	1E+06	1E+07	2E+07	2E+07	3E+07	5E+07	5E+07	8E+07	8E+07	6E+07	9E+06	9E+06	2E+07	2E+07	2E+07	3E+07
Dino	1E+10	3E+07		1E+06	1E+06	1E+06	3E+07	3E+07	3E+07	7E+07	6E+07	2E+08	2E+08	1E+08	2E+08	2E+08	2E+08	1E+08	2E+08	2E+08	1E+08
Alt	3E+09	1E+04		1E+03	6E+03	7E+03	6E+03	7E+03	8E+03	1E+04	9E+03	1E+04	3E+03	3E+06	2E+04	1E+03	5E+06	1E+07	1E+07	2E+07	2E+07
Mar	6E+09	6E+06		1E+07	5E+06	5E+06	1E+07	3E+06	1E+07	8E+06	1E+07	2E+07	2E+07	2E+07	1E+07	2E+07	2E+07	2E+07	2E+07	2E+07	2E+07
Ver	8E+09	5E+06		1E+07	1E+07	1E+07	1E+07	1E+07	1E+07	1E+07	1E+07	8E+06	2E+07	2E+07	2E+07	1E+06	3E+05	3E+05	7E+05	1E+06	6E+05
AN10	1E+08	8E+05		5E+06	6E+06	6E+06	2E+05	2E+05	3E+05	1E+06	8E+05	1E+06	2E+06	2E+06	3E+06	3E+06	4E+06	3E+06	3E+05	5E+05	4E+05
E.coli	1E+08	1E+05		1E+03	1E+02	1E+02	1E+00	1E+00	1E+00												

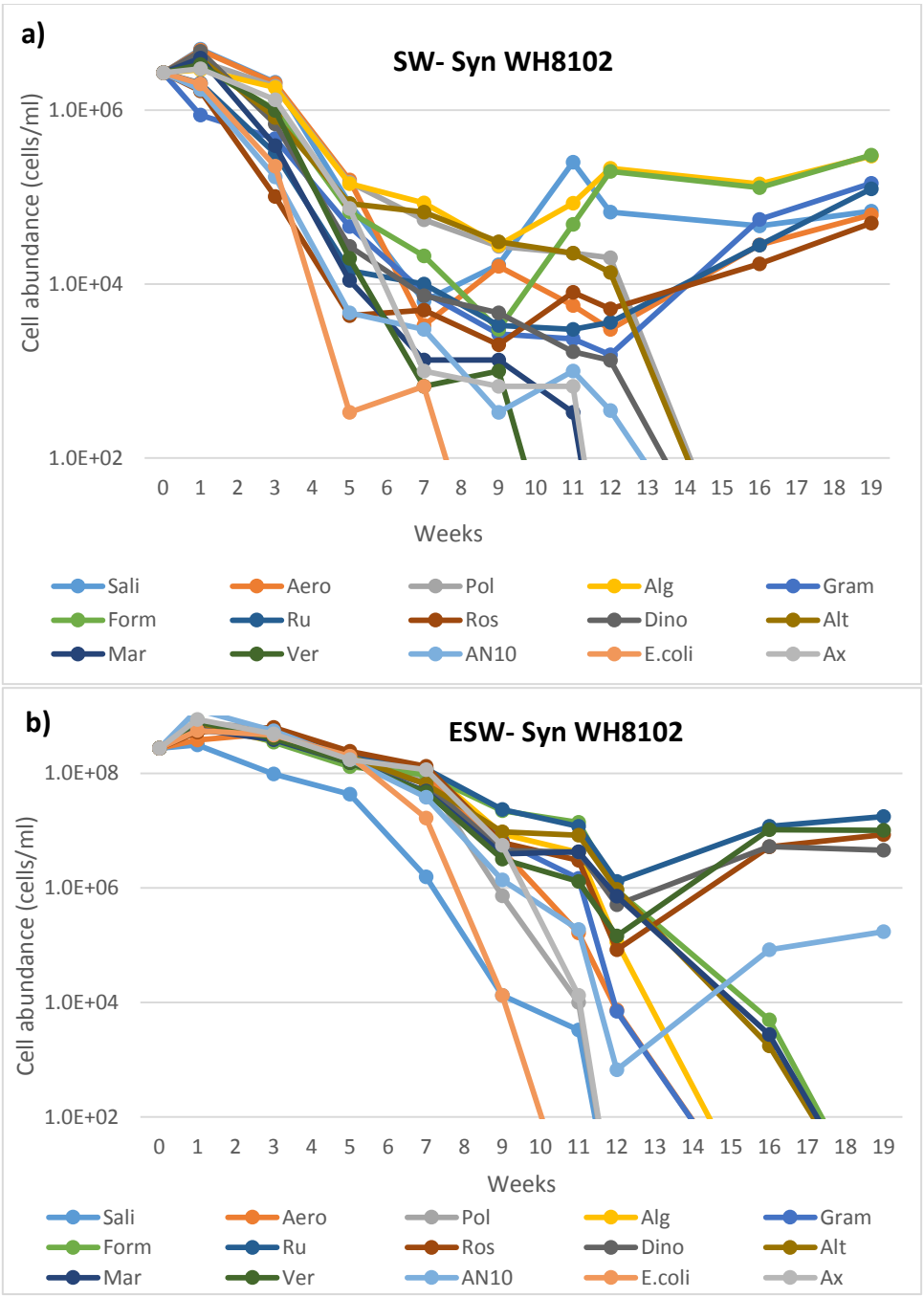
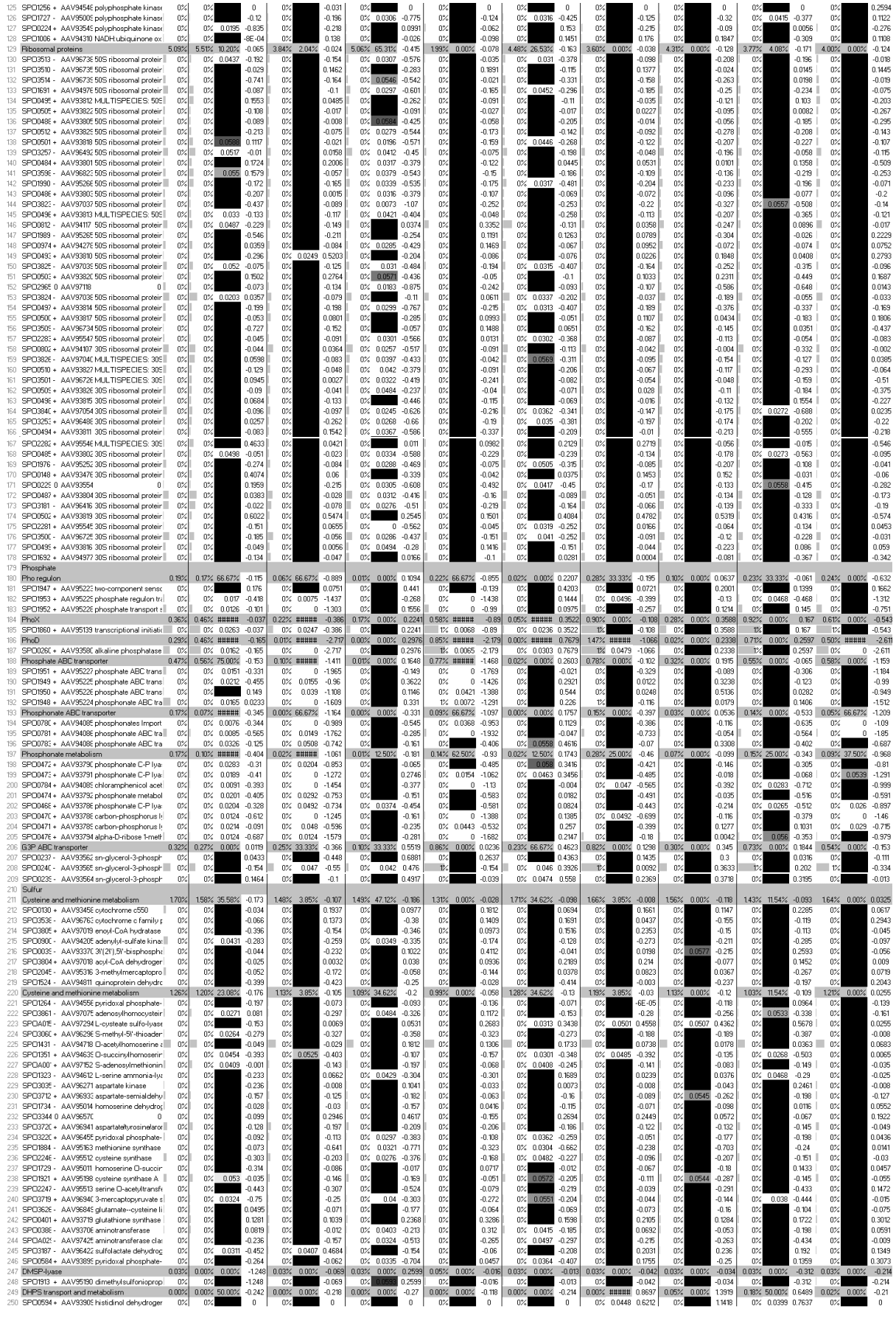
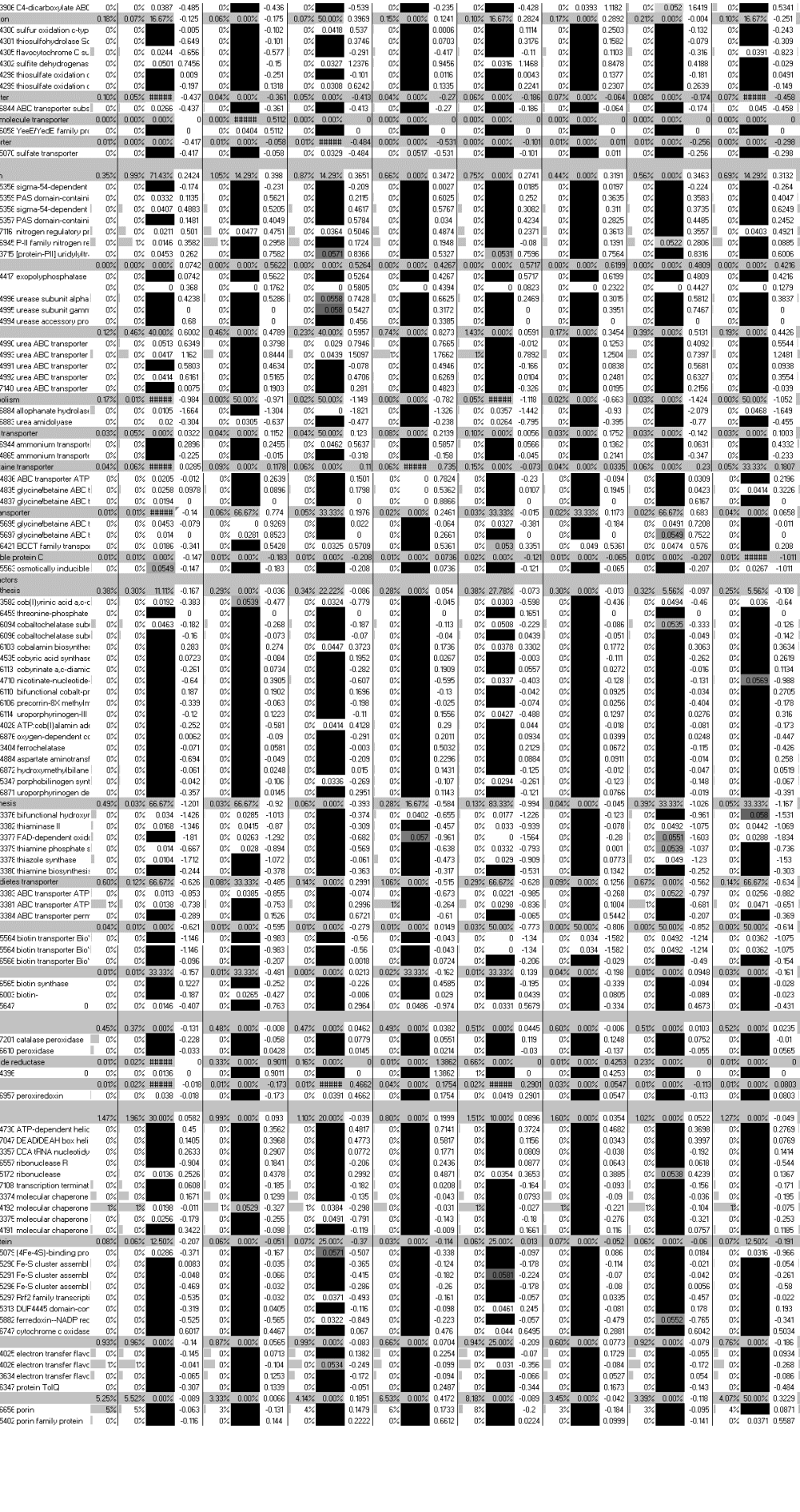


Figure 7.1: Population curves over 19 weeks of the Synechococcus WH8102 in co-cultures with 14 heterotrophic species (the colour of each heterotrophic treatment represented on the bottom of the figure) and without any heterotroph (axenic culture; grey line), incubated in oligotrophic seawater (a; SW) and in enriched media (b; ESW).

Table 7.10: Proteins of R. pomeroyi categorised into metabolic pathways, presented in Fig. 4.3.4.1, including the relative abundance (RA), significant difference (Sign) and the Log_{10}fold change (FD) in mono-culture and 9 co-culture treatments.

1		R. pom	Prochlo			Syn WH7803			Syn WH8102			Micr. sp.			M. pusilla			Ostr			E.hux			Thalas			Phae		
2	Central metabolic processes	RA	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC
3	Glycolysis/gluconeogenesis	2.63%	2.78%	20.8%	-0.091	1.89%	8.33%	-0.142	1.71%	20.83%	-0.021	2.79%	4.17%	-0.041	2.28%	12.50%	-0.014	3.06%	0.00%	0.0458	2.19%	4.17%	-0.062	2.09%	0.00%	-0.064	2.10%	4.17%	-0.071
4	SPO0701 - AAV94007 type I glyceraldehyde	0%	0%	0.0259	0.6069	0%		0.1665	0%		0.3647	0%		0.4135	0%		0.0869	0%		0.1078	0%		0.0531	0%		0.2803	0%		-0.172
5	SPO0875 - AAV94180 aldehyde dehydroger	0%	0%		-0.31	0%		-0.175	0%		0.1563	0%		0.0522	0%		-0.072	0%		-0.047	0%		-0.137	0%		0.1258	0%		-0.04
6	SPO2198 + AAV95464 type I glyceraldehyde	0%	0%		-0.428	0%		-0.182	0%		-0.288	0%		-0.077	0%		-0.072	0%		0.1002	0%		-0.178	0%		-0.109	0%		0.1792
7	SPO3878 + AAV97092 type I glyceraldehyde	0%	0%	0.0195	-0.206	0%	0.0391	-1.54	0%		-0.007	0%	0	-1.96	0%		0.103	0%		-0.106	0%		0.0919	0%		-0.233	0%		-1.162
8	SPO0864 + AAV94169 glucokinase	0%	0%		0.4021	0%		0	0%	0	0.6873	0%		0.2886	0%		0.1709	0%		0.4483	0%	0.0481	0.5196	0%		0	0%	0.0311	0.3626
9	SPO0946 - AAV94251 phosphomannomutas	0%	0%	0.0486	-0.136	0%		-0.179	0%		-0.13	0%		0.0064	0%		-0.126	0%		-0.072	0%		-0.123	0%		-0.339	0%		-0.151
10	SPO2046 - AAV95317 glucose-6-phosphate	0%	0%		-0.052	0%		-0.179	0%		-0.016	0%		-0.247	0%		0.1501	0%		-0.157	0%		0.0376	0%		0.0635	0%		-0.108
11	SPO3369 + AAV96595 aldehyde dehydroger	0%	0%		0.0444	0%		-0.108	0%		-0.205	0%		-0.121	0%		-0.121	0%		-0.086	0%		-0.067	0%		-0.066	0%		-0.003
12	SPO3382 - AAV96609 aldehyde dehydroger	0%	0%		0.0767	0%		0.0489	0%		-0.17	0%		-0.189	0%		-0.004	0%		0.006	0%		0.0948	0%		-0.104	0%		0.0316
13	SPO0235 + AAV93560 aldehyde dehydroger	0%	0%		0.044	0%		0.0086	0%	0.0294	-0.395	0%		-0.084	0%	0.0284	-0.398	0%		0.0946	0%		-0.363	0%		0.2406	0%		0.2515
14	SPO2621 + AAV95866 triose-phosphate ison	0%	0%		-0.222	0%		0.3518	0%		0.6272	0%		0.5334	0%		0.3536	0%		0.432	0%		-0.439	0%		0.0458	0%		-0.2
15	SPO1733 - AAV95013 fructose-bisphosphat	0%	0%		0.0416	0%		-0.049	0%	0.0292	-0.313	0%		-0.002	0%		-0.133	0%		-0.05	0%		-0.15	0%		-0.028	0%		0.0388
16	SPO1899 + AAV95177 fructose bisphosphat	0%	0%	0.0575	-0.2	0%		-0.171	0%		-0.036	0%		0.156	0%	0.0462	-0.232	0%		0.0391	0%		-0.126	0%		-0.171	0%		-0.032
17	SPO2235 + AAV95501 phosphoglycerate kir	0%	0%		-0.013	0%		0.0614	0%		0.0844	0%		0.0551	0%		0.0654	0%		0.0415	0%		-0.047	0%		0.1659	0%		-0.056
18	SPO3600 - AAV96824 pyruvate kinase	0%	0%		0.1482	0%		-2E-04	0%	0.043	0.2697	0%		0.2574	0%		0.1464	0%		-0.027	0%		0.0042	0%		0.1305	0%		-0.046
19	SPO1813 - AAV95092 acetate--CoA ligase	1%	1%	0.0325	-0.108	1%	0.0255	-0.394	0%	0.0558	-0.196	1%		-0.207	1%		-0.161	1%		-0.19	1%		-0.259	1%		-0.214	1%		-0.286
20	SPO2791 - AAV96032 AMP-dependent synt	0%	0%	0.0405	-0.449	0%		-0.574	0%		-0.299	0%		-0.392	0%		-0.314	0%		0.4257	0%		-0.073	0%		-0.403	0%		-0.192
21	SPO1889 + AAV95168 alcohol dehydrogena	0%	0%		-0.276	0%		-0.017	0%		-0.518	0%		0.2367	0%		0.035	0%		-0.027	0%		-0.01	0%		-0.017	0%		0.0894
22	SPO1508 - AAV94795 PQQ-dependent deh	1%	0%		-0.06	0%		-0.309	0%		0.4146	1%		0.0415	0%		0.3441	1%		0.0187	0%		-0.049	0%		-0.485	0%		-0.2
23	SPO3810 + AAV97024 2,3-bisphosphoglycer	0%	0%		-0.169	0%		-0.069	0%	0.0424	-0.602	0%		-0.04	0%		-0.064	0%		0.0533	0%		-0.037	0%		-0.449	0%		-0.071
24	SPO2474 - AAV95728 enolase	0%	0%		0.0411	0%		-0.06	0%		-0.047	0%		0.1001	0%		-0.052	0%		-0.01	0%		-0.093	0%		-0.024	0%		0.0263
25	SPO0097 - AAV93428 aldehyde dehydroger	0%	0%		-0.077	0%		0.0189	0%		0.2398	0%		0.0902	0%	0.0577	0.2088	0%		0.1393	0%		0.0269	0%		-0.039	0%		-0.039
26	SPO1970 - AAV95246 histidine phosphatas	0%	0%		-0.747	0%		-0.168	0%		-0.423	0%		0.0753	0%	0.046	-0.296	0%		-0.158	0%		-0.225	0%		-0.07	0%		-0.035
27	SPO3850 + AAV97064 S-(hydroxymethyl)glu	0%	0%		-0.131	0%		0.1042	0%		0.304	0%		0.0413	0%		0.0358	0%		0.1246	0%		0.0582	0%		0.1697	0%		0.1115
28	Pyruvate metabolism	1.68%	1.24%	14.29%	-0.208	1.34%	4.76%	-0.062	1.62%	9.52%	-0.226	1.00%	0.00%	-0.135	1.33%	28.57%	-0.128	1.33%	0.00%	-0.024	1.71%	0.00%	-0.093	1.82%	4.76%	-0.176	1.12%	0.00%	0.0385
29	SPO2918 + AAV96159 acetyl-CoA acetyltran	0%	0%		-0.054	0%		-0.115	0%	0.0348	-0.429	0%		0.0058	0%		-0.211	0%		-0.062	0%		0.1074	0%		-0.203	0%		0.0243
30	SPOA042 - AAV97556 acetyl-CoA acetyltran	0%	0%		-0.25	0%		0.2825	0%	0.0534	0.331	0%		-0.308	0%		0.1096	0%		0.3508	0%		0.114	0%		-0.224	0%		0.2645
31	SPO0773 + AAV94078 acetyl-CoA acetyltran	0%	0%		-0.217	0%		-0.186	0%		-0.202	0%		-0.271	0%		-0.156	0%		0.0909	0%		-0.083	0%		-0.103	0%		0.0082
32	SPO3408 - AAV96635 acetyl-CoA C-acyltrar	0%	0%		-0.278	0%		-0.011	0%		-0.114	0%		-0.01	0%		-0.097	0%		0.011	0%		-0.048	0%		-0.087	0%		-0.16
33	SPO0142 + AAV93470 acetyl-CoA C-acyltrar	1%	0%		-0.171	0%		0.161	1%		0.0229	0%		-0.069	0%		0.0617	1%		0.2234	1%		0.3156	1%		-0.205	0%		0.34
34	SPO1013 + AAV94316 acetyl-CoA acetyltran	0%	0%		-0.485	0%		-0.06	0%		-0.186	0%		-0.346	0%		0.0895	0%		0.0353	0%		0.1029	0%		-0.205	0%		0.0647
35	SPO2932 + AAV96173 NADP-dependent ma	0%	0%	0.0285	-0.392	0%		-0.399	0%		-0.359	0%		-0.384	0%	0.0305	-0.579	0%		-0.08	0%	0.0556	-0.551	0%		-0.417	0%		0.1143
36	SPO0012 + AAV93343 NADP-dependent ma	0%	0%		-0.181	0%		-0.14	0%		0.0041	0%		-0.011	0%		-0.102	0%		-0.043	0%		-0.16	0%		-0.306	0%		-0.009
37	SPO1359 + AAV94647 pyruvate, phosphate	0%	0%		-0.027	0%		-0.072	0%		-0.048	0%		0.0368	0%		-0.09	0%		-0.269	0%		0.0029	0%		-0.002	0%		-0.121
38	SPO1010 - AAV94314 acetyl-CoA carboxyla	0%	0%		-0.07	0%		0.0562	0%		-0.124	0%		-0.005	0%		-0.108	0%		-0.054	0%		-0.02	0%		-0.042	0%		-0.014
39	SPO3616 + AAV96840 acetyl-CoA carboxyla	0%	0%		0.0527	0%		-0.022	0%		-0.053	0%		0.0423	0%		-0.075	0%		-0.074	0%		-0.162	0%		0.0762	0%		0.0247
40	SPO3817 + AAV97031 acetyl-CoA carboxyla	0%	0%		-0.188	0%		0.1243	0%		-0.531	0%		-0.026	0%		-0.093	0%		-0.081	0%		-0.061	0%		-0.183	0%		0.1166
41	SPO0115 + AAV93445 bifunctional enoyl-Co	0%	0%		0.1319	0%		0.0799	0%		-0.778	0%		-0.54	0%	0.0365	0.2962	0%		-0.097	0%		-0.115	0%		-0.331	0%		0.139
42	SPO1011 - AAV94315 acetyl-CoA carboxyla	0%	0%		-0.427	0%		-0.104	0%	0.0532	-0.472	0%		-0.342	0%	0.0463	-0.234	0%		-0.345	0%		-0.177	0%		-0.407	0%		-0.089
43	SPO1172 - AAV94468 alpha-hydroxy-acid o	0%	0%		0.188	0%		0.4161	0%		0.5092	0%		0.3496	0%		0.2242	0%		0.1138	0%		0.2075	0%		0.4206	0%		-0.062
44	SPO3168 + AAV96403 hydroxyacylglutathior	0%	0%		-0.349	0%		-0.026	0%		-0.17	0%		0.1358	0%		-0.126	0%		0.0825	0%		0.0573	0%		-0.004	0%		0.2196
45	SPO0813 - AAV94118 alpha-hydroxy-acid o	0%	0%	0.0517	0.0263	0%		-0.077	0%		-0.437	0%		-0.503	0%	0.0305	-0.523	0%		0.2907	0%		-0.324	0%		-0.423	0%		0.2085
46	SPO0422 - AAV93740 2-isopropylmalate syr	0%	0%		-0.306	0%		-0.226	0%	0.056	-0.36	0%		-0.474	0%		-0.147	0%		-0.157	0%		-0.185	0%		-0.227	0%		-0.08
47	SPO2130 - AAV95399 citramalate synthase	0%	0%		-0.57	0%		-0.233	0%		-0.543	0%		0.0335	0%		-0.247	0%		-0.112	0%		-0.208	0%		-0.039	0%		0.0758
48	SPO1598 - AAV94885 acyl-CoA dehydroger	0%	0%	0.0258	-0.213	0%		-0.289	0%		-0.231	0%		-0.096	0%	0.0495	-0.206	0%		-0.174	0%		-0.241	0%		-0.129	0%		-0.008
49	SPO1094 + AAV94394 methylmalonyl-CoA c	0%	0%	0.0099	-0.601	0%	0.0412	-0.454	0%	0.029	-0.578	0%		-0.057	0%	0.0338	-0.477	0%		-0.159	0%	0.0528	-0.523	0%	0.0419	-0.666	0%		-0.25
50	TCA cycle	5.27%	3.96%	25.00%	-0.322	4.32%	0.00%	-0.207	4.47%	20.83%	-0.18	3.94%	4.17%	-0.267	3.80%	20.83%	-0.146	4.38%	0.00%	0.0167	5.82%	0.00%	-0.147	4.18%	16.67%	-0.224	4.12%	0.00%	0.0233
51	SPO2240 + AAV95506 pyruvate dehydrogen	0%	0%	0.0367	-0.37	0%		-0.393	0%	0.0475	-0.597	0%		-0.438	0%		-0.391	0%		-0.16	0%		-0.498	0%		-0.568	0%		-0.033
52	SPO2241 + AAV95507 pyruvate dehydrogen	0%	0%		-0.305	0%		-0.133	0%		0.0306	0%		-0.048	0%		-0.117	0%		0.0689	0%		-0.152	0%		-0.112	0%		0.0372
53	SPOA031 + AAV97451 transketolase	0%	0%		-0.058	0%		-0.209	0%		-0.083	0%	0.0322	-0.509	0%	0.0314	-0.444	0%		0.0504	0%		-0.348	0%		-0.148	0%		0.1206
54	SPO0709 - AAV94015 phosphoenolpyruvate	0%	0%		-0.176	0%		-0.061	0%		0.0675	0%		-0.026	0%		0.0419	0%		0.0092	0%		-0.01	0%		-0.057	0%		-0.071
55	SPO2222 - AAV95488 dihydrolipoyl dehydro	0%	0%	0.0579	-0.296	0%		-0.182	0%		-0.157	0%		-0.187	0%		-0.213	0%		-0.061	0%		-0.29	0%		-0.203	0%		0.0145
56	SPO2242 + AAV95508 pyruvate dehydrogen	0%	0%	0.0325	-0.264	0%		-0.22	0%		-0.272	0%		-0.247	0%		-0.204	0%		-0.092	0%		-0.277	0%		-0.271	0%		-0.015
57	SPO1171 - AAV94467 pyruvate carboxylase	0%	0%		0.1364	0%		0.1146	0%		-0.003	0%		0.0304	0%		-0.016	0%		-0.083	0%		0.0295	0%		0.0599	0%		0.0073
58	SPO2157 + AAV95425 citrate synthase	1%	1%		0.0304	1%		0.0655	1%		-0.149	0%		-0.11	1%		-0.146	1%		-0.104	1%		-0.02	1%		-0.005	1%		-0.02
59	SPO3792 - AAV97012 acetoin:2,6-dichloropl	0%	0%	0.0276	-1.411	0%		-0.862	0%	0.0375	-0.772	0%		-1.028	0%	0.0558	-0.38	0%		0.0965	1%		-0.306	0%	0.0414	-0.673	0%		-0.082
60	SPO3791 - AAV97011 alpha-ketoacid dehyc	1%	0%		-0.818	0%		-0.413	0%		0.0481	0%		-0.479	0%		0.164	1%		0.51	1%		0.0357	0%		-0.272	0%		0.1868
61	SPO3790 - AAV97010 acetoin dehydrogena	0%	0%	0.0371	-1.268	0%		-1.138	0%		-0.411	0%		-1.013	0%		-0.255	0%		0.0868	0%		-0.267	0%	0.0379	-0.948	0%		0.0458
62	SPO3793 - AAV97013 acetoin catabolism pr	0%	0%		-0.761	0%		-0.77	0%		-0.537	0%		-0.996	0%		-0.272	0%		0.1803	0%		-0.308	0%		-0.34	0%		0.0759
63	SPO2312 + AAV95574 aconitate hydratase A	0%	0%		-0.252	0%		-0.078	0%		0.171	0%		0.0998	0%		0.0395	0%		0.1061	0%		-0.004	0%		-0.038	0%		-0.063
64	SPOA031 + AAV97447 isocitrate dehydroger	0%	0%		-0.15	0%		-0.006	0%		-0.147	0%		-0.057	0%		-0.107	0%		-0.005	0%		-0.013	0%		-0.082	0%		0.0666
65	SPO0340 - AAV93660 dihydrolipoyl dehydro	0%	0%		-0.164	0%		-0.093	0%		-0.195	0%		-0.286	0%	0.0467	-0.202	0%		-0.069	0%		-0.157	0%		-0.132	0%		0.0179
66	SPO0343 - AAV93661 dihydrolipoyllysine-re	0%	0%		-0.194	0%		-0.132	0%		-0.214	0%		-0.123	0%		-0.131	0%		-0.047	0%		-0.128	0%		-0.155	0%		0.0617
67	SPO0344 - AAV93662 2-oxoglutarate dehydi	0%	0%	0.027	-0.247	0%		-0.231	0%	0.0464	-0.26	0%		-0.239	0%	0.0578	-0.179	0%		-0.109	0%		-0.194	0%	0.042	-0.332	0%		-0.04
68	SPO0346 - AAV93664 succinate--CoA ligase	0%	0%		-0.219	0%		-0.126	0%	0.0432	-0.298	0%		-0.225	0%	0.0409	-0.266	0%		-0.032	0%		-0.219	0%		-0.18	0%		-0.008
69	SPO0347 - AAV93665 ADP-forming succina	0%	0%		-0.064	0%		-0.065	0%		0.0007	0%		-0.006	0%		-0.051	0%		0.0599	0%		-0.106	0%		-0.058	0%		0.1166
70	SPO0360 + AAV93678 succinate dehydroge	0%	0%		-0.094	0%		-0.03	0%		-0.161	0%		-0.219	0%	0.0443	-0.183	0%		-0.069	0%		-0.043	0%		-0.246	0%		0.0543
71	SPO0361 + AAV93679 succinate dehydroge	0%	0%	0.0393	-0.136	0%		-0.113	0%	0.0314	-0.438	0%		-0.31	0%	0.0441	-0.282	0%		-0.07	0%		-0.17	0%	0.0439	-0.379	0%		0.0385
72	SPO1498 - AAV94785 fumarate hydratase	0%	0%		-0.112	0%		-0.06	0%		-0.218	0%		-0.235	0%		-0.045	0%		-0.119	0%		-0.082	0%		-0.333	0%		-0.116
73	SPO1905 - AAV95183 fumarate hydratase, c	0%	0%		-0.534	0%		0.0641	0%		-0.016	0%		0.0194	0%		0.0269	0%		0.1272	0%		-0.045	0%		-0.01	0%		0.1465
74	SPO0349 - AAV93667 malate dehydrogenas	0%	0%		-0.005	0%		0.0974	0%		0.2996	1%		0.2263	1%		0.1076	0%		0.1273	0%		0.0483	0%		0.0971	0%		0.0177
75	Glyoxylate and dicarboxylate metabolism	3.47%	2.08%	41.18%	-0.367	1.97%	23.53%	-0.185	2.32%	23.53%	-0.111	2.95%	5.88%	-0.11	3.01%	23.53%	-0.169	2.77%	0.00%	-0.064	2.85%	5.88%	-0.17	2.63%	35.29%	-0.339	1.95%	0.00%	-0.068
76	SPO0326 - AAV93644 acetyl-CoA C-acetyltra	1%	1%		-0.107	1%		-0.028	1%		0.1244	1%		0.1041	1%		0.0555	1%		0.0321	1%		0.0255	1%		-0.11	1%		-0.025
77	SPO0325 - AAV93643 beta-ketoacyl-ACP re	1%	0%	0.0272	-0.272	0%		-0.332	0%		-0.22	0%		-0.282	0%	0.0584	-0.29	0%		-0.118	0%		-0.211	0%		-0.371	0%		-0.026
78	SPO1565 - AAV94852 CoA ester lyase	0%	0%		-0.115	0%		0.1299	0%		0.2013	0%		0.2076	0%		0.1846	0%		0.2987	0%		-0.237	0%		-0.307	0%		-0.042
79	SPO0355 + AAV93673 hypothetical protein	0%	0%		-0.402	0%		-0.239	0%		-0.033	0%		-0.077	0%	0.0385	-0.302	0%		-0.17	0%		-0.142	0%	0.041	-0.565	0%		-0.045
80	SPO0932 + AAV94237 methylmalonyl-CoA e	0%	0%		0.2371	0%		-0.033	0%		0.1677	0%		-0.013	0%		-0.066	0%		-0.184	0%		-0.298	0%		0.258	0%		0.2708
81	SPO1105 + AAV94405 methylmalonyl-CoA n	0%	0%	0.0156	-0.539	0%	0.0188	-0.486	0%	0.027	-0.498	0%		-0.032	0%	0.0311	-0.42	0%		-0.196	0%		-0.468	0%	0.0255	-0.666	0%		-0.322
82	SPO1101 + AAV94401 acetyl/propionyl/meth	0%	0%	0.0135	-0.561	0%	0.0271	-0.465	0%	0.0284	-0.635	0%		-0.07	0%	0.044	-0.542	0%		-0.228	0%	0.0482	-0.494	0%	0.0379	-0.822	0%		-0.264
83	SPO3529 - AAV96754 serine hydroxymethyl	0%	0%		-0.191	0%		0.6375	0%		0.1692	0%		-0.238	0%		0.1416	0%		0.1737	0%		0.3194	0%		0.0076	0%		0.4782
84	SPOA005 + AAV97199 glycine dehydrogena	0%	0%		-0.014	0%		-0.011	0%	0.037	-0.312	0%		-0.174	0%		-0.064	0%		-0.117	0%		-0.1	0%		-0.166	0%		0.0021
85	SPOA005 + AAV97197 glycine cleavage syst	0%	0%		-0.034	0%		0.0381	0%	0.0479	-0.245	0%		-0.002	0%		-0.127	0%		0.0534	0%		-0.12	0%		-0.109	0%		0.0211
86	SPO0352 + AAV93670 CoA ester lyase	0%	0%		-0.572	0%		-0.217	0%		0.3377	0%		0.1036	0%		-0.256	0%		-0.025	0%		0.0539	0%		-0.436	0%		-0.159
87	SPO0370 + AAV93688 crotonyl-CoA carboxy	1%	0%	0.0188	-0.772	0%		-0.527	0%		-0.404	0%		-0.548	0%		-0.356	0%		-0.109	0%		-0.29	0%	0.041	-0.736	0%		-0.263
88	SPO0693 - AAV94000 acyl-CoA dehydroger	0%	0%	0.0184	-0.609	0%	0.04	-0.386	0%		-0.064	0%		-0.255	0%		-0.134	0%		-0.174	0%		-0.152	0%	0.0408	-0.433	0%		-0.209
89	SPO3608 + AAV96832 CoA ester lyase	0%	0%		-0.333	0%		-0.241	0%		0.2268	0%		0.0803	0%		-0.088	0%		-0.065	0%		-0.169	0%		-0.173	0%		-0.123
90	SPO0368 - AAV93686 protein meaA	0%	0%	0.0272	-1.053	0%		-0.276	0%		-0.003	0%		-0.109	0%		-0.08	0%		-0.135	0%		-0.037	0%		-0.424	0%		-0.23
91	SPO2839 - AAV96080 malate synthase G	0%	0%		-0.267	0%		-0.177	0%		-0.236	0%		-0.036	0%		-0.051	0%		-0.028	0%		-0.143	0%		-0.198	0%		-0.039
92	SPO3152 + AAV96387 dehydratase	0%	0%	0.0157	-0.633	0%	0.027	-0.528	0%		-0.464	0%	0.0462	-0.53	0%	0.0292	-0.476	0%		-0.103	0%	0.0551	-0.431	0%	0.0373	-0.504	0%		-0.18
93	Oxidative phosphorylation	2.97%	3.45%	14.29%	-0.109	3.10%	2.86%	-0.057	3.18%	14.29%	-0.058	3.95%	0.00%	-0.058	3.28%	20.00%	-0.013	3.55%	0.00%	-0.038	2.99%	2.86%	-0.044	3.53%	5.71%	-0.012	4.00%	0.00%	0.0303
94	SPO0272 + AAV93592 cytochrome b	0%	0%		-0.123	0%		-0.035	0%		-0.096	0%		0.1518	0%		0.0462	0%		-0.021	0%		-0.217	0%		0.2133	0%		-0.043
95	SPO0273 + AAV93593 cytochrome c	0%	0%		-0.18	0%		0.1133	0%		-0.645	0%		-0.264	0%	0.0498	-0.227	0%		-0.067	0%		-0.146	0%		-0.129	0%		0.0201
96	SPO0271 + AAV93591 ubiquinol-cytochrome	0%	0%		-0.262	0%		0.0055	0%		0.1429	0%		0.2478	0%		0.024	0%		0.1185	0%		0.054	0%		0.0095	0%		0.1129
97	SPO1383 - AAV94671 cytochrome c oxidase	0%	0%		-0.383	0%		-0.08	0%		-0.351	0%		-0.037	0%		0.0192	0%		-0.003	0%		-0.21	0%		-0.062	0%		-0.091
98	SPO3526 - AAV96751 cytochrome-c oxidase	0%	0%		-0.245	0%		-0.215	0%		-0.19	0%		-0.245	0%		-0.1	0%		-0.056	0%		-0.498	0%		-0.048	0%		0.1925
99	SPO3525 - AAV96750 cytochrome-c oxidase	0%	0%		0.1269	0%		-0.168	0%		-0.213	0%		-0.228	0%		-0.191	0%		0.07	0%		-0.02	0%		-0.069	0%		-0.193
100	SPO3523 - AAV96748 cytochrome-c oxidase	0%	0%		-0.319	0%		-0.072	0%	0.0334	-0.605	0%	0.0579	-0.32	0%	0.0411	-0.184	0%		-0.172	0%	0.0589	-0.226	0%		0.0192	0%		-0.097
101	SPO3076 - AAV96311 cytochrome c oxidase	0%	0%	0.0473	-0.042	0%	0.0395	-0.274	0%		-0.144	0%		-0.149	0%	0.047	-0.229	0%		-0.094	0%	0.0472	-0.322	0%		-0.236	0%		-0.257
102	SPO3073 - AAV96308 cytochrome c oxidase	0%	0%		-0.279	0%		-0.375	0%		-0.53	0%		-0.269	0%		-0.176	0%		-0.353	0%		-0.187	0%		-0.206	0%		0.2916
103	SPO3147 + AAV96382 manganese-depende	0%	0%	0.0254	-0.288	0%		-0.238	0%		0.0902	0%		-0.12	0%	0.0448	-0.227	0%		-0.164	0%		-0.167	0%		-0.39	0%		-0.066
104	SPO2785 - AAV96026 NADH-quinone oxido	0%	0%	0.0517	-0.055	0%		-0.179	0%		0.0038	0%		-0.074	0%		-0.098	0%		-0.088	0%		0.1215	0%	0.0304	0.6072	0%		-0.025
105	SPO2782 - AAV96023 NADH-quinone oxido	0%	0%		-0.145	0%		-0.04	0%		-0.194	0%		-0.291	0%		-0.113	0%		-0.105	0%		-0.113	0%		-0.233	0%		-0.061
106	SPO2774 - AAV96015 NADH-quinone oxido	0%	0%		-0.038	0%		0.0195	0%		0.0957	0%		-0.083	0%		0.0656	0%		-0.024	0%		0.0925	0%		-0.091	0%		-0.016
107	SPO2772 - AAV96013 NADH-quinone oxido	0%	0%		-0.145	0%		0.1686	0%		0.1506	0%		0.1341	0%		0.2986	0%		0.1347	0%		-0.044	0%		-0.035	0%		0.1991
108	SPO2770 - AAV96011 NADH-quinone oxido	0%	0%		-0.068	0%		-0.082	0%	0.0377	-0.581	0%		-0.067	0%		-0.169	0%		-0.074	0%		-0.074	0%		-0.079	0%		0.0248
109	SPO2767 - AAV96008 NADH:ubiquinone ox	0%	0%		-0.228	0%		-0.006	0%		-0.169	0%		-0.158	0%	0.0514	-0.236	0%		-0.138	0%		-0.003	0%		0.0398	0%		0.1117
110	SPO2765 - AAV96006 NADH-quinone oxido	0%	0%		0.037	0%		-0.224	0%		0.3855	0%		-0.137	0%		0.3861	0%		-0.025	0%		0.3562	0%		0.0419	0%		0.3753
111	SPO2764 - AAV96005 NADH-quinone oxido	0%	0%		-0.167	0%		0.022	0%	0.0559	-0.43	0%		-0.022	0%		-0.105	0%		-0.149	0%		-0.053	0%		-0.357	0%		0.2661
112	SPO2763 - AAV96004 NADH-quinone oxido	0%	0%		-0.371	0%		-0.047	0%		-0.036	0%		-0.031	0%		-0.119	0%		-0.137	0%		-0.144	0%		-0.149	0%		0.0429
113	SPO3772 - AAV96993 complex I NDUFA9 su	0%	0%	0.0419	0.7636	0%		0.7092	0%	0.0321	1.2479	0%		0.5382	0%	0.0356	0.9783	0%		0.8847	0%		0.8573	0%		0.9869	0%		-0.23
114	SPO2780 - AAV96021 NADH dehydrogenas	0%	0%		-0.17	0%		-0.1	0%		-0.1	0%		-0.076	0%		0.0269	0%		-0.039	0%		0.0147	0%		-0.18	0%		0.0324
115	SPO2784 - AAV96025 NADH-quinone oxido	0%	0%		-0.047	0%		-0.165	0%		-0.131	0%		-0.078	0%		-0.05	0%		-0.025	0%		-0.066	0%		-0.251	0%		-0.011
116	SPO2777 - AAV96018 NADH-quinone oxido	0%	0%		0.0369	0%		-0.042	0%		0.0072	0%		-0.054	0%		0.035	0%		0.0221	0%		0.0097	0%		0.0624	0%		-0.124
117	SPO3165 - AAV96400 ATP synthase subun	0%	0%		-0.054	0%		-0.181	0%		0.0714	0%		0.0359	0%		-0.031	0%		-0.082	0%		-0.189	0%		-0.067	0%		0.0507
118	SPO3236 - AAV96471 F0F1 ATP synthase s	0%	0%		-0.107	0%		0.1311	0%	0.0478	0.8348	0%		0.1049	0%		0.5632	0%		-0.122	0%		0.093	0%		0.6053	0%		0.052
119	SPO3233 - AAV96468 ATP synthase subun	0%	0%		-0.134	0%		-0.135	0%		-0.191	0%		-0.136	0%	0.0468	-0.244	0%		-0.155	0%		-0.101	0%		-0.084	0%		0.0318
120	SPO3234 - AAV96469 F0F1 ATP synthase s	0%	0%		-0.104	0%		0.0163	0%		0.0037	0%		0.019	0%		-0.019	0%		-0.075	0%		-0.046	0%		-0.029	0%		0.1821
121	SPO3164 - AAV96399 ATP synthase subun	1%	1%		0.0023	1%		-0.033	1%		0.0236	1%		-0.066	1%		-0.047	1%		-0.066	1%		0.0564	1%		-0.055	1%		0.0375
122	SPO3162 - AAV96397 ATP synthase subun	1%	1%		0.072	1%		0.0713	1%	0.0559	0.3328	2%		0.1707	1%		0.1692	1%		0.122	1%		0.1904	1%		0.2453	2%		0.0943
123	SPO3161 - AAV96396 ATP synthase epsilor	0%	0%		0.1654	0%		-0.076	0%		-0.037	0%		-0.169	0%		-0.168	0%		-0.15	0%		-0.194	0%		0.183	0%		0.0285
124	SPO3163 - AAV96398 ATP synthase subun	0%	0%	0.0271	-0.102	0%		-0.175	0%		-0.076	0%		-0.068	0%		-0.203	0%		-0.123	0%		-0.14	0%		-0.016	0%		-0.078





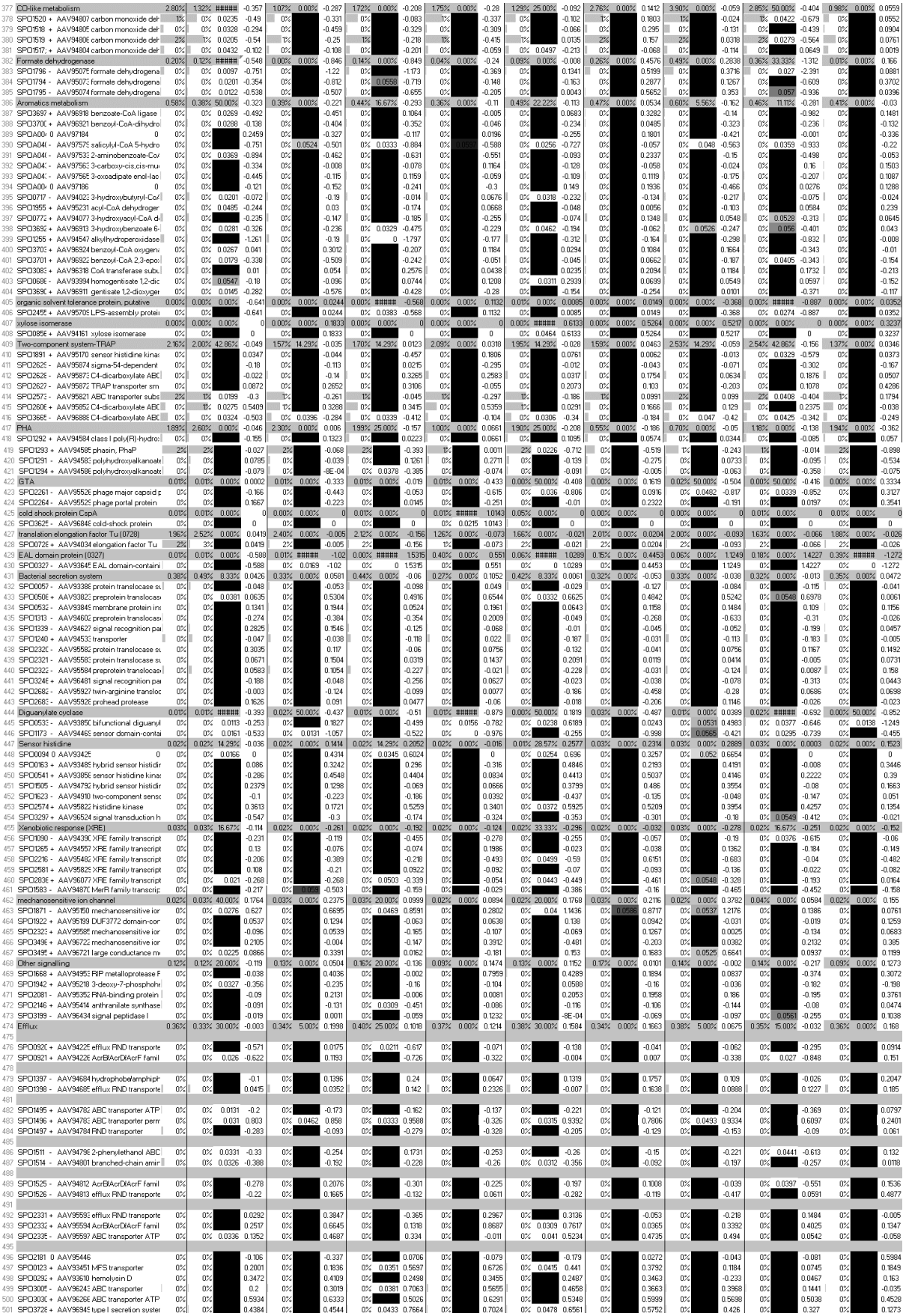


Table 7.11: Relative abundance of transporters of R. pomeroyi represented in Figures 4.3.5.1 to 3.

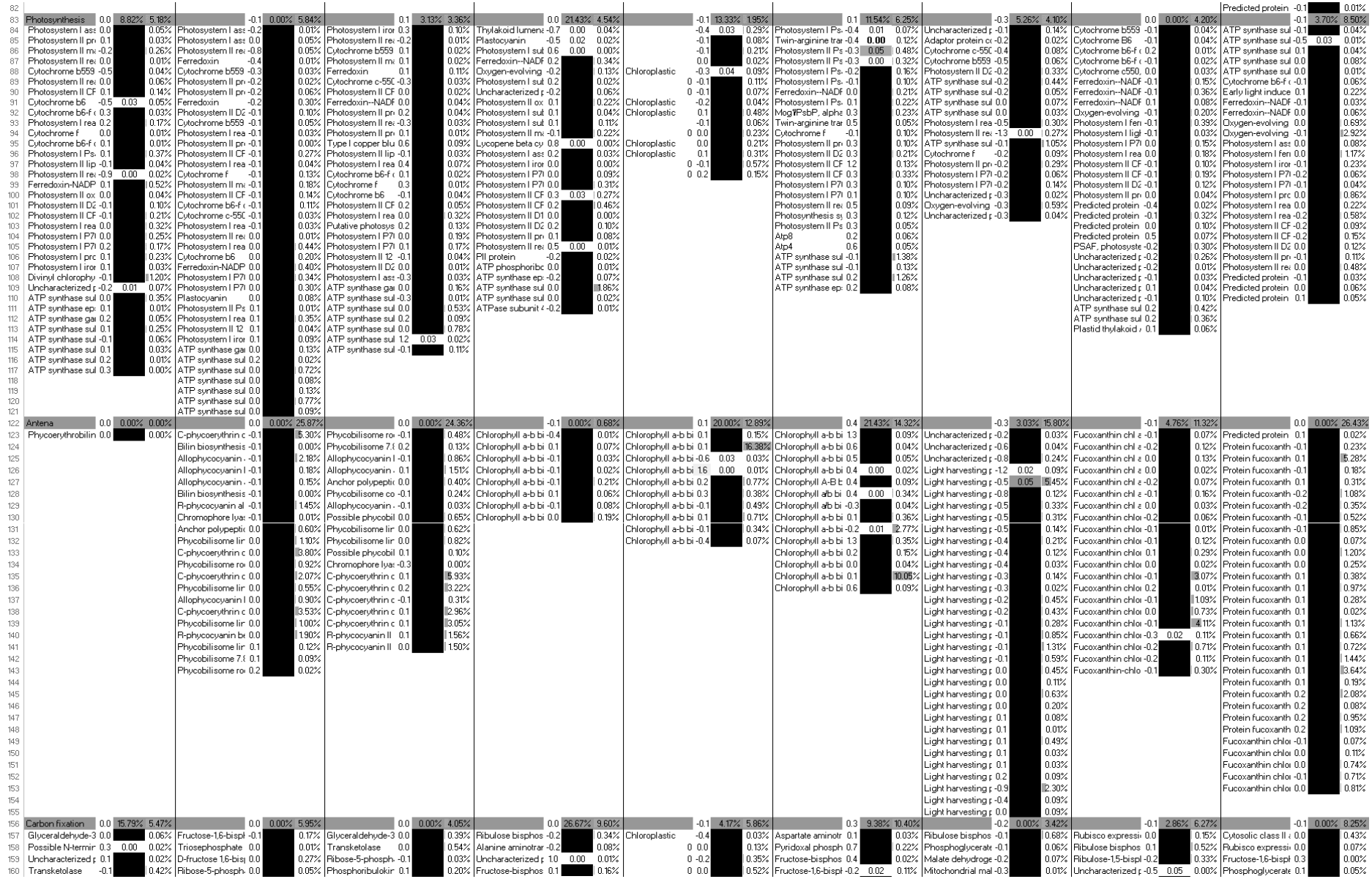
Relative abundance of transpo	R. pom	Prochl	SynWH	SynWH	Micr. s	M.pusil	Ostr	E.huxl	Thal	Phae
Amino acids										
Branched chain AA (0825)	0.10%	0.11%	0.21%	0.15%	0.15%	0.07%	0.16%	0.14%	0.15%	0.19%
Branched chain AA (1017-8,21)	0.31%	0.12%	0.05%	0.11%	0.17%	0.24%	0.21%	0.54%	0.13%	0.06%
Branched chain AA (1830)	0.05%	0.07%	0.07%	0.06%	0.12%	0.19%	0.06%	0.06%	0.06%	0.10%
Branched chain AA (1846,48-50)	0.98%	0.39%	0.49%	0.56%	0.65%	0.58%	0.77%	1.08%	0.79%	0.33%
Branched chain AA (1938-9)	0.42%	0.49%	0.77%	0.49%	0.56%	0.60%	0.45%	0.42%	0.48%	0.66%
Branched chain AA (2534)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Branched chain AA (3290-1,5)	0.67%	0.30%	0.44%	0.54%	0.49%	0.49%	0.56%	1.38%	0.62%	0.21%
Branched chain AA (3705-9)	1.62%	0.74%	0.99%	1.30%	5.44%	1.00%	3.39%	3.55%	4.07%	0.41%
Branched chain AA (A0300)	0.02%	0.05%	0.10%	0.10%	0.10%	0.21%	0.07%	0.03%	0.05%	0.26%
Alanine (2370)	0.02%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.02%	0.01%	0.02%
Gln (3040,3)	0.00%	0.01%	0.01%	0.01%	0.00%	0.00%	0.00%	0.01%	0.02%	0.01%
Gln (3335)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Glu (2658,61)	0.02%	0.02%	0.15%	0.10%	0.17%	0.06%	0.20%	0.05%	0.49%	0.33%
Glu (0519-22)	2.02%	3.69%	3.39%	3.15%	5.25%	5.00%	2.51%	2.77%	2.41%	3.88%
His/Glu/Gln/Arg/opine (1304-7)	0.03%	0.04%	0.04%	0.02%	0.02%	0.04%	0.02%	0.03%	0.02%	0.04%
peptide/opine/nickel (0098,0101)	0.13%	0.09%	0.14%	0.13%	0.29%	0.13%	0.19%	0.19%	0.19%	0.22%
peptide/opine/nickel (1543,5-7)	1.30%	1.48%	2.90%	2.89%	1.28%	1.22%	1.61%	1.36%	1.95%	2.13%
peptide/opine/nickel (2554)	0.00%	0.00%	0.00%	0.05%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
peptide/opine/nickel (2813-6)	1.68%	2.92%	3.68%	2.33%	2.14%	2.01%	1.84%	1.93%	2.47%	1.70%
peptide/opine/nickel (2995-6,8)	0.40%	0.44%	0.54%	0.39%	0.47%	0.41%	0.59%	0.42%	0.79%	0.59%
oligopeptide (0558,60-1)	0.10%	0.07%	0.11%	0.11%	0.08%	0.07%	0.13%	0.09%	0.13%	0.09%
oligopeptide (0702,6)	0.25%	0.21%	0.35%	0.18%	0.38%	0.13%	0.51%	0.19%	0.42%	0.46%
oligopeptide (1210-3)	0.31%	0.61%	1.30%	0.98%	1.19%	0.72%	0.67%	0.37%	0.72%	1.11%
oligopeptide/dipeptide (1644,7)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	0.04%	0.00%	0.00%
oligopeptide/dipeptide (1656-9)	0.75%	0.53%	0.97%	0.95%	0.72%	0.51%	0.92%	0.86%	0.88%	0.58%
oligopeptide/dipeptide (3534,7)	0.07%	0.07%	0.08%	0.09%	0.08%	0.09%	0.09%	0.08%	0.08%	0.05%
oligopeptide/dipeptide (3774,8)	0.30%	0.15%	0.33%	0.65%	0.19%	0.23%	0.32%	0.31%	0.33%	0.40%
Other AA	0.05%	0.02%	0.03%	0.04%	0.04%	0.04%	0.04%	0.05%	0.04%	0.05%
Carbohydrates										
Xylose transporter	0.03%	0.03%	0.02%	0.03%	0.03%	0.04%	0.05%	0.03%	0.03%	0.04%
Acetate (1810)	0.05%	0.05%	0.05%	0.02%	0.04%	0.05%	0.05%	0.03%	0.04%	0.04%
Isethionate (2358)	0.00%	0.00%	0.00%	0.00%	0.02%	0.11%	0.01%	0.01%	0.00%	0.00%
CH/G3P (3783,7)	0.41%	0.09%	0.08%	0.08%	0.15%	0.06%	0.34%	0.47%	0.25%	0.07%
CH/G3P (1820,3)	0.12%	0.11%	0.00%	0.00%	0.68%	0.00%	0.98%	0.02%	0.36%	0.39%
CH/G3P (1835,9)	0.05%	0.03%	0.05%	0.04%	0.05%	0.04%	0.06%	0.05%	0.06%	0.05%
CH (0376,8-9)	0.30%	0.41%	0.45%	0.37%	0.29%	0.28%	0.24%	0.26%	0.41%	0.55%
CH (0608-10)	0.16%	0.27%	0.28%	0.14%	0.13%	0.06%	0.09%	0.31%	0.16%	0.06%
CH (1465)	0.08%	0.08%	0.17%	0.10%	0.07%	0.04%	0.05%	0.06%	0.07%	0.11%
CH (1756)	0.10%	0.13%	0.11%	0.10%	0.06%	0.18%	0.05%	0.08%	0.07%	0.09%
CH (1773)	0.07%	0.03%	0.04%	0.04%	0.02%	0.02%	0.03%	0.06%	0.05%	0.03%
CH (1814-5)	0.13%	0.08%	0.22%	0.08%	0.09%	0.09%	0.09%	0.19%	0.13%	0.07%
CH (3693,5)	0.17%	0.07%	0.08%	0.09%	0.13%	0.10%	0.11%	0.22%	0.14%	0.08%
CH (A0280)	0.03%	0.03%	0.06%	0.04%	0.06%	0.04%	0.09%	0.05%	0.06%	0.04%
CH (A0374)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	0.00%	0.00%
Other CH (2605)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Other CH (2803)	0.00%	0.00%	0.01%	0.01%	0.01%	0.01%	0.00%	0.00%	0.00%	0.01%
CH unknown (A0166)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	0.00%	0.00%	0.00%
Unknown (2186-7)	1.71%	1.76%	2.01%	1.43%	0.90%	1.10%	1.20%	1.28%	1.51%	1.61%
Other	0.14%	0.12%	0.12%	0.10%	0.09%	0.11%	0.13%	0.17%	0.18%	0.07%
Other										
Phosphate (1948-51)	0.47%	0.56%	0.10%	0.01%	0.77%	0.02%	0.78%	0.32%	0.55%	0.58%
Phosphonate (0780-1,3)	0.17%	0.07%	0.00%	0.00%	0.09%	0.00%	0.15%	0.03%	0.14%	0.05%
G3P (0237,39-40)	0.32%	0.27%	0.25%	0.10%	0.86%	0.23%	0.82%	0.30%	0.73%	0.54%
DHPS (0591,4)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.05%	0.18%	0.02%
Sulfonate (3620)	0.10%	0.05%	0.04%	0.05%	0.04%	0.06%	0.07%	0.08%	0.07%	0.04%
S-containing molecule (2817)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Urea (1707a,07-10)	0.12%	0.46%	0.46%	0.23%	0.74%	1.43%	0.17%	0.39%	0.19%	0.48%
Ammonium (1578)	0.00%	0.00%	0.01%	0.00%	0.00%	0.00%	0.00%	0.01%	0.00%	0.00%
Ammonium (3723)	0.02%	0.05%	0.03%	0.04%	0.07%	0.09%	0.03%	0.03%	0.03%	0.06%
Unknown inorganics (1785,88,91)	0.26%	0.52%	0.63%	0.53%	0.30%	0.38%	0.33%	0.33%	0.42%	0.55%
Mg (2955)	0.00%	0.00%	0.01%	0.00%	0.00%	0.00%	0.01%	0.01%	0.00%	0.00%
Mn (3365-6)	0.02%	0.02%	0.02%	0.03%	0.01%	0.04%	0.01%	0.01%	0.00%	0.02%
Salt efflux (1424,2083)	0.01%	0.01%	0.01%	0.01%	0.02%	0.02%	0.01%	0.01%	0.01%	0.01%
Fe (3287)	0.08%	0.17%	0.06%	0.02%	0.38%	0.13%	0.08%	0.05%	0.03%	0.04%
TMAO-glycine betaine (1548-50)	0.04%	0.06%	0.09%	0.06%	0.06%	0.15%	0.04%	0.06%	0.05%	0.09%
Glycine betaine (2441,3)	0.00%	0.01%	0.03%	0.03%	0.00%	0.00%	0.00%	0.00%	0.02%	0.00%
Glycine betaine (3186)	0.01%	0.00%	0.02%	0.02%	0.02%	0.02%	0.01%	0.02%	0.02%	0.01%
Spermidine/putrescine (1606-7,9)	0.06%	0.05%	0.04%	0.33%	0.06%	0.05%	0.05%	0.05%	0.04%	0.08%
Spermidine/putrescine (A0381-2)	0.01%	0.00%	0.01%	0.01%	0.04%	0.01%	0.01%	0.11%	0.02%	0.00%
Spermidine/putrescine/polyamine (3	0.49%	0.60%	0.54%	0.56%	0.46%	0.72%	0.32%	0.44%	0.43%	0.48%
Putrescine/polyamine (3468-9)	0.12%	0.17%	0.27%	0.19%	0.20%	0.17%	0.16%	0.16%	0.21%	0.26%
Other osmolites	0.10%	0.08%	0.10%	0.07%	0.09%	0.12%	0.09%	0.13%	0.14%	0.09%
N-acetylTaurine (0660)	0.28%	0.26%	0.32%	0.23%	0.30%	0.38%	0.31%	0.30%	0.41%	0.48%
Taurine (0674-6)	0.12%	0.11%	0.13%	0.08%	0.14%	0.16%	0.13%	0.13%	0.17%	0.21%
Thiamine-intermediete (0050,2-3)	0.60%	0.12%	0.08%	0.14%	1.06%	0.29%	0.09%	0.67%	0.14%	0.14%
Biotin (2302)	0.04%	0.01%	0.01%	0.01%	0.01%	0.03%	0.00%	0.00%	0.00%	0.01%
Biotin unkown (3339)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
BMP (0648, 2802)	0.46%	0.91%	1.21%	1.03%	0.70%	0.70%	0.68%	0.48%	0.75%	1.05%
Nucleotide (0650-1)	0.02%	0.02%	0.03%	0.03%	0.01%	0.01%	0.02%	0.02%	0.02%	0.02%
Unknown (2446)	0.00%	0.00%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
Other	0.27%	0.29%	0.29%	0.30%	0.22%	0.35%	0.26%	0.24%	0.27%	0.25%

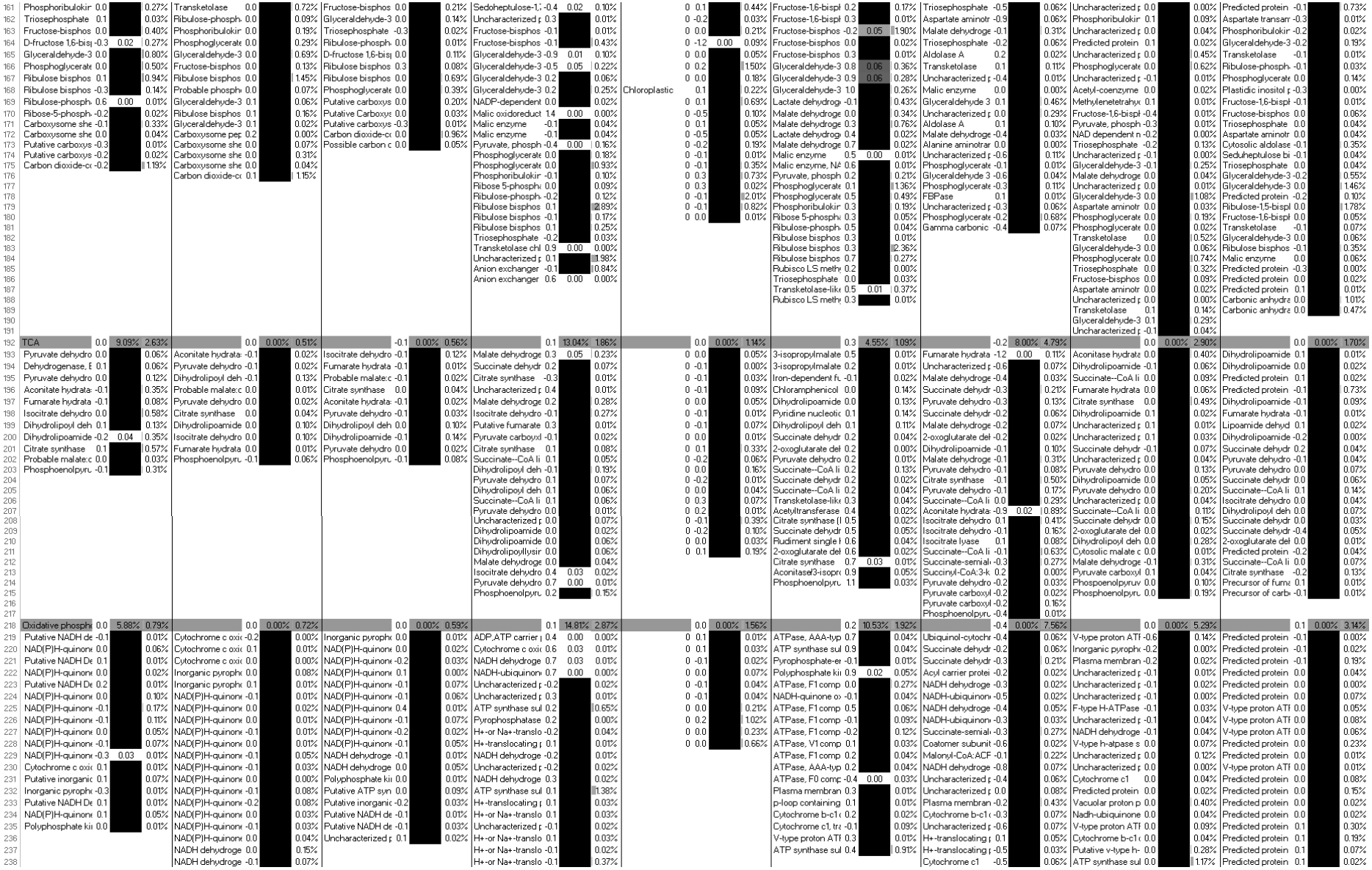
Table 7.12: Proteins of R. pomeroyi categorised used to construct the metabolic map presented in Fig. 4.3.6.1, including the relative abundance (RA), significant difference (Sign) and the Log_{10}fold change (FD) in mono-culture and 9 co-culture treatments.

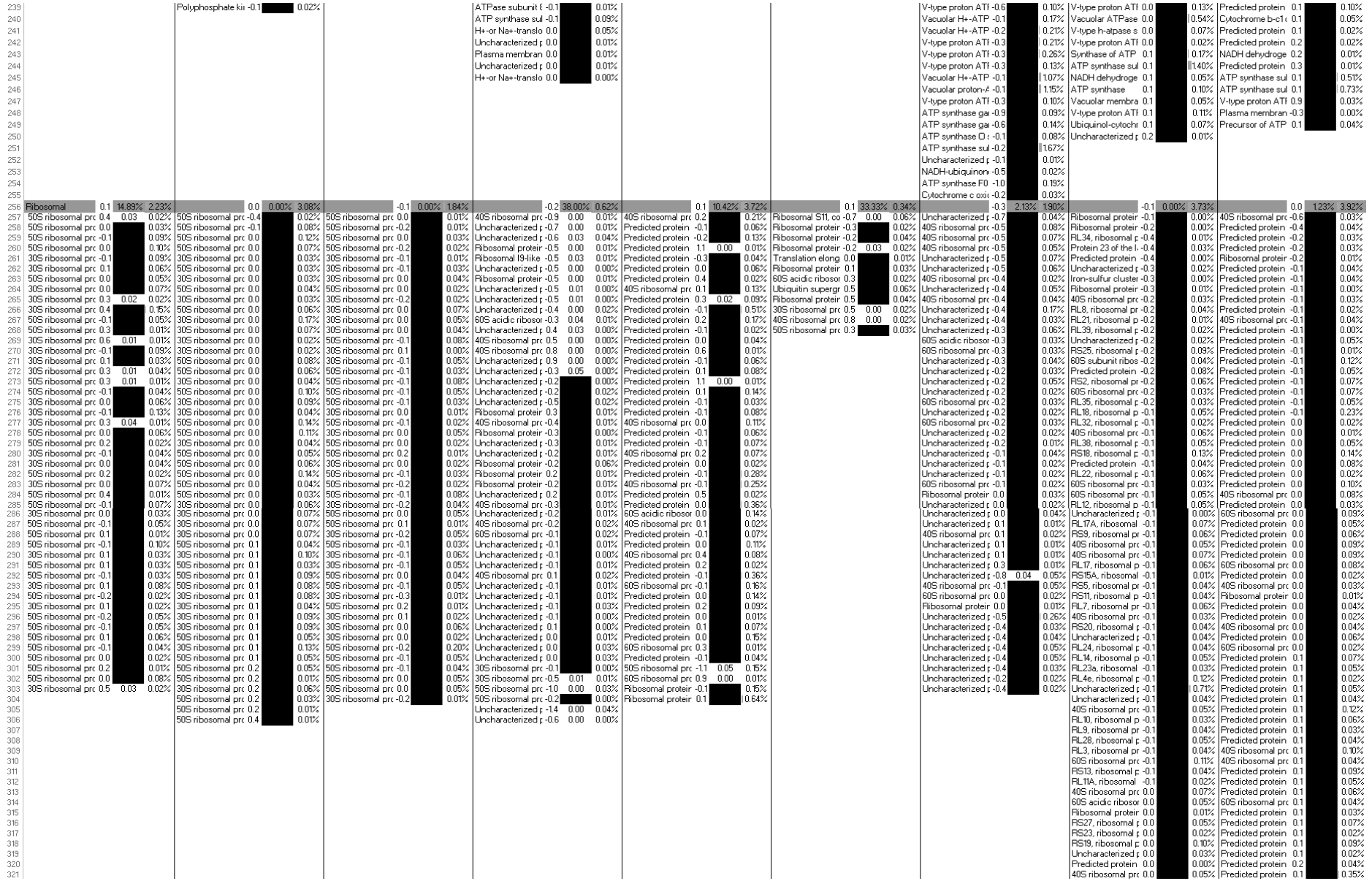
				R. pom	Prochloro			WH7803			WH8102			Microm			M. pusilla			Ostreo			E hux			Thalasio			Phaeod		
				RA	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC	RA	Sign	FC
3	xylose isomerase			0.00%	0.00%	0.00%	0.000	0.00%	0.00%	0.363	0.00%	0.00%	0.183	0.00%	0.00%	0.000	0.00%	0.00%	0.000	0.00%	100.00%	0.613	0.00%	0.00%	0.526	0.00%	0.00%	0.522	0.00%	0.00%	0.000
4	SPO0856 +	AAV94161	xylose isoı	0.00%	0.00%		0.000	0.00%		0.363	0.00%		0.183	0.00%		0.000	0.00%		0.000	0.00%	0.0	0.613	0.00%		0.526	0.00%		0.522	0.00%		0.000
5	Xylose transporter			0.03%	0.03%	50.00%	0.2	0.02%	0.00%	0.0	0.03%	0.00%	0.1	0.03%	0.00%	0.2	0.04%	0.00%	0.2	0.05%	50.00%	0.5	0.03%	0.00%	0.1	0.03%	0.00%	0.3	0.04%	0.00%	0.3
6	SPO0861 +	AAV94166	xylose AB	0.03%	0.03%		-0.098	0.02%		-0.088	0.03%		-0.003	0.03%		0.130	0.04%		0.164	0.05%		0.279	0.03%		0.055	0.03%		0.113	0.04%		0.190
7	SPO0862 +	AAV94167	xylose AB	0.00%	0.00%	0.0	0.551	0.00%		0.132	0.00%		0.216	0.00%		0.194	0.00%		0.202	0.00%	0.0	0.716	0.00%		0.241	0.00%	0.0	0.562	0.00%		0.408
8	glucokinase			0.00%	0.00%	0.00%	0.402	0.00%	0.00%	0.000	0.00%	0.00%	0.000	0.00%	100.00%	0.687	0.00%	0.00%	0.289	0.00%	0.00%	0.171	0.00%	0.00%	0.448	0.00%	100.00%	0.520	0.00%	0.00%	0.000
9	SPO0864 +	AAV94169	glucokina	0.00%	0.00%		0.402	0.00%		0.000	0.00%		0.000	0.00%	0.0	0.687	0.00%		0.289	0.00%		0.171	0.00%		0.448	0.00%	0.0	0.520	0.00%		0.000
10	gap-1			0.01%	0.05%	0.00%	0.607	0.03%	100.00%	0.474	0.02%	0.00%	0.166	0.02%	0.00%	0.365	0.03%	0.00%	0.413	0.01%	0.00%	0.087	0.01%	0.00%	0.108	0.01%	0.00%	0.053	0.03%	0.00%	0.280
11	SPO0701 -	AAV94007	glyceralde	0.01%	0.05%	0.1	0.607	0.03%	0.0	0.474	0.02%		0.166	0.02%		0.365	0.03%		0.413	0.01%		0.087	0.01%		0.108	0.01%		0.053	0.03%		0.280
12	gap-4			0.16%	0.15%	0.00%	-0.206	0.04%	100.00%	-0.631	0.01%	100.00%	-1.540	0.15%	0.00%	-0.007	0.00%	100.00%	-1.960	0.22%	0.00%	0.103	0.12%	0.00%	-0.106	0.21%	0.00%	0.092	0.11%	0.00%	-0.233
13	SPO3878 +	AAV97092	glyceralde	0.16%	0.15%		-0.206	0.04%	0.0	-0.631	0.01%	0.0	-1.540	0.15%		-0.007	0.00%	0.0	-1.960	0.22%		0.103	0.12%		-0.106	0.21%		0.092	0.11%		-0.233
14	glycolate oxidase			0.06%	0.02%	0.00%	-0.5	0.02%	33.33%	-0.5	0.05%	0.00%	-0.1	0.02%	0.00%	-0.5	0.02%	0.00%	-0.4	0.01%	33.33%	-0.6	0.32%	33.33%	1.0	0.02%	0.00%	-0.5	0.00%	33.33%	-1.0
15	SPO3478 +	AAV96704	glycolate c	0.01%	0.00%		-0.140	0.00%		-0.462	0.01%		0.125	0.00%		-0.378	0.00%		-0.060	0.00%		-0.647	0.11%	0.0	1.397	0.00%		-0.615	0.00%		-0.622
16	SPO3479 +	AAV96705	glycolate c	0.04%	0.01%		-0.758	0.02%		-0.371	0.04%		-0.092	0.01%		-0.411	0.02%		-0.376	0.01%	0.1	-0.547	0.16%		0.648	0.02%		-0.329	0.00%	0.0	-1.619
17	SPO3480 +	AAV96706	glycolate c	0.01%	0.00%		-0.613	0.00%	0.0	-0.785	0.01%		-0.214	0.00%		-0.771	0.00%		-0.770	0.00%	0.0	-0.743	0.05%		0.851	0.00%		-0.424	0.00%		-0.830
18	serine--glyoxylate transaminase			0.00%	0.02%	100.00%	1.455	0.00%	0.00%	0.110	0.00%	0.00%	0.330	0.00%	100.00%	0.663	0.02%	0.00%	1.273	0.00%	0.00%	0.067	0.00%	0.00%	0.132	0.01%	0.00%	0.902	0.00%	0.00%	0.414
19	SPO0A014 +	AAV97280	serine--gly	0.00%	0.02%	0.0	1.455	0.00%		0.110	0.00%		0.330	0.00%	0.0	0.663	0.02%		1.273	0.00%		0.067	0.00%		0.132	0.01%		0.902	0.00%		0.414
20	acetoin dehydrogenase			1.11%	0.25%	0.00%	-1.065	0.48%	50.00%	-0.433	0.42%	0.00%	-0.795	0.62%	25.00%	-0.418	0.27%	0.00%	-0.879	0.89%	0.00%	-0.186	1.93%	0.00%	0.218	0.73%	0.00%	-0.212	0.37%	50.00%	-0.558
21	SPO3792 -	AAV97012	acetoin de	0.44%	0.07%		-1.411	0.12%	0.0	-0.600	0.10%		-0.862	0.07%	0.0	-0.772	0.07%		-1.028	0.19%	0.1	-0.380	0.51%		0.096	0.22%		-0.306	0.10%	0.0	-0.673
22	SPO3791 -	AAV97011	acetoin de	0.52%	0.16%		-0.818	0.32%		-0.158	0.28%		-0.413	0.49%		0.048	0.17%		-0.479	0.62%		0.164	1.26%		0.510	0.45%		0.036	0.24%		-0.272
23	SPO3790 -	AAV97010	acetoin de	0.10%	0.01%		-1.268	0.03%	0.0	-0.561	0.02%		-1.138	0.03%		-0.411	0.02%		-1.013	0.05%		-0.255	0.10%		0.087	0.05%		-0.267	0.01%	0.0	-0.948
24	SPO3793 -	AAV97013	acetoin ca	0.05%	0.01%		-0.761	0.02%		-0.412	0.01%		-0.770	0.02%		-0.537	0.01%		-0.996	0.02%		-0.272	0.06%		0.180	0.02%		-0.308	0.02%		-0.340
25	succinate dehydrogenase			0.25%	0.23%	0.00%	-0.1	0.25%	50.00%	-0.1	0.25%	0.00%	-0.1	0.15%	50.00%	-0.3	0.15%	0.00%	-0.3	0.17%	100.00%	-0.2	0.21%	0.00%	-0.1	0.22%	0.00%	-0.1	0.15%	50.00%	-0.3
26	SPO0360 +	AAV93675	succinate	0.20%	0.19%		-0.094	0.22%		-0.009	0.21%		-0.030	0.13%		-0.161	0.13%		-0.219	0.14%	0.0	-0.183	0.17%		-0.069	0.19%		-0.043	0.13%		-0.246
27	SPO0361 +	AAV93675	succinate	0.05%	0.04%		-0.136	0.03%	0.0	-0.238	0.04%		-0.113	0.02%	0.0	-0.438	0.03%		-0.310	0.03%	0.0	-0.282	0.04%		-0.070	0.03%		-0.170	0.02%	0.0	-0.379
28	glutamate dehydrogenase			0.20%	0.11%	0.00%	-0.299	0.10%	0.00%	-0.325	0.10%	0.00%	-0.361	0.06%	100.00%	-0.452	0.11%	0.00%	-0.230	0.13%	0.00%	-0.187	0.15%	0.00%	-0.082	0.08%	0.00%	-0.407	0.16%	0.00%	-0.126
29	SPO1743 +	AAV95023	glutamate	0.20%	0.11%		-0.299	0.10%	0.1	-0.325	0.10%		-0.361	0.06%	0.0	-0.452	0.11%		-0.230	0.13%		-0.187	0.15%		-0.082	0.08%		-0.407	0.16%		-0.126
30	glutamine synthetase			1.39%	2.48%	0.00%	-0.054	2.97%	14.29%	0.137	2.79%	0.00%	0.033	3.00%	0.00%	-0.057	2.35%	0.00%	0.018	2.03%	14.29%	0.048	2.03%	0.00%	0.144	2.00%	0.00%	-0.001	2.45%	14.29%	-0.206
31	SPO2607 +	AAV95853	gamma-gl	0.04%	0.10%		0.307	0.07%		0.186	0.07%		0.202	0.02%		-0.275	0.07%		0.187	0.03%		-0.146	0.04%		0.008	0.04%		0.020	0.02%		-0.280
32	SPO0765 +	AAV94070	glutamine	0.01%	0.00%		-0.486	0.01%		-0.026	0.01%		-0.144	0.00%		-0.440	0.01%		-0.108	0.01%		-0.043	0.01%		0.141	0.00%		-0.128	0.00%	0.0	-0.672
33	SPO1300 -	AAV94585	glutamine	0.04%	0.03%		-0.233	0.04%		-0.091	0.04%		-0.094	0.02%		-0.215	0.03%		-0.182	0.03%		-0.128	0.04%		-0.004	0.03%		-0.160	0.04%		-0.056
34	SPO1302 -	AAV94591	glutamine	0.00%	0.00%		0.154	0.00%		0.195	0.00%		-0.238	0.00%		0.249	0.00%		0.243	0.00%	0.0	0.422	0.00%		0.212	0.00%		0.143	0.00%		-0.016
35	SPO1825 -	AAV95104	glutamine	0.00%	0.00%		-0.461	0.00%		0.087	0.00%		0.023	0.01%		-0.083	0.00%		-0.300	0.00%		0.012	0.01%		0.178	0.00%		-0.038	0.00%	0.1	-0.668
36	SPO2295 +	AAV95557	glutamine	1.27%	2.31%		0.287	2.80%		0.407	2.62%		0.372	2.92%		0.477	2.22%		0.346	1.92%		0.245	1.89%		0.289	1.89%		0.265	2.35%		0.319
37	SPO1573 -	AAV94860	glutamine	0.03%	0.04%		0.057	0.05%	0.0	0.198	0.04%		0.112	0.02%		-0.109	0.03%		-0.058	0.03%		-0.028	0.05%		0.187	0.03%		-0.114	0.03%		-0.071
38	Gln transporter			0.00%	0.01%	0.00%	0.0	0.01%	66.67%	0.4	0.01%	0.00%	0.3	0.01%	0.00%	0.0	0.01%	0.00%	0.1	0.00%	0.00%	-0.1	0.01%	0.00%	0.0	0.02%	0.00%	0.4	0.01%	0.00%	0.1
39	SPO3040 +	AAV96276	polar amir	0.00%	0.00%		-0.001	0.01%	0.0	0.604	0.01%		0.594	0.00%		-0.182	0.00%		0.023	0.00%		-0.276	0.01%		0.439	0.00%		0.097	0.00%		0.196
40	SPO3043 +	AAV96275	glutamine	0.00%	0.00%		0.000	0.00%		0.000	0.00%		0.000	0.00%		0.000	0.00%		0.000	0.00%		0.000	0.00%		0.000	0.02%		1.248	0.00%		0.000
41	SPO3335 +	AAV96562	glutamine	0.00%	0.00%		0.010	0.00%	0.0	0.454	0.00%		0.204	0.00%		0.074	0.00%		0.300	0.00%		-0.049	0.00%		-0.307	0.00%		-0.133	0.00%		0.042
42	glutamate synthase			0.06%	0.07%	0.00%	0.092	0.18%	50.00%	0.485	0.17%	0.00%	0.433	0.13%	0.00%	0.354	0.13%	0.00%	0.373	0.09%	0.00%	0.235	0.13%	0.00%	0.402	0.12%	0.00%	0.297	0.14%	0.00%	0.347
43	SPO3768 -	AAV96985	glutamate	0.02%	0.03%		0.214	0.08%	0.0	0.636	0.07%		0.594	0.04%		0.421	0.04%		0.424	0.03%		0.198	0.04%		0.457	0.05%		0.496	0.05%		0.408

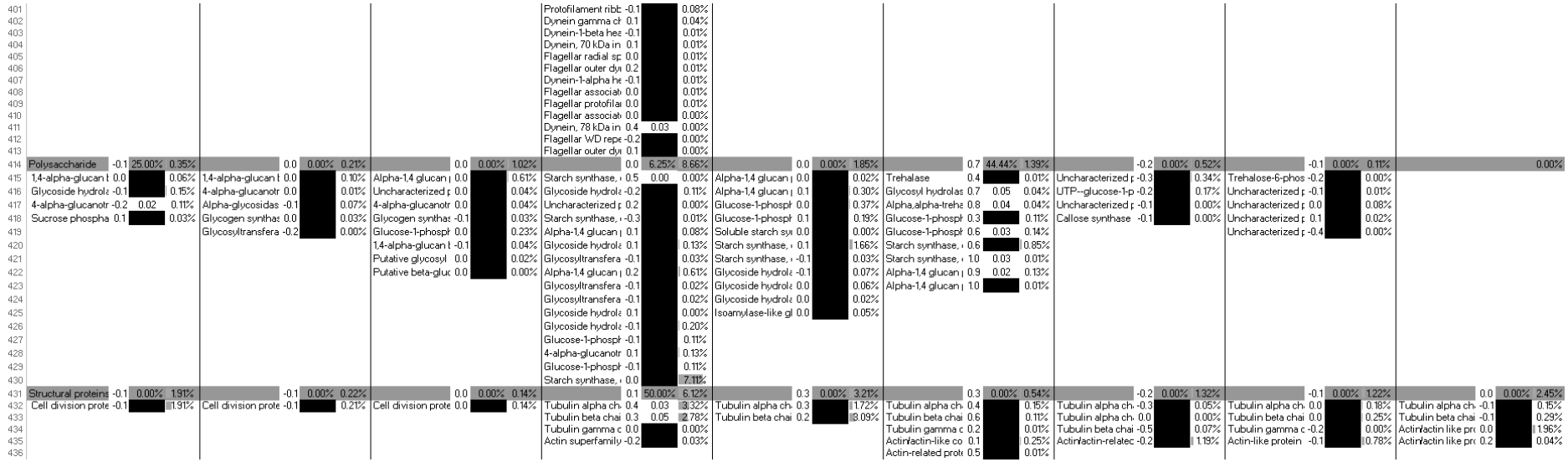
Table 7.13: Proteins of nine phototrophs categorised into metabolic pathways, presented in Fig. 5.3.2.1 and 2, including the relative abundance (RA), significant difference (Sign) and the Log$_{10}$fold change (FD).

Prochlorococcus				*Synechococcus WH7803*				*Synechococcus WH8102*				*Micromonas*				*M. pusilla*				*Ostreococcus*				*E. huxleyi*				*Thalassiosira*				*Phaeodactylum*			
Fasta Header	FD	Sign	RA Ax	Fasta Header	FD	Sign	RA Ax	Fasta Header	FD	Sign	RA Ax	Fasta Header	FD	Sign	RA Ax	Fasta Header	FD	Sign	RA Ax	Fasta Header	FD	Sign	RA Ax	Fasta Header	FD	Sign	RA Ax	Fasta Header	FD	Sign	RA Ax	Fasta Header	FD	Sign	RA Ax
P transporter	0.2	0.00%	1.13%		0.1	0.00%	0.37%		0.0	0.00%	5.80%		0.3	66.67%	0.23%		0.0	0.00%	0.03%		0.7	50.00%	1.83%		0.0	0.00%	0.28%		0.0	0.00%	0.74%		-0.1	0.00%	0.33%
Phosphate transp	0.3		0.00%	Phosphate import	0.0		0.04%	Phosphate-bindir	0.0		5.57%	Phosphate:Na+ sy	0.6	0.00	0.15%	Phosphate:Na+ sy	0.0		0.03%	Phosphate transp	0.1		1.83%	Phosphate transp	0.0		0.02%	Sodium phosphat	0.0		0.73%	Predicted protein	-0.1		0.01%
Phosphate transp	0.2		0.00%	Phosphate transp	0.1		0.00%	Phosphate-bindir	0.0		0.11%	Phosphate:Na+ sy	0.6	0.00	0.02%					Major facilitator su	1.3	0.03	0.04%	Phosphate transp	0.1		0.01%					Predicted protein	0.0		0.32%
Phosphate import	0.2		0.05%	Phosphate transp	0.1		0.00%	Phosphate-bindir	0.1		0.12%	Phosphate:Na+ sy	-0.1		0.07%									Putative inorganic	-0.2		0.24%								
Phosphate-bindir	0.1		1.07%	Phosphate-bindir	0.1		0.01%																												
				Phosphate-bindir	0.2		0.32%																												
Not found			0.00%				0.00%		-0.1	0.00%	0.73%				0.00%				0.00%				0.00%		0.0	0.00%	8.11%		0.1	0.00%	0.98%		0.0	0.00%	0.08%
								Putative alkaline p	-0.4		0.70%																Alkaline phospha	0.0		8.10%	Uncharacterized p	0.1		0.85%	Predicted protein	0.1		0.07%
								Putative alkaline p	0.1		0.04%																				Alkaline phospha	0.4		0.08%	Predicted protein	-0.2		0.01%
																															Predicted protein	0.0		0.01%				
																															Predicted protein	0.0		0.03%				
																															Uncharacterized p	0.0		0.00%				
Phytase	0.1	0.00%	0.70%				0.00%		-0.2	0.00%	0.03%				0.00%				0.00%		0.5	0.00%	0.02%				0.00%		0.0	0.00%	0.72%		0.2	0.00%	2.30%
Putative secreted	0.1		0.69%					Conserved hypoth	-0.2		0.03%									Phytase-like dom	0.5		0.02%					Alkaline phospha	0.0		0.53%	Predicted protein	0.2		2.30%
																															Uncharacterized p	0.0		0.18%				
Phosphonate trans	-0.3	50.00%	0.02%		0.1	0.00%	0.00%		-0.2	0.00%	0.00%				0.00%				0.00%				0.00%				0.00%				0.00%				0.00%
Putative phospho	-0.3	0.05	0.01%	ABC-type phosph	0.1		0.00%	Putative phospho	-0.2		0.00%																								
Putative phospho	-0.4	0.04	0.00%																																
Ammonium transp	-0.2	0.00%	0.63%		-0.1	0.00%	0.10%		-0.2	0.00%	0.01%				0.00%		0.5	0.00%	0.00%		0.5	0.00%	0.56%				0.00%		0.1	0.00%	0.00%		-0.2	0.00%	0.11%
Ammonium transp	-0.2		0.63%	Ammonium transp	-0.1		0.10%	Ammonium transp	-0.2		0.01%					Ammonium transp	0.5		0.01%	Ammonium transp	-0.1		0.44%					Ammonium transp	0.1		0.00%	Ammonium transp	-0.2		0.11%
																				Ammonium transp	1.1		0.11%												
Glutamine synthet	-0.3	#####	5.27%		-0.1	0.00%	1.45%		0.0	0.00%	1.46%		0.3	50.00%	0.18%		0.3	0.00%	0.13%		0.5	0.00%	0.82%		-0.3	0.00%	0.27%		0.0	0.00%	1.16%		-0.2	0.00%	0.64%
Glutamine synthe	-0.3	0.05	5.26%	Glutamine synthe	0.0		1.03%	Glutamine synthe	0.0		1.46%	Glutamine synthe	0.5	0.02	0.08%	Glutamine synthe	0.3	0.00	0.19%	Glutamine synthe	0.5		0.79%	Glutamine synthe	-0.3		0.11%	Glutamine synthe	0.0		0.04%	Glutamine synthe	-0.2		0.01%
				Glutamate--ammc	-0.1		0.42%					Glutamine synthe	0.1		0.10%									Glutamine synthe	-0.4		0.17%	Uncharacterized p	0.0		1.11%	GLNA, glutamine	-0.2		0.63%
Glutamate synthas	0.0	0.00%	0.23%		-0.1	0.00%	0.07%		-0.2	0.00%	0.04%		0.2	0.00%	0.59%		-0.2	0.00%	0.05%		0.8	0.00%	0.08%		-0.4	0.00%	0.01%		-0.1	0.00%	0.11%		-0.1	0.00%	0.04%
Ferredoxin-deper	0.0		0.23%	Ferredoxin-deper	-0.1		0.07%	Ferredoxin-deper	-0.2		0.04%	Glutamate syntha	0.1		0.46%	Glutamate syntha	-0.2		0.07%	Glutamate syntha	0.8		0.08%	Ferredoxin-deper	-0.4		0.01%	Glutamate syntha	-0.1		0.11%	Ferredoxin-deper	-0.1		0.01%
												Glutamate syntha	0.2	0.06	0.13%																Synthase of gluta	0.0		0.03%
Glutamate dehydrogenase			0.00%				0.00%				0.00%				0.00%				0.00%				0.00%				0.00%		0.0	0.00%	0.19%		-0.1	0.00%	0.02%
																															Glutamate dehydr	0.0		0.14%	Glutamate dehydr	-0.1		0.02%
																															Glutamate dehydr	0.0		0.05%				
Nitrilase	-0.2	#####	0.07%		0.0	0.00%	0.05%		-0.2	0.00%	0.06%				0.00%				0.00%				0.00%				0.00%				0.00%				0.00%
Possible nitrilase	-0.2	0.02	0.07%	Possible nitrilase	0.0		0.05%	Probable nitrilase	-0.3		0.04%																								
								Possible nitrilase	-0.1		0.02%																										
Nitrate transporter			0.00%		-0.1	0.00%	0.07%		-0.1	0.00%	0.21%				0.00%				0.02%				0.00%		-1.2	#####	0.09%		-0.1	0.00%	0.04%		-0.2	0.00%	0.01%
				Formate/nitrate tra	-0.3		0.00%	Nitrate transporter	-0.1		0.04%													Putative nitrate tra	-1.2	0.00	0.08%	Nitrate transporter	0.0		0.03%	Predicted protein	-0.2		0.01%
				Nitrate permease I	0.0		0.07%	Nitrate transporter	-0.2		0.05%																	Nitrate transporter	-0.1		0.01%				
								ABC transporter, r	-0.1		0.12%																										
								ABC transporter, r	0.1		0.00%																										
Nitrate/nitrite reductase			0.00%		-0.2	0.00%	0.09%		0.3	50.00%	0.02%				0.00%		0.5	0.00%	0.02%				0.00%				0.00%		-0.1	0.00%	0.00%		-0.5	0.00%	0.00%
				Nitrate reductase	-0.1		0.02%	Nitrate reductase	0.6	0.03	0.00%					Nitrate reductase	0.8		0.02%											Nitrate reductase	-0.1		0.00%	Nitrate reductase	-0.5		0.00%
				Ferredoxin--nitrite	-0.3		0.07%	Ferredoxin--nitrite	0.1		0.02%					Nitrite reductase f	0.2		0.01%											Nitrite reductase-f	0.0		0.00%				
Urea transporter	-0.1	20.00%	11.20%				0.00%		0.0	0.00%	0.82%				0.00%				0.00%				0.00%		-0.5	0.00%	0.05%				0.00%		0.2	0.00%	0.01%
Putative urea ABC	-0.3	0.02	10.99%					Putative urea binc	0.1		0.58%													Putative urea tran	-0.5		0.05%					Urea transporter	0.2		0.01%
Putative urea ABC	-0.3		0.01%					Putative ATP-bin	-0.1		0.02%																										
Putative membrar	-0.1		0.01%					Putative ATP-bin	-0.1		0.01%																										
Putative ATP binc	-0.1		0.07%					Putative urea ABC	0.1		0.22%																										
Putative ATP-bin	0.0		0.11%																																
Urease	-0.1	0.00%	0.67%				0.00%		0.0	0.00%	0.06%		-0.1	0.00%	0.03%				0.00%		0.6	0.00%	0.02%		-0.4	0.00%	0.13%		-0.1	0.00%	0.07%		-0.1	0.00%	0.02%
Urease subunit al	-0.1		0.52%					Urease subunit al	-0.1		0.05%	Urease	-0.1		0.03%					Urease	0.6		0.02%	Urease	-0.4		0.12%	Urease	0.0		0.06%	Predicted protein	-0.1		0.02%
Urease subunit be	0.0		0.09%					Urease accessory	0.0		0.00%																	Urease accessory	-0.1		0.01%				
Urease subunit ga	-0.3		0.06%																																
Cyanate transpore	-0.2	33.33%	2.16%				0.00%		0.0	0.00%	0.22%				0.00%				0.00%				0.00%				0.00%				0.00%				0.00%
Putative cyanate /	-0.2		1.77%					Putative cyanate /	-0.1		0.07%																								
Putative cyanate /	-0.4	0.04	0.01%					Putative cyanate /	0.0		0.15%																								
Putative cyanate /	0.0		0.37%																																
Cyanate hydratase	-0.2	#####	0.34%		-0.2	0.00%	0.72%		-0.5	0.00%	0.08%				0.00%				0.00%				0.00%				0.00%		-0.1	0.00%	0.00%			0.00%	0.00%
Cyanate hydratase	-0.2	0.02	0.34%	Cyanate hydratase	-0.2		0.72%	Cyanate hydratase	-0.5		0.08%																	Cyanate hydratase	-0.1		0.00%	Cyanate hydratase	0.0		0.00%
FeMetals transpor	-0.1	0.00%	0.60%		0.1	0.00%	0.59%		-0.1	0.00%	0.79%				0.00%				0.00%				0.00%				0.00%		-0.2	0.00%	0.03%		-0.1	0.00%	0.00%
Magnesium trans	-0.1		0.31%	ABC-type Fe3+ tra	0.0		0.53%	Magnesium trans	-0.2		0.01%	CorA metal ion tra	0.3	0.04	0.00%													Uncharacterized p	-0.1		0.03%	Predicted protein	-0.1		0.00%
Possible cobalt tra	-0.4		0.01%	Possible ABC-typ	0.4		0.00%	Possible cobalt tra	-0.5		0.01%	CorA metal ion tra	0.2	0.00	0.00%													Uncharacterized p	-0.1		0.00%				
Putative iron ABC	0.1		0.00%	ABC-type Mn2+ tr	0.0		0.05%	ABC transporter, /	0.3		0.06%																	Copper transporte	-0.3		0.00%				
Putative iron ABC	0.2		0.21%	ABC-type Mn2+ tr	0.0		0.00%	Putative iron ABC	0.0		0.72%																								
ABC transporter, /	0.0		0.00%	Magnesium trans	0.0		0.01%	ABC transporter, s	0.0		0.00%																								
ABC transporter, /	-0.3		0.04%																																
ABC transporter, s	-0.1		0.03%																																
Catalase			0.00%		0.0	0.00%	0.11%				0.00%				0.00%				0.00%				0.00%				0.00%				0.00%				0.00%
				Catalase-peroxida	0.0		0.10%																														
Superoxide dismu	-0.1	0.00%	0.59%		-0.1	0.00%	0.88%		0.0	0.00%	0.45%		-0.1	0.00%	0.06%				0.00%		0.5	0.00%	0.35%		-0.1	0.00%	0.11%		0.0	0.00%	0.16%		0.0	0.00%	0.22%
Putative nickel-co	-0.1		0.59%	Superoxide dismu	0.0		0.87%	Putative nickel-co	0.0		0.45%	Superoxide dismu	0.0		0.04%					Superoxide dismu	0.1		0.30%	Superoxide dismu	-0.1		0.11%	Superoxide dismu	-0.1		0.10%	Superoxide dismu	-0.1		0.20%
				Superoxide dismu	-0.1		0.01%					Superoxide dismu	-0.3		0.02%					Superoxide dismu	0.9		0.06%					Superoxide dismu	0.0		0.06%	Superoxide dismu	0.0		0.02%
Peroxidase	-0.1	0.00%	0.62%		-0.1	0.00%	0.03%		0.0	0.00%	1.38%		-0.1	33.33%	0.20%		-0.1	0.00%	0.10%		0.4	33.33%	0.07%		-0.3	25.00%	0.18%		-0.1	0.00%	0.47%		0.0	0.00%	0.79%
Glutathione perox	-0.1		0.04%	Glutathione perox	-0.1		0.03%	Glutathione perox	0.0		0.02%	Glutathione perox	0.0		0.06%		-0.2		0.05%	Haem peroxidase,	1.0	0.00	0.01%	Putative cytochror	-0.2		0.11%	Uncharacterized p	-0.1		0.01%	Predicted protein	-0.1		0.09%
Thioredoxin pero	-0.1		0.58%					Thioredoxin pero	0.0		1.36%	Uncharacterized p	-0.4	0.02	0.12%		0.0		0.10%	Haem peroxidase,	0.3		0.05%	Glutathione perox	-0.6	0.02	0.04%	Uncharacterized p	-0.3		0.01%	Predicted protein	-0.1		0.46%
												Uncharacterized p	0.0		0.02%					Haem peroxidase,	-0.1		0.02%	Putative cytochror	-0.2		0.03%	Ascorbate peroxic	-0.1		0.19%	Glutathione perox	0.0		0.07%
																								Uncharacterized p	-0.2		0.01%	Ascorbate peroxic	-0.1		0.15%	L-ascorbate perox	0.1		0.06%
																												Uncharacterized p	-0.1		0.09%	Ascorbate peroxic	-0.2		0.04%
																												Cytochrome C per	-0.1		0.03%	Glutathione perox	0.0		0.04%
																															Predicted protein	0.0		0.02%
																															Predicted protein	0.0		0.01%








Protofilament ribb -0.1 0.08%
Dynein gamma ch 0.1 0.04%
Dynein-1-beta hea -0.1 0.01%
Dynein, 70 kDa in 0.1 0.01%
Flagellar radial sp 0.0 0.01%
Flagellar outer dyn 0.2 0.01%
Dynein-1-alpha he -0.1 0.01%
Flagellar associat 0.0 0.01%
Flagellar protofila 0.0 0.01%
Flagellar associat 0.0 0.00%
Dynein, 78 kDa in 0.4 0.03 0.00%
Flagellar WD repe -0.2 0.00%
Flagellar outer dyn 0.1 0.00%
Polysaccharide -0.1 25.00% 0.35%
1,4-alpha-glucan b 0.0 0.06%
Glycoside hydrola -0.1 0.15%
4-alpha-glucanotr -0.2 0.02 0.11%
Sucrose phospha 0.1 0.03%
1,4-alpha-glucan b 0.0 0.10%
4-alpha-glucanotr 0.0 0.01%
Alpha-glycosidas -0.1 0.07%
Glycogen synthas 0.0 0.03%
Glycosyltransfera -0.2 0.00%
Alpha-1,4 glucan p 0.0 0.00% 1.02%
Uncharacterized p 0.0 0.04%
4-alpha-glucanotr 0.0 0.04%
Glycogen synthas -0.1 0.03%
Glucose-1-phospt 0.0 0.23%
1,4-alpha-glucan b -0.1 0.04%
Putative glycosyl 0.0 0.02%
Putative beta-gluc 0.0 0.00%
Starch synthase, r 0.5 0.00 0.00%
Glycoside hydrola -0.2 0.11%
Uncharacterized p 0.2 0.00%
Starch synthase, r -0.3 0.01%
Alpha-1,4 glucan p 0.1 0.08%
Glycoside hydrola 0.1 0.13%
Glycosyltransfera -0.1 0.03%
Alpha-1,4 glucan p 0.2 0.61%
Glycosyltransfera -0.1 0.02%
Glycosyltransfera -0.1 0.02%
Glycoside hydrola 0.1 0.00%
Glycoside hydrola -0.1 0.20%
Glucose-1-phospt -0.1 0.11%
4-alpha-glucanotr 0.1 0.13%
Glucose-1-phospt -0.1 0.11%
Starch synthase, r 0.0 7.11%
Alpha-1,4 glucan p 0.0 0.00% 1.85%
Alpha-1,4 glucan p 0.0 0.02%
Alpha-1,4 glucan p 0.1 0.30%
Glucose-1-phospt 0.0 0.37%
Glucose-1-phospt 0.1 0.19%
Soluble starch syn 0.0 0.00%
Starch synthase, r 0.1 1.66%
Starch synthase, r -0.1 0.03%
Glycoside hydrola -0.1 0.07%
Glycoside hydrola 0.0 0.06%
Glycoside hydrola 0.0 0.02%
Isoamylase-like gl 0.0 0.05%
Trehalase 0.4 0.01%
Glycosyl hydrolas 0.7 0.05 0.04%
Alpha,alpha-treha 0.8 0.04 0.04%
Glucose-1-phospt 0.3 0.11%
Glucose-1-phospt 0.6 0.03 0.14%
Starch synthase, r 0.6 0.85%
Starch synthase, r 1.0 0.03 0.01%
Alpha-1,4 glucan p 0.9 0.02 0.13%
Alpha-1,4 glucan p 1.0 0.01%
Uncharacterized p -0.3 0.34%
UTP--glucose-1-p -0.2 0.17%
Uncharacterized p -0.1 0.00%
Callose synthase -0.1 0.00%
Trehalose-6-phos -0.2 0.00%
Uncharacterized p -0.1 0.01%
Uncharacterized p 0.0 0.08%
Uncharacterized p 0.1 0.02%
Uncharacterized p -0.4 0.00%
0.00%
Structural proteins -0.1 0.00% 1.91%
Cell division prote -0.1 1.91%
Cell division prote -0.1 0.21%
Cell division prote 0.0 0.14%
Tubulin alpha ch 0.4 0.03 3.32%
Tubulin beta chai 0.3 0.05 2.78%
Tubulin gamma c 0.0 0.00%
Actin superfamily -0.2 0.03%
Tubulin alpha ch 0.3 1.72%
Tubulin beta chai 0.2 8.09%
Tubulin alpha ch 0.4 0.15%
Tubulin beta chai 0.6 0.11%
Tubulin gamma c 0.2 0.01%
Actin/actin-like co 0.1 0.25%
Actin-related prot 0.5 0.01%
Tubulin alpha ch -0.3 0.05%
Tubulin alpha ch 0.0 0.00%
Tubulin beta chai -0.5 0.07%
Actin/actin-relatec -0.2 1.19%
Tubulin alpha ch 0.0 0.18%
Tubulin beta chai 0.0 0.25%
Tubulin gamma c -0.2 0.00%
Actin-like protein -0.1 0.78%
Tubulin alpha ch -0.1 0.15%
Tubulin beta chai -0.1 0.29%
Actin/actin like pr 0.0 1.96%
Actin/actin like pr 0.2 0.04%