

A smooth sea never made a skilled sailor.

Franklin D. Roosevelt (1882 – 1945)

Development of environmental impact assessment methods for marine sourced products

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Ontwikkeling van methoden voor de beoordeling van de ecologische duurzaamheid van mariene producten.

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Summary

To sustain the needs of growing world population, seas and oceans are becoming heavily exploited. Initially exploited for food and transportation, offshore marine areas are nowadays supplying energy and minerals. Whilst the extraction of terrestrial natural resources led to major environmental consequences (*i.e.* biodiversity loss), it is crucial to ensure the global environmental sustainability of marine products on their entire life cycle. Life Cycle Assessment (LCA) methods have the potential to provide such information and to identify hotspots of environmental impacts in the value chain of the product under analysis. At the endpoint level, LCA results consider impacts on three areas of protection (AoP): human health, ecosystem quality and natural resources. However, LCA methods have been traditionally applied to industrial processes and thus, are limited to include site-specific aspects (*e.g.* disturbance of the local ecosystem) in the scope of the assessment. The application of LCA to assess the environmental sustainability of marine products including ecosystem-specific life cycle impact assessments (LCIAs) in the evaluation of impacts belonging to the three AoP. Moreover, quantitative data on mass and energy flows associated to the entire life cycle of the products (production / extraction of raw materials and their processing to final commodities) are required to perform global environmental sustainability assessments. The overall objective of this PhD is to reinforce LCA capacity to assess the global sustainability of marine products. Two operational frameworks are proposed to include site-specific aspects related to the sourcing of marine raw materials, and data related to the processing of wet biomass are provided. In this way, the evaluation of the global environmental sustainability of marine products through LCA will be more inclusive and meaningful for comparative assessments with terrestrial alternatives.

The PhD starts with a general introduction (**Chapter 1**) divided into four sections. First, an overview of marine activities is provided. The most important marine activities in terms of economic importance are described and the concept of *the industrial revolution of the seas and oceans* is introduced. This refers to the growing importance of the marine-sourced materials and energy for the global economy. Indeed, the importance of the marine economy is expected to follow a two-fold increase by 2030. On a longer time horizon, the potential recovery of deep-sea minerals might significantly increase our dependence on marine commodities. The second section provides background information related to the classification of natural resources and their link with ecosystem services. Natural resources are classified according to renewability, exhaustibility and their form at the moment of extraction (biotic / abiotic). Marine natural resources are presented according to this classification and in the context of ecosystem services. Deep-sea minerals are extensively presented as they might become substantial for our economy in a near future. The ecological pressures on marine

ecosystems are discussed in the third section. Direct drivers of impact caused by the marine economy are highlighted, such as the reduction of commercial fish stock size. The fourth section introduces the global concepts of LCA and the development of site-specific LCIA pathways to assess changes in local ecosystem quality, measured through biodiversity related metrics. The main limitations for global environmental sustainability assessments of marine products are exposed. The needs for site-specific marine LCIA and further data regarding the processing of marine raw materials are highlighted.

Chapter 2 quantifies trade-offs amongst seaweed farming and wild catches fisheries. Both are considered as marine natural resources and marine ecosystem services. The reduction in fisheries yields caused by the harvesting of net primary production (NPP) (*i.e.* seaweed) is estimated through a trophic food web approach. A site-specific LCIA framework relying on the seasonal ecosystem NPP, seaweed biomass growth and fish landings is proposed to assess the Lost Potential Yield (LPY) of the area under study. LPY are reported in terms of biomass, economic value and eco-exergy, a metric measuring the genomic complexity of the organisms. The framework is illustrated for the Greater North Sea and shows a net positive contribution of seaweed farming in terms of marine natural resources (*i.e.* the production of seaweed exceeds the decrease in fisheries landings for the three LPY metrics). Further research could consist in the development of additional impact pathways to NPP reduction (*e.g.* habitat provision) and on the consideration of ecosystem carrying capacity.

The following chapter (**Chapter 3**) develops a site-specific LCIA framework to assess impacts of deep seafloor disturbance on regional and global biodiversity as proxy for ecosystem quality. Changes in ecosystem quality are measured through a biodiversity-related metric: the potentially disappeared fraction of species (PDF), expressing relative changes in species richness caused by the intervention. The framework builds on existing LCIA assessing impacts on ecosystem quality from land-use (*i.e.* land transformation and occupation). According to existing literature, the framework identifies three kinds of impacts: transformation, occupation and permanent impacts that can be summed to obtain the total impact on regional and global ecosystem quality. The regional biodiversity impacts are first assessed and converted to global biodiversity impacts considering the vulnerability and the scarcity of the ecosystem impacted. The framework is operationalized in a case study consisting to polymetallic nodules mining in the Clarion Clipperton Fracture Zone (CCZ). Despite the very limited knowledge on benthic recovery from deep-sea mining, the framework shows consistency with existing LCA characterization models for biodiversity. The total impact on regional and global biodiversity is mostly influenced by the permanent impact on biodiversity because of the absence of recovery of a significant fraction of species. This framework can be

integrated into LCA studies in order to understand the global environmental sustainability of deep-sea activities.

Next to the development of additional LCIA, the availability of detailed and transparent datasets is another challenge to assess the global environmental sustainability of marine products. **Chapter 4** computes mass and energy flows associated with the harvesting and the processing of microalgae under eight biorefinery scenarios to produce lipids, proteins, energy and dried biomass. Two cell disruption methods are tested and two solvents for lipid extraction are compared. Complete flowsheets are provided for each step of the downstream processing of the raw biomass. The chapter highlights the impact of the cell disruption method on the total energy demand but also, the influence amongst downstream processes in a cascade design. Lipid extraction has influence on protein extraction, this latter improving energy production as it has a more favourable carbon to nitrogen ratio. In addition, lipids are extracted with a conventional solvent (hexane) for some scenarios and with a biobased solvent (2-methyltetrahydrofuran) for other scenarios. The azeotropic distillation required for the recovery of the biobased solvent (and thus its extra energy demand) shows that solvent selection is crucial to control the total energy demand of the process, but lipid profiles will vary according to solvent properties.

The last chapter (**Chapter 5**) consists of the conclusions and perspectives of the manuscript. Whilst the conclusions discuss the main outcomes of the three (published) research chapters (**Chapter 2, Chapter 3 and Chapter 4**), the perspective section opens a discussion on the requirement for an exhaustive classification of marine ecosystems. In a similar way as for the terrestrial ecosystems, such classification will facilitate the development of databases for marine ecosystem attributes and hence, the implementation of site-specific LCIA. Furthermore, the section discusses alternatives to species richness related metrics to monitor changes in the ecosystem quality. Different types of biodiversity are defined according to the combination of biodiversity level (*i.e.* genetic, species, communities and landscape) and biodiversity attribute (*i.e.* composition, structure, function). Consequently, it is not possible to grasp the entire complexity of biodiversity through a single indicator such as species richness in LCA methods. The use of potential additional indicators for ecosystem quality and the main challenges arising from it are discussed. Finally, the discussion highlights the importance of aligning the scope of LCA studies with the descriptors used by European policy makers to assess the environmental status of marine ecosystems (under the Marine Strategy Framework Directive, MSFD). It emphasizes the needs for additional marine LCIA to consider all descriptors identified by the MSFD (11) in LCA studies of marine products. The challenge of integrating marine ecosystem services in the scope of LCA studies is considered. Because of the complexity of quantifying ecosystem services and their link with biodiversity, the use of

regional biodiversity as midpoint indicator for ecosystem services is proposed. Finally, the section concludes by discussing the challenge of evaluating the total cumulative impact caused by different stressors on a given marine ecosystem. Whilst existing LCIAAs do not consider interactions amongst each other, it is relevant to make use of ecological risk assessment tools to model the final ecosystem response to various disturbances occurring in parallel.

To conclude, this work has emphasized two main challenges for the global environmental sustainability assessment of marine products: the implementation of site-specific LCIA frameworks and the development of datasets regarding further processing of the harvested products.

Samenvatting

Om in de behoeften van de groeiende wereldbevolking te voorzien, worden zeeën en oceanen meer en meer geëxploiteerd. Aanvankelijk was dit vooral voor voedsel en transport, nu leveren offshore zeegebieden energie en in de toekomst allicht aanzienlijke hoeveelheden mineralen. De winning van natuurlijke hulpbronnen op het land heeft grote gevolgen voor het milieu (d.w.z. verlies van biodiversiteit), maar het is ook van cruciaal belang om de wereldwijde ecologische duurzaamheid van deze mariene producten gedurende hun hele levenscyclus te waarborgen. Daarom hebben methoden voor levenscyclusanalyse (LCA) het potentieel om dergelijke informatie te verstrekken en hotspots van milieueffecten in de waardeketen van het geanalyseerde product te identificeren. Op het eindpuntniveau houden LCA-resultaten rekening met effecten op drie “beschermingsgebieden” (*Areas of Protection, AoPs*): menselijke gezondheid, ecosysteemkwaliteit en natuurlijke hulpbronnen. LCA-methoden worden echter traditioneel toegepast op industriële en terrestrische processen en zijn daarom vaak beperkt tot het opnemen van locatiespecifieke en terrestrische effecten (bijv. verstoring van het lokale ecosysteem). LCA heeft verdere ontwikkeling om de ecologische duurzaamheid van mariene producten te beoordelen, dit met het opnemen van specifieke mariene ecosysteemeffecten, en dit in relatie tot drie AoPs. Bovendien zijn kwantitatieve gegevens over massa- en energiestromen die verband houden met de gehele levenscyclus van de producten (niet alleen productie/winning van grondstoffen maar ook hun verwerking tot eindproducten) vereist om tot holistische milieuduurzaamheidsbeoordelingen te komen.

De algemene doelstelling van dit doctoraat is om LCA als methodologie te versterken zodat de milieuduurzaamheid van maritieme producten beter beoordeeld kan worden. Er worden twee operationele kaders voorgesteld om locatie-specifieke aspecten op te nemen die verband houden met de winning van mariene grondstoffen, en er worden gegevens verstrekt met betrekking tot de verwerking van biomassa. Op deze manier zal de evaluatie van de wereldwijde ecologische duurzaamheid van mariene producten door middel van LCA inclusiever en zinvoller zijn voor vergelijkende beoordelingen met terrestrische alternatieven.

Het doctoraat begint met een algemene inleiding (**Hoofdstuk 1**), verdeeld in vier delen. Eerst wordt een overzicht van de maritieme activiteiten gegeven. De belangrijkste maritieme economische activiteiten worden beschreven en het concept van de industrialisatie van de zeeën en oceanen wordt geïntroduceerd. Dit verwijst naar het groeiende belang van materialen en energie uit de zee voor de wereldeconomie. Verwacht wordt dat het belang van de mariene economie tegen 2030 een verdubbeling zal kennen. Op langere termijn zou het potentieel van diepzeemineralen onze afhankelijkheid van mariene grondstoffen aanzienlijk kunnen vergroten. Het tweede deel bevat achtergrondinformatie over de classificatie van

natuurlijke hulpbronnen en hun verband met ecosysteemdiensten. Natuurlijke hulpbronnen worden ingedeeld naar hernieuwbaarheid, uitputbaarheid en hun vorm op het moment van winning (biotisch/abiotisch). Mariene natuurlijke hulpbronnen worden gepresenteerd volgens deze classificatie en in de context van ecosysteemdiensten. Diepzeemineralen worden uitgebreid besproken omdat ze in de nabije toekomst belangrijk kunnen worden voor onze economie. De ecologische druk op mariene ecosystemen wordt besproken in de derde paragraaf. Directe druk veroorzaakt door de mariene economie wordt benadrukt, zoals de vermindering van de grootte van commerciële visbestanden. Het vierde deel introduceert de globale concepten van LCA en de ontwikkeling van locatiespecifieke LCIA-routes om veranderingen in de kwaliteit van lokale ecosystemen te beoordelen, gemeten aan de hand van metrische gegevens over biodiversiteit. De belangrijkste beperkingen voor mondiale milieuduurzaamheidsbeoordelingen van mariene producten worden blootgelegd. De behoeften aan locatiespecifieke maritieme LCIA's en verdere gegevens met betrekking tot de verwerking van mariene grondstoffen worden benadrukt.

Hoofdstuk 2 kwantificeert de afwegingen tussen de zeewierteelt en de klassieke visserij. Beide worden beschouwd als mariene natuurlijke hulpbronnen en als mariene ecosysteemdiensten. De vermindering van de visserijopbrengsten als gevolg van het oogsten van de netto primaire productie (NPP) (d.w.z. zeewier) wordt geschat op basis van een trofische voedselwebbenadering. Een locatiespecifiek LCIA-raamwerk dat steunt op het seizoensgebonden ecosysteem NPP, de groei van zeewierbiomassa en de aanvoer van vis wordt voorgesteld om de verloren potentiële opbrengst (VPO) van het onderzochte gebied te beoordelen. VPO wordt gerapporteerd in termen van biomassa, economische waarde en eco-exergie, een maatstaf die de genomische complexiteit van de organismen meet. Het raamwerk wordt geïllustreerd voor de Grote Noordzee en toont een netto positieve bijdrage van zeewierteelt in termen van natuurlijke rijkdommen van de zee (d.w.z. de productie van zeewier overtreft de afname van de aanvoer van visserij voor de drie VPO-maatstaven). Verder onderzoek zou kunnen bestaan uit de ontwikkeling van aanvullende impactpaden voor de reductie van kerncentrales (bv. habitatvoorziening) en de overweging van de draagkracht van ecosystemen.

Het volgende hoofdstuk (**Hoofdstuk 3**) ontwikkelt een locatiespecifiek LCIA-raamwerk om de effecten van verstoring van de diepzeebodem op regionale en mondiale biodiversiteit te beoordelen als maatstaf voor de kwaliteit van ecosystemen. Veranderingen in de kwaliteit van ecosystemen worden gemeten aan de hand van een biodiversiteit gerelateerde maatstaf: de potentieel verdwenen fractie van soorten (PDF), die relatieve veranderingen in soortenrijkdom als gevolg van de interventie uitdrukt. Het raamwerk bouwt voort op bestaande LCIA's die de impact op de kwaliteit van ecosystemen beoordelen door landgebruik (d.w.z. landtransformatie

en bezetting). Volgens de bestaande literatuur identificeert het raamwerk drie soorten effecten: transformatie, bezetting en permanente effecten die kunnen worden opgeteld om de totale impact op de regionale en mondiale ecosysteemkwaliteit te verkrijgen. De regionale effecten op de biodiversiteit worden eerst beoordeeld en omgezet in wereldwijde effecten op de biodiversiteit, rekening houdend met de kwetsbaarheid en de schaarste van het getroffen ecosysteem. Het raamwerk is geoperationaliseerd in een case study die bestaat uit de winning van polymetallische knollen in de Clarion Clipperton Fracture Zone (CCZ). Ondanks de zeer beperkte kennis over het benthisch herstel na diepzeemijnbouw, vertoont het raamwerk consistentie. De totale impact op de regionale en mondiale biodiversiteit wordt grotendeels beïnvloed door de permanente impact op de biodiversiteit gezien een aanzienlijk deel van de soorten niet herstelt. Dit raamwerk kan worden geïntegreerd in LCA-studies om de wereldwijde ecologische duurzaamheid van diepzeeactiviteiten beter te begrijpen.

Naast de ontwikkeling van aanvullende LCIA's is de beschikbaarheid van gedetailleerde en transparante datasets een andere uitdaging om de wereldwijde ecologische duurzaamheid van maritieme producten te beoordelen. **Hoofdstuk 4** berekent massa- en energiestromen die verband houden met het oogsten en verwerken van microalgen onder acht bio-raffinage scenario's om lipiden, eiwitten, energie en gedroogde biomassa te produceren. Twee methoden voor het verbreken van cellen worden getest en twee oplosmiddelen voor lipide-extractie worden vergeleken. Voor elke stap van de stroomafwaartse verwerking van de biomassa worden volledige stroomschema's verstrekt. Het hoofdstuk belicht de impact van de celdisruptie methode op de totale energievraag, maar ook de invloed van stroomafwaartse processen in een cascadeontwerp. Lipide-extractie heeft invloed op de eiwitextractie; deze laatste verbetert de energieproductie omdat het een gunstigere koolstof-stikstofverhouding heeft. Daarnaast worden lipiden voor sommige scenario's geëxtraheerd met een conventioneel oplosmiddel (hexaan) en voor andere scenario's met een bio-based oplosmiddel (2-methyltetrahydrofuraan). De azeotropische destillatie die nodig is voor het terugwinnen van het bio-based oplosmiddel (en dus de extra energievraag) laat zien dat de keuze van het oplosmiddel cruciaal is om de totale energiebehoefte van het proces te beheersen, waarbij de lipidenprofielen zullen variëren volgens de oplosmiddeleigenschappen.

Het laatste hoofdstuk (**Hoofdstuk 5**) bevat de conclusies en perspectieven van het manuscript. Terwijl de conclusies de belangrijkste resultaten van de drie (gepubliceerde) onderzoekshoofdstukken (**Hoofdstuk 2, Hoofdstuk 3 en Hoofdstuk 4**) bespreken, opent de perspectiefsectie een discussie over de vereiste voor een uitputtende classificatie van mariene ecosystemen. Op dezelfde manier als voor de terrestrische ecosystemen, zal een dergelijke classificatie de ontwikkeling van databases voor kenmerken van mariene ecosystemen vergemakkelijken en dus de implementatie van locatiespecifieke LCIA's. Verder bespreekt de

sectie alternatieven voor metrische gegevens over soortenrijkdom om veranderingen in de ecosysteemkwaliteit te volgen. Verschillende soorten biodiversiteit worden gedefinieerd volgens de combinatie van biodiversiteitsniveau (d.w.z. genetisch, soorten, gemeenschappen en landschap) en biodiversiteitsattribuut (d.w.z. samenstelling, structuur, functie). Bijgevolg is het niet mogelijk om de gehele complexiteit van biodiversiteit te vatten via één enkele indicator, zoals soortenrijkdom in LCA-methoden. Het gebruik van mogelijke aanvullende indicatoren voor de kwaliteit van ecosystemen en de belangrijkste uitdagingen die daaruit voortvloeien, worden besproken. Ten slotte benadrukt de bespreking het belang van het afstemmen van de reikwijdte van LCA-onderzoeken met de descriptorren die door Europese beleidsmakers worden gebruikt om de milieutoestand van mariene ecosystemen te beoordelen (onder de Mariene Strategie Kaderrichtlijn, KMS). Het benadrukt de noodzaak van aanvullende maritieme LCIA's om alle descriptorren die door de KMS (11) worden geïdentificeerd in LCA-onderzoeken van mariene producten in aanmerking te nemen. De uitdaging om mariene ecosysteemdiensten te integreren in de scope van LCA-studies wordt overwogen. Vanwege de complexiteit van het kwantificeren van ecosysteemdiensten en hun verband met biodiversiteit, wordt voorgesteld om regionale biodiversiteit als middelpuntindicator voor ecosysteemdiensten te gebruiken. Ten slotte wordt dit gedeelte afgesloten met een bespreking over de uitdaging van het evalueren van de totale cumulatieve impact veroorzaakt door verschillende stressoren op een bepaald marien ecosysteem. Hoewel bestaande LCIA's geen rekening houden met onderlinge interacties, is het relevant om gebruik te maken van ecologische risicobeoordelingsinstrumenten om de uiteindelijke ecosysteemrespons op verschillende parallel optredende verstoringen te modelleren.

Concluderend heeft dit werk de nadruk gelegd op twee belangrijke uitdagingen voor de wereldwijde beoordeling van de ecologische duurzaamheid van mariene producten: de implementatie van locatiespecifieke LCIA-kaders en de ontwikkeling van datasets met betrekking tot de verdere verwerking van de oogst.

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List of abbreviations

AC	Avoided costs
AoP	Area of protection
BCE	Before the common era
BM	Bead mill
CCZ	Clarion Clipperton Fracture Zone
CE	Common era
CEFAS	Centre for Environment, Fisheries and Aquaculture Science
CEVA	Centre d'étude et de valorisation des algues
CF	Characterization factor
CICES	Common International Classification of Ecosystem Services
CNS	Central part of the North Sea
DISCOL	Disturbance and recolonization experiment
DNA	Deoxyribonucleic acid
DW	Dried weight
EBM	Ecosystem based management
EC	European Commission
EEZ	Exclusive economic zone
EF	Effect factor
ES	Ecosystem services
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FD	Functional diversity
Fe-Mn	Ferromanganese
FF	Fate factor
FL	Fish landings
FW	Fresh weight
GWh	Gigawatt hours
HANPP	Human appropriated net primary production
HPH	High pressure homogenization
HRT	Hydraulic retention time
HVAC	Heating ventilation and air conditioning

ICES	International Council for the Exploration of the Seas
IEA	International Energy Agency
ISA	International Seabed Authority
ISO	International Organisation for Standardization
IUCN	International Union for the Conservation of Nature
LCA	Life cycle assessment
LCI	Life cycle inventory
LCIA	Life cycle impact assessment
Lh.D.	Lipids hexane extraction, drying the biomass
Lh.E.	Lipids hexane extraction, methane and fertilizers production
Lh.P.E.	Lipids hexane extraction, proteins extraction, methane and fertilizers production
Lm.P.E.	Lipids 2-MeTHF extraction, proteins extraction, methane & fertilizers production
LHV	Lower heating value
LPY	Lost potential yield
LW	Living weight
MEA	Millennium Ecosystem Assessment
MGR	Marine genetic resources
MJ	Megajoules
MSFD	Marine Strategy Framework Directive
MW	Megawatt
MSY	Maximum sustainable yield
MV	Market value
NDI	Naturalness degradation indicator
NIOZ	Royal Netherlands Institute for Sea Research
NNS	North part of the North Sea
NPP	Net primary production
OECD	Organisation for Economic Co-operation and Development
PAF	Potentially affected fraction of species
PDF	Potentially disappeared fraction of species
PEF	Product Environmental Footprint
REE	Rare-earth elements

RNA	Ribonucleic acid
ROFI	Region of freshwater influence
SAR	Species area relationship
SDG	Sustainable development goal
SETAC	Society of Environmental Toxicology and Chemistry
SNS	Southern part of the North Sea
SMS	Seafloor massive sulfide
SPI	Soy protein isolate
TAG	Triacylglyceride
TEEB	The Economics of Ecosystems and Biodiversity
TLW	Ton of living weight
TMP	Theoretical methane potential
UN	United Nations
UNEP	United Nations Environment Programme
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNCLOS	United Nations Convention for the Law of the Sea
UNFC	United Nations Framework Classification for Resources
WEF	World Economic Forum
WTO	World Trade Organization
WTP	Willingness to pay
WF	Weighting factor
WWF	Worldwide Fund for Nature
WWTP	Wastewater treatment plant
2-MeTHF	2-Methyltetrahydrofuran

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CHAPTER 1

General introduction and research objectives

1.1. An overview of marine activities

About 71% (360 million km²) of the Earth's surface is covered by seas and oceans forming a vast marine environment that has a crucial role in human well-being and in global economic development (Eakins and Sharman, 2010). The use of oceans by humans is not a recent event, starting with search for food on shore and nearshore areas during prehistoric times. Long lasting overseas travels and oceans were the next step. Although an early occurrence (e.g. Polynesians through the Pacific by 3000 BCE, Vikings crossing the Atlantic by 1000 CE), they were especially driven in 16th to 19th centuries, aiming to chart coasts, to find new lands and richness (Matisoo-Smith and Robins, 2004; Hudson, 2005). Scientific explorations started at the end of the 18th century with James Cook's voyages (1768 - 1779) followed a little later by HMS Beagle (1831 - 1836) and HMS Challenger (1872 - 1879) expeditions (Lincoln, 2001). The exploitation of marine ecosystems has intensified with drilling for oil and gas in the 20th century and has diversified into new activities complementing the continental production. Our exploitation of marine resources is nowadays worldwide and becoming more sophisticated, e.g. the development of deep-water drilling at depth of 2450 meters in the Gulf of Mexico (Stow, 2017).

Fisheries, maritime transport and submarine cables were first set up, but the use of seas and oceans has progressively extended to aquaculture production, oil, gas, minerals extraction and offshore wind energy supply (Smith, 2000). **Marine fishery production** is worldwide and relatively stable since the late 20th century with 84.4 million tons (living weight, LW) caught in 2018 (FAO, 2020b). On the other hand, marine and inland aquaculture productions are rising in response to the growing demand. **Marine aquaculture production** represents today 17.2% (LW) of the total fishery outputs (excluding seaweeds and seagrasses) (FAO, 2020b) and its total production is expected to double between 2010 and 2030, driven by Asian countries (World Bank, 2013; OECD, 2016). In addition, seaweed aquaculture, traditionally farmed in Asia for food purposes, has gained interest worldwide because the exploited seaweed species and their related applications have diversified to value added products such as nutraceuticals and pharmaceuticals ingredients (Seghetta *et al.*, 2016; Zhou, 2017; Ferdouse *et al.*, 2018). While the growth of commercial production from harvesting remains minor, seaweed aquaculture jumped from 13.4 million tons (fresh weight, FW) per year in 2005 to 32.4 million tons (FW) in 2018 (Ferdouse *et al.*, 2018; FAO, 2020b). This growth is expected to continue especially because of the interest of sectors that aim for higher value applications such as for the production of pharmaceutical or cosmetics ingredients (Buschmann *et al.*, 2017). Besides fisheries and aquaculture, seas and oceans are experiencing increased **maritime transportation** since the middle of the 20th century due to intense trans-continental commercial activities. Nowadays, maritime transportation continues to follow a long-term

growth, especially in the Pacific ocean (UN, 2019). **Submarines cables** laid on the seafloor are involved in communications (telegraph, telephony, optical telecommunication) or in energy transportation (power cables). Communication cables were first locally installed at the end of the 19th century (telegraph cables), forming since that early time a complex expanded network (Carter *et al.*, 2009). Today, owing to the development of new technologies (e.g. optical fibers) the deployment of new cables ensures most (up to 99%) of worldwide telecommunications (Pilipetskii, 2019). Power cables have developed during the 20th century and are now crucial in local supply of electric power (e.g. from continents to islands) or in transferring electric power produced offshore (wind farms) (Ardelean and Minnebo, 2015). The **offshore energy sector** has experienced substantial growth in the last decade with more than a quarter of total oil and gas supply currently produced offshore (IEA, 2019b). This contribution is expected to increase in the coming years because of decreasing exploitation costs of offshore extraction and growing demand for natural gas (IEA, 2018). In addition, the total worldwide production capacity of offshore wind farms has grown from 2,134 MW in 2009 to 28,308 MW in 2019 (representing 0.3% of global energy supply) and this value is expected to double by 2025 (IEA, 2019b; Sönnichsen, 2020). **Sea-floor mining** also developed since decades, with the extraction of various mineral resources. Gravels, and sands (and diamonds) are extracted from marine aggregates in coastal areas (usually at depths less than 50 m) (Baker *et al.*, 2016) while metals (manganese, cobalt, nickel, copper) start to be prospected in deep-sea (Sparenberg, 2019). With the sand crisis just around the corner (Smith, 2018), the extraction of marine aggregates is expected to become more intense and to occur at greater depths (Bide and Mankelow, 2014). One can expect mining activities to be no longer limited to coastal and nearshore areas because mining of phosphate, cobalt-rich crusts, massive sulfides or manganese nodules become a real option to supply materials for the development of the renewable energy sector (Cuyvers *et al.*, 2018). Last but not least, about 40% of the world population is living in coastal areas and population growth induces rising pressure on nearshore environments. Moreover, some coastal and nearshore areas face another type of economic fast-growing activity, namely coastal tourism and leisure. This sector is developing since the middle of the last century and presents the highest growth amongst all marine economic activities (Hall, 2001; OECD, 2016).

The increasing intensity of commercial activities in marine environments led authors to refer to the “*industrialization of seas and oceans*” or “*industrial revolution of the seas and oceans*” (Smith, 2000; Salcido, 2008; Golden *et al.*, 2017). This indicates that seas and oceans are now facing an intensification of human interventions in a similar way to terrestrial environments with the industrial revolution that started in the middle of the nineteenth century. This vision is supported by the positions of the Organization for Economic Co-operation and Development

(OECD) and by the European Union (EU) which decided to increase research and development projects in marine sectors for their potential to drive the global economic growth (OECD, 2016; EC, 2018b). The contribution of marine economic activities (including all downstream processing of marine outputs) is estimated at USD 1.5 trillion (in 2010, best estimates available), representing 2.5% of world gross value added¹ (GVA) (OECD, 2016). The oil and gas offshore extraction and the coastal tourism are by far the largest contributors in terms of total economic value, representing respectively 34% and 26% of the total GVA of marine activities in 2010. For most conservative scenarios, the GVA from marine activities is expected to double by 2030 and will still be dominated by the same sectors (OECD, 2016).

The expansion of the blue economy has raised concerns on the sustainable use of the seas and oceans to avoid similar environmental damages as the industrial revolution did for terrestrial environments (McCauley *et al.*, 2015; Golden *et al.*, 2017). Today, politics refer to the “*blue economy*” or “blue growth” when discussing management practices for sustainable growth in the marine economy (EC, 2018b; Rayner *et al.*, 2019). The protection of marine life is also part of the 17 sustainable development goals (SDG) set by the United Nations (UN, 2020). Goal 14, “*Conserve and sustainably use the oceans, seas and marine resources for sustainable development*”, consists of multiple actions that are addressed under coastal and marine policies (EC, 2019). In this context, the present PhD focuses on marine production systems and more precisely, on the development of scientific knowledge regarding the development and application of environmental sustainability assessment methods. The next section provides an overview of marine resources and introduces ecosystem services in order to better underline the scope of the research.

¹ The gross value added (GVA) is measured by the total price of the outputs subtracted by the total price of the intermediate products. It represents the total value of goods and services produced in a given area. The gross domestic product (GDP) is obtained by summing the GVA with the total taxes and subtracting subsidies (Eurostat, 2019).

1.2. Natural resources and ecosystem services

1.2.1. A definition for natural resources and ecosystem services

Before detailing the different flows of valuable outputs extracted from seas and oceans and the consequences for involved ecosystems, the global concepts related to the exploitation of the natural environment are reviewed. This section discusses the state of the art related to terminologies and classifications for natural resources and ecosystem services. It allows to understand the complexity of natural resources, the targeted resources considered in this PhD research and their dependence on ecosystem health.

1.2.1.1. Delimiting the scope of natural resources

The OECD defines natural resources as “*natural assets (raw materials) occurring in nature that can be used for economic production or consumption*” (OECD, 2001). On the other hand, the World Trade Organization (WTO) considers that natural resources are scarce, have direct economic value and, consist of raw materials or slightly transformed materials (WTO, 2010). This perspective including transformed materials focuses on resources in their commercial form. In addition, the scarcity and the direct economic value aspects exclude commodities that are not traded in markets such as seawater or air. The complexity in defining natural resources results from the different contexts in which they are considered: international trade focuses on the economic value as key aspect. This criterion is replaced by their utility to create economic value in other definitions including a wider spectrum of elements (Fischer-Kowalski *et al.*, 2011; Sonderegger *et al.*, 2017). In this second perspective, some elements that are not scarce or marketable are considered as natural resource (e.g. seawater) because of their intervention in the production process of marketable goods. This thesis adopts this broader definition for discussing natural resources.

Meanwhile, natural resources may be further classified from different perspectives. A first classification focuses on the type of natural resources at the time of extraction. It differentiates between biotic and abiotic natural resources. Biotic natural resources are living organic materials until the extraction from their natural environment (e.g. wood), whilst abiotic resources are inorganic materials (e.g. minerals) or dead organic materials (e.g. fossil fuel) at the moment of extraction (Lindeijer *et al.*, 2002; Klinglmair *et al.*, 2014). The other classification focuses on the renewability of natural resources for their classification and therefore differentiates amongst stock, fund and flow resources (Lindeijer *et al.*, 2002). Stock resources occur in finite amounts because of their non-renewability on humans' time perspective (e.g. fossil fuel). Consequently, they are considered as exhaustible because they are continuously depleted by extraction. On the contrary, fund resources are considered as renewable as they come from ecosystems with biotic resources (e.g. wood) or from physical processes related to

abiotic resources (e.g. groundwater). The abundance of these resources increases or decreases according to the balance of extraction rate versus regeneration rate. However, fund resources are always considered as exhaustible because they might be depleted in case of overexploitation (De Haes, 2002). Flow resources are non-exhaustible but limited in total amount (e.g. wind, land). They are not dissipated during their extraction and they are considered as renewable when occurring periodically at the same location (Sonderegger *et al.*, 2017).

The two classifications for natural resources are valid but their application depends on the perspective to highlight. However, they do not exclude each other, and their conciliation is shown in Figure 1- 1. Abiotic materials belong to fund, flow and stock resources depending on their renewability and exhaustibility. For example, coral sand reserves and availability can increase or decrease depending on the management practices and thus considered as fund resources. On the other hand, other abiotic resources such as tidal energy are considered as flow resources because they are continuously supplied independently of their extraction (non-exhaustible), but occur in limited amount. Finally, some abiotic resources such as minerals or fossil fuels are considered as stock resources because their renewability is not ensured on human time perspective.

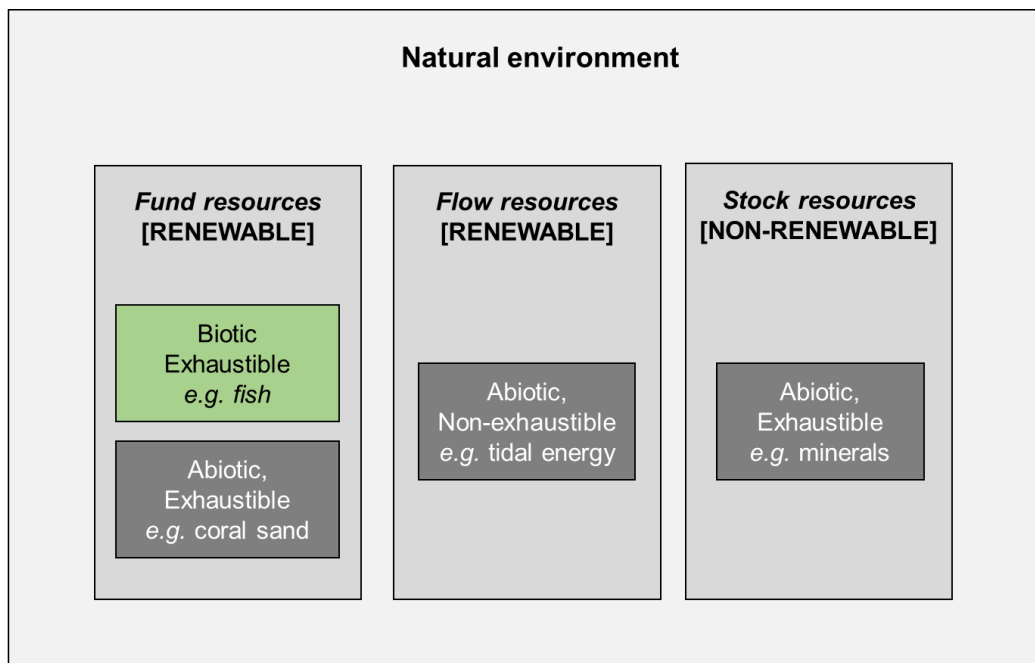


Figure 1- 1 Classification of natural resources, examples are provided in italics.

In this global vision, ecosystems are presented as a natural capital because they generate revenues from provisioning fund biotic resources but also, from maintaining the quality of

abiotic fund and flow resources such as clean water or fertile land (MEA, 2005). Moreover, they can be depreciated in case of unsustainable management (Costanza and Daly, 1992) leading to exhaustibility of biotic fund resources (Power, 2010). The self-maintenance of this natural capital (*i.e.* ecosystems) and its provision of renewable natural resources are ultimately related to solar energy inputs (Costanza and Daly, 1992).

Biotic fund resources are frequently not exhausted due to the relatively fast regeneration capacity of ecosystem functioning. However, they may become exhausted if exploitation exceeds the maximum sustainable yield level (MSY), *i.e.* the harvest generating the highest economic return without compromising future harvests. This notion has been introduced for fisheries management in the 1950's (Schaefer, 1954, 1959) and rapidly extended to forestry and hunting management² (Walter, 1980; Robinson and Redford, 1994).

The terminology regarding stock resources extraction can be further detailed according to the recommendations from the *United Nations Framework Classification for Fossil Energy and Mineral Resources* (UNFC) (UN, 2009). The UNFC differentiates amongst reserves, remaining resources and total resources. Reserves consist of the fraction of natural resources for which the exploitation is economically viable given the current technology level. Remaining resources are proven and non-proven stocks of valuable elements that cannot be exploited yet because of technology limitation (increasing the exploitation costs) or, because of the current status of geological knowledge. The term total resources is used when referring to reserves and resources on the same time (UN, 2009). For the sake of consistency with fund resources terminology, the present thesis refers to stock resources to designate total stock resources in UNFC terms.

Apart from the extraction of biotic fund resources from ecosystems, biomass may also be produced from farming systems (*i.e.* agriculture and aquaculture). The present thesis does not consider the outputs from farming systems as biotic fund resources despite their similarity in their composition. Rather than being generated by the natural capital (*i.e.* ecosystems), this kind of biomass is provided by agroecosystems³ (part of the technosphere) consisting of planned and unplanned species diversities (Power, 2013). The functioning of agroecosystems (and hence, the production of biomass) is ensured by multiple production factors such as abiotic resources (*e.g.* mineral fertilizers, occupied land or sea surfaces), the manufactured

² The exploitation of fisheries above their MSY is further detailed in 1.3.

³ Agroecosystems consists of ecosystems modified by humans to produce agricultural products, *i.e.* biomass (Conway, 1987). Therefore, this definition does not include soilless production systems since they are not connected to ecosystems anymore. This manuscript considers aquaculture as part of the agroecosystems it relies on ecosystems for some aspects (water quality, pest control) but some aquaculture systems disconnected from ecosystem functioning (*e.g.* microalgae production in photobioreactor) are no longer considered as agroecosystems.

capital (e.g. agricultural equipment) and the human capital (e.g. employees). Meanwhile, conventional agricultural practices may impact ecosystem functioning and reduce therefore the production of biotic fund resources (Schaller, 1993). This phenomenon is also observed for aquaculture (Buschmann *et al.*, 2006; Clavelle *et al.*, 2019) and illustrated by a case study in the thesis (**Chapter 2**). While agroecosystems management practices may induce ecosystem disservices, the productivity of agroecosystems strongly relies on ecosystem functioning (Power, 2010; Garbach *et al.*, 2014). The dependence of socio-economic systems on ecosystems functioning is referred to as “ecosystem services” (MEA, 2005). Natural resources can be considered from the perspective of ecosystem services to highlight their dependence on ecosystem functioning and their vulnerability to human disturbance (Curtin and Pallezo, 2010).

1.2.1.2. Ecosystem services and their influence on natural resources

The field of ecosystem services has received growing scientific interest since the late 1990's (Fisher *et al.*, 2009). The main contribution to this transdisciplinary research is the publication in 2005 of the Millennium Ecosystem Assessment (MEA), a consortium of more than 1300 scientists that investigated the relations between society and natural ecosystems (MEA, 2005). This research area identifies and quantifies the benefits delivered from ecosystems to socio-economic systems (*i.e.* ecosystem services) in order to facilitate decision-making and to understand trade-offs between the development of an economic activity and the subsequent ecosystem service losses (Daily, 1997). Ecosystem services are intrinsically related to biodiversity patterns that determine ecosystem functioning (Figure 1- 2). Socio-economic systems make use of ecosystem services for their different value aspects (e.g. direct economic value for goods provided by ecosystems) and influence long-term provision of ecosystem services through impacting biodiversity.

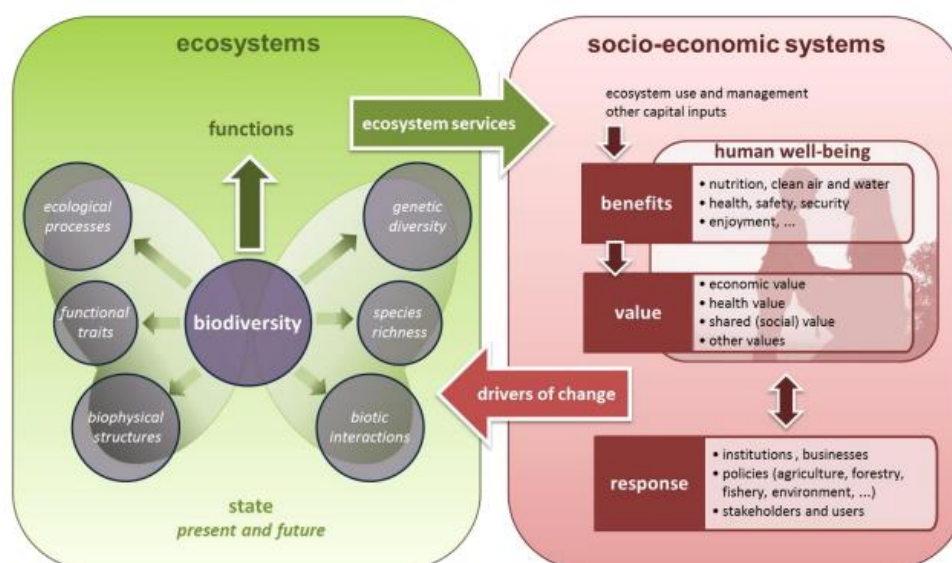


Figure 1- 2 Conceptual framework for ecosystem services and ecosystem functioning, Maes et al. (2012).

Such natural benefits are classified in four main categories: (1) provisioning services of products obtained from ecosystems (e.g. food, genetic resources, heating wood, etc.); (2) regulating services of benefits obtained from the regulation of ecosystem processes (e.g. water purification, pest control, pollination, etc.); (3) cultural services of non-material benefits (e.g. aesthetic, recreation, education, etc.); (4) supporting services needed for the production of the three other types of ecosystem services (e.g. soil formation, primary production and nutrients cycling) (MEA, 2005). However, the classification of ecosystem services is slightly different amongst classification frameworks that have been proposed later. Today, three main classification frameworks are co-existing: the MEA, The Economics of Ecosystems and Biodiversity (TEEB) and, the Common International Classification of Ecosystem Services (CICES) (Sukhdev and Kumar, 2008; CICES, 2021). The CICES considers three types of ecosystem services: (1) provisioning services, (2) cultural services and (3) regulating and maintenance services (including both regulating and supporting services in MEA terms) (CICES, 2021). Similarly, the TEEB considers three categories of ecosystem services: (1) provisioning services, (2) cultural services and (3) regulating services (overlapping with supporting services in MEA terms). The ambiguity regarding ecosystem services classification has already been pointed out (La Notte et al., 2017). However, the aim of these frameworks is similar and proposes a detailed classification in order to quantify and map ecosystem services at different spatial scales. In order to understand how ecosystem properties determine the supply of ecosystem services, the cascade framework from Haines-Young and Potschin (2009) is widely adopted and identifies five connected aspects: ecosystem properties, functions, services, benefits, and values (Haines-Young and Potschin, 2009) (Figure 1- 3). This allows one to understand how a change at a given level will affect the value of the

ecosystem services. Figure 1- 3 illustrates the concept for net primary production (ecosystem property) and income from fisheries (ecosystem service value).

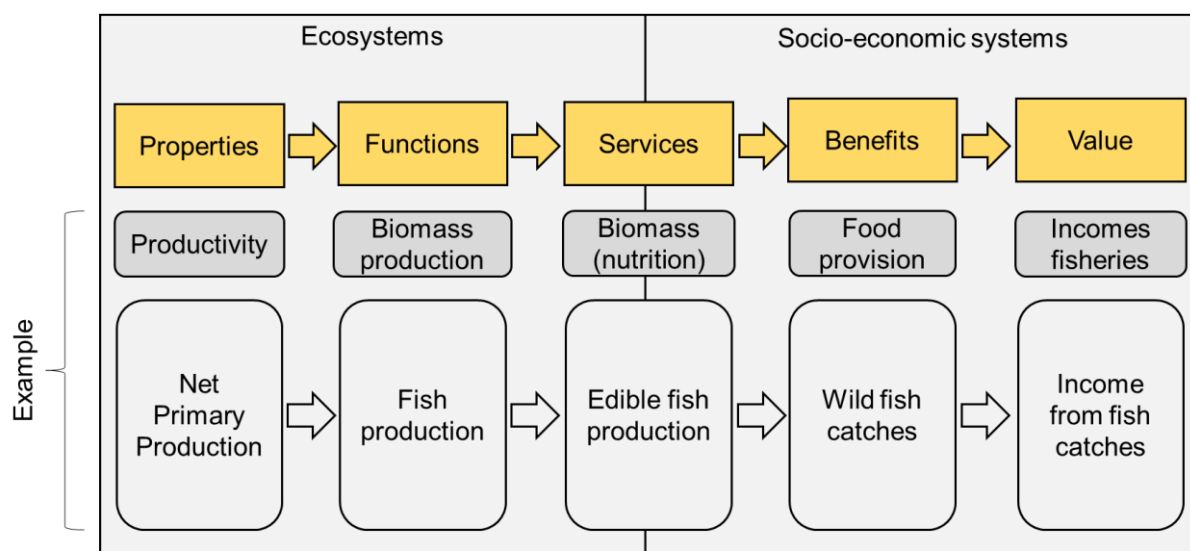


Figure 1- 3 Cascade framework for ecosystem services and illustration for fisheries, adapted from Haines-Young and Potschin, (2009); de Groot *et al.*, (2010).

In order to understand trade-offs amongst the loss of ecosystem services engendered by gains in other economic sectors, endpoint indicators for ecosystem services are quantified in monetary terms when possible. The market value, for most of ecosystem services other than provisioning services, is difficult to estimate because those are not sold on the market of goods and services (e.g. pollination, erosion prevention) (Costanza *et al.*, 1998). Therefore, their economic assessment is performed through indirect market valuation methods such as willingness to pay or hedonic pricing (De Groot *et al.*, 2002).

Ecosystem services and their classifications are becoming central in decision making for environmental policies. The 2020 EU Biodiversity Strategy has emphasized on mapping ecosystems and their services belonging to member states are one of its main targets (EC, 2011; Maes *et al.*, 2012). In this perspective, many economic activities are strongly relying on ecosystem services, and ecosystem management must be performed in order to deliver services in a sustainable way (Geneletti, 2011).

1.2.2. Understanding natural resources and ecosystem services

The previous paragraphs introduced classification of natural resources and the importance of ecosystem functioning to deliver services. The concept of MSY for biotic resources exhaustibility has been pointed to and agroecosystems have been defined. Figure 4 represents in a simplified scheme the concepts brought in 1.2.1.1 and 1.2.1.2. The figure considers two main compartments: the man-made and the natural environments. The man-made

environment, consisting of human and manufactured capital, is sustained by inputs from the natural environment. Both biotic and abiotic resources, extracted from the natural environment to ensure the functioning of the man-made environment, consist of ecosystem services according to the extended version of CICES (v5.1) (CICES, 2021). More specifically, biotic fund resources (e.g. wild fish) are provided by ecosystem functioning and consist of ecosystem provisioning services (Figure 1- 1, Figure 1- 4). On the other hand, the quality of some abiotic fund resources relies on ecosystem functioning to provide regulating services (e.g. soil formation). Other abiotic fund resources do not rely on ecosystem functioning at all (e.g. groundwater supply). Ecosystem regulating services are also playing a key role in the quality of some flow resources such as freshwater, seawater or air that are intrinsically abiotic, but they strongly rely on ecosystem functioning for their maintenance. Considering this first approach, biotic fund resources consist of ecosystem provisioning services while ecosystem regulating services are crucial to ensure the quality of some abiotic fund and flow resources. The provision of flow and stock resources does not belong to ecosystem functioning and thus, should not be classified as ecosystem services. However, the extended classification of the CICES framework (v5.1) considers all abiotic materials (from fund, flow and stock resources) as ecosystem provisioning services because they are supplied from the natural environment (Figure 1- 4).

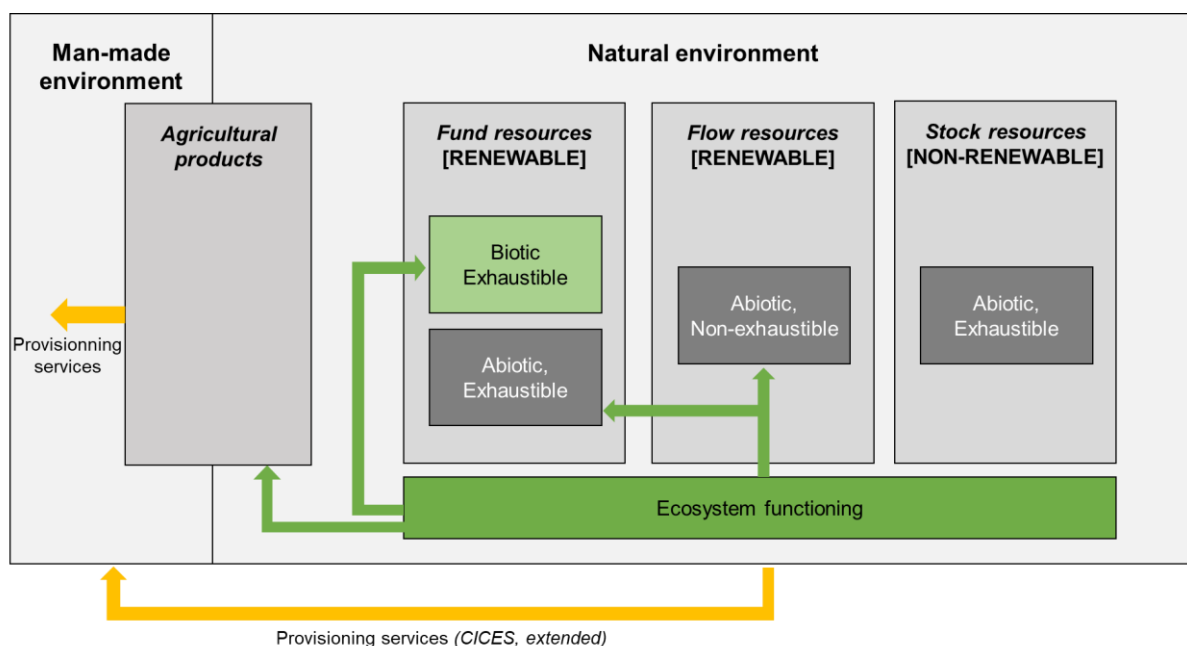


Figure 1- 4 Interactions between natural resources, ecosystem functioning and ecosystem services. Green arrows represent ecosystem functioning importance to sustain the quality or quantity of natural resources; yellow arrows represent ecosystem provisioning services, consisting in the extraction of natural resources from the natural environment.

Ecosystem services are known to play a key role in agroecosystems that rely both on the man-made environment (e.g. labour, energy, fertilizers) and on the natural environment (Figure 1-

4). Ecosystem regulating services, such as pollination for crops or clean water provision for aquaculture, have a direct influence on the productivity of the agroecosystem. Similarly as for biotic fund resources, the production from agroecosystems is considered as an ecosystem provisioning service despite the intervention of the man-made environment in the production (MEA, 2005; Power, 2010; Wratten *et al.*, 2013).

1.2.3. Marine natural resources and ecosystem services

In this paragraph, marine natural resources are listed (Table 1- 1) and classified according to the previous classification (Figure 1- 1). It allows one to understand which types of marine natural resources are considered in this PhD research and how they interact with each other. Furthermore, it highlights the two-sided relation between ecosystem functioning and natural resources extraction that is discussed further in section 1.3. Three topics are successively considered here: marine natural resources that are currently exploited, marine natural resources that have not been commercially exploited so far, and background information on marine ecosystem services.

Table 1- 1 Tentative and non-exhaustive classification of marine resources and their exploitation status. MGR, marine genetic resources. () Including methane hydrates; (**) wave energy harvesting buoys and breakwater systems.*

Categories	Type	Exhaustibility	Resources	Exploitation	References
Fund resources	Biotic	Exhaustible	Fish / shellfish	Yes	FAO, 2020
			Seaweed	Yes	Ferdouse <i>et al.</i> , 2018
			MGR	No	Leary <i>et al.</i> , 2009
			Active compounds	Yes	Suleria <i>et al.</i> , 2016
			Coral reef	Yes	Naseer, 1997
	Abiotic	Exhaustible	Coral sand	Yes	Daley <i>et al.</i> , 2006
Stock resources	Abiotic	Exhaustible	Oil and gas*	Yes	Salcido, 2008
			Minerals	No	Miller <i>et al.</i> , 2018
			Aggregates	Yes	Charlier <i>et al.</i> , 1992
Flow resources	Abiotic	Non-exhaustible	Tidal	Yes	Johnstone <i>et al.</i> , 2013
			Waves**	Yes	Levitan, 2014
			Dissolved minerals	Yes	Thys, 2003
			Seawater	Yes	Cisternas <i>et al.</i> , 2018

1.2.3.1. Overview of currently exploited marine natural resources

Marine fund resources

Compared to terrestrial environments, the exploitation of marine resources is limited to a few sectors but their contribution to the global supply of natural resources is crucial for the stability of the society. For example, the annual fish production from marine fisheries exceeds by far the annual production of poultry or cattle (Béné *et al.*, 2016). In addition, fish provides essential nutrition to more than one billion humans (The World Bank, 2011) and many low income countries rely mostly on seafood products for animal proteins ingestion (Garcia and Rosenberg, 2010; Kawarazuka and Béné, 2011). At the world population level, marine wild fisheries delivered in 2017 more than 10% of the total animal proteins ingested (FAO, 2020b). Another type of marine fund resources that is commercially exploited consists of seaweed. However, most of world seaweed production (*i.e.* 96%) is now produced from marine aquaculture practices and Asian countries are contributing to most of the supply but their range of applications is similar as for harvested seaweed (White and Wilson, 2015; Ferdouse *et al.*, 2018). Traditionally used for fertilizers and feedstock in Northern Europe (Kain and Dawes, 1987) or consumed for food in Asia, seaweed applications are now far more diversified (Ferdouse *et al.*, 2018). For example, the food processing and cosmetic industries make use of phycocolloids from seaweed for their emulsifying properties. On the other hand, seaweed extracts are gaining interest in the pharmaceutical and nutraceutical sectors (O'Connor, 2017). The isolation, characterization and clinical trials of marine active compounds for drugs production started in the 1950's and experienced renewed interest from the industry (Alves *et al.*, 2018). They mostly consist of secondary metabolites from marine fauna and flora (terpenoids, alkaloids, sugars). Although their industrial applications are still limited, they are expected to grow significantly with the increasing number of described compounds (Nair *et al.*, 2015; Suleria *et al.*, 2016; Alves *et al.*, 2018). Another type of fund resources extracted from the marine environments consists of living corals. The exploitation of coral reef, called coral mining, is rather limited (mainly in the Indian Ocean) and used to deliver primary materials for construction and also for diverse applications such as jewels or aquarium landscape (Brown, 2011). All those marine fund resources cited above consist of biotic material and can be considered as provisioning ecosystem services. Marine fund resources also include abiotic material such as coral sand but its harvesting is rather symbolic (Daley and Girggs, 2006).

Marine stock resources

Amongst marine stock resources, offshore oil and gas has started in the late nineteenth century with first drilling activities on the US nearshore (AOGHS, 2010). Nowadays, offshore fossil energy contributes significantly to the global energy supply and represents now more than one quarter of total oil and gas annual production (IEA, 2019b). As many exploited oil and gas fields

are becoming exhausted, companies are moving progressively to deep-water production (*i.e.* higher water depths than 400 meters or more) (Lange *et al.*, 2014). So far, offshore fossil energy is the largest contributor to the total value of resources extracted from marine environments (OECD, 2016). Marine aggregates (*i.e.* non-metallic unconsolidated materials consisting of sands, gravels, granulates, shells) also have a commercial interest especially for the construction and beach replenishment sectors. In northern Europe, they face intense commercial exploitation with about 50 million cubic meters exploited annually (Velegrakis *et al.*, 2010; EMODNET, 2020).

Marine flow resources

The main component of marine flow resources is the seawater itself presenting a large panel of applications such as in the mining sector (Cisternas and Gálvez, 2018) or in the HVAC sector. The dissolved minerals (*e.g.* salt, magnesium) from the sea are considered as flow resources in this thesis because of their perpetual occurrence / non-exhaustibility regardless of the extraction intensity. The production of energy from oceans does not present many commercial applications yet because of technological, environmental and financial barriers (Magagna and Uihlein, 2015). So far, tidal barrage is the most advanced technology with two large-scale tidal barrages being operational (France and South Korea, respective annual capacity 500 GWh and 550 GWh) and some smaller projects in China, Canada and Australia (Kim, 2016; EDF, 2019). Other set ups to recover tidal energy such as tidal turbines are being implemented but are still limited to testing projects (Edmond, 2020). Similarly, the commercial applications of wave energy recovery are limited. Energy recovery from wave breakwater systems is being commercially exploited in Spain (bay of Biscay) and produces annually 246 kWh of renewable electricity but buoys are being more intensively studied (Uihlein and Magagna, 2016; Ibarra-Berastegi *et al.*, 2018). Compared to the annual production of electricity from hydropower (4,325,111 GWh) or from wind energy (1,273,409 GWh) in 2018 (IEA, 2020), the production of electricity from oceans energy is still limited with slightly more than 1,000 GWh produced from few commercial plants. However, technologies to produce electricity from tidal and wave energy are expected to achieve commercial applications, the European Union intending to supply 10% of its total electricity demand with ocean energy by 2050 (EC, 2014; Collombet, 2020).

1.2.3.2. Overview of marine natural resources with potential commercial interest

Marine fund resources

Marine environments also contain fund resources that are not yet exploited but that present a growing economic interest. Amongst them, marine genetic resources (MGR) namely genetic material from marine organisms (DNA, RNA, amino-acid sequences) inform sequence data

expressing products of interest (in pharmaceutical, molecular biology, cosmetics and bioremediation sectors) (Arrieta and Duarte, 2013). An increasing amount of MGR-related patents are being released each year (UN, 2016; Oldham *et al.*, 2017) but their commercial exploitation is still facing challenges. Most of MGR are related to organisms living in extreme environments such as hydrothermal vents, polar or abyssal environments (Arrieta *et al.*, 2010). Therefore, most of these areas are located beyond exclusive economic zones (EEZ) and thus experience ambiguity regarding their exploitation that is currently limited to bioprospecting⁴ (Jørem and Tvedt, 2014). The international legislation regarding the exploitation of areas beyond EEZ is driven by the United Nations Convention on the Law of the Sea (UNCLOS) (see also next paragraph) (UN, 1982). Overall, the convention is facing legal gaps regarding the exploitation of MGR and the conservation of marine biodiversity (Kristina *et al.*, 2018). However, the United Nations are currently developing additional regulations that should support the development of MGR exploitation (Rabone *et al.*, 2019; Humphries *et al.*, 2020).

Marine stock resources

This part is extensively discussed because it deals with a resource for which the exploitation is very close to be operational, that is highly controversial and that might significantly affect the global economy through its magnitude of supply (Van Dover, 2011; OECD, 2016; Beaulieu *et al.*, 2017; Paulikas *et al.*, 2019).

The transition in the energy sector increases pressure on the terrestrial minerals stocks (Moss *et al.*, 2013). The European Commission monitors the criticality of raw materials according to their economic importance and their supply risk. In 2020, the most recent report identifies 30 metals considered as critical (EC, 2020). Therefore, the exploitation of deep-sea mineral resources is becoming more attractive for the supply of critical minerals such as lithium and cobalt (Volkmann and Lehnert, 2018) and thus appears to play a role in driving the sustainable economic development (Paulikas *et al.*, 2019)⁵. Deep-sea mineral resources are known since more than a century with their first recovery from the Challenger expedition (1873 – 1875) (Baturin *et al.*, 1998), but their exploitation is still uneconomic due to major technical and legal barriers. Deep-sea minerals resources consist of ferromanganese (Fe-Mn) crusts, polymetallic nodules and seafloor massive sulfides (SMS). Fe-Mn crusts are enriched in cobalt, tellurium,

⁴ Bioprospecting has been defined by the United Nations as “*The search for biological compounds of actual or potential value to various applications, in particular commercial applications.*”(UN, 2007).

⁵ In the context of growing demand for minerals and increased prices, DSM appears as a strategic option amongst a broader portfolio to increase a nation’s influence on world politics. The production of minerals from new producing nations will shift them from “mineral resources importer” profile to “mineral resources exporter” / “resource nation” (Carver *et al.*, 2020).

molybdenum, bismuth, platinum, tungsten, zirconium, niobium, yttrium and REE (rare-earth elements). Polymetallic nodules are enriched in nickel, copper, cobalt, molybdenum, zirconium, lithium, yttrium and REE (Hein *et al.*, 2013). The metal composition of SMS is highly variable, some being enriched in iron and thus, without any economic value while others contains higher concentrations of copper and zinc amongst trace elements such as gold or silver (Monecke *et al.*, 2014; Petersen *et al.*, 2016a). Both resources result from the precipitation of dissolved minerals, but their formation processes are diverse. Fe-Mn crusts derive from hydrogenous processes caused by upwelling currents on the flank and summits of seamounts and ridges; polymetallic nodules arise from sedimentary and chemical processes occurring under abyssal conditions, with precipitation of dissolved metals around a nucleus (small debris); SMS formation results from deep seawater circulation within the crusts, accumulating sulfides from rocks and expelled in hydrothermal vents (Hein and Koschinsky, 2009; Cuyvers *et al.*, 2018) (Figure 1- 5).

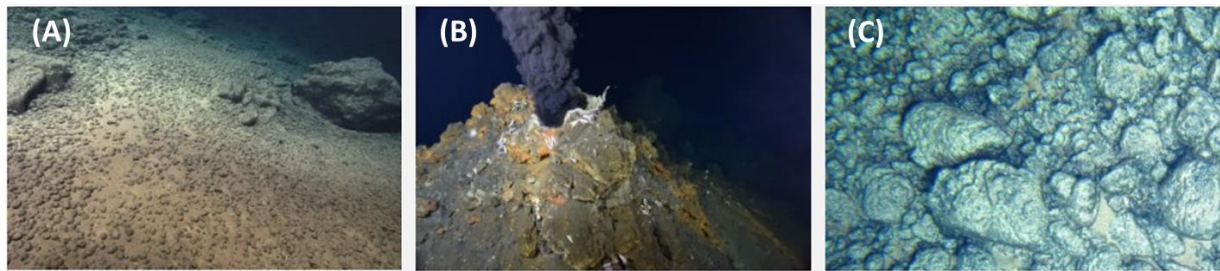


Figure 1- 5 (A) Polymetallic nodules field, (B) hydrothermal vent and SMS, (C) Fe-Mn crusts. Source ISA, (2020).

Consequently, the repartition of these resources is spread over all seas and oceans. The majority of Fe-Mn crusts are found between 800 and 2500 meters depth in the South Pacific related to its highest seamounts occurrence (Hein and Koschinsky, 2009). Polymetallic nodules are mostly observed in abyssal plains (4000 – 6000 meters depth) and occur in all seas and oceans (McKelvey *et al.*, 1983; Petersen *et al.*, 2016a). However, most of polymetallic nodules resources are found in three abyssal areas in the Pacific Ocean: the Clarion Clipperton Fracture Zone (CCZ), the Peru Basin and the Penrhyn Basin (Petersen *et al.*, 2016) (Figure 1- 6). The CCZ contains by far the largest stock of polymetallic nodules resource, it covers a total area of 5.2 million km² where 4.2 million km² are of commercial interest. This later area presents nodule densities typically ranging between 10 and 15 kg m⁻² (Morgan *et al.*, 2010; Cuyvers *et al.*, 2018). SMS are related to hydrothermal vents and thus appear along mid-ocean ridges at the limits of ancient and active tectonic plates. Most of them occur at depths of more than 2500 meters (Cuyvers *et al.*, 2018). Amongst these three types of resources, polymetallic nodules are the most abundant covering an estimated total area of 38 million km², while Fe-Mn crusts and SMS cover respectively 1.7 and 3.2 million km² (Figure 1- 6).

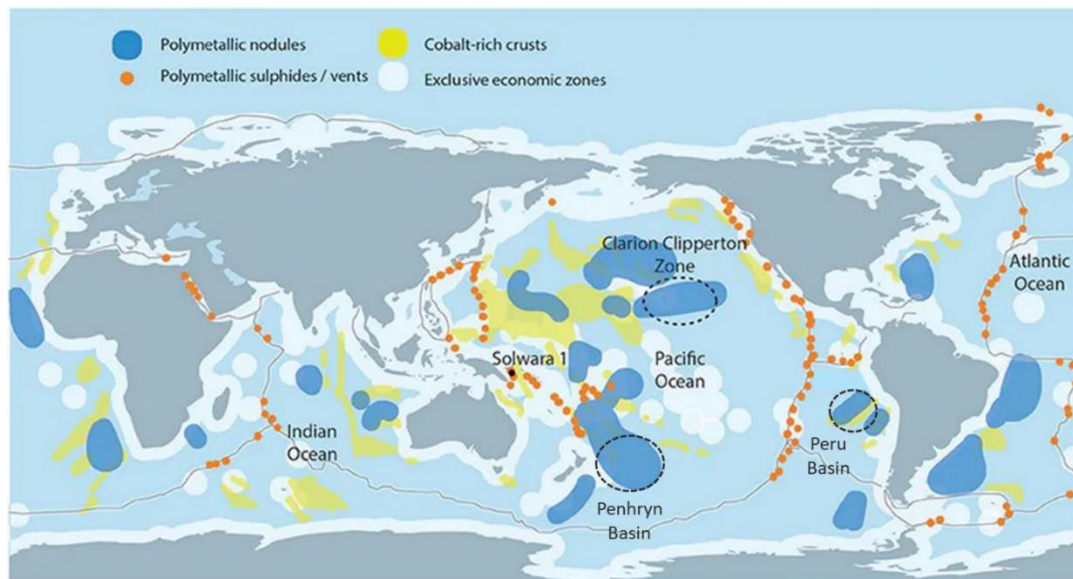


Figure 1- 6 Geographic repartition of marine mineral resource deposits and of exclusive economic zones. The dashed lines delimit the areas of the largest nodules fields discovered so far (Clarion Clipperton Zone, Penhryn Basin, Peru Basin). Adapted from Miller *et al.* (2018).

Despite the knowledge gaps on deep-sea mineral resources abundance (Glover and Smith, 2003; Henderson *et al.*, 2017), the stock of deep-sea minerals is expected to be larger than terrestrial stocks for the following elements: cobalt, bismuth (both critical metals), nickel, yttrium, tellurium and manganese (Banakar, 2010; Hein *et al.*, 2013; Henderson *et al.*, 2017). The stock of polymetallic nodules occurring in the CCZ itself is estimated to 34 billion wet metric tons. This represents 6 billion tons of manganese, 270 million tons of nickel, 234 million tons of copper, and 46 million tons of cobalt (Morgan, 2000). In the context of growing world population and the forthcoming energy transition, such resource estimates explain the growing interest from governments and companies for deep sea mining (Petersen *et al.*, 2016). However, most of those resources are located beyond EEZ and thus regulated under the UNCLOS (e.g. the CCZ being entirely located in international waters). The International Seabed Authority (ISA was created in 1994 and mandated by the UNCLOS for the supervision of the exploration and exploitation of deep-sea mineral resources located beyond EEZ. So far, the ISA has delivered 30 contracts to governments or companies for exploration in marine areas containing mineral resources. These consist of respectively 18, 7 and 5 exploration contracts for polymetallic nodules, SMS and Fe-Mn crusts. Amongst the 18 exploration contracts for polymetallic nodules, 16 concern areas located in the CCZ. Each exploration contract delivered from the ISA is valid for 15 years and can be further extended to 5 years. After this period, the contractor is not allowed renewing its exploration contract anymore and

must continue within an exploitation license or must stop its activities⁶. The exploration areas are similar for each contractor and consist of 75,000 km² for polymetallic nodules, 15,000 km² for SMS and 20 km² for Fe-Mn crusts (ISA, 2020). The ISA has also set-up so-called “reserved areas” that can only be contracted later by developing countries to ensure their access to these resources (ISA, 2019).

So far, the commercial exploitation of deep-sea minerals is limited to Japan that has initiated the exploitation of hydrothermal vents in its national waters (Henriques, 2019). In addition, the “Solwara 1 project” was close to commercial exploitation; it was driven by the Canadian private-held company Nautilus Minerals (Filer and Gabriel, 2018). It planned to start commercial operations for early 2019 after 20 years of exploration. The project aimed at mining SMS at 1600 m depth in the Bismarck Sea, in an area located in the EEZ of Papua New Guinea (Figure 1- 6). Under the pressure from NGOs and governments regarding the uncertainty of the environmental impacts, Papua New Guinea government decided to resign from the contract and engage a moratorium on deep-sea mining (Filer and Gabriel, 2018; The Guardian, 2019; DSM Observer, 2020). Technology is ongoing but mining at depths of 4000 m – 6000 m (e.g. in case of polymetallic nodules mining) remains a major technological challenge (Atmanand and Ramadass, 2017). The on-site operations consist of a remotely operated collector shipping minerals to a vessel through a pipe under hydraulic pressure. The commercial fraction is separated from water and sediments on the vessel and is then shipped for further processing onshore (Atmanand and Ramadass, 2017; Miller *et al.*, 2018). The mining technology is becoming mature for commercially viable exploitation of deep-sea mineral resources (Wedding *et al.*, 2015; Atmanand and Ramadass, 2017; Volkmann and Lehnen, 2018) and spatial analysis of resources abundance are being performed by contractors (Volkmann and Lehnen, 2018). However, the high uncertainty regarding the environmental impacts remains a consequent barrier to undergo commercial exploitation (Levin *et al.*, 2016; Niner *et al.*, 2018).

1.2.3.3. Marine ecosystem services

It has been estimated that oceans and seas account for more than 60% of total ecosystem services value supplied from the biosphere, mostly explained by the contribution of nutrients cycling in coastal areas (Costanza *et al.*, 1997, 1998)⁷. However, food provision (*i.e.* fisheries and offshore aquaculture) is by far the most intensively studied marine ecosystem service (Liquete *et al.*, 2013). The range of marine ecosystem services goes far beyond the delivery

⁶ So far, the first 15-years exploration contracts (5) that have been signed in 2001 have been extended in 2016 for 5 additional years. These all belong to governmental bodies, companies have contracted from 2010 (ISA, 2020).

⁷ Surprisingly, marine and terrestrial ecosystems present equal contributions to total earth's net primary production (NPP, 1.04E+11 tons per year) (Field *et al.*, 1998) and, the total terrestrial biomass exceeds the total marine biomass because of plants' contribution (Bar-On *et al.*, 2018).

of natural resources (*i.e.* provisioning services) with dependence amongst services: regulating and maintenance services such as water purification, nutrients cycling, habitat provision and pest / disease control (Table 1- 2) are directly influencing provisioning services. Because of water circulation, many marine ecosystem services are respectively generated and utilized in distinct locations and therefore, spatial dimension is crucial in their evaluation (Drakou *et al.*, 2017).

The understanding of ecological processes leading to the supply of ecosystem services has gained importance over the last two decades. This has been reinforced with the implementation of the EU Marine Strategy Framework Directive (2008/56/EC) that requires Member States to set up programs to achieve good environmental status of their marine waters (*i.e.* clean, healthy and productive seas and oceans). Therefore, EBM principles and hence, ecosystem services approach are advocated for sustainable use of the marine environment (EC, 2013). Marine ecosystem services assessment is relevant in marine spatial planning in order to highlight global economic trade-offs in the development of commercial activities that are no longer considered from a direct *costs-revenues* perspective (Lester *et al.*, 2013; Börger *et al.*, 2014). However, the quantification of ecosystem services provided by a given marine area faces major challenge related to ecological knowledge (Hattam *et al.*, 2015).

Table 1- 2 Overview of marine ecosystem services, adapted from Liqueste *et al.* (2013) and Barbier (2017). The classification of ecosystem services relies on the CICES framework.

Provisioning services	Regulating and maintenance services	Cultural services
Food provision	Water purification	Symbolic and aesthetic values
Water provision	Air quality regulation	Recreation and tourism
Raw materials	Coastal protection	Cognitive effects
Genetic materials	Climate regulation	Educational opportunities
	Weather regulation	
	Nutrients cycling	
	Habitat provision	
	Pest and disease control	

Most of marine ecosystem services are not directly marketable (Table 1- 2) and thus, their valuation is determined based on their support to valuable production activities (*e.g.* life maintenance) or based on their role in the protection of economic assets (*e.g.* coastal protection) (Barbier, 2017). This implies to understand how ecosystem functions affect the delivery of the final service and to quantify variation in the final service due to marginal change in ecosystem functioning. However, performing such assessments is not straightforward and leads to high uncertainty in the quantification of the cause-effect chain. For these reasons,

global monetary valuation of marine ecosystem services performed by Costanza *et al.* (1997) and de Groot *et al.* (2012) were criticized (Pendleton *et al.*, 2016). To overcome this issue, the use of indicators for ecosystem processes contributing to a given service is recommended instead of quantifying the contribution of each process involved in the delivery of the service (e.g. reduction of waves energy for coastal protection) (Hattam *et al.*, 2015). Marine biodiversity indicators are also recommended as proxy to assess changes in ecosystem services supply (Broszeit *et al.*, 2017) but their scope does not cover all kind of ecosystem services (e.g. air quality regulation) (Balvanera *et al.*, 2014).

1.3. Anthropogenic pressures caused by marine resources exploitation

Through direct (e.g. catches from fisheries) or indirect means (e.g. coastal eutrophication from agriculture), humans influence marine ecosystem functioning (Bracken *et al.*, 2008; Curtin and Prellezo, 2010; Gamfeldt *et al.*, 2015). Halpern *et al.* (2008) monitor changes in 17 drivers of anthropogenic pressure on marine ecosystems. The study reveals that all marine ecosystems are affected by human activities, even deep-sea benthic areas undergo pressures from increased pollution, ocean acidification or temperature change. In a similar way, as for terrestrial ecosystems, species composition of marine ecosystems is changing because of human activities (Sala and Knowlton, 2006). When looking specifically to pressures from natural resources exploitation, the most obvious example consists of fisheries impact on fish stocks caused by industrialization of the sector in the second half of the nineteenth century (Pauly *et al.*, 2005). Fisheries were exploited for long time without considering the MSY, causing decline in targeted fish stocks but also in non-commercial fish stocks because of by-catches or food-web interactions (Alverson *et al.*, 1994; Pauly *et al.*, 2005). Moreover, overfishing has also lead to a reduction of the average trophic level of commercial species, this phenomenon is also called *fishing down marine food webs* and clearly illustrates the increase of over-exploited fish stocks fraction (Figure 1- 7) (Pauly *et al.*, 1998, 2002; Myers and Worm, 2003). In addition, some fishing practices such as benthic trawling induce disturbance on seafloor ecosystems and thus affect species assemblages (Jennings *et al.*, 2001). The fraction of fish stocks sustainably exploited represented 65.8% of total catches in 2017 (FAO, 2020b) (Figure 1- 7). However, fisheries management is heterogeneous worldwide: some nations such as European countries and the United States have implemented effective management to avoid overfishing, while others do not have any regulation with the consequence of growing fraction of overexploited fish stocks (Hilborn, 2016) (Figure 1- 7). For these reasons, marine aquaculture is presented as a solution to ensure seafood production for the growing world population without increasing the intensity of wild catches fisheries. This sector experiencing substantial growth (FAO, 2020b) presents ecological implications at large scale for wild fish stocks such as nutrients pollution, spreading diseases and releasing chemicals (Arechavala-Lopez *et al.*, 2013; Froehlich *et al.*, 2017). For example, the expansion of the salmonid aquaculture in Ireland, Scotland, Atlantic Canada and Pacific Canada co-occurs with the significant reduction in wild stocks of salmon in Northern marine areas (Ford and Myers, 2008).

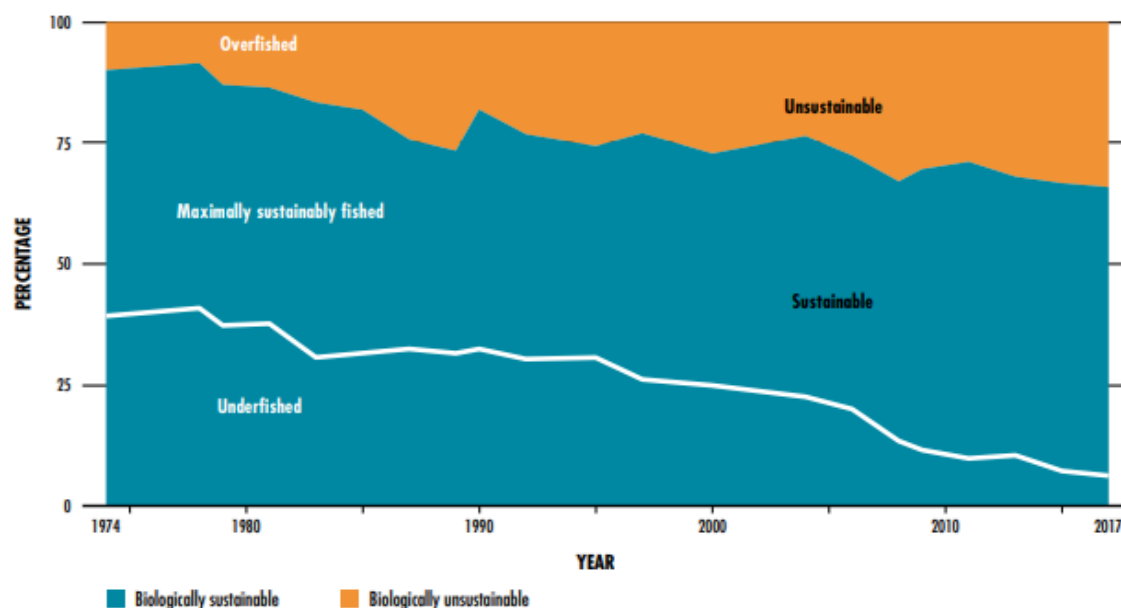


Figure 1- 7 Global trends in the proportions of wild fish stocks that are overexploited, sustainably exploited and underexploited for the period 1974 – 2017. Source FAO (2020).

A second clear evidence of direct human disturbance in marine ecosystems consists of the exploitation of offshore oil and gas. First, it may influence ecosystem processes in the surroundings of the platform through processed cutting releases, drilling mud discharges, acoustic disturbance emissions and artificial light generations (Moore *et al.*, 2000; Netto *et al.*, 2009; Cordes *et al.*, 2016). Extreme events such as oil spills (e.g. the platform Deepwater Horizon in Mexico Bay, 2010) have been drastically reduced (*i.e.* respectively 25.3 and 3.3 spills per year on average during 1970 – 1979 and 2000 – 2009) (Patin, 2013) but they still present major environmental consequences. Acknowledging the impacts on the entire coastal ecosystems, oil spills may also affect fisheries production because of reducing survival amongst fish eggs and larvae (Rooker *et al.*, 2013; Langangen *et al.*, 2017). In addition, deep-sea megafauna communities have been affected by the Deepwater Horizon oil spill (Mcclain, Nunnally and Benfield, 2019).

In addition to existing pressures caused indirectly by human activities, mining represents a major threat to the deep-sea ecosystems. Large-scale deep-sea mining operations are expected to induce major disturbance to deep-sea ecosystems (Miller *et al.*, 2018). The extraction of polymetallic nodules, Fe-Mn crusts and SMS will remove the substrate necessary for the development of benthic communities. In addition, sediments deposition (*i.e.* plumes) may significantly extend the impacted area compared to the area directly concerned by mining operations (Aleynik *et al.*, 2017; Volz *et al.*, 2018). However, overall, the environmental effects are still poorly understood for large-scale commercial operations because previsions rely on small-scale benthic disturbance experiments with short time perspective (Wedding *et al.*, 2015;

Tilot *et al.*, 2018). On the other hand, meta-analysis of existing datasets from disturbance experiments shows that deep-sea benthic communities will experience substantial changes in their composition and relative abundance of organisms; some organisms will not be able to recover in the absence of hard substrates (Vanreusel *et al.*, 2016; Gollner *et al.*, 2017; Jones *et al.*, 2017).

Combined with other stressors that do not belong to marine natural resources extraction (e.g. nutrient pollutions, marine littering, coastal infrastructures), marine biodiversity is declining in a similar way as in terrestrial ecosystems (McCauley *et al.*, 2015). The loss of biodiversity is expected to induce substantial changes in marine ecosystem functions and thus in services of key importance such as water quality, fisheries, coastal protection (Duarte, 2000; Sala and Knowlton, 2006; Worm *et al.*, 2006). Consequently, the sustainability of marine ecosystem services are seriously compromised (US Commission Policy, 2004; MEA, 2005). For these reasons, the concept of ecosystem-based management (EBM, also referred as integrated ocean management or ecosystem approach) gained importance for driving marine activities in a sustainable way (Palumbi *et al.*, 2009). EBM for oceans considers the complexity of marine ecosystems in the implementation of marine activities. It includes aspects such as connectivity, spatial heterogeneity, biochemistry, water circulation and productivity to develop a global understanding for a given marine area and to implement marine spatial planning (Arkema *et al.*, 2006; Crowder and Norse, 2008; Halpern *et al.*, 2008). The approach aims to preserve ecosystems resilience and their capacity to deliver services through protecting biodiversity (Levin and Lubchenco, 2008; Morishita, 2008; Long *et al.*, 2015).

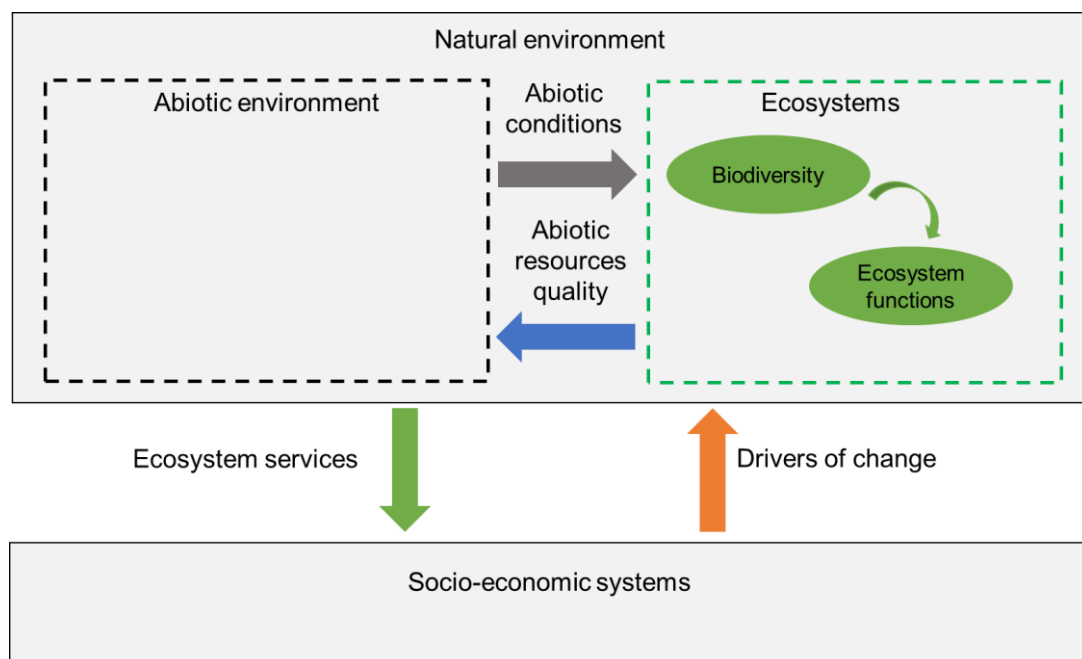


Figure 1- 8 Schematic representation of interactions between socio-economic systems and natural environment. Ecosystem services are considered according to the extended version of CICES.

Figure 1- 8 summarizes the main concepts brought in section 1.3. Through continuous inputs of materials from natural environments, socio-economic systems affect ecosystem functioning. Drivers of change for ecosystems arise from the extraction of abiotic and biotic resources and from other anthropogenic pressures such as air pollution. Biodiversity relying on abiotic and biotic conditions may change with consequences on ecosystem functions and services. On the other hand, this simplified relation between biodiversity and ecosystem services is not linear and is challenged in case of introduction of non-native species (Schwartz *et al.*, 2000; Balvanera *et al.*, 2006; Haines-Young and Potschin, 2009). The implementation of EBM follows this global perspective to understand cumulative impacts caused by different activities (Halpern *et al.*, 2008).

There is potential to make use of EBM principles to assess the total environmental impact of a product or a service. So far, the existing decision supporting tools for environmental management such as Life Cycle Assessment (LCA) methods poorly address *in situ* impacts on ecosystem functioning and report impacts from a global perspective. Including EBM perspective in LCA has a potential to extend the scope of the total environmental impact measured and is particularly relevant to understand the sustainability of natural resources exploitation. Cause-effect chains for natural resources extraction and, for agroecosystems production on ecosystem services can be included in life cycle thinking methods to better address the complexity of ecosystems functioning in the global results. The next section (1.4) describes the key principles of LCA and emphasizes on their limitation in the consideration of marine impact categories.

1.4. Life cycle thinking and impact assessment methods

LCA is an internationally standardized methodology to assess quantitatively the environmental performance (*i.e.* total impact) of a product or a service (defined under ISO 14000 series). The objective of LCA depends of the study scope: (1) LCA is applied to existing products / services to understand hotspots of environmental impacts associated to their life cycle and foresee actions to reduce them, (2) LCA is applied to multiple alternative products / services to identify the solution with the lowest environmental impact and, (3) LCA is applied during the development phase of the product / service in order to identify best manufacturing practices in terms of total environmental impact (*i.e.* prospective LCA for ecodesign) (Guinée, 2002; ISO, 2006; Millet *et al.*, 2007). The implementation of the method is organized in four steps: (1) study goal and scope definition, identifying the element to analyse (*i.e.* the functional unit: the quantity of the product or service under study) and defining the system boundaries, (2) development of a Life Cycle Inventory (LCI) consisting to develop an exhaustive dataset of elementary flows (*i.e.* materials and energy inputs and outputs) associated with the functional unit on its entire life, from cradle to grave, (3) the impact assessment, multiplying elementary flows from the LCI with specific factors (*i.e.* characterization factors, CF)⁸ to convert them in impacts according to specific cause-effect chains (*i.e.* life cycle impact assessment, LCIA) and, (4) systematic interpretation of the three previous stages with global conclusions and recommendations as main outcomes (Figure 1- 9) (ISO, 2006). The stages are interdependent (double sided arrows in Figure 1- 9): the completion of one stage informs on how to complete other stages.

⁸ Characterization factor (CF): factor derived from a characterisation model which is applied to convert an assigned life cycle inventory analysis result to the common unit of the impact category indicator (ISO 14040).

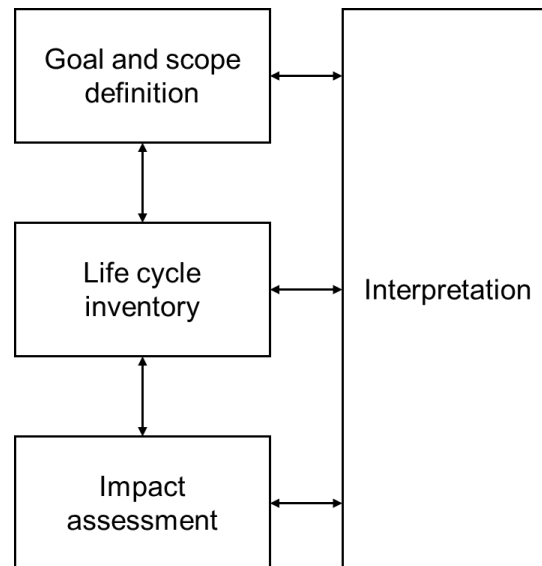


Figure 1- 9 The four stages of Life Cycle Assessment according to ISO 14040.

This PhD research focuses on two stages to implement LCA of marine products: the life cycle inventory and the impact assessment. The following paragraphs introduce these stages in order to highlight LCA limitations to perform environmental impact assessment of marine products/services.

1.4.1. Life cycle inventory

The goal and scope being defined, the practitioner performs a data collection to establish the elementary flows related to the functional unit. In a first step, it requires to model the processes associated with the product / service entire life in order to identify and quantify the elementary flows. Each elementary flow is then connected to a database containing information on its manufacturing processes. For example, if the production of the functional unit requires electricity, the amount of electricity in the inventory flow is connected to a database containing information regarding the country-specific electricity mix (*i.e.* generation). In this way, the total environmental impact considers upstream processes required to produce inputs (*i.e.* inventory flows) for the functional unit. Depending on the inventory flows, different databases are available, some being specific to sectors such as agriculture and food sectors (*e.g.* Agri-footprint) or dealing with industrial processes from various sectors (*e.g.* Ecoinvent) (Wernet *et al.*, 2016; Durlinger *et al.*, 2017). In addition to databases, the scientific literature also reports information regarding production processes from various sectors for specific technologies (Finnveden *et al.*, 2009).

While LCI are available for the production and processing of various commercially exploited marine commodities (*e.g.* fisheries, Avadí and Fréon, 2013), a main challenge consists in the development of LCI for processes that are still under development. Amongst products derived

from the marine environment, compounds extracted from wet biomass (e.g. seaweed⁹) or from marine minerals present high expectations as sustainable source of raw materials. However, the evaluation of their environmental sustainability through LCA requires the use of process engineering principles and scale factors to model their potential value chain at a commercial scale. Seaweeds are considered as sustainable feedstock to produce different compounds from processes designs such as anaerobic digestion for methane production, fermentation for bioethanol production and chemical extraction for proteins and lipids production (Marquez *et al.*, 2014; Kadam *et al.*, 2015; Balina *et al.*, 2017). The LCA literature related to seaweeds production and processing received growing interest during last decade to explore different cultivation systems or processing scenarios (Romagnoli *et al.*, 2010; Langlois *et al.*, 2012; Aitken *et al.*, 2014; Taelman *et al.*, 2015; van Oirschot *et al.*, 2017; Vijay Anand *et al.*, 2018; Parsons *et al.*, 2019). On the other hand, none of these studies addresses the concepts of biorefinery and cascade processes to extract multiple value-added compounds from wet biomass. Applying biorefineries designs for seaweed biomass has a potential to enhance the environmental sustainability and economic feasibility of the products as the environmental footprint and costs of upstream processes are shared amongst products. (Van Hal *et al.*, 2014; Herrero and Ibáñez, 2015; Dickson *et al.*, 2020). However, the biorefinery design possibilities are diverse and their quantitative assessment is required to develop LCI allowing environmental sustainability studies. Therefore, the first step consists of modelling biorefinery applications at a commercial scale to develop exhaustive LCI databases and understand trade-offs amongst cascades processes.

1.4.2. Life cycle impact assessment

The evaluation of the total environmental impact relies on the LCI and the impact assessment method selected. Depending on the method and the scope of the assessment, impacts can be pooled at different levels (midpoint and endpoint categories) to summarize the total impact in a limited number of impact categories (endpoint categories). Endpoint indicators facilitate decision making, they gather different midpoint impact categories based on midpoint-specific damage pathways. Besides its main advantage of summarizing information, the endpoint approach has some inconvenience such as reducing transparency and increasing uncertainty. Different LCA methods were developed; they differ in their cause-effect chains (LCIA) and impact categories. For example, the European Commission developed its own LCA method, the Product Environmental Footprint (PEF or generally the EF framework), relying on multiple

⁹ Despite being mostly consumed as whole or processed to extract hydrocolloids (White and Wilson, 2015), seaweed biomass is gaining attention for its value-added bioactive compounds such as pharmaceuticals and nutraceuticals (e.g. specific sugars, lipids and amino acids) (Hafting *et al.*, 2015; Peng *et al.*, 2015).

LCIA models that are recommended by the scientific community to assess impacts at midpoint level (Table 1- 3) (Fazio *et al.*, 2018). Other methods such as “ReCiPe” further aggregate the results of midpoint impact categories in three endpoint impact categories, called areas of protection (AoP): human health, ecosystem quality (*i.e.* also called natural environment and ecosystem health) and natural resources (Huijbregts *et al.*, 2016).

Table 1- 3 Midpoint impact categories considered in the Product Environmental Footprint method, Fazio *et al.*, (2018).

Midpoint impact category
Climate change
Ozone depletion
Human toxicity, cancer effects
Human toxicity, non- cancer effects
Particulate matter/Respiratory inorganics
Ionising radiation, human health
Photochemical ozone formation
Acidification
Eutrophication, terrestrial
Eutrophication, aquatic freshwater
Eutrophication, aquatic marine
Ecotoxicity (freshwater)
Land use
Water scarcity
Resource use, minerals and metals
Resource use, energy carriers

However, environmental impacts of many elementary flows (*e.g.* land transformation) differ according to their geographical location and thus, their assessment requires spatially differentiated LCI and LCIA (Patouillard *et al.*, 2016; Bulle *et al.*, 2019). The regionalization of cause effect chains is a main challenge in LCA to improve the spatial representativity of the results (Patouillard *et al.*, 2018): many regional aspects are still overlooked in LCA such as impacts on regional biodiversity (Curran *et al.*, 2016). Overall, the difficulty to address regional parameters in LCA arises from the complexity of modelling site-specific ecological processes / interactions under specific disturbance (type, intensity) (De Baan *et al.*, 2013a; Chaplin-Kramer *et al.*, 2017). This constitutes a main barrier for the integration of EBM principles (*i.e.* ecological complexity) in the LCA of biotic products.

Globally, the development of spatially differentiated LCIA remains a main challenge in LCA. Some LCIA such as local biodiversity impact assessment, are strongly relying on the geographical location where the harmful substance is emitted (or where the harmful intervention takes place) and where the impact occurs. Therefore, the proper modelling of these impacts requires the development of specific FFs to address the geographic repartition of the harmful substance and CFs to address ecosystem heterogeneity. Potting and Hauschild (2006) identified three levels of spatial differentiation in LCIA: (1) site-generic level that does

not rely on any geographical consideration, *i.e.* a generic environment is impacted; (2) site-dependent level considering a regional / country scale approach identifying the area where the substance is emitted and higher spatial resolution (few kilometres) to differentiate the impacted area and; (3) specific level site considering a specific location for which the substance (or intervention) is emitted and modelling the impact in the surrounding of the source. The integration of EBM in LCA (*c.f.* 1.3) is strongly relying on ecosystem-specific parameters and thus, requires developing LCIA specific- site.

1.4.2.1. Biodiversity impact assessment in traditional LCA

Biodiversity loss is considered as a major threat for humanity according to the magnitude of its economic consequences and its likelihood (WEF, 2020). Therefore, including biodiversity aspects in the scope of LCA is relevant to assess the environmental sustainability of a product or service. Only few LCA methods include regional biodiversity impact because of the complexity of its LCIA that is site- and disturbance-specific. For example, the European Commission does not report biodiversity metrics in its PEF method but includes six midpoint impact categories influencing biodiversity: climate change, eutrophication - aquatic freshwater, eutrophication - aquatic marine, acidification, water use and land use. The PEF guidance recommends assessing biodiversity impact separately and reporting the results as “additional environmental information” (EC, 2018a). On the other hand, other LCA methods such as ReCiPe include LCIA for biodiversity and report impacts in the AoP natural environment (impacts reported as “damage to ecosystems”) using biodiversity metric (Figure 1- 10). Depending on the method, impacts are measured in terms of “Potentially Disappeared Fraction of species per annual equivalent” (PDF x yr, *i.e.* the proportion of species disappearing because of the activity) or in “total species lost per annual equivalent”.

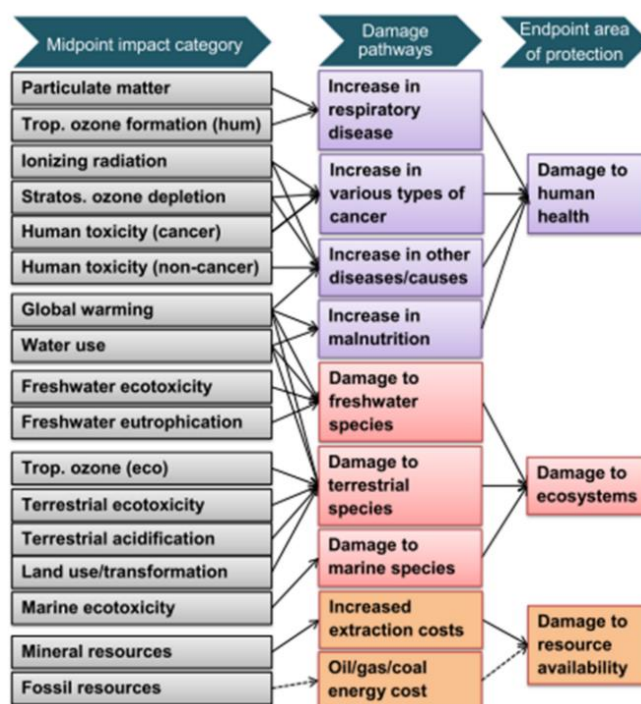


Figure 1- 10 Cause effect chains for midpoint to endpoint impact assessment in ReCiPe 2016, Huijbregts *et al.* (2016).

The ReCiPe method assesses global biodiversity impacts at the endpoint level (*i.e.* in “damage to ecosystems”) based on nine LCIA pathways, each linking a specific midpoint category to global biodiversity impacts through three damage pathways (freshwater, marine and terrestrial species) (Figure 1- 10, Table 1- 4). All LCIA pathways rely on key groups (*e.g.* vascular plants or freshwater fishes) as proxy for the assessment of the total biodiversity impact on the natural environment. In addition, LCIA for water-use impacts on terrestrial biodiversity (Pfister *et al.*, 2009) relies on NPP as proxy for vascular plants diversity. Depending on the spatial differentiation level in the LCIA, the modelling of biodiversity impacts may be site-generic or site-dependent (Table 1- 4). In site-generic approaches, biodiversity impacts are inferred from general relationships and do not belong to specific responses from areas in which the impacts are taking place. Additional site-generic approaches consider regional aspects (*e.g.* region-specific water scarcity level for “water use”) but the biodiversity response still relies on general relationships. In site-dependent approaches, the biodiversity response varies according to the geographic location affected. However, the level of spatial differentiation is varying depending on the LCIA, *e.g.* ecoregion¹⁰ level for “photochemical ozone formation” or biome¹¹ level for

¹⁰ Ecoregions are defined as large units of land with their specific assemblage of natural communities and, with boundaries based on the original extent of natural communities prior to land-use change (Olson *et al.*, 2006).

¹¹ Biomes, also called major habitats types, are defined as different regions of the world presenting similar ecological condition, habitat structure, and, containing similar patterns of biodiversity (levels, species adaptations) (Olson and Dinerstein, 1998).

“terrestrial acidification” (Table 1- 4). The results for the three damage pathways are aggregated to “damage to ecosystems” considering globally averaged species density in freshwater, marine and terrestrial ecosystems (Huijbregts *et al.*, 2016). Contrastingly, LC-impacts (*i.e.* another LCA method) reports three endpoint indicators for ecosystem quality: terrestrial biodiversity, freshwater biodiversity and marine biodiversity. Those are not aggregated into a single metric for ecosystem quality because of the lack of consensus regarding the weighting amongst the three different types of ecosystems (Verones *et al.*, 2020).

Table 1- 4 LCIA models for biodiversity impacts in ReCiPe 2016.

Damage pathway	Midpoint impact	Spatial differentiation	Model	Remark
Damage to freshwater species	Global warming	Site-generic	Hanafiah <i>et al.</i> , (2011); Xenopoulos <i>et al.</i> , (2005)	Logarithm curve of freshwater fish species loss according to river discharge reduction
	Water use	Site-dependent	Hanafiah <i>et al.</i> , (2011)	Logarithm curve of freshwater fish species loss according to river discharge reduction
	Freshwater ecotoxicity	Site-generic	Van Zelm <i>et al.</i> , (2009); Van De Meent and Huijbregts, (2005)	Log-normal species (fish, <i>daphnia</i> and algae) distribution according to contaminant concentration
	Freshwater eutrophication	Site-dependent	Helmes <i>et al.</i> , (2012); Azevedo <i>et al.</i> , (2013)	Log-logistic function of heterotroph species diversity according to phosphorus level (based on observations from temperate regions)
Damage to terrestrial species	Global warming	Site-generic	Urban, (2015)	Linear response of global species diversity related to temperature increase
	Water use	Site-dependent	Pfister <i>et al.</i> , (2009)	Logistic response of vascular plant species diversity to water stress, net primary production is used as proxy
	Photochemical ozone formation	Site-dependent	Van Zelm <i>et al.</i> , (2016); van Goethem <i>et al.</i> , (2013)	Linear response of vascular plant species diversity to ozone concentration, impacts are evaluated according to the proportion of forests and grasslands per region
	Terrestrial ecotoxicity	Site-generic	Van Zelm <i>et al.</i> , (2009)	<i>Impact inferred from freshwater ecotoxicity</i>
	Terrestrial acidification	Site-dependent	Roy <i>et al.</i> , (2014)	Logistic response of vascular plant species diversity to soil acidity [H ⁺] at biome level
	Land use	Site-dependent	De Baan <i>et al.</i> , (2013a); Curran <i>et al.</i> , (2014)	Global average of species-area relationship (<i>i.e.</i> logistic curve) for vascular plants and 5 faunal groups for 4 land-use classes at ecoregion level
Damage to marine species	Marine ecotoxicity	Site-generic	Van Zelm <i>et al.</i> , (2009)	<i>Impact inferred from freshwater ecotoxicity</i>

1.4.2.2. Specific methods accounting for land-use impacts on biodiversity in LCA

Amongst drivers of biodiversity loss (*i.e.* midpoint impacts in Figure 1- 10 and in Table 1- 4), land-use is considered as the main contributor to world biodiversity decline (WEF, 2020). Multiple methods have been developed to compute sets of regional CFs for biodiversity impacts caused by land-use interventions, considered as elementary flows in the LCI (Weidema and Lindeijer, 2001; Michelsen, 2008; De Baan *et al.*, 2013a; Verones *et al.*, 2013; Chaudhary *et al.*, 2015; Winter *et al.*, 2018). Most methods start from a similar framework differentiating amongst transformation, occupation and permanent biodiversity impacts on ecosystem quality caused by two interventions from the LCI: land transformation [m^2] and land occupation [$\text{m}^2 \times \text{yr}$] (Milà i Canals *et al.*, 2007). The impact assessment relies on three dimensions: the difference in ecosystem quality levels (*i.e.* biodiversity), the time spent for this difference to occur and, the area impacted (Figure 1- 11). If a transformation is not followed by an occupation period, the impacted area will gradually reverse due to natural regeneration. The impact resulting from transformation reflects the difference in the biodiversity level just after land transformation has occurred (B_1) and the biodiversity after the ecosystem recovery period (B_p). This difference is integrated on the recovery time of the ecosystem to evaluate the impact (Figure 1- 11). Similarly to the transformation impact, the occupation impact consists of the difference in biodiversity between transformation and post-recovery levels, integrated over the affected area and over the occupation time. The framework also differentiates permanent biodiversity impacts consisting to permanent loss of biodiversity because of the absence of recovery for some species (Milà i Canals *et al.*, 2007; Koellner *et al.*, 2013a).

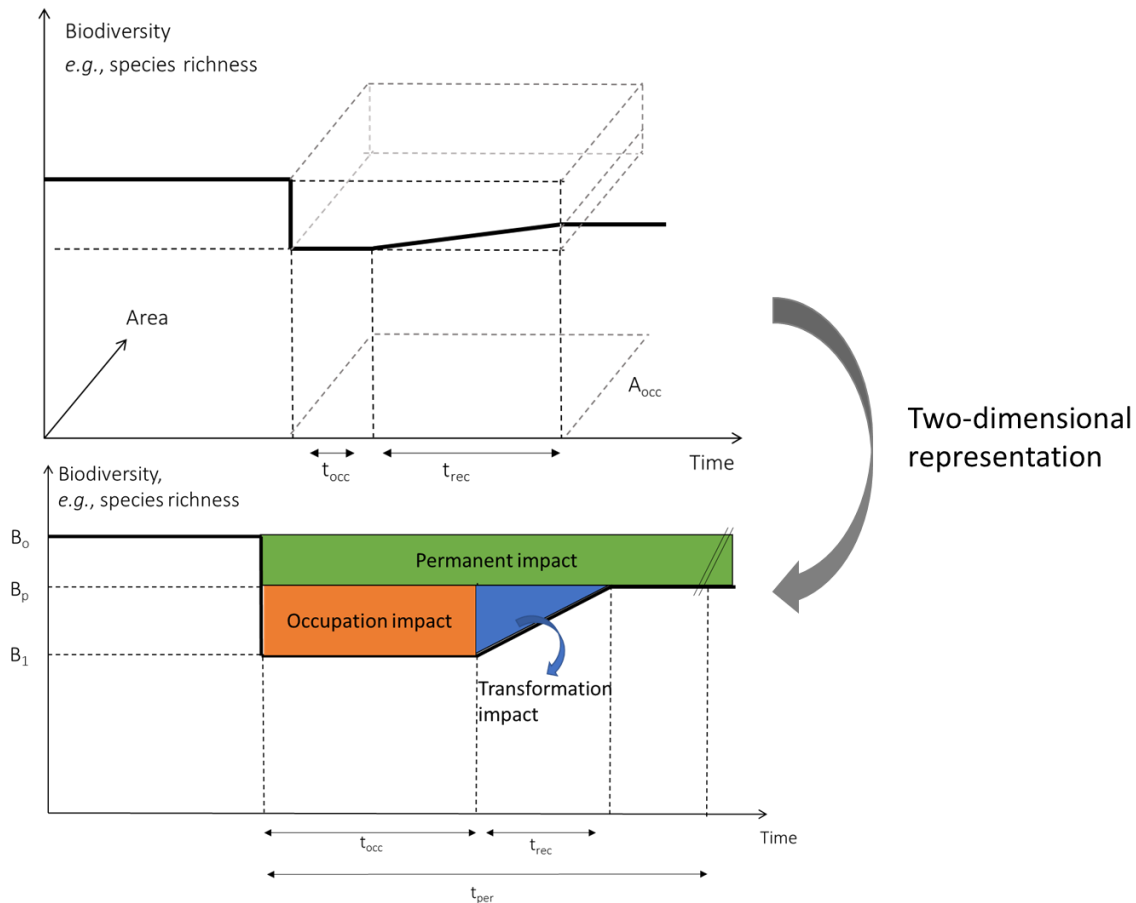


Figure 1- 11 Three-dimensional and two-dimensional representation of biodiversity impacts according to the framework from Milà i Canals *et al.* (2007).

ReCiPe relies on CFs from De Baan *et al.* (2013a) regarding regional biodiversity impacts caused by land-use (Table 1- 4). This method follows the framework from Milà i Canals *et al.* (2007) illustrated in Figure 1- 11 and considers four classes of land-use practices: agriculture, pasture, managed forest and urban area. The method relies on ecoregions as reference unit to assess impacts on plants', birds', amphibians', reptiles' and mammals' biodiversity (*i.e.* species richness) from species-area relationship (SAR) modelling. Regional biodiversity impacts, belonging to transformation and occupation interventions, are accounted by the loss of non-endemic species and considered as reversible. On the other hand, the authors model the loss of endemic species to assess global biodiversity impacts that are considered as permanent impacts. Both regional and global biodiversity impacts are assessed, accounting respectively for the loss of non-endemic and endemic species. Such as for other methods, CFs represent the marginal loss of species richness per disturbed square meter.

CFs that are multiplied with transformed area as elementary flow [m^2] are expressed in $\text{PDF} \times \text{yr} \times \text{m}^{-2}$ while CFs that are multiplied with occupied area as elementary flow [$\text{m}^2 \times \text{yr}$] are expressed in $\text{PDF} \times \text{m}^{-2}$.

De Baan *et al.* (2013a) consider species endemism to assess global biodiversity impacts, providing information on the magnitude of the impact related to world biodiversity: impacts occurring in ecosystems with high species endemism are more harmful than those occurring in ecosystems with limited fraction of endemic species. Next to De Baan *et al.* (2013a), other methods assessing biodiversity impacts from land-use differ in their evaluation of global impacts, relying on various indicators (Table 1- 5). Each method has its own combination of indicators related to regional or species attributes and, tackling scarcity or vulnerability aspects. These parameters are retrieved from exhaustive databases for terrestrial ecosystems or species such as the WorldWide Fund for Nature (WWF) Wildfinder for ecosystem threat level and existing area or, the International Union for the Conservation of Nature (IUCN) for species threat level.

*Table 1- 5 Indicators considered to assess global impacts on terrestrial biodiversity according to different methods. (SC) scarcity indicators, (VU) stands for vulnerability indicators. (1) Probability of the habitat to become unsuitable according to specific parameters in Verones *et al.* (2013). (2) Fitting parameter in species-area relationship (SAR) represents the sensitivity of species richness to a reduction in the area of the initial habitat. (3) Species endemism is measured through species distribution in Verones *et al.* (2013) while it is measured in terms of endemic species number in Cowell (1998) and Winter *et al.* (2018).*

Indicator			Cowell (1998)	Weidema & Lindeijer (2001)	Michelsen (2008)	Verones <i>et al.</i> , (2013)	Winter <i>et al.</i> , (2018)	Chaudhary <i>et al.</i> , (2015)
Region	SC	Potential area		X	X		X	
	SC	Existing area	X	X				
	VU	Threat level			X		X	
	VU	Habitat loss risk ¹				X		
Species	SC	Endemism ³	X			X	X	X
	VU	Z ²		X				
	VU	Threat level				X		X
	-	Species richness	X	X		X	X	X
	VU	Productivity	X					

None of these methods were applied to marine environments because of knowledge gaps regarding their indicators but also, regarding SAR modelling. Except for coastal and shelf ecosystems (Spalding *et al.*, 2007), marine ecosystems and their specific attributes are not detailed in similar database as for terrestrial ecosystems in the WWF Wildfinder. However, a specific method for seafloor disturbance impact on coastal marine biodiversity has been recently developed for European marine coastal areas (Woods and Verones, 2019). Following the state of the art for land-use impacts on biodiversity (Milà i Canals *et al.*, 2007), the method develops CFs for occupation and transformation impacts on regional biodiversity and relies on observations from disturbance experiments rather than SAR models (Woods and Verones, 2019). In a similar way as for terrestrial ecosystems, the implementation of LCA to address

biodiversity aspects is not only limited to seafloor disturbance but, should also consider other drivers such as fisheries, invasive species, eutrophication, acidification or plastic debris (Woods *et al.*, 2016).

1.4.2.3. Specific methods for land-use impacts on ecosystem services in LCA

In a similar way as for biodiversity, ecosystem services are also affected by land-use and their impact assessment has potential to be included in LCA (Bare, 2011). However, this requires developing site-specific LCIA that consider the services provided by each ecosystem and their quantitative relation with its functions. Such exhaustive assessments need to integrate ecological models for ecosystem functions (e.g. InVEST or GUMBO) in LCA (Chaplin-Kramer *et al.*, 2017). The integration of these dynamic models in LCA faces main barriers related to the detail level of the LCI but also, related to the incompatibility with current LCIA models (Arbault *et al.*, 2014). Acknowledging the main challenges of integrating ecosystem services in LCA, the Life Cycle Initiative¹² proposed a framework to integrate ecosystem services in LCA (Rugani *et al.*, 2019) based on the cascade framework for ecosystem services (Haines-Young and Potschin, 2009) (Figure 1- 3). However, the operationalization of the framework requires exhaustive datasets regarding ecosystem processes, function and services to understand how they are affected by various human interventions (in terms of intensity and type). The existing impact categories in traditional LCA methods such as ReCiPe do not ensure full coverage of ecosystem services (Alejandre *et al.*, 2019; Rugani *et al.*, 2019). In order to ensure an optimal coverage of ecosystem services categories in LCA without performing exhaustive assessments, Alejandre *et al.*, (2019) propose to prioritize the development of LCIA for specific ecosystem services according to their total monetary value. Therefore, 15 impact categories are identified in order to prioritize the development of CFs for ecosystem services in LCA: (1) biomass provision, (2) genetic material resources, (3) mineral resources, (4) non-mineral resources, (5) water provision, (6) mediation of wastes / toxics / nuisances, (7) mediation of smell / noise / visual impacts, (8) regulation of flows / extreme events, (9) habitat gene pool maintenance, (10) pest / disease control, (11) soil quality, (12) water conditions, (13) atmospheric conditions / composition, (14) maintenance of abiotic conditions and, (15) aesthetic / cultural / educational / scientific value. Categories (3), (4), (5) and (13) are assessed in ReCiPe 2016 (Figure 1- 10), and the remaining impact categories must be addressed through new LCIA in order to implement LCA applicability to ecosystem services. Besides this guideline identifying the most relevant ecosystem services for LCA, previous research has developed multiple LCIA and CFs belonging to different categories of ecosystem services impacted by specific drivers. For example, impacts from land-use on biotic production potential

¹² The Life Cycle Initiative is a consortium aiming to harmonize LCA knowledge.

(i.e. biomass provision) or on soil erosion prevention have been addressed by multiple methods using proxy indicators such as soil organic carbon level (Brandão and Milà i Canals, 2013; Núñez *et al.*, 2013; Taelman *et al.*, 2016; Boone *et al.*, 2018). Other LCIA indicators were proposed for impacts of land-use on freshwater regulation (Saad *et al.*, 2013), on food provision and carbon sequestration (Othoniel *et al.*, 2019), on biomass provision, freshwater recharge, erosion prevention, water filtration and climate regulation (Cao *et al.*, 2015) or, for the impact of groundwater and seawater extractions on food provision, tourism / recreation, flood protection and carbon sequestration (Blanco *et al.*, 2018). The development of LCIA pathways for ecosystem services faces main challenges related to its site-specificity requiring datasets for high spatial resolution, to feedback loops and interrelations amongst ecological processes and, to the development of global aggregation methods avoiding double counting amongst impacts on ecosystem services (Chaplin-Kramer *et al.*, 2017; Alexandre *et al.*, 2019; Rugani *et al.*, 2019).

The assessment of marine ecosystem services impacts in LCA is limited by data gaps regarding marine ecosystem functioning. However, some CFs for marine impact categories are somehow related to ecosystem services such as CFs for marine NPP decrease as indicator for biotic resources production (Langlois *et al.*, 2014a; Taelman *et al.*, 2014; Langlois *et al.*, 2015). On the other hand, some reviews of marine ecosystem services provide a good overview for the development of specific LCIA indicators (Liquete *et al.*, 2013).

1.4.3. Challenges for marine LCA products: the post-harvest processing

As discussed in previous paragraphs, LCA presents gaps in modelling while it comes to detailed level of spatial resolution in the cause-effect chain (Chaplin-Kramer *et al.*, 2017). This was illustrated for biodiversity impacts and for the development of *ad-hoc* methods in land-use. On the other hand, biodiversity impacts from sea-use are very limited in LCA and therefore the method overlooks crucial aspects in the environmental sustainability of marine products.

In addition, other LCIA requiring regional approach are not modelled at all, limiting the integration of EBM principles in LCA. For example, a framework for invasive species release has been proposed for freshwater environments (Hanafiah *et al.*, 2013), but other categories such as noise emission, turbidity generation, artificial structure creations or changes in food web dynamics are not addressed in LCA in an operational manner¹³. They affect the ecosystem functioning and hence, the availability of natural resources through the ecosystem service perspectives. Therefore, including EBM principles in LCA should rely on advanced level of understanding for regional ecosystem functioning. In addition, endpoint indicators for

¹³ Some specific approaches have been proposed, *e.g.* Middel and Verones (2017), but they are not yet implemented in commonly used LCA methods.

ecosystem services in LCA might consist to monetary valuation of the impact for consistency with the cascade framework and the Life Cycle Initiative framework (De Groot *et al.*, 2002; Haines-Young and Potschin, 2009; Rugani *et al.*, 2019). Using monetary valuation for ecosystem services would facilitate the analysis of trade-offs amongst commercial activities and the value of ecosystem services (Costanza *et al.*, 2014).

It is also necessary to develop transparent LCI databases with detailed information on their reliability (Hellweg and Milà i Canals, 2014). This is particularly relevant to understand the environmental sustainability of marine products according to varying post-harvest processing scenarios. Overall, the development of LCIs can be achieved in two ways: through measuring inputs / outputs flows of an existing process or through retrieving data from literature and modelling the process before its effective implementation. In the second approach, the identification of impact hotspots allows to test multiple production scenarios in terms of total environmental impact. In this perspective, the processing of aquatic biomass should be further documented to perform LCA with transparent background information regarding the processes involved. Such prospective modelling of processes provides key information for sustainability assessments of promising valorisation routes for aquatic biomass.

A first challenge for the processing of aquatic biomass such as microalgae and seaweed consists of efficient dewatering to avoid degradation and to increase yields of downstream processes (Milledge and Heaven, 2013; Kadam *et al.*, 2015). Various drying methods were developed and depend on the desired output properties for downstream processes. Microalgae and seaweed are extensively studied for their various value-added potential applications. On the other hand, the isolation of their target compounds requires energy intensive methods such as cell disruption techniques (Günerken *et al.*, 2015; Kadam *et al.*, 2015). It is therefore essential to understand trade-offs amongst downstream processes in order to find a balance between low costs and efficient production yields. Many researches have studied mass and energy flows for hypothetical processes at advanced technology readiness levels and their results should be considered carefully when used in LCIs because of assumptions on production scale and on efficiency (Grierson *et al.*, 2013; Passell *et al.*, 2013; Thomassen *et al.*, 2017). The sustainability assessment of aquatic biomass processing and its comparison to potential terrestrial-based alternatives must rely on transparent background information in order to determine if the processes designed can be implemented at large scale under existing technology readiness levels. In a similar way, the potential commercial exploitation of deep-sea minerals has been investigated through LCA, based on pilot-scale processes (Paulikas *et al.*, 2020). Besides metal criticality and economic aspects, LCA is relevant to compare the potential of deep-sea mining compared to terrestrial mining in terms of environmental sustainability. However, LCA results must be combined with risk

assessment at the production site, in order to understand the total environmental sustainability of deep-sea mining (UNEP, 2007). It is necessary to develop detailed LCI for mass and energy flows from extraction to metal production and test multiple processing routes. Globally, the application of LCA to assess environmental performance of hypothetical production relies on modelling and assumptions to obtain the LCI. With increased interest in marine products to substitute / complement terrestrial production (OECD, 2016), LCA has a potential to provide key information regarding their environmental sustainability, but its application must rely on proper LCI to highlight existing barriers and advantages for the development of marine value-chains.

Finally, it is crucial not to overlook the commercial value of marketable products compared to their input requirements when developing new valorisation routes for raw materials. For example, the production of biofuel from microalgae is facing main challenges in terms of commercial viability because of the high energy demand in downstream processing compared to the low-added value of the production (Sorguven and Özilgen, 2010; Milledge and Heaven, 2013, 2014). Therefore, performing environmental sustainability and economic sustainability assessments are essential in the market perspectives for new products (Figure 1- 12). The development of LCI for the production and / or harvesting of raw materials and for downstream processing (post-harvest) must be performed together with insights on the market potential of the final products.

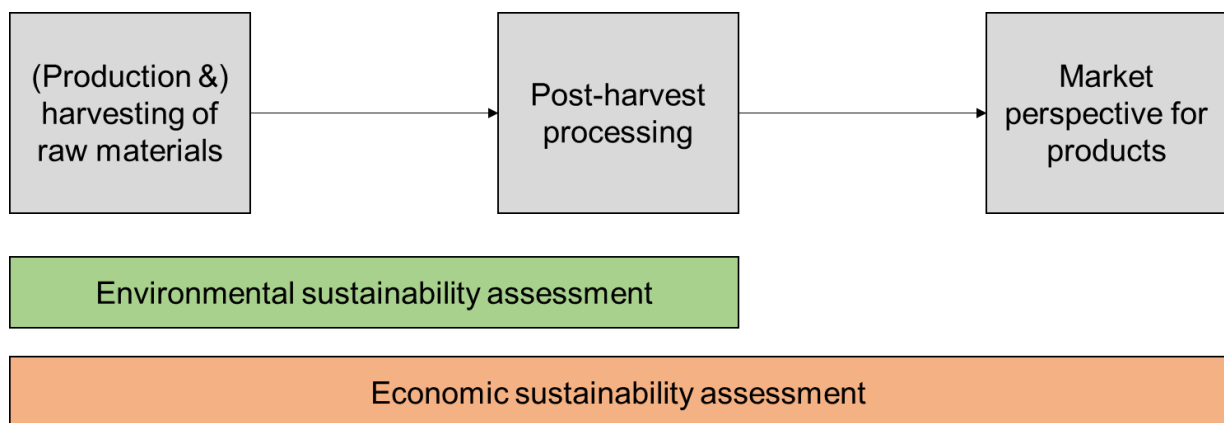


Figure 1- 12 Simplified representation of value chain for aquatic products and scope of the environmental and economic sustainability assessments.

1.5. Research objectives

This PhD research aims to facilitate the sustainability assessment of marine product by providing frameworks (and their application to a case study) to better account for environmental impacts related to marine activities (**Chapter 2** and **Chapter 3**) and, by developing LCI for the processing of aquatic biomass (**Chapter 4**).

- (1) **Chapter 2** proposes and operationalizes a framework to account for impact of seaweed farming on fisheries. It provides indicators to balance the production of marine biomass with its externalities on a marine ecosystem service (*i.e.* fisheries). The framework is applied to a hypothetical case study in the North Sea in order to develop site-generic CFs for biotic resources depletion (*i.e.* fish landings) from the perspective of Table 1- 4. The model considers regional aspects (regional NPP) but relies on a generic impact pathway (trophic foodweb).
- (2) As **Chapter 2**, **Chapter 3** proposes and operationalizes a framework to account for deep seafloor disturbance on biodiversity in LCA. The framework is operationalized to a case study for deep-sea polymetallic nodules mining in the CCZ in order to develop regionalized CFs. In light of Table 1- 4, those are site-specific and focus on regional biodiversity aspects as midpoint impact category.
- (3) **Chapter 4** focuses on the LCI of post-harvest aquatic biomass in order to supply transparent datasets. The processing of aquatic biomass for added value compounds is investigated through varying valorisation scenarios.

Chapter 2 and **Chapter 3** consist of site-specific LCIA methods focusing on the production stage of marine raw materials (Figure 1- 13). So far, site-specific LCIA methods for regional ecosystem functioning are mostly focusing on terrestrial and freshwater ecosystems due to data limitation for marine ecosystems. **Chapter 2** and **Chapter 3** focus on impacts on ecosystem functioning and develop new impact characterization models for specific indicators. Combined with traditional LCA methods, these LCIA methods provide information on the impact magnitude observed for aspects that are not considered so far in LCA marine products as fish landings and global biodiversity. The application of the LCIA to traditional LCA method depends on the scope of the study. However, they are of relevance to identify economical trade-offs for seaweed products and for marine interventions having influence on benthic communities (mining, fisheries, dredging, marine infrastructures). In a second step, this PhD research develops reliable scientific knowledge regarding the processing stage of marine raw materials and summarizes it in an exhaustive LCI (Figure 1- 13). **Chapter 4** concerns innovative valorisation routes having potential for driving the development of marine products. With these three chapters focusing on the initial stages of marine products value chain (with two LCA steps: LCI

and LCIA), the PhD documents and weights the use of LCA for marine products, a method that is still poorly addressed compared to terrestrial alternatives.

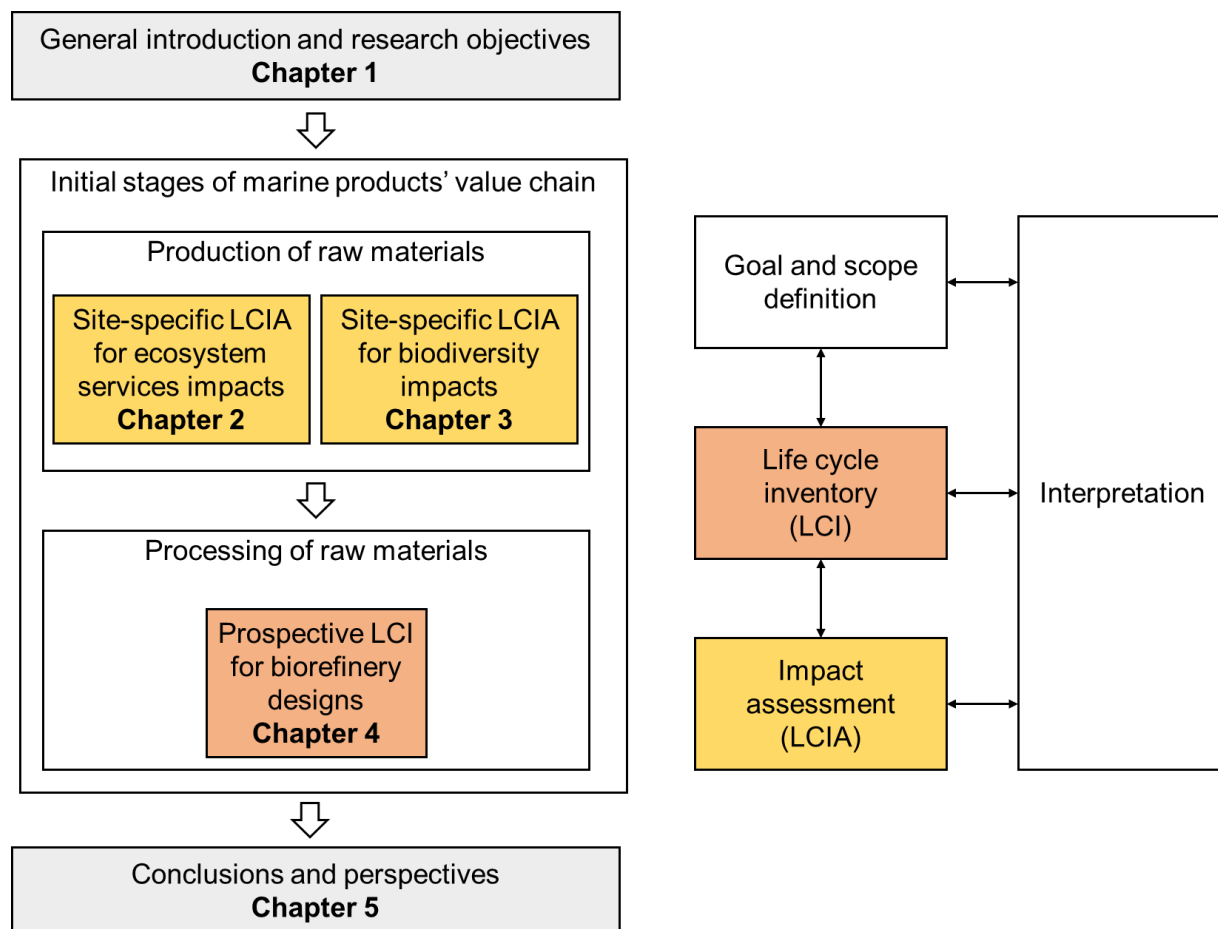


Figure 1- 13 Development of LCA knowledge for marine products, general structure of the manuscript and organisation of the three research chapters.

CHAPTER 2

Development of potential yield loss indicators to assess the effect of seaweed farming on fish landings

Redrafted from

Préat, N., De Troch, M., van Leeuwen, S., Taelman, S. E., De Meester, S., Allais, F., & Dewulf, J. (2018). Development of potential yield loss indicators to assess the effect of seaweed farming on fish landings. *Algal research*, 35, pp.194-205.

Abstract

In recent years, several indicators have been proposed to assess the effect of human activities on ecosystems provisioning capacity. Some of these methods focus on the Net Primary Production (NPP) available for ecosystem functioning through the comparison between the Human Appropriated Net Primary Production (HANPP) and the ecosystem's initial NPP at a given reference year. While some approaches have been proposed for marine ecosystems, most of the HANPP studies focus on terrestrial systems. This study highlights the relation between the HANPP methods and the production of natural resources in marine ecosystems. The linkage between current overfishing and future fish provisioning (ecosystem service) is well known. However, less studied before, is the relation between seaweed aquaculture and fish provisioning through the marine food web. Seaweed growth requires nutrients and light that will consequently be no longer available for natural phytoplankton production. As seaweed is periodically harvested, a fraction of the ecosystem's NPP (HANPP) is no longer available for ecosystem production. The HANPP of aquaculture reduces the ecosystem carrying capacity and thus affects commercial fish stocks. Therefore, an integrative approach is proposed in this study to assess the potential effect of seaweed farming on fish landings in the Greater North Sea. Three indicators are proposed to assess the Lost Potential Yield (LPY) in fish landings: LPYB, LPYV and LPYE, accounting respectively for reduction in biomass, monetary value and eco-exergy. For these three aspects, the LPY results remains smaller than the seaweed production, meaning that the overall natural resources balance for seaweed farming is positive. Besides HANPP, seaweed farming is expected to affect fish stocks through additional environmental mechanisms that must be quantified.

2.1. Introduction

The pressures on natural resources are increasing as a consequence of the current trend in human population size. Since oceans cover the majority of the surface area of our planet, the diversification and the regulation of marine resources extraction are crucial to make the most of ocean production potential. The recent development of the aquaculture sector is one way of diversification and does not only consist of fish and shellfish production. Indeed, the production of aquatic plants (mostly seaweed) accounts for 27.8% (Fresh Weight, FW) of the total current aquaculture production (Zhou, 2017). Due to its wide range of applications (food, feed, nutraceuticals or biofuels) (White and Wilson, 2015) and its role in eutrophication reduction (Seghetta, Tørring, *et al.*, 2016; Augyte *et al.*, 2017), seaweed farming is no longer limited to Asia (Buschmann *et al.*, 2017). Several pilot cultivation test have been performed in the North Sea to grow the sugar kelp *Saccharina latissima* (Linneaus) (Laminariales, Phaeophyceae), a brown seaweed widely distributed in the European Atlantic (Peteiro and Freire, 2013; Taelman *et al.*, 2015; van der Molen *et al.*, 2018). The kelp is found on subtidal rocky substrates and is composed of a large frond, a stipe and a holdfast.

Nevertheless, the large-scale cultivation of seaweed affects the properties of the surrounding ecosystem by shading (Taelman *et al.*, 2015), artificial reef creation (Langhamer, 2012) and nutrients uptake (Seghetta, Tørring, *et al.*, 2016; Augyte *et al.*, 2017). Even in the eutrophic coastal areas, the phytoplankton productivity is limited by nutrients availability during the growth season, when light is not a constraint for growth (Conley *et al.*, 2009). Therefore, seaweed farming without the addition of nutrients is expected to reduce the magnitude of phytoplankton blooms in spring and summer. In other words, the production of seaweed biomass replaces a fraction of the phytoplankton biomass. While phytoplankton productivity has been proven to affect fisheries yield (Chassot *et al.*, 2007, 2010; Capuzzo *et al.*, 2017), large-scale seaweed farming has the potential to reduce fish landings, but this has not been documented so far to our knowledge. Thus, a good methodology is needed to tackle this issue. The existing frameworks for natural resources and ecosystem services are helpful to initiate a first intuitive approach for the impact assessment of seaweed farming on fisheries yield.

Many definitions for natural resources have been proposed and the lack of consensus can lead to misunderstandings while assessing pressures on the natural capital. This issue was considered by an experts group from the United Nation Environment Programme (UNEP) and from the Society of Environmental Toxicology and Chemistry (SETAC) proposing a common definition : “*Natural resources are material and non-material assets occurring in nature that are at some point in time deemed useful for humans*” (Sonderegger *et al.*, 2017, 2018). This broad definition allows for a distinction between biotic and abiotic resources; both can be considered

in terms of renewability and finiteness according to their stock and extraction rate. Within biotic resources, Crenna *et al.* (2018) differentiate between *naturally occurring biotic resources* (e.g. wild fish) and *biotic resources resulting from human intervention* (e.g. fish from aquaculture). The production of naturally occurring biotic resources can be addressed from an ecosystem services perspective. The Millennium Assessment (MEA, 2005) defines ecosystem services as “*the benefits that people obtain from the ecosystem*”. Three frameworks have been proposed in parallel for ecosystem services classification and all of them consider the production of biotic resources as a provisioning service (Maes *et al.*, 2012). Since these frameworks were developed, the socio-ecological link between human activities and the provision of ecosystem services has been investigated (MEA, 2005; Carpenter *et al.*, 2009; Villamagna *et al.*, 2013). The human activities influence natural processes (Vitousek *et al.*, 1997; O'Neill and Kahn, 2000) through direct (e.g. land transformation) and indirect (e.g. changes in biogeochemical fluxes) pressure and thus reduce the intensity and diversity of ecosystem services.

The global economy relies on resource consumption and as abiotic resources are finite, the concept of bio-based economy has progressively emerged. It is defined as the “*economic activities resulting from the production, use and development of biological products and processes*” (OECD, 2009). The shift toward a bio-based economy does not mean that our society is automatically more sustainable. The renewability of the naturally occurring biotic resources depends on their extraction rate and their regeneration period (Langlois *et al.*, 2014a; Crenna *et al.*, 2018). On the other hand, the biotic resources resulting from human interventions affect the ecosystem functioning and thus the ecosystem capacity to sustain services such as provision of naturally occurring biotic resources (Foley *et al.*, 2005). Since most of the services are not marketable, the evaluation of the ecosystem capacity to provide services is challenging. The net primary production (NPP) of an ecosystem is stated to be a good proxy to assess the flow of services (Costanza *et al.*, 1998; Gaston, 2000; Richmond *et al.*, 2007). The NPP is the net amount of carbon assimilated by primary producers and is available at the basis of the ecosystem food web. The higher trophic level abundance in the ecosystem depends on the NPP, which determines the energy available in the trophic chain. This was illustrated for fish by Capuzzo *et al.* (2017) who highlighted the correlation between marine NPP and fish abundance. The decreasing NPP trend in the North Sea was associated with a reduction in fish stock. This clearly shows the link between NPP and ecosystem services since fish abundance affects fisheries yield in the marine environment. In this case, a NPP decrease affects negatively the naturally occurring biotic resources in upper trophic levels and thus, ecosystem provisioning services.

Because NPP is considered as a proxy for the flow of ecosystem services, the concept of Human Appropriated Net Primary Production (HANPP) is relevant to study the sustainability of biotic resource production (both naturally occurring and resulting from human intervention). The HANPP is quantified as the difference between the NPP in the pristine environment and the NPP in the anthropic environment (Vitousek *et al.*, 1986; Imhoff *et al.*, 2004). As global agricultural crop production is increasing to support the world population growth using more bio-sourced products (Cirera and Masset, 2010; Kearney, 2010; Tilman *et al.*, 2011), the global HANPP is increasing (Krausmann *et al.*, 2013) and thus, ecosystems are more affected and less effective in the intensity and diversity of goods and services. HANPP has been mapped for terrestrial systems based on satellite data (Imhoff *et al.*, 2004; Haberl *et al.*, 2007). However, because the oceans currently provide 2 % of the calories ingested per capita (European Commission, 2017), it is equally relevant to assess potentially declining NPP values in marine ecosystems due to human activities, especially as this contribution is expected to increase with global food demand. Pauly and Christensen (Pauly and Christensen, 1995) quantified the amount of primary production required to sustain fisheries based on the landings and the trophic levels. With this methodology, HANPP resulting from fisheries can be estimated from the fish biomass uptake. Most of the studies assessing pressures from fisheries on ecosystems consider Pauly and Christensen equation for the evaluation (Cashion *et al.*, 2016). Similar approaches have been developed for the shading impact of seaweed aquaculture on ecosystem functioning (Langlois *et al.*, 2015; Taelman *et al.*, 2015).

In parallel to NPP, ecosystems health can be studied from a thermodynamic point of view because natural systems tend to move away from the thermodynamic equilibrium by storing energy in biomass (Schneider and Kay, 1994). The ecological law of thermodynamics was formulated by Jørgensen (1997): ecosystems receiving inputs of exergy (solar radiation) will utilise it to move away from the thermodynamical equilibrium. In this sense, ecosystems can be considered as biological self-organized systems (Kutsch *et al.*, 2001). Svirezhev and Steinborn (2001) combined these perspectives with the Kullback measure, defining the increment of information related to ecosystem development. The authors brought the concept of ecosystems as thermodynamic machines moving away from the thermodynamic equilibrium by (1) storing energy available for mechanical work, and (2) producing and storing information. From this perspective, Jørgensen *et al.* (2000) formulated the following theory: *“specific exergy (exergy/energy ratio) increases throughout succession to maturity, in early stages mainly due to mass accrual, and in the later stages to gains in information and organization. During senescence, storage, entropy production, specific dissipation, and specific exergy all decrease, reflecting a declining ecosystem returning toward equilibrium.”*¹⁴ In this perspective,

¹⁴ This section has been added after the publication of the paper and does not appear in the published version.

a thermodynamic method called “eco-exergy” has been developed, weighting the energy in the living biomass with their respective genome size (Jørgensen *et al.*, 1995). Eco-exergy assesses how far a system is from the reference state, a system made of detritus (*i.e.* the dead organic matter). The eco-exergy is measured in term of detritus equivalent and can be converted in Joules considering the averaged energy content of detritus (Jørgensen *et al.*, 1995; Jørgensen *et al.*, 2010). This methodology is mainly used for marine ecosystem studies: *e.g.* to develop a recovery indicator of the benthic community exposed to fish trawling (Libralato *et al.*, 2006), as a goal function in ecosystem modelling (Jørgensen *et al.*, 2002; Jørgensen and Nielsen, 2007) and as an index for ecosystem health assessment (Marques *et al.*, 1997; Silow and In-Hye, 2004; Austoni *et al.*, 2007). Eco-exergy discerns different kind of biomass according to their genetic complexity and therefore, this approach is relevant to assign specific values to the biotic resources extracted.

While NPP- and eco-exergy-based developments assess human pressures on ecosystem health, Emanuelsson *et al.* (2014) consider the consequences on ecosystem provisioning capacity. This study uses a population dynamic model for fish stocks to compute the “Lost Potential Yield” (LPY) indicator. The LPY estimates the reduction in caught fish due to current overfishing compared to a sustainable fishing scenario. The calculation of the potential losses in naturally occurring biotic resources extraction due to bad management is particularly relevant to support policy decisions since it shows concretely the impact for human society.

Since the potential impact of seaweed production on fisheries yield has not been assessed yet, this study aims to model the NPP from the seaweed *Saccharina latissima* in aquaculture and develop indicators to assess the potential reductions in fisheries yield due to large-scale seaweed farming in a given zone. This study balances seaweed production (HANPP) and possible reduction in naturally occurring biotic resources (fish landings) in term of biomass, monetary value and eco-exergy. The seaweed productivity is modelled from experimental data and the values are benchmarked to phytoplankton productivity. The impact of seaweed farming on the fisheries yield for the ten most fished species in the North Sea is estimated from phytoplankton depletion.

2.2. Materials and methods

Similarly to Emanuelsson *et al.* (2014), we propose to estimate the potential losses in naturally occurring biotic resources due to the production of biotic resources resulting from human intervention. A method is proposed for the evaluation of the effect from seaweed production on fish landings through the LPY indicator in term of biomass (LPY_B), monetary value (LPY_V) and eco-exergy (LPY_E).

2.2.1. Spatial scale of the analysis

The International Council for the Exploration of the Sea (ICES) identifies ecoregions within the European seas in order to develop an ecosystem approach for the management of the zone. Each ecoregion is composed by several sub-areas for which ICES statistics for fish nominal catches are available. The Greater North Sea ecoregion covers seven sub-areas. The present study focuses on the three largest sub-areas from the Greater North Sea: the Northern North Sea (NNS), the Central North Sea (CNS) and the Southern North Sea (SNS). The study is limited to these sub-areas as the landings are comparable in term of species catches while this is not the case for the other sub-areas from the ecoregion. The North Sea as defined in this study covers a total area of 611,927.19 km² (NNS, CNS, SNS).

2.2.2. Modelling Phytoplankton Net Primary Production

The NPP from phytoplankton is estimated from an existing simulation of the GETM-ERSEM-BFM model applied to the North Sea. This model considers the hydrodynamic aspects (GETM: General Estuarine Transport Model) and the biogeochemical fluxes (ERSEM-BFM, developed at the NIOZ and Cefas institutes from the original ERSEM model) for the estimation of net primary production. More information on the applied model and used hindcast simulation is available in van Leeuwen *et al.* (2013, 2015). The simulation computes the NPP values for the entire North Sea domain: here, the results for two geographic areas (delimited by the red boxes in Figure 2- 1) are extracted and analysed for the period 1985 – 2005. The total NPP for the North Sea (NPP_{NS}) is estimated by spatially averaging the depth-integrated daily NPP values of the total North Sea area, and taking the mean over the selected 21-years period (averaged seasonal signal). Seaweed farming is limited to coastal areas, as they are richer in nutrients compared to more offshore areas in the North Sea due to their proximity to land-based sources (e.g. rivers, direct discharges, groundwater). In order to compare seaweed growth with the phytoplankton growth in the surrounding waters, the daily NPP values are extracted for a particular coastal environment and the total North Sea area (Figure 2- 1) from the hindcast simulation. The coastal environment under study is located along the Dutch coastline and influenced by nutrient loadings from the Rhine and Meuse rivers.

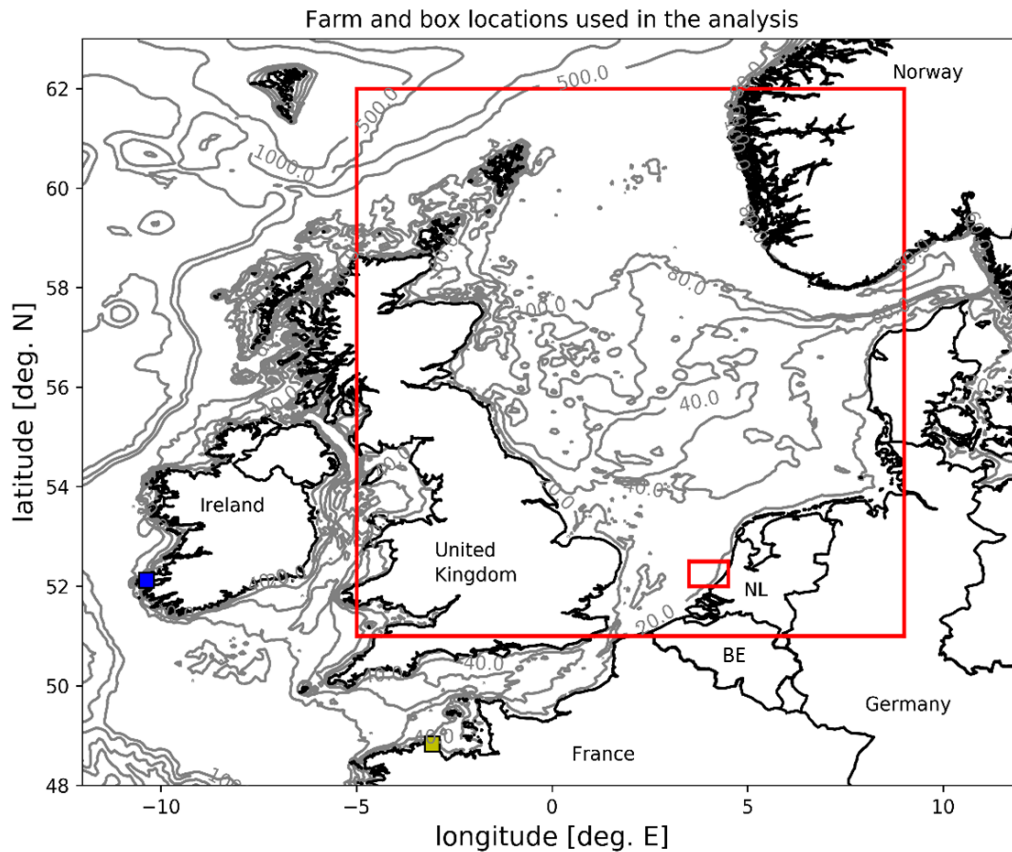


Figure 2- 1 Location of the farms and model domain for the North Sea and the coastal area. The blue and yellow squares indicate respectively the Irish and French farms. The large box indicates the North Sea area and the small box shows the boundaries of the coastal area under study. The grey lines indicate the depth.

2.2.3. Modelling Seaweed Net Primary Production

The growth of *S. latissima* is monitored in two farming systems by regular measurements of the frond area per meter of cultivation rope and the frond area specific fresh weight (data from Taelman *et al.* (2015), personal communication). One farming system was located in Ventry Harbour (Ireland, Celtic Sea) and was operated by Dingle Bay Seaweed. The other farm was located in Pleubian (France, Greater North Sea) and was operated by the CEVA (*Centre d'Etudes et de Valorisation des Algues*), see Figure 2- 1 for locations. As there is no data available for seaweed growth within the three ICES sub-zones, it was not possible to model seaweed NPP in these regions. However, these two locations have been chosen for their geographical proximity to the North Sea. The farms consist of floating ropes that are spaced by 2 (France) or 9 meters (Ireland). The cultivation period started on the 15th of December 2013 with the deployment of seaweed, which was harvested 30th of June 2014. The ropes are seeded and transferred to the sea after a hatchery phase of respectively 36 (France) and 23 days (Ireland). Detailed information on the farming designs is available in Taelman *et al.* (2015). Initially reported per meter of cultivation rope, the seaweed frond growth is first converted to frond area per square meter of sea surface. The growth curves (frond area per

sea area) are modelled from respectively 6 (France) and 5 (Ireland) observations at sea site. The frond area per sea area (y) is estimated from the days after deployment (x) by the sigmoidal equation 2-1 with three parameters. Sigmaplot Version 13 (Systat Software, San Jose, CA) is used to compute the parameters a , b and x_0 from the observations.

$$y = \frac{a}{(1 + \exp - (\frac{x - x_0}{b}))} \quad (2-1)$$

In parallel, the evolution of frond area-specific fresh weight (d) according to the days after immersion (x) is modelled from 7 observations for both farms (equation 2-2). Sigmaplot is used to estimate the parameter m based on a linear regression curve (equation 2-2). The linear relationship has only been verified for the farming period, during seaweed early life-cycle stages.

$$d = m \cdot x \quad (2-2)$$

The results from seaweed growth [$\text{m}^2_{\text{frond, FW}} \times \text{m}^{-2}_{\text{sea surface}}$] and frond area-specific fresh weight [$\text{g}_{\text{frond, FW}} \times \text{m}^{-2}_{\text{frond}}$] are combined in order to model the seaweed biomass growth [$\text{g}_{\text{frond, FW}} \times \text{m}^{-2}_{\text{sea surface}}$]. The daily biomass increase is computed from the biomass growth estimations. The moisture and carbon content of *S. latissima* reported in literature are applied here to the daily biomass increase in order to estimate the NPP. The carbon content represents 4.02% of the seaweed fresh biomass (Schiener *et al.*, 2015). The carbon and water content are assumed to be stable during the farming period. The seaweed NPP values account for the daily increase in seaweed carbon weight per sea area. The yields (total fresh biomass reported to the farm area) are similar for both locations and therefore, the average daily NPP values are computed from the two locations. We model the seaweed NPP for the period starting from 15th December to 31st May, which corresponds to the usual seaweed farming season in the North Sea. When harvested after the end of May, seaweed fronds get more easily colonized by epiphytic organisms which affects the frond quality and the overall biomass yield (Peteiro and Freire, 2013).

2.2.4. Development of Lost Potential Yield indicators

2.2.4.1. Lost Potential Yield in biomass (LPY_B) and monetary value (LPY_V)

Shelf seas like the North Sea are rich in nutrients in winter, due to low biological activity. In spring, when light and temperature increase to allow for photosynthetic activity by plankton, this pool of nutrients fuels the spring bloom of the planktonic community, until one or more nutrients become limiting (Conley *et al.*, 2009; Lenhart *et al.*, 2010; Passy *et al.*, 2013). Seaweed growth reduces nutrient levels and thus, the phytoplankton maximal productivity

(Miller *et al.*, 2011). Therefore, seaweed harvesting limits the NPP by phytoplankton, resulting in the Human Appropriated Net Primary Production (HANPP).

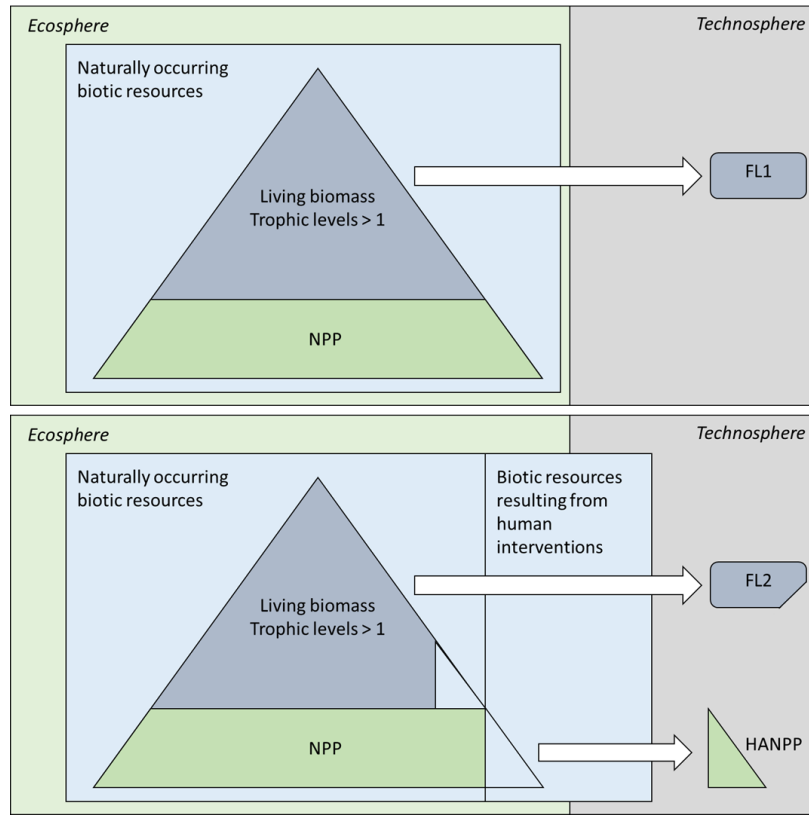


Figure 2- 2 System boundaries for biotic resources from the marine environment according to the framework from Crenna *et al.* (2018). (a) Representation of the system without seaweed production. The fish landings (FL1) are influenced by the net primary production (NPP) of the natural resources. (b) Representation of the system while seaweed is produced (HANPP), the fish landings (FL2) are smaller than in situation (a). The difference between FL1 and FL2 represents the lost potential yield (LPY).

Phytoplankton NPP supports the fish landings (Chassot *et al.*, 2007, 2010; Capuzzo *et al.*, 2017), thus for a given ecosystem, the reduction of NPP induces potential loss in fish landings (Figure 2- 2). Therefore, an empirical model for the lost potential yield in fish fresh biomass (LPY_B) is proposed in equation 2-3. The LPY indicators are developed according to the principle of HANPP and ecosystem functioning. The equation is derived from Pauly and Christensen (Pauly and Christensen, 1995) for the primary production required to sustain fisheries. More information about the calculations is available in supplementary material A1.

$$LPY_B = \frac{HANPP}{NPP_{NS}} \times FL \quad (2-3)$$

An LPY_B [$\text{kg living weight} \times \text{yr}^{-1} \times \text{kg}^{-1} \text{C}_{\text{eq}} \text{yr}^{-1}$] indicator is calculated for each of the top-10 species with the highest landings in the 3 sub-areas assuming that phytoplankton abundance limits the population growth (Capuzzo *et al.*, 2017). These species (Table 2- 1) represent 87 % (weight based) of the total fish catches in the 3 sub-areas. For each species, the mean fish landings for the period 2011 – 2015 are calculated from the ICES nominal catches database

(Table 2- 1) (ICES, 2017). The NPP_{NS} calculations are explained in 2.2.3, while the HANPP calculation was mentioned in the first paragraph of this section. The lost potential yield in monetary value (LPY_V) is obtained by multiplying the LPY_B with the species market value. The European fish market values (Table 2- 1) were found in the EUROSTAT database for the year 2015 and for the category “*fresh whole*” (EUROSTAT, 2017). The European market value of *S. latissima* was estimated from the category “*brown seaweed*” in EUROSTAT (54.32 € x T⁻¹ FW). The LPY_V are compared with the market value of *S. latissima* produced.

The main hypothesis of the LPY estimations consists in considering NPP as the limiting factor for fish population growth. The fish population dynamics (described by the ratio spawning stock biomass on the production of juveniles) are influenced by the fishing pressure, causing truncation in the population age structure. This is particularly the case for overfished species where the population growth results from ecosystem productivity and fishing history (Britten *et al.*, 2016). Among the 10 species considered in this study, 7 are sustainably exploited and at fully reproductive capacity in the North Sea according to the ICES (ICES, 2018). Haddock, cod and blue whiting are overexploited in the North Sea and consequently, the calculation of their LPY value from the HANPP approach might be oversimplified.

Table 2- 1 Fish landings and European market price for the 10 most fished species in the Greater North Sea. The fish landings (averaged over 2011-2015) are provided by ICES database and expressed in tons of live weight [TLW]. The EUROSTAT data portal was used to compute the European Market Values based on estimates for 2015, the category “*fresh, whole*” was considered (Austoni *et al.*, 2007).

Common name	Averaged landings [TLW]	European market value [EUR x T ⁻¹]
Atlantic herring	388 E+03	358
Atlantic mackerel	293E+03	792
Sand eels	273E+03	230
Sprat	152E+03	240
European plaice	720E+02	1345
Saithe (Pollock)	690E+02	1228
Norway pout	446E+02	241
Haddock	318E+02	1516
Cod	286E+02	2578
Blue whiting	178E+02	389

2.2.4.2. Lost Potential Yield in eco-exergy (LPY_E)

The eco-exergy for a population is determined by multiplying the total biomass concentration by its respective weighting factor. The eco-exergy is estimated at population level and then summed to compute the total eco-exergy of the ecosystem (equation 2-4) (Jørgensen *et al.*, 1995).

$$Ex/R.T = \sum_{i=1}^n \beta_i \cdot C_i \quad (2-4)$$

Here, R is the gas constant ($8.314 \text{ J} \times \text{mol}^{-1} \times \text{K}^{-1}$), T is the standard temperature (298 K), β_i is the weighting factor and C_i is the species biomass concentration [$\text{g} \times \text{L}^{-1}$]. The eco-exergy $Ex_{R.T}$ is measured in Detritus (dead organic matter) equivalent [$\text{g}_{\text{detritus}} \times \text{L}^{-1}$]. The average exergy value of the Detritus is the reference level instead of the thermodynamic equilibrium. Therefore $Ex_{R.T}$ measures the deviation of the ecosystem from a state without living biomass. The weighting value β_i is computed from the genome size following the equations 2-5 and 2-6 (Fonseca *et al.*, 2000).

$$\beta_i = \frac{\ln P_i}{Ex_{det}} \quad (2-5)$$

$$P_i = 20^{-\left(\frac{C^*}{2}\right)\left(\frac{bp}{3}\right)} \quad (2-6)$$

P_i is the probability of producing detritus with the corresponding genetic information of species i , C^* is the haploid genome size of the species i (C-value in picograms, computed from the diploid genomes sizes shown in Table 2- 2), bp is the number of base pairs per pg of DNA (1 pg = 9.8×10^5 bp). β_i is normalized in detritus equivalent considering the reference value for detritus ($Ex_{det} = 7.43 \times 10^5$). The exponential term is the number of nucleotide triplets in genome and thus, its maximum coding capacity. More information about Ex_{det} and maximum coding capacity calculation are provided in Fonseca *et al.* (2000).

The eco-exergy approach gives different weights to different biomass types according to their genome complexity, *i.e.* the potential loss in genetic information associated with seaweed production can be calculated. Equation 2-4 has been adapted by considering C_i as fish biomass [kg] instead of concentration and the eco-exergy is measured in kilograms of detritus equivalent. The lost potential yield in term of eco-exergy LPY_E is estimated for each of the 10 fish species by multiplying its LPY_B [kg] by the respective β value. The LPY_E is then measured in kilograms of detritus equivalent. The LPY_E values are compared with the eco-exergy value of *S. latissima* produced.

Table 2- 2 Inventory data for the diploid genome size (2C values, $C = 2C/2$) for 10 fished species from the Greater North Sea and for *Sacharina latissima*. When no information on species genome size was reported in the literature, the genome size from species from the same family was considered ("Species for genome" column). *An exception is made for *Ammodytes* spp. where there is no record for genome size for any species from the family (Ammodytidae, order Perciformes). The averaged 2C value of the order Perciformes (available on Animal Genome Size Database) was calculated based on 633 entries (2C values from species belonging to the order Perciformes). (A) Hardie and Herbert (2004), (B) Hinegardner and Rosen (1972), (C) Gregory (2018), (D) Kapraun (2005).

Common name	Scientific name	2C [pg]	Species for genome	Source
Atlantic herring	<i>Clupea harengus</i>	0.98	<i>Clupea pallasii</i>	A
Atlantic mackerel	<i>Scomber scombrus</i>	0.97	<i>Scomber scombrus</i>	B
Sand eels	<i>Ammodytes</i> spp.	0.94	Perciformes*	C
Sprat	<i>Sprattus sprattus</i>	0.98	<i>Clupea pallasii</i>	A
European plaice	<i>Pleuronectes platessa</i>	0.70	<i>Pleuronectes americanus</i>	B
Saithe (Pollock)	<i>Pollachius virens</i>	0.93	<i>Gadus morhua</i>	A
Norway pout	<i>Trisopterus esmarkii</i>	0.93	<i>Gadus morhua</i>	A
Haddock	<i>Melanogrammus aeglefinus</i>	0.93	<i>Gadus morhua</i>	A
Cod	<i>Gadus morhua</i>	0.93	<i>Gadus morhua</i>	A
Blue whiting	<i>Micromesistius poutassou</i>	0.93	<i>Gadus morhua</i>	A
Kelp	<i>Saccharina latissima</i>	0.65	<i>Saccharina latissima</i>	D

2.3. Results and discussion

2.3.1. Seaweed productivity in the two locations

The values of a [$\text{m}^2_{\text{frond}} \times \text{m}^{-2}_{\text{sea}}$], b (in days) and x_0 (in days) for equation 2-1 computed by Sigmaplot (using the non-linear least squares regression curve fitting) are respectively 5.11 (± 0.12 SE), 20.67 (± 1.21 SE) and 130.70 (± 1.61 SE) for France; 6.63 (± 0.21 SE), 26.90 (± 1.01 SE) and 151.70 (± 2.14 SE) for Ireland. The resulting growth curves from equation 2-1 are in accordance with experimental results (Figure 2- 3). Despite the divergence in the modelled growths per meter of cultivation rope ($8.79 \text{ m}^2_{\text{frond}} \times \text{m}^{-1}_{\text{rope}}$ for France and $40.04 \text{ m}^2_{\text{frond}} \times \text{m}^{-1}_{\text{rope}}$ for Ireland at harvesting), the growth reported per sea area occupation is similar for both sites with 4.29 and $4.39 \text{ m}^2_{\text{frond}} \times \text{m}^{-2}_{\text{sea}}$ in France and Ireland, respectively. The cultivation ropes are spaced closer together in France than in Ireland and the growth of *S. latissima* in France reaches the stationary phase sooner than in Ireland. The best-fitted model for the frond area-specific fresh weight (d) observations as function of the days after immersion (x) follows the linear equation 2-2. The slope coefficient m is estimated to be 2.41×10^{-3} ($\pm 4.83 \times 10^{-5}$) $\text{g}_{\text{frond}} \times \text{m}^{-2}_{\text{frond}} \times \text{day}^{-1}$. The biomass growth curves combine the growth equations for the area and for the frond area-specific fresh weight (Figure 2- 3). The yield of seaweed fresh biomass per sea surface are respectively 1.78 and $1.73 \text{ kg} \times \text{m}^{-2}_{\text{sea}}$ for France and for Ireland.

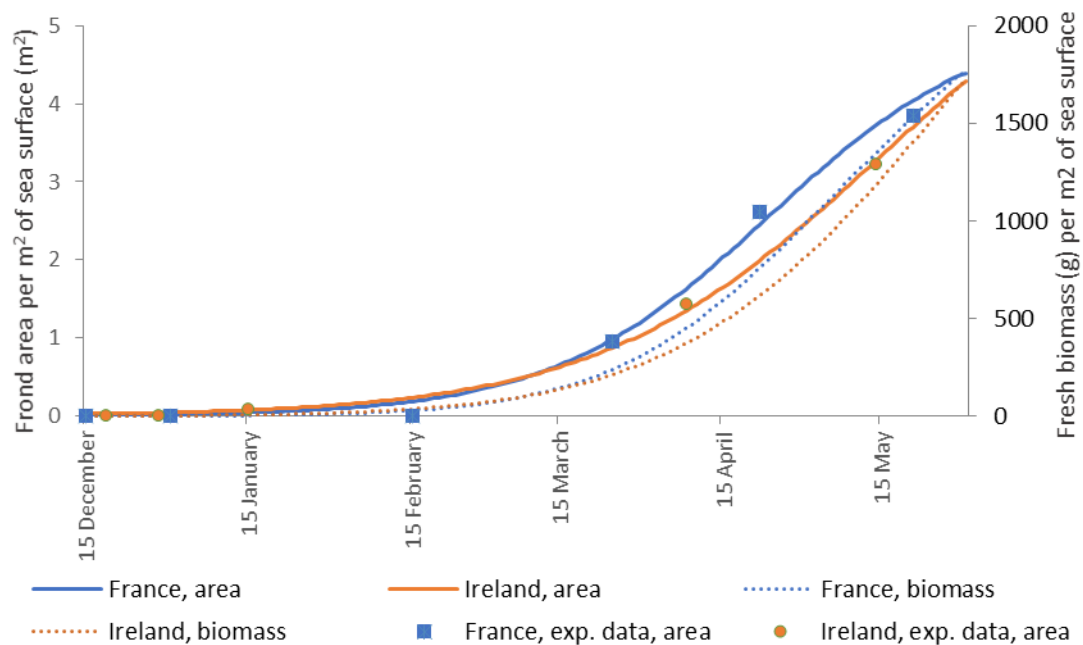


Figure 2- 3 Growth curves and experimental data of *S. latissima* farmed in France and Ireland. The farming period started on 15th of December and ended on 31st of May (after 168 days). The data are adapted from Taelman et al. (2015) that reported the growth of the frond area (m^2) per meter of cultivation rope.

The HANPP from seaweed farmed in France reached a maximal value of $2.20 \text{ gC}_{\text{eq}} \times \text{m}^{-2} \times \text{d}^{-1}$ after 143 days (6th of May) while in Ireland the maximal HANPP value of $2.32 \text{ gC}_{\text{eq}} \times \text{m}^{-2} \times \text{d}^{-1}$ occurred after 170 days of immersion (2nd of June). Figure 2- 4 shows the non-site specific HANPP curve (average HANPP values for both locations) which reached its peak of $1.68 \text{ gC}_{\text{eq}} \times \text{m}^{-2} \times \text{d}^{-1}$ on the 15th of May after 151 days of farming. The lower value for the average HANPP peak is due to the productivity time lag between the two farms: the HANPP in Ireland is still low ($1.20 \text{ gC}_{\text{eq}} \times \text{m}^{-2} \times \text{d}^{-1}$) when the HANPP in France is at its maximum (Figure A- 1).

The difference in the maximum seaweed NPP observed in the two farms is reduced but a shift of 23 days in maximum NPP occurs despite a similar date for deployment at sea. This time shift is due to the different times spent in the hatchery (36 days in France and 23 days in Ireland) and abiotic differences between the two sites (e.g. light conditions and exposure to water currents). For both sites, the maximum NPP occurrence (6th of May in France; 2nd of June in Ireland) coincides with the observations on the seasonal biomass of *S. latissima* in Northern latitudes. The growth rate of natural populations starts to increase in October to reach its maximal value in May (Sogn Andersen *et al.*, 2011; Nielsen *et al.*, 2014). Site-specific differences directly affecting seaweed and phytoplankton growth include latitude (daylight hours and intensity), local riverine sources (nutrients), temperature, salinity, waves exposure and circulation patterns (Peteiro and Sánchez, 2012; Peteiro and Freire, 2013; Nielsen *et al.*, 2014). The phytoplankton blooms decrease the light availability for deeper layers and thus, the photosynthesis rate of seaweed (Kavanaugh *et al.*, 2009). For seaweed farming, the shading effect is limited by the depth of the cultivation ropes, which does not exceed 1.0 m to 1.5 m below sea surface. The reduction in nutrient concentrations may also affect the seaweed growth but perennial seaweed such as *S. latissima* are less affected by nutrient availability than phytoplankton and fast-growing green seaweed (Pedersen and Borum, 1996).

In addition to competition with phytoplankton, the colonization of the fronds by epiphytic algae also affects seaweed growth and this process relies on the above-mentioned site-specific parameters (Harrison and Hurd, 2001; Leonardi *et al.*, 2006; Marinho-Soriano *et al.*, 2009; Andersen *et al.*, 2011; Peteiro and Freire, 2013). Nevertheless, the harvest occurs before the major part of the frond is colonized (Park and Hwang, 2012). Therefore, the characterization of seaweed growth rate and yield relies on site-specific aspects and farming practices, as shown in van der Molen *et al.* (2018) and Taelman *et al.* (2015).

2.3.2. Seaweed versus phytoplankton productivity

The simulated, annually averaged productivity for phytoplankton NPP is highest for the coastal North Sea environment and shows three productivity peaks (Figure 2- 4). The first peak, also called “spring bloom”, occurs in early June when the temperature and day length increase

enough to allow for primary production within the turbid riverine plume. The two following peaks (the “summer blooms”) result largely from the regenerated nutrients from the first bloom and consist of different phytoplankton species (*c.f.* ROFI results in van Leeuwen *et al.*, 2015). On average, the maximum phytoplankton NPP occurs at the end of May during the diatom-dominated spring bloom and reaches $3.68 \text{ gC}_{\text{eq}} \times \text{m}^{-2} \times \text{d}^{-1}$. Despite being farmed in a coastal environment, seaweed present a lower NPP than the phytoplankton NPP in the coastal area. The spring bloom from the latter represents 158% of the maximum seaweed NPP in Ireland and 219% of the two sites-averaged seaweed maximal NPP value (Figure 2- 4). Seaweed growth starts approximately one month before the phytoplankton growth and is reduced during the first phytoplankton bloom. The spatially averaged NPP curve for phytoplankton in the North Sea is steeper than the coastal NPP curve. The North Sea NPP (total surface average) reaches a maximum value of $1.46 \text{ gC}_{\text{eq}} \times \text{m}^{-2} \times \text{d}^{-1}$ at the end of April. The growing season for phytoplankton is spread from mid-March to mid-October (both coastal and open North Sea), with the spring bloom starting in the south and steadily moving northwards with increasing light levels.

The seaweed growing season starts in January, when light intensity and water temperature are not sufficient to support phytoplankton growth. *Saccharina latissima* can support a broad range of temperatures without affecting its photosynthetic efficiency (Andersen *et al.*, 2013). Moreover, the pigments content in *S. latissima* adapts according to the light conditions in order to keep a constant photosynthetic efficiency (Borum *et al.*, 2002). The temperature and light tolerances explain the early growth of *S. latissima*, which benefits from the nutrient accumulation during winter (Figure 2- 4). The NPP for the coastal area considered in this study is mostly dominated by diatoms and *Phaeocystis sp.* algae (Peperzak *et al.*, 1998; Lancelot *et al.*, 2007). In the coastal area, three phytoplankton blooms occur due to the sensitive response of phytoplankton growth to the local conditions. Each of the coastal phytoplankton blooms has its own species assemblage (van Leeuwen *et al.*, 2015). In other words, each bloom is explained by seasonal growths from divergent species, each with their own specific circumstances for optimal growth. Because seaweed harvesting occurs before the summer season (from June to September), it is not possible to determine in this study if they are subject to periodic growth within one year such as phytoplankton. Nevertheless, previous studies on *S. latissima* growth measurements over longer periods have shown that the NPP increases only once a year (Andersen *et al.*, 2011; Nielsen *et al.*, 2014). The North Sea area includes five distinct hydrodynamic regimes (van Leeuwen *et al.*, 2015) with their own biogeochemical specificities; the coastal area is included in the regime “*region of freshwater influence*” or ROFI. This regime is characterized by short periods of saline stratification as tidal and riverine influences dominate alternatively depending on the circumstances (*e.g.* spring tide, onshore

winds versus high river discharge, offshore winds, etc.). As the North Sea encompasses the five hydrodynamically different regimes (plus the areas which cannot be classified due to high inter-annual variability) its phytoplankton NPP fluctuates less and in smaller magnitudes than the averaged phytoplankton NPP for the coastal area (which is more sensitive to local drivers like Rhine discharge or wind direction). For the farming sites (both located in coastal areas), the NPP from seaweed is compared to the phytoplankton NPP in a coastal area. Nevertheless, the biogeochemical parameters and thus the phytoplankton NPP may diverge in the coastal areas. In order to model the NPP in both farming sites, the GETM-ERSEM-BFM would have to be extended to the desired geographical zones. Although a shelf-wide set-up exists of the coupled model (*c.f.* van der Molen *et al.*, 2018), new simulations were outside the scope of this work. Validation results also showed that the set-up used in van der Molen *et al.* (2018) overestimates production in coastal areas, which is the focus of the current study.

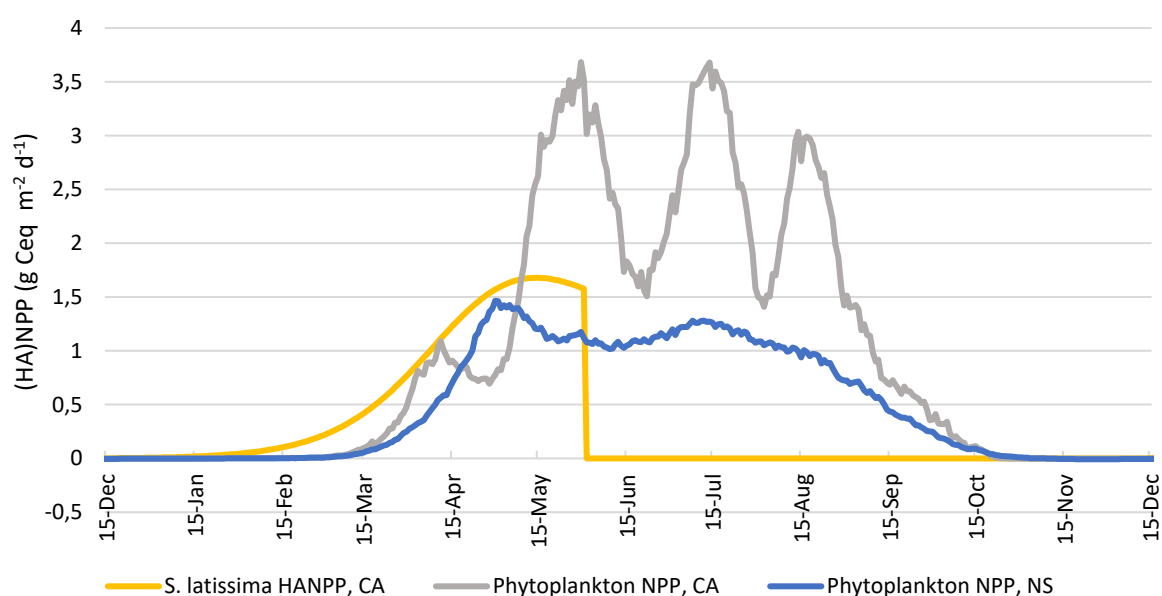


Figure 2- 4 Daily (HA)NPP values for *S. latissima* and for phytoplankton in the North Sea. The *S. latissima* HANPP is estimated from the growth (frond area) and frond area specific fresh weight of seaweed farms in Ireland (NUI) and France (CEVA). The fresh biomass is converted in carbon equivalent assuming a carbon concentration of 4% of seaweed fresh weight [42]. The phytoplankton NPP in the North Sea coastal area and total area are depth-integrated daily estimates, averaged over the period 1985 – 2005 and generated by the GETM-ERSEM-BFM North Sea hindcast, CEFAS, UK. CA: Coastal Area; NS: North Sea.

2.3.3. Lost Potential Yield indicators

The LPY indicators for the 10 most caught fish species are listed in and are computed based on the total fish landings in the sub areas NNS, CNS and SNS. The LPY values (Table 2- 3) are calculated for the production of 1 kg of *Saccharina latissima* (FW). The variation in the LPY_B between species results from the divergence in fish landings biomass (equation 2-3 and Table 2- 1). Atlantic herring has the highest LPY_B value (0.14 g) because it has the highest fish landings for the period 2011 - 2015. The LPY_V and LPY_E divergences between species are

also explained by the fish landings biomass but are balanced by the differences between species in monetary value (LPY_V) and the genome content (LPY_E). Atlantic mackerel presents the highest LPY_V value: its European market value ($791.75 \text{ €} \times \text{TLW}^{-1}$) is 2.21 higher than for Atlantic herring ($357.80 \text{ €} \times \text{TLW}^{-1}$), which compensates for the lower landing values (292,821.8 TLW versus 388,518.8 TLW). Cod is the species with the highest European market value ($2577.98 \text{ €} \times \text{TLW}^{-1}$) but is less heavily fished (9th of the 10th most fished species) compared to the other species. The LPY_V of cod is therefore higher than for sand eels (respectively $2.69 \times 10^{-5} \text{ €}$ and $2.29 \times 10^{-5} \text{ €}$) which is the third most fished species but has the lowest European market value ($230.32 \text{ €} \times \text{TLW}^{-1}$). The calculation of the LPY_E requires a first calculation step for the weighting factors. Except for European plaice which has a weighting factor (β) of 232, both β estimations are included in the same range (from 308 to 324) and thus, the genetic complexity is similar for both species (see C in appendices). This is explained by assumptions on the reference species for the genome size when data was lacking (Table 2- 2). The weighting factor computed for *S. latissima* is 215. The LPY_E is the highest for Atlantic herring ($4.60 \times 10^{-2} \text{ kg det}_{eq}$) which is the most fished species and also has the highest weighting factor of 324. Its weighting factor is based on the Pacific herring (*Clupea pallasii*) genome size because the genome size of Atlantic herring has not been reported in literature so far. The lowest fished species, cod and blue whiting, present the lowest LPY_E values. They also have the lowest weighting factor, meaning that the genome sizes are the smallest for the two species.

When considering the 10 most caught fish species together, the total LPY_B represents 0.5 g of fish in term of fresh weight while 1000 g of seaweed is produced. Compared with seaweed production (1000 g), the potential loss in fish landings biomass is negligible (0.05 % of seaweed biomass). The consideration of the European market value balances the results because of the low European market value for fresh seaweed ($54.32 \text{ €} \times \text{TFW}^{-1}$) compared to fish. The total LPY_V accounts for 0.5% of the value of seaweed produced ($5.43 \times 10^{-2} \text{ €}$). The total LPY_E is negligible when compared to *S. latissima* (0.07% of seaweed eco-exergy). The divergence in the weighting factors for *S. latissima* and the fish species does not balance the high seaweed biomass production and the relatively low LPY_B . The net gain for the technosphere (Table 2- 3) compares the production of harvested seaweed with the potential losses of fish landings. The net gains for the technosphere are almost equal to the seaweed production because the LPY indicators are relatively low compared to the seaweed production converted in their equivalent units. This is due to the low value of the HANPP from seaweed compared with the North Sea total NPP (equation 2-3).

Table 2- 3 Lost potential yield (LPY) per kilogram (fresh weight) of *Saccharina latissima* (sugar kelp) harvested in the North Sea. The LPY is expressed in term of fresh weight (FW) biomass (LPY_B), monetary value (LPY_V) and eco-exergy (LPY_E). The LPY_V considers European market price for fish (fresh, whole) in 2015. The LPY_E is estimated based on the respective biomass (LPY_B) and the weighting factor (β), the last one considers DNA content. The monetary value and the eco-exergy for 1 kg of *S. latissima* are provided in the row "Sugar kelp". The net gain for the technosphere (Net Gain Tech.) are listed in the last row.

Fish landings	LPY_B [kg, FW]	LPY_V [EUR]	LPY_E [kg_{det}]
Atlantic herring	1.42E-04	5.07E-05	4.60E-02
Atlantic mackerel	1.07E-04	8.45E-05	3.43E-02
Sand eels	9.96E-05	2.29E-05	3.10E-02
Sprat	5.56E-05	1.33E-05	1.80E-02
European plaice	2.63E-05	3.53E-05	6.08E-03
Saithe (Pollock)	2.52E-05	3.09E-05	7.74E-03
Norway pout	1.63E-05	3.93E-06	5.00E-03
Haddock	1.16E-05	1.76E-05	3.57E-03
Cod	1.04E-05	2.69E-05	3.21E-03
Blue whiting	6.50E-06	2.53E-06	2.00E-03
<i>Total</i>	5.00E-04	2.89E-04	1.57E-01
Aquaculture landings	Biomass [kg, FW]	Value [EUR]	Eco-exergy [kg_{det}]
Sugar kelp	1.00E+00	5.43E-02	2.15E+02
Net extracted biomass	Net biomass [kg, FW]	Net value [EUR]	Net eco-exergy [kg_{det}]
Net Gain Tech.	9.99E-01	5.40E-02	2.15E+02

The comparison of the results for the LPYs indicators and the associated seaweed production shows that the potential losses for the 10 most fished species are negligible in the three measurement units. The LPYs remain low even when they are summed for the 10 most fished species, accounting for 87 % (weight based) of the fisheries catches in the North Sea. The relatively low phytoplankton NPP decrease compared to the total North Sea NPP explains the small values of LPYs (equation 2-3). Such results show that seaweed farming is an interesting option to increase the natural resources extraction in the North Sea since the overall balance with fish landings is positive.

Table 2- 4 Scenarios for the lost potential yield in biomass and seaweed production according to the total seaweed-farmed area in the North Sea (only the cultivation of *S. latissima* is assumed). The farmed area does not consider the area used for the anchoring system. The total size of the ICES sub areas North North Sea, Central North Sea and South North Sea is considered (611,927 km²) when reporting the total farmed area to the North Sea area (Fraction of the North Sea). A yield of 1.75 kg FW m⁻² (cultivation at sea from 15th of December to 31st of May) is assumed for the estimation of the seaweed production (kg of Fresh Weight). The LPY_B results consider the 10 most fished species in the North Sea. The landings from the sub areas above mentioned are averaged for the period 2011 – 2015 for the calculation of the LPY_B [kg of Living Weight] and their relative value to the landings (Fraction of the landings).

Total farmed area [ha]	Fraction of the North Sea [%]	Seaweed production [kg FW]	LPY _B [kg, LW]	Fraction of the landings [%]
1.00E+00	1.75E-06	17.51E+03	8.75E+00	6.39E-07
34.54E+02	56.44E-04	6.05E+07	3.02E+04	2.21E-03
61.19E+04	1.00+E00	1.07E+10	5.36E+06	0.39E+00

Nevertheless, the large-scale cultivation of seaweed (where thousands of tons are produced) may considerably affect the fish landings in the zone. Table 2- 4 shows the LPY_B results according to 3 hypothetical scenarios for the farmed area in the North Sea. While seaweed farms represent a total area of 1 hectare in the North Sea, the LPY_B remains negligible compared to the total landings. The second scenario considers that 1% of the Belgian part of the North Sea is farmed (3454 hectares) to produce 60,500 tons (FW) of seaweed. In this case, the fish landings decrease by 30 tons (LW). The LPY_B is finally calculated for an overestimated area, when 1% of the North Sea area is farmed with seaweed. In this case, the LPY_B represents 0.39% of the fish landings. For comparison, the total seaweed production from aquaculture in the 27-EU countries reached 261 tons (FW) in 2019 (FAO, 2020a), far behind China with 13,924,535 tons (FW) in 2015 (Ferdouse *et al.*, 2018). This value corresponds to the quantity of seaweed produced if 1% of the North Sea is farmed with seaweed (Table 2- 4)¹⁵.

We quantify the fish depletion with the LPY_B indicator but some fished species are more endangered by their stock reduction than species that are more resilient. It is therefore important to identify threshold values for each species. The fisheries Maximum Sustainable Yield (MSY) (Schaefer, 1954) for each species in the North Sea appears attractive to normalize the LPY_B results. This indicator reports the maximum fisheries catches allowed without affecting further fish provisions. The limitation of such an approach consists in the bias introduced by the correlation between the LPY_B and the MSY. The LPY are computed from fish landings (equation 2-3), which depend themselves on the MSY (used to establish the fisheries quotas). Therefore, the MSY was not considered in this study. The MSY itself is affected by large-scale seaweed aquaculture due to the impact on fish stocks reduction. Alvarenga *et al.* (2013) proposed to assess land use efficiency through the “overall net annual exergy production (ΔEP)”. This method considers the amount of exergy in the biomass

¹⁵ This section has been added after the publication of the paper and does not appear in the published version.

produced, in the NPP from the initial environment and in the human inputs. The methodology from Alvarenga *et al.* (2013) can be extended to seaweed farming and sea use efficiency through the consideration of the LPY_B indicator to balance the seaweed biomass produced. The $\Delta EP-LPY_B$ methodology can be applied in case studies to provide insights on the best scenario to produce seaweed and fish biomass in an efficient way in terms of natural resources and input requirements. The implementation of the methodology with LPY_B might be relevant for further case studies assessing the overall performance of specific farming practice.

The LPY_V comparison with the market value of *S. latissima* (Net Gain Tech. in Table 2- 3) is influenced by the recent increase for the European seaweed market price, from 41.31 EUR x T^{-1} in 2014 to 54.31 EUR x T^{-1} in 2015 (Austoni *et al.*, 2007). The calculation of eco-exergy for the LPY_E is based on several assumptions on species genome size as no reliable data were available. Moreover, the eco-exergy calculation considers the whole genome size and thus, includes the non-coding fraction of the genome. The correlation between genome size and organism complexity is not straightforward: the amount of non-coding sequences is variable between species. This phenomenon is known as the C-value paradox: less complex species can present a bigger genome than more complex species because of their amount of non-coding sequences (Thomas, 1971). Taft *et al.* (2007) have shown that in most of the cases, the proportion of non-coding sequences increases with the complexity of the organism. Therefore, the principle of eco-exergy for organism complexity is still valid but the C-value paradox can explain inconsistency in the results. Once compared with the eco-exergy of *S. latissima* produced, the total LPY_E are negligible because of the similarity in the weighting factors (β) between fish and seaweed. This is explained by the relatively big genome size of *S. latissima* compared to other seaweed species (Kaprana, 2005) and the C-value paradox. The three LPY s indicators are sensitive to the fish landings, the LPY_V is influenced by the European market value of the products and the species genome size affects the LPY_E .

2.3.4. Uncertainties on seaweed productivity and phytoplankton depletion

The seaweed growth was first modelled in terms of wet biomass and then converted into carbon equivalents considering the total carbon content of *S. latissima* (26.8% DW). Our study does not consider seasonal variation in total carbon content that potentially affects the NPP values. *S. latissima* is known to store carbon reserves that are used during wintertime (Nielsen *et al.*, 2014). Therefore, the total carbon content of *S. latissima* increases from January / February to August / September (Sjøtun, 1993; Peteiro and Freire, 2013). The colonization of perennial seaweed by epiphytic organisms weakens the fronds and thus, induces apical frond losses. Nevertheless, the colonization is limited to the distal part of the frond for farmed seaweed harvested before summer (Park and Hwang, 2012). For mature seaweed, the apical

frond losses exceed the growth during the period July – November (Nielsen *et al.*, 2014). No study is available on the apical frond losses rate during seaweed farming, but the phenomenon is supposed to be limited because of the narrow fronds colonization at harvesting. As it is computed from the fresh standing biomass, the seaweed NPP can be underestimated by the non-consideration of the apical frond losses. Finally, the sigmoidal curve adopted for the model can oversimplify the seaweed growth. Nevertheless, the results are in accordance with a more exhaustive model for the growth of *S. latissima* (Broch and Slagstad, 2012).

The seaweed growth reduces the nutrient enrichment in coastal water during wintertime because of their uptake. The reduction in nutrient levels affects the magnitude of the phytoplankton blooms, when nutrients are the limiting factor for phytoplankton growth (Breton *et al.*, 2006; Conley *et al.*, 2009). Because it is assumed that seaweed production replaces the same amount of phytoplankton production in terms of the carbon equivalent, the reduction in phytoplankton biomass is probably underestimated. Indeed, our approach does not consider the cascade effect between the blooms since the two last blooms are partially supported by the regenerated nutrients from the first bloom (van Leeuwen *et al.*, 2013). Finally, it is worthwhile to mention that the impact is not localised at the farm site but is spread according to water circulation. In their study, van der Molen *et al.* (2018) do not find significant changes in phytoplankton productivity near seaweed farms. This is explained by the relatively small sizes of the farms and the water flows on the site, moving away and spreading the nutrients-depleted waters out of the zone.

In addition, the evaluation of phytoplankton NPP relies on the 1985 – 2005 period and hence, does not consider more recent conditions. De-eutrophication has been described in the North Sea, explained by the reduction of nutrients levels in regions of freshwater influence (Burson *et al.*, 2016). Nevertheless, the North Sea NPP has increased these last decades, probably explained by increase in temperature, changes in wind and light regimes and physiological acclimation and therefore, existing models NPP should be reconsidered (Desmit *et al.*, 2020; Xu *et al.*, 2020). This re-evaluation of the North Sea NPP might change the LPY results; those could be overestimated in case of increase in North Sea NPP.¹⁶

2.3.5. Impact pathways of seaweed farming on fish stocks

Our model focuses on one impact pathway of seaweed farming on fish landings through the reduction in the phytoplankton productivity. Nevertheless, other causality chains are expected and have to be considered (Table 2- 5). The large-scale seaweed farming in soft bottom environments such as the North Sea involves major changes in the surrounding ecosystem.

¹⁶ This section has been added after the publication of the paper and does not appear in the published version.

The immersion of hard structures (e.g. seaweed farm) in sandy marine ecosystems creates artificial reefs. This effect has been studied for the installation of wind turbines in the North Sea studies on ecosystem (Whilhemsson and Malm, 2008; Lindeboom *et al.*, 2011; De Troch *et al.*, 2013; Reubens *et al.*, 2013). Whitmarsh *et al.* (2008) highlight the potential positive effect of artificial reefs (concrete blocks) on the economic performance of fisheries in Portugal. However, this is less straightforward for seaweed farming because of the harvests which limit the immersion time to 5 - 6 months per year. It has been shown that seaweed farms attract fish by concentrating food and providing a shelter habitat but their impact on local fish biomass production seems limited (Eklöf *et al.*, 2006) while their effect on local fish diversity is not clear (Bergman *et al.*, 2001). Because fish tend to concentrate around the seaweed farm (Eklöf *et al.*, 2006), it can be expected that the reproduction rate will increase leading to more fish production. This potential positive impact for fisheries is balanced with the development of fishing exclusion zones at the farm location. Thus, the concentration of fish around the seaweed farm and the fishing exclusion zone are expected to increase, but it is unsure how this will affect the fish landings. The effect on local marine biodiversity is still unclear as it concerns both the water column and bottom substrate. The sediments enrichment with organic matter is expected to change the benthic species assemblage, as it is the case for wind turbines (Coates *et al.*, 2014). Finally, the nutrient bio-extraction from seaweed farming can decrease eutrophication levels in nutrients-rich coastal zones (Augyte *et al.*, 2017). This would reduce the magnitude of the toxic algae bloom, and thus the levels of toxins it releases that affect fish mortality in the local area (Hallegraeff, 1993).

Table 2- 5 The potential impact pathways of seaweed farming on fish community size. (+), (-) and (±) indicates respectively positive, negative and undetermined effects on fish community size. The LPY indicators developed in this study relies on the pathway shown in *italic characters*.

Intervention	Changes in ecosystem patterns	Causalities	Effects on community size
<i>Seaweed farming</i>	Artificial reef creation	Food concentration	+
		Protection	+
		Attraction	+
		Spawning support	+
		Trophic structure changes	±
	<i>Nutrients levels reduction</i>	<i>Phytoplankton productivity</i>	-
		Toxic algae blooms mitigation	+

With more than 90% of NPP transferred to upper trophic levels, phytoplankton productivity is a key factor in the structure of the ecosystem (Duarte and Cebrián, 1996). The correlation between phytoplankton NPP and fisheries yields has been highlighted at both regional and global scales (Ware and Thomson, 2005; Chassot *et al.*, 2007, 2010). However, the magnitude of this correlation varies according to ecological conditions and the trophic food web structure. For example, eutrophication (defined as increased NPP and accumulation of organic matter)

leads to an increase in NPP that is caused by bloom of few phytoplankton opportunistic species (Vallina *et al.*, 2014). As some phytoplankton species are not edible, this could reduce food availability for grazers and hence, affect the magnitude of primary production transfer (Micheli, 1999). In addition, the species composition and abundance in coastal areas are affected by the proliferation of harmful algae blooms and local hypoxia conditions caused by eutrophication (Rabalais *et al.*, 2009; Smith and Schindler, 2009).

2.4. Conclusions

This work is a first attempt to describe how seaweed production may affect the fisheries sector (wild catches). Our model computes indicators (LPY, Lost Potential Yield) assessing the effect of seaweed production on fish landings. We identify the reduction in phytoplankton productivity as a plausible causality between seaweed farming and fish landings. The harvesting of seaweed reduces nutrients levels and therefore, decreases phytoplankton maximum productivity. The production of 1 kg (FW) of *S. latissima* in the North Sea may potentially reduce the fisheries landings by 0.5 g (FW) when considering the 10 most fished species in the North Sea. It represents a loss of 0.03 cents (€) while the market value of *S. latissima* is about 5 cents [€] per kilo. These results favour the production of seaweed in term of natural resources extraction (more seaweed biomass extracted than potentially lost in terms of fish catches) but they have to be benchmarked with threshold values. Indeed, if phytoplankton productivity becomes too low to support the current food web, the species composition in the ecosystem, and thus the fish landings, might substantially differ. Since the LPYs proposed here are valid for the North Sea eco-region, the development suggested here can be extended to other zones by considering the local parameters. Finally, it is worth mentioning that our results rely on one causality, namely the decrease in phytoplankton production. We identify other potential cause-effect chains that probably occur in parallel and affect the global impact on fish landings. Moreover, it is important to mention that our study focuses on selected commercial species but seaweed farming has an impact across the whole ecosystem. While NPP is associated to ecosystem functioning and biodiversity, a significant reduction in NPP will affect ecosystem patterns. Since resource efficiency is crucial in the economy, it is relevant to understand how human activities may affect further resources provision. The current sustainability assessment studies for seaweed farming do not consider the potential effects on the fish stocks since this is the first study assessing the effect of such activity on fish provision. The Life Cycle Assessment (LCA) methodology is widely accepted as an efficient tool to assess the overall environmental sustainability of human-derived products. This method considers the pressures on three areas of concern (called “*Areas of Protection*”), one of them being the natural resources. It is recommended to integrate the LPY_B factor in the calculation of the pressure on natural resources in order to extend the scope of LCA studies dealing with seaweed farming.

CHAPTER 3

Development of a life cycle impact assessment framework accounting for biodiversity in deep seafloor ecosystems: a case study on the Clarion Clipperton Fracture Zone.

Redrafted from

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Abstract

The transformation of ecosystems is known to be a major driver of biodiversity loss. Consequently, supporting tools such as life cycle assessment methods (LCA) include this aspect in the evaluation of a product's environmental performance. Such methods consist of quantifying input and output flows to assess their specific contributions to impact categories. Therefore, land occupation and transformation are considered as inputs to assess biodiversity impacts amongst others. However, the modelling of biodiversity impact in deep seafloor ecosystems is still lacking in LCA. Most of the LCA methods focus on terrestrial biodiversity and none of them can be transposed to benthic deep sea because of knowledge gaps. This manuscript proposes a LCA framework to assess biodiversity impacts in deep seafloor ecosystems. The framework builds upon the existing methods accounting for biodiversity impacts in terrestrial and coastal habitats. A two-step approach is proposed, assessing impacts on regional and on global biodiversity. While the evaluation of regional biodiversity impacts relies only on the benthic communities' response to disturbance, the global perspective considers ecosystem vulnerability and scarcity. Those provide additional perspective for the comparison of impacts occurring in different ecosystems. The framework is operationalised to a case study for deep-sea mining in the Clarion Clipperton Fractures Zone (CCZ). Through the large variety of data sources needed to run the impact evaluation modelling, the framework shows consistency and manages the existing limitations in the understanding of deep seafloor ecosystems, although limitations for its application in the CCZ were observed mainly due to the lack of finer scaled habitat maps and data on connectivity. With growing interest for commercial activities in the deep-sea and hence, increased environmental research, this work is a first attempt for the implementation of LCA methods to deep-sea products.

3.1. Introduction

The loss of biodiversity, the diversity amongst genes, species or ecosystems, is considered as a major risk for humanity (WEF, 2020). The decline of biodiversity is accelerating faster than ever (IPBES, 2019) with a species extinction rate estimated between 0.01 and 0.1% per year (WWF, 2018). From an economic perspective, this damage induces a significant reduction of ecosystem services (Costanza *et al.*, 2014, 1998) that are supported by biodiversity (Haines-Young and Potschin, 2009). Five major drivers are recognized for their effect on the world biodiversity loss: (1) the transformation of terrestrial and marine ecosystems to man-made environments, (2) the exploitation of natural resources (e.g. animals and plants), (3) the pollution of land and marine areas with contaminated waste, (4) the introduction of invasive species and, (5) the consequences of climate change (WEF, 2020).

For these reasons, supporting tools for environmental informed decision making such as Life Cycle Assessment (LCA) tackle biodiversity aspects in their evaluation. LCA quantifies life cycle inventory (LCI) flows associated to a product life and convert them into environmental impacts, through their multiplication with specific coefficients called characterization factors (CF). Therefore, *land use (land occupation)* and *land use change (land transformation)* are part of the product LCI flows. They are considered by a diverse set of options of life cycle impact assessment (LCIA) methods in order to assess impacts on ecosystems and thus, on biodiversity. To avoid dealing with the complexity of biodiversity, changes in proxy indicators such as the net primary productivity, soil organic carbon or water flows are proposed to assess impacts from land-use on the natural environment (Curran *et al.*, 2016; Milà i Canals *et al.*, 2007; Taelman *et al.*, 2016; Teillard *et al.*, 2016; Teixeira *et al.*, 2016). However, other LCIA methods account for land-use impact on ecosystems through changes in biodiversity metrics (Chaudhary *et al.*, 2015; De Baan *et al.*, 2013a; Michelsen, 2008; Verones *et al.*, 2013; Weidema and Lindeijer, 2001). In these LCIA, biodiversity impacts are assessed in terms of potentially disappeared fraction of species (PDF, *i.e.* the relative loss of species richness), at regional and global scale. The global perspective weights the regional impacts according to the ecosystem in which they take place considering its specific attributes such as species/ecosystem vulnerability, species endemism, total species richness or ecosystem scarcity. This brings additional information to compare the biodiversity impacts amongst activities occurring in different regions and gives higher weight to impacts occurring in scarcer and more vulnerable ecosystems.

Similarly as for terrestrial ecosystems, a set of CFs has been proposed to assess regional biodiversity impacts caused by seafloor interventions in European marine coastal ecosystems identified by the World Wildlife Fund (WWF) (Woods and Verones, 2019). However, its

application to other ecosystems such as deep seafloor ecosystems has not been reported so far and the method does not provide CFs for global biodiversity impact assessment. Though, with the growing threat of anthropogenic marine disturbance such as benthic trawling, mining, tailings disposal, marine litter accumulation or oil and gas extraction (Santos *et al.*, 2019), there is a need to assess also the effects on biodiversity loss at the deep seafloor. There is potential to adapt and extent the method from Woods and Verones (2019) to address this issue and on top, to assess global biodiversity impacts despite the lower data availability on deep-sea ecosystems.

Therefore, the objective of this study is twofold. First, this study proposes a LCIA framework for regional and global biodiversity impact assessments caused by interventions on the deep seafloor. Secondly, it aims at an implementation of the framework for a particular deep-sea area, *i.e.* at the Clarion Clipperton fracture zone, as this is an area that is subject of exploration for deep-sea mining. While existing frameworks are preferred for case studies assessing land-use impact on terrestrial biodiversity, the application of this new framework for deep-sea environments is relevant in case of comparative LCA of commodities sourced from the deep-sea versus terrestrial mines. This way, it aims to facilitate the use of LCA to understand which of the deep-sea activities or its terrestrial/marine alternatives are the least harmful in terms of total environmental impacts.

3.2. Methodology

3.2.1. Overview of the methods considered for the development of the framework

This section introduces the methods used to establish the LCIA framework, *i.e.* (i) the cause-effect chain from Langlois *et al.* (2014) is extended to assess global biodiversity; (ii) the framework from Milà i Canals *et al.* (2007) is followed to differentiate amongst impacts, (iii) the method from Woods and Verones (2019) is chosen to establish CFs for regional biodiversity impacts from deep seafloor occupation and transformation, (iv) the recommendations from Koellner *et al.* (2013) are followed to consider the permanent impact and finally, (v) a unique CF for global biodiversity impacts¹⁷ is proposed considering vulnerability and scarcity aspects (Figure 3- 1).

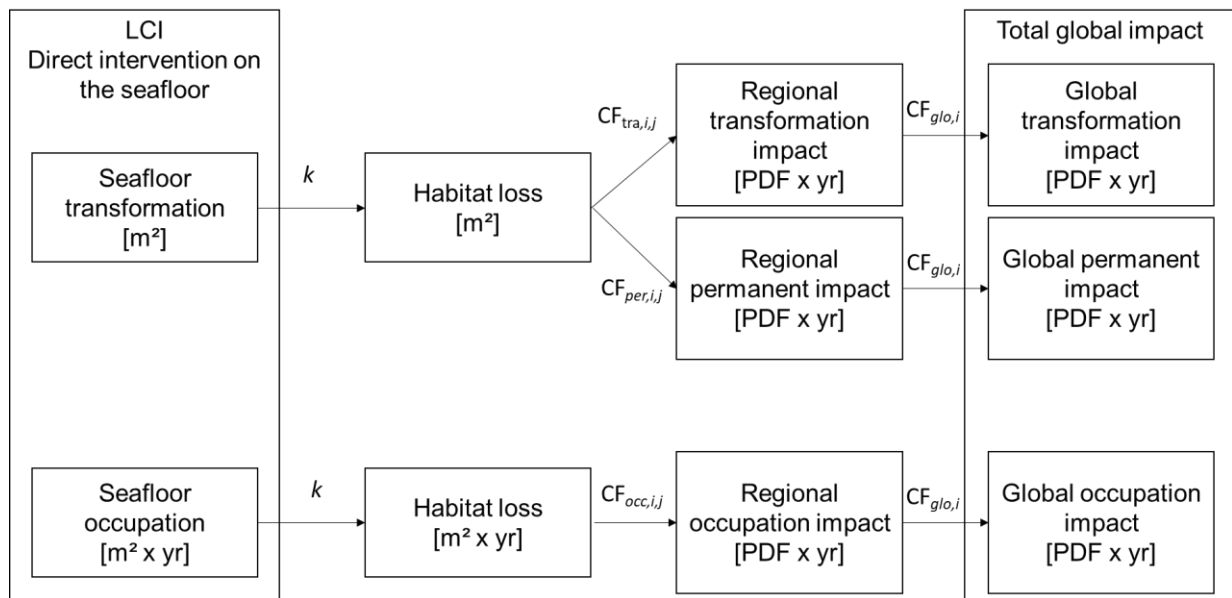


Figure 3- 1 Framework to assess biodiversity impacts caused by deep seafloor intervention in Life Cycle Assessment.

3.2.2. Existing frameworks for marine biodiversity impacts in LCA

The previous work from Langlois *et al.* (2014) is followed as a first guideline for the development of the framework. The authors identify multiple impact pathways from various interventions referred to sea use (shading, seafloor destruction, artificial reef creation, biotic resource uptake, invasive species introduction and noise). Amongst them, the present method

¹⁷ For consistency with the published version, the terminology “global characterization factor” is maintained in this manuscript. However, this global characterization factor is not multiplied with the LCI and hence, it deviates from the conventional characterization factors as with ISO standards.

focuses on the impact pathway of seafloor destruction - hereafter called *direct intervention on the seafloor* on regional biodiversity. This includes two LCI flows: *seafloor transformation* and *seafloor occupation* as starting elements for the LCIA framework. However, the framework is extended to global biodiversity (Figure 3- 2), following the recommendations from UNEP-SETAC Life Cycle Initiative (Jolliet *et al.*, 2018) to consider ecosystem-specific attributes in the evaluation of the biodiversity impact. Although biodiversity is defined at three levels (genes, species, ecosystems) and based on three attributes (composition, structure, function), the method follows UNEP-SETAC advise to use a species richness metric for biodiversity impact assessment (Jolliet *et al.*, 2018). For terrestrial biodiversity, the UNEP-SETAC recommends the countryside species-area relationship (SAR) method from Chaudhary *et al.* (2015). This method focuses on land-use (*i.e.* land transformation and land occupation) impacts on regional and global biodiversity, in which SAR models are fitted to specific ecosystems. Regional biodiversity impacts are assessed based on SAR models for non-endemic species while global biodiversity impacts rely on SAR models for endemic species. This method has been applied to terrestrial ecosystems only and has not been found suitable for deep seafloor ecosystems because of low data availability regarding species-area curves. Woods and Verones (2019) develop a framework to assess regional biodiversity impacts of anthropogenic disturbance in marine coastal ecosystems. The model considers the relative biodiversity loss due to anthropogenic disturbance compared to the initial biodiversity level (*e.g.* the authors set it to 50% for benthic trawling according to literature). The area of the ecosystem following the WWF classification for coastal ecoregions and the biodiversity recovery time are also required to assess the relative biodiversity loss. The recovery time is ecosystem-specific and is modelled according to hydrodynamic energy flows at the seafloor, substrate characteristics and stock of recolonize fauna. The present study follows a similar approach as Chaudhary *et al.* (2015), differentiating amongst regional and global biodiversity impacts. More precisely, a two-step modelling approach is proposed: (1) the impacts of habitat loss on regional biodiversity are assessed following principles from Woods and Verones (2019) and, (2) the regional biodiversity impacts are converted to global biodiversity impacts to allow comparison amongst various ecosystems. However, the development of CFs differs from Chaudhary *et al.* (2015) because it relies on disturbance experiments for the evaluation of regional CFs and ecosystems' patterns for the evaluation of global CFs.

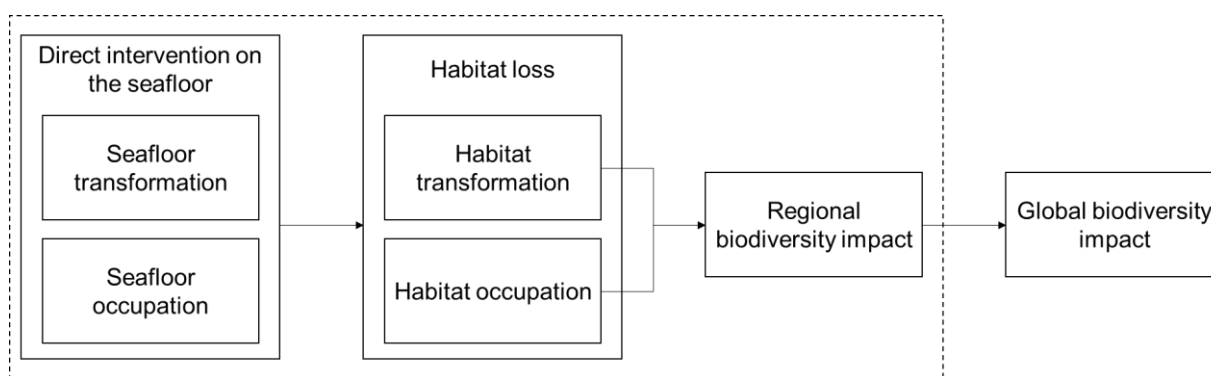


Figure 3- 2 Impact of seafloor intervention on regional biodiversity, adapted from Langlois *et al.* (2014) (dotted line) and extended to global biodiversity impact following UNEP-SETAC guidelines.

3.2.3. Impact of deep seafloor intervention on regional biodiversity

3.2.3.1. Proxy organisms for biodiversity impacts assessment

The evaluation of the biodiversity impact must rely on the benthic response of proxy organisms such as for terrestrial LCIA models. Therefore, changes in the deep-sea benthic megafauna composition caused by deep seafloor interventions are proposed here as benchmark to monitor biodiversity impacts. The definition of deep-sea benthic megafauna does not rely on taxonomic considerations but is rather defined on a size criterion: the group includes organisms being larger than 1 cm (Grassle *et al.*, 1975; Tilot, 2006). Organisms amongst deep-sea benthic megafauna mostly consist of cnidarians, echinoderms and sponges (Ramirez-Llodra *et al.*, 2010). Epifauna such as sponges, octocorallians or stalked crinoids are only found in nodule areas on which they can settle. Nodules are spherical mineral concretions ranging from 5 to 10 cm (*c.f.* 3.4.1) and represent the only naturally occurring hard substrates within these abyssal plains for attachment. Globally, the abundance and taxonomic richness of megafauna increases with nodule abundance (Tilot *et al.*, 2018). Deep-sea bacteria account for most of deep-sea benthic biomass (Rex *et al.*, 2006). However, megafaunal assemblages represent a significant fraction of the benthic deep-sea biomass (Haedrich and Rowe, 1977). The group is known to influence carbon cycling and mineralization in the ecosystem (Rex *et al.*, 2006; Sharma and Rao, 1992; Sibuet *et al.*, 1984; Sibuet and Lauwrence, 1981; Smith *et al.*, 1997). In addition, some megafaunal organisms are acting as ecosystem engineers through the process of bioturbation (*i.e.* reworking of the sedimentary deposits) or providing habitat for other organisms (Durden *et al.*, 2017; Smith *et al.*, 2008). Because of its slow metabolic growth, its low colonization rate and its influence on nodules ecosystems, the group species composition is used as an indicator for the recolonization of the biological communities after disturbance in nodule areas (Bluhm 2001; Tilot *et al.*, 2018).

3.2.3.2. Impact of occupying the deep seafloor on regional biodiversity

Similar to most of the other LCIA methods for land use impact on terrestrial biodiversity, Woods and Verones (2019) transpose the framework from Milà i Canals *et al.* (2007) to assess impacts on marine biodiversity caused by seafloor intervention in coastal regions. Milà i Canals *et al.* (2007) differentiate three types of impacts caused by seafloor intervention (*i.e.* occupation, transformation and permanent impacts) (Figure 3- 3). The assessment of the seafloor occupation impact ($OI_{i,j}$) consists of multiplying the occupied area (A_{occ}) [m^2] with the occupation time (t_{occ}) [yr] (both part of the LCI) and, with a CF. The last is specific to the ecosystem (i) and the intervention type (j) ($CF_{occ,i,j}$) and is measured in terms of marginal potentially disappeared fraction of species [$PDF \times m^{-2}$] (equation 3-1). Consequently, the occupation impact is measured in $PDF \times yr$. According to Woods and Verones (2019), the benthic response to area occupation is divided by the area of the ecosystem (A_i) to obtain the marginal benthic response (equation 3-2). The benthic response is measured in terms of relative species loss ($\frac{\Delta SR_{i,j}}{SR_{i,o}}$) [PDF] and, is specific to an intervention type j occurring in ecosystem i . The numerator $\Delta SR_{i,j}$ measures the temporary loss of species richness and is obtained by subtracting the species richness level during occupation ($SR_{i,j}$) to the permanent species richness level after regeneration has occurred ($SR_{i,p}$). The denominator consists in the species richness at the undisturbed state ($SR_{i,o}$). The benthic response is negative in case of activities inducing a loss of species richness or, is positive if the activity results in an increase of species richness (*e.g.* restoration of damaged ecosystems).

$$OI_{i,j} = CF_{occ,i,j} \times t_{occ} \times A_{occ} \quad (3-1)$$

$$CF_{occ,i,j} = \frac{\Delta SR_{i,j}}{SR_{i,o}} \times \frac{1}{A_i} = \frac{SR_{i,p} - SR_{i,j}}{SR_{i,o}} \times \frac{1}{A_i} \quad (3-2)$$

Where $OI_{i,j}$ is the occupation impact of disturbance j in ecosystem i , $CF_{occ,i,j}$ is the characterization factor for occupation impact assessment, t_{occ} is the occupation time, A_{occ} is the occupied area, $\Delta SR_{i,j}$ is the difference between species richness after regeneration ($SR_{i,p}$) and species richness at disturbance ($SR_{i,j}$), $SR_{i,o}$ is the initial species richness of ecosystem i and A_i is the total area of ecosystem i .

3.2.3.3. Impact of transforming the deep seafloor on regional biodiversity

The previous section focuses on the LCIA for deep seafloor occupation leading to occupation impacts according to Milà i Canals *et al.* (2007). The framework identifies another two impacts, transformation ($TI_{i,j}$) and permanent ($PI_{i,j}$) ones [$PDF \times yr$], caused by the transformation of an ecosystem (i) due to an intervention (j). The magnitude of these impacts relies on the recovery

rate of the benthic communities once the area is abandoned. Seafloor transformation is known to have higher impacts within ecosystems that are characterized by low recovery rates, resulting in lower biodiversity levels compared to pre-disturbance levels. Seafloor transformation is more impactful in ecosystems with low recovery rate and thus low biodiversity levels compared to pre-disturbance levels. These impacts are evaluated by multiplying a CF (specific to ecosystem i and intervention type j) [PDF $\times$ yr $\times$ m⁻²] with the area transformed (A_{tra}) [m²] (equation 3-3, equation 3-4). The characterization factor for transformation impact ($CF_{tra,i,j}$) relies on the regeneration time of the benthic response (t_{reg}) [yr] to reach the secondary succession steady state. A linear recovery is assumed for the evaluation of the CF (equation 3-5) (Koellner *et al.*, 2013a).

$$TI_{i,j} = CF_{tra,i,j} \times A_{tra} \quad (3-3)$$

$$PI_{i,j} = CF_{per,i,j} \times A_{tra} \quad (3-4)$$

$$CF_{tra,i,j} = \frac{1}{2} \times CF_{occ,i,j} \times t_{reg} \quad (3-5)$$

Where $TI_{i,j}$ and $PI_{i,j}$ are respectively the transformation and permanent impacts caused by disturbance j in ecosystem i , $CF_{tra,i,j}$ and $CF_{per,i,j}$ are respectively characterization factors for transformation and permanent impacts assessment and t_{reg} is the regeneration time.

The permanent impact considers irreversible changes in benthic communities resulting from deep seafloor transformation: the benthic response does not recover anymore at the secondary succession state. Because of uncertainty regarding endemic species in the deep sea, this framework assesses permanent impact as irreversible damage due to incomplete recovery of nodule-associated communities (Koellner *et al.*, 2013a; Milà i Canals *et al.*, 2007) instead of loss of endemic species such as in Chaudhary *et al.* (2015) and in de Baan *et al.* (2013a). The magnitude of the CF for permanent impact ($CF_{per,i,j}$) is identified by the loss of species richness at the undisturbed state ($SR_{i,o}$) and species richness at the secondary steady state ($SR_{i,p}$) (equation 3-6). The evaluation of the CF for permanent impact on regional biodiversity follows the recommendation from UNEP-SETAC (Koellner *et al.*, 2013b). Similarly as for CF_{occ} , the permanent change ($\Delta SR_{i,p}$) in species richness is measured relatively to species richness at the undisturbed state and divided by the area of the ecosystem (A_i) [m²]. The difference in species richness levels occurs on an infinite time due to the steady state assumption of the secondary succession. Therefore, a fixed time horizon (t_{per}) of 500 years is selected for the evaluation of $CF_{per,i,j}$ according to the modelling of biodiversity recovery periods in Curran *et al.* (2016) discussed in De Baan *et al.* (2013b). In this context it is worth to mention that the method from Woods and Verones (2019) for marine coastal ecosystems do not

consider permanent impacts and can lead to underestimation of the total biodiversity impact depending on the permanent loss of biodiversity.

$$CF_{per,i,j} = \frac{\Delta SR_{i,p}}{SR_{i,o}} \times \frac{1}{A_i} \times t_{per} \quad (3-6)$$

Where $\frac{\Delta SR_{i,p}}{SR_{i,o}}$ is the relative permanent loss of biodiversity in ecosystem i , A_i is the area of the ecosystem and t_{per} is the integration time (500 years).

Figure 3- 3 represents the three impacts evaluated in this study according to the framework from Milà i Canals *et al.* (2007) for land use impact on terrestrial ecosystem quality. It illustrates the three dimensions of the LCIA model: the benthic response, the time perspective and the area impacted. Transformation (TI) and permanent (PI) impacts are estimated by multiplying the respective CF (considering the marginal relative benthic response integrated over time) with the area transformed (life cycle inventory flows). The occupation impact (OI) is obtained by multiplying its specific CF (considering the marginal relative benthic response) with the area occupied and the occupation time (life cycle inventory flows).

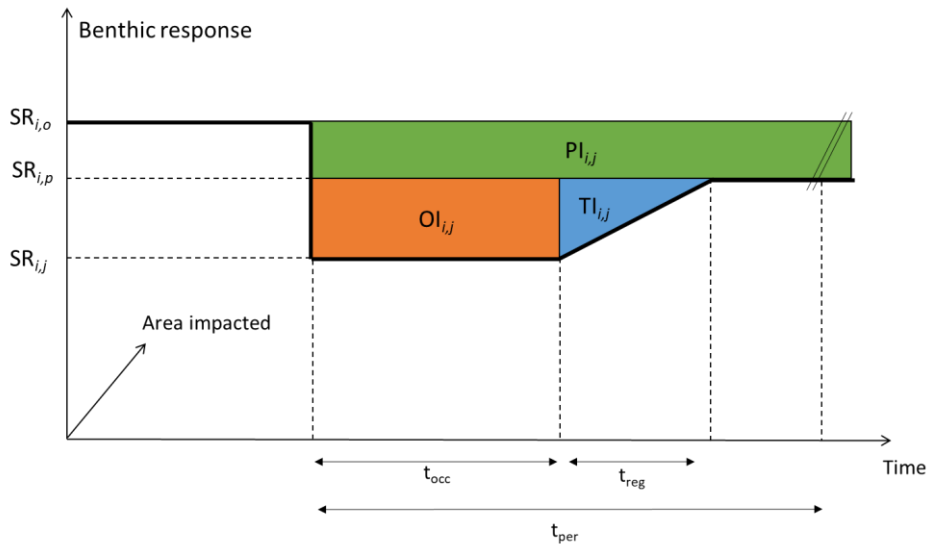


Figure 3- 3 Representation of deep seafloor interventions impacts on biodiversity according to the framework from Milà i Canals *et al.* (2007) for terrestrial ecosystems. $SR_{i,o}$, $SR_{i,p}$ and $SR_{i,j}$ stands respectively for the benthic response (species richness) in ecosystem i for the undisturbed, the permanent and the after disturbance situations. $PI_{i,j}$, $OI_{i,j}$ and $TI_{i,j}$ represents respectively permanent, occupation and transformation impacts of intervention j in ecosystem i . t_{occ} : occupation time, t_{reg} : regeneration time, t_{per} : permanent time.

3.2.4. Impact of deep seafloor intervention on global biodiversity

In this section, a unitless CF for global biodiversity impacts ($CF_{glo,i}$) is developed to consider specific attributes of the ecosystem (i) impacted in the LCIA model. Therefore, this CF consists of a weighting factor for the regional biodiversity impacts according to the ecosystem in which they occur (Curran *et al.*, 2016; Jolliet *et al.*, 2018). The CF is multiplied with each of the three

regional biodiversity impacts ($OI_{i,j}$, $TI_{i,j}$ and $PI_{i,j}$) [PDF x yr] to obtain the three types of global impact (equations 3-7, 3-8, 3-9). The total global biodiversity impact ($BI_{glo,i,j}$) [PDF x yr] is obtained through summing up global occupation, transformation and permanent impacts, respectively $OI_{glo,i,j}$, $TI_{glo,i,j}$ and $PI_{glo,i,j}$ [PDF x yr] (equation 3-10).

$$OI_{glo,i,j} = CF_{glo,i} \times OI_{i,j} \quad (3-7)$$

$$TI_{glo,i,j} = CF_{glo,i} \times TI_{i,j} \quad (3-8)$$

$$PI_{glo,i,j} = CF_{glo,i} \times PI_{i,j} \quad (3-9)$$

$$BI_{glo,i,j} = OI_{glo,i,j} + TI_{glo,i,j} + PI_{glo,i,j} \quad (3-10)$$

Where $OI_{glo,i,j}$, $TI_{glo,i,j}$, $PI_{glo,i,j}$ and $BI_{glo,i,j}$ are respectively global occupation, transformation, permanent and total biodiversity impacts caused by disturbance j in ecosystem i , $CF_{glo,i}$ is the global characterization factor to assess global impacts from regional occupation ($OI_{i,j}$), transformation ($TI_{i,j}$) and permanent ($PI_{i,j}$) impacts.

The method recommended by UNEP-SETAC for the evaluation of the global biodiversity impact (Chaudhary *et al.*, 2015) relies on species' endemism and species' threat level for the evaluation of CF_{glo} . So far, such information at species level is not available for deep seafloor ecosystems that remain largely unexplored and therefore, still present high discovery rates of new species and habitats (Amon *et al.*, 2016; Ramirez-Llodra *et al.*, 2010). On the other hand, other LCIA models rely on ecosystem conservation status (called abusively threat status), retrieved from WWF (Wildfinder database), rather than species' threat level, or they consider ecosystem scarcity for the evaluation of global biodiversity impacts (Michelsen, 2008; Weidema and Lindeijer, 2001; Winter *et al.*, 2018). So far, the final conservation statuses from WWF evaluations are only available for terrestrial and some coastal ecosystems (WWF, 2006; Spalding *et al.*, 2007). However, the WWF guidelines for the assessment of the final conservation status are available (Dinerstein *et al.*, 1995) and might be transposed to deep seafloor environments. In addition to ecosystem vulnerability, ecosystem scarcity indicators rely on the comparison between the area of the ecosystem under study with the area of the largest ecosystem (Weidema and Lindeijer, 2001; Winter *et al.*, 2018). Because none of the existing LCIA models are applicable for global biodiversity impact assessment in deep seafloor ecosystems, this section proposes a hybrid approach inspired by existing methods (Weidema and Lindeijer, 2001; Michelsen, 2008). This echoes the suggestion from Curran *et al.* (2016) and UNEP-SETAC (Jolliet *et al.*, 2018) to develop CFs that consider ecosystem vulnerability in the assessment of global biodiversity impact. $CF_{glo,i}$ is obtained through multiplying a vulnerability score with a scarcity score that are both specific for ecosystem i (equation 3-11).

The following subsections detail the calculation of the ecosystem vulnerability (EV_i) and ecosystem scarcity (ES_i) scores according to the existing methods.

$$CF_{glo,i} = EV_i \times ES_i \quad (3-11)$$

Where $CF_{glo,i}$ is the global characterization factor for ecosystem i , EV_i is the ecosystem vulnerability score and ES_i is the ecosystem scarcity score.

3.2.4.1. Ecosystem vulnerability

The WWF method is applied to the specific deep seafloor ecosystem to evaluate its final conservation status in contrary to LCIA methods for global biodiversity impact assessment which retrieve this information from the Wildfinder database. The WWF method evaluates two statuses and combines them in one final conservation status: (1) a *snapshot conservation status* that is the consequence of past disturbance events and, (2) a *threat status (sensu stricto)* relying only on disturbance events expected within 20 years. The snapshot conservation status is estimated by summing the scores obtained for six evaluation criteria: (a) the loss of original habitat, (b) the number and size of large blocks of the original habitat, (c) the degree of fragmentation, (d) the degree of degradation, (e) the conversion rate of the remaining habitat and, (f) the degree of protection. A score is assigned for each criterion according to benchmarks set by WWF and the sum of all criteria is converted to the respective snapshot conservation status amongst five levels (Table 3- 1). In a second step, the threat status is assessed similarly as for the snapshot conservation status, but it relies on 20 years-projections for three criteria: (a) conversion threat, (b) degradation threat and, (c) wildlife exploitation. The threat status varies amongst three levels in function of the total threat score (Table 3- 1). The snapshot conservation status and the threat status are combined in the final conservation status in the following way: (1) if the threat status is *low threat*, the final conservation status is similar to the snapshot conservation status, (2) if the threat status is *medium threat*, the final conservation status is similar or one level higher to the snapshot conservation status (up to experts' judgement) and, (3) if the threat status is *high threat*, the final conservation status is one level higher than the snapshot conservation status (e.g. from *relatively stable* to *vulnerable*). The reader is invited to consult the literature (Dinerstein *et al.*, 1995; Olson *et al.*, 2006) for detailed information on the WWF method.

Table 3- 1 Evaluation of the snapshot conservation / threat status according to snapshot conservation / threat scores (Dinerstein *et al.*, 1995).

Snapshot conservation score	Corresponding snapshot conservation status
0 to 6	<i>Relatively intact</i>
7 to 36	<i>Relatively stable</i>
37 to 64	<i>Vulnerable</i>
65 to 88	<i>Endangered</i>
89 to 100	<i>Critical</i>
Threat score	Corresponding threat status
0 to 19	<i>Low threat</i>
20 to 69	<i>Medium threat</i>
70 to 100	<i>High threat</i>

The final conservation status assessed through the WWF method is converted to an ecosystem vulnerability score (EV_i) according to the method from Chaudhary *et al.* (2015) that attributes scores ranging from 0.2 to 1.0. The final conservation status varying between five levels (Table 3- 1), EV_i increases by 0.2 for each increment of the final conservation status. Consequently, the final conservation status *relatively intact* corresponds to an EV_i of 0.2 and the final conservation status *critical* corresponds to an EV_i of 1.0.

3.2.4.2. Ecosystem scarcity

The evaluation of ecosystem scarcity score (ES_i) follows the method from Michelsen (2008), comparing the area of the terrestrial ecosystem under study (A_i) with the area of the largest terrestrial ecosystem (A_{max} , Sahara Desert) (equation 3-12).

$$ES_i = 1 - \frac{A_i}{A_{max}} \quad (3-12)$$

Where ES_i is the scarcity score of the ecosystem i , A_i is the area of the ecosystem i and A_{max} is the area of largest ecosystem.

Nevertheless, the method cannot be transposed directly to deep seafloor ecosystems because it requires to identify the largest ecosystem through a standard framework for ecosystem definition and a detailed mapping of all ecosystems. The WWF facilitates this exercise by providing an exhaustive list of 825 terrestrial ecoregions (*i.e.* ecosystem in WWF terminology) and their attributes in its Wildfinder database (Olson *et al.*, 2006). Although some attempts have been made (Agostini *et al.*, 2009), no unified classification for deep seafloor ecosystems is available so far. To avoid any confusion and to be able to compare ES_i for both terrestrial and marine ecosystems, the total area of Earth (A_e , 510,072,000 km²) is used as benchmark rather than the area (A_{max}) of the largest ecosystem (as the latter could not be defined for deep

seafloor ecosystems). In order to increase the sensitivity of the indicator, the logarithm of both A_i and A_e is used because these are not in the same order of magnitude (equation 3-13).

$$ES_i = 1 - \frac{\log(A_i)}{\log(A_e)} \quad (3-13)$$

Where A_e is the earth area [km²].

The logarithmic scale is used for its non-linear behaviour, in order to deal with the large variability amongst ecosystems areas. On the other hand, this approach limits the sensitivity of ES_i for comparisons amongst ecosystems which are in the same order of scale.

3.3. Requirements towards the operationalisation of the framework

A framework is proposed to assess deep seafloor intervention impacts on regional and global biodiversity in LCA (Figure 3- 3). Because the LCIA framework would be site- and intervention-specific, its implementation requires to collect the proper information for the evaluation of the impact. The identification of the ecosystem boundaries is the first step necessary for the development of the LCIA and refers to existing classifications of deep-sea regions. Those being fragmentary and defined for higher levels than ecosystem, additional information (reports, scientific literature) is required to identify the extent of the ecosystem under study. In addition, the intervention must be fully understood and described in order to assess its effect on habitat loss: an area impact factor (k) is estimated considering the total area of habitat loss compared to the area where the intervention takes place. Once the ecosystem is being identified and the intervention is being described, a set of specific parameters is necessary to calculate the CF of the LCIA method (Table 3- 2). The development of the CFs for regional biodiversity impacts relies on the literature monitoring the recovery of species richness from benthic disturbance experiments and the total area of the ecosystem. It requires to identify three biodiversity levels (pre-disturbance, at disturbance and post-disturbance at steady state) and the regeneration time to reach the permanent species richness level (steady state). CF_{glo} is assessed through two indicators: ecosystem vulnerability and ecosystem scarcity. While the last relies on the area of the ecosystem compared to the earth total area, the evaluation of ecosystem vulnerability according to the WWF method relies on quantitative and qualitative criteria. These are not focusing on any biodiversity status but rather on the evolution of specific attributes from the ecosystem. The implementation of the WWF method requires to gather information retrieved from experts' consultations and reports. Moreover, the evaluation of some criteria may require the use geographic information systems (GIS) when the information is not reported so far. The CF for global biodiversity is always smaller than 1.0 and provides information on the ecosystems' area and vulnerability allowing a comparison of regional biodiversity impacts occurring in distinct ecosystems.

Table 3- 2 Set of input parameters required for the LCIA of deep seafloor intervention impacts on local and global biodiversity and potential sources of information. Except for the criteria market with * (qualitative), all criteria are based on quantitative approaches.

Parameters	Input for	Sources
Species richness before occupation (initial) ($SR_{i,0}$)	$CF_{occ,i,j}$, $CF_{tra,i,j}$, $CF_{per,i,j}$	Disturbance experiments
Species richness during occupation ($SR_{i,j}$)	$CF_{occ,i,j}$, $CF_{tra,i,j}$	Disturbance experiments
Species richness after regeneration ($SR_{i,p}$)	$CF_{occ,i,j}$, $CF_{tra,i,j}$, $CF_{per,i,j}$	Disturbance experiments
Area of the ecosystem (A_i)	$CF_{occ,i,j}$, $CF_{tra,i,j}$, $CF_{per,i,j}$, CF_{glo}	GIS, classification schemes
Regeneration time (t_{reg})	$CF_{tra,i,j}$	Disturbance experiments
Proportion of habitat loss (in WWF for EV_i)	$CF_{glo,i}$	Reports
Habitat block size (in WWF for EV_i)	$CF_{glo,i}$	GIS, reports
Habitat fragmentation* (in WWF for EV_i)	$CF_{glo,i}$	GIS, reports
Habitat degradation* (in WWF for EV_i)	$CF_{glo,i}$	Reports, expert opinions
Annual habitat conversion rate (in WWF for EV_i)	$CF_{glo,i}$	Reports
Proportion of protected areas (in WWF for EV_i)	$CF_{glo,i}$	GIS, reports
Prospective habitat conversion (in WWF for EV_i)	$CF_{glo,i}$	Reports, expert opinions
Prospective degradation threat* (in WWF for EV_i)	$CF_{glo,i}$	Reports, expert opinions
Prospective wildlife exploitation* (in WWF for EV_i)	$CF_{glo,i}$	Reports, expert opinions

3.4. Operationalisation of the framework: a case study for deep-sea mining at the Clarion Clipperton Fracture Zone

3.4.1. Scope of the case study and LCI

An interesting metal source from the deep-sea consists of polymetallic manganese nodules being mostly composed of manganese, copper, nickel and cobalt (Petersen *et al.*, 2016). Although they have been discovered in the late nineteenth century, their commercial exploitation has not been started so far because of the lack of legal framework (*i.e.* most of the resources being in international waters) and their high exploitation costs (Cuyvers *et al.*, 2018). With increasing demand for metal commodities, the recovery of polymetallic nodules is becoming a possibility for the next decades. The United Nations established in 1982 a convention on the law for the deep-sea and founded in 1994 the International Seabed Authority (ISA) to support the regulatory framework. The Clarion Clipperton Fracture Zone (CCZ) is so far the largest discovered reserve of polymetallic nodules and covers approximately 6,000,000 km² in international waters within the North-East Pacific Basin (from 5 to 17°N and from 115 to 157°W) (ISA, 2012) (Figure 3- 4). No commercial mining operation has taken place so far, but mining exploration is being performed by contractors on the so-called contract areas (yellow areas in (Figure 3- 4). On the other hand, the ISA has set-up reserved areas (purple areas in Figure 3- 4) that can be mined later by developing countries.

The case study operationalizes the framework to assess regional and global biodiversity impacts caused by the harvesting of 1 ton of nodules dry weight (DW) in the CCZ. Therefore, the LCI flows required for the impact assessment (transformed and occupied area) are identified prior to determine input parameters for the evaluation of the characterization factors. Those are estimated based on the average nodule abundance in mining sites. An average nodule abundance of 12 kg DW m⁻² is considered according to resources estimates for the Eastern CCZ (Volkman and Volker, 2017; Volkman and Lehn, 2018). The LCI flows (transformed and occupied area) are estimated for this value, despite high spatial heterogeneity and uncertainty regarding the cut-off value for mining an area (Morgan *et al.*, 2010). For this nodule abundance value, the average transformed area in mining sites is 83.33 m² per ton of nodules DW and the average occupied area is negligible because each mining site is visited only once (*i.e.* a punctual disturbance event occurs considering that the collector is efficient to collect most of the nodules in a single visit).

3.4.2. Input parameters to develop CFs

3.4.2.1. Preliminary steps

(A) Ecosystem identification and description

In a first step, the implementation of the method requires to identify the ecosystem under study. A high heterogeneity in terms of bathymetry is observed with soft sediment abyssal plains (up to 6000 m depth), ridges, abyssal hills and seamounts (Volz *et al.*, 2018; Wedding *et al.*, 2013). The CCZ experiences substantial gradients (north-south, east-west) in overlying primary productivity and therefore a similar pattern is also found in terms of food fluxes to the abyssal seafloor (Cuyvers *et al.*, 2018; Smith *et al.*, 2006). Therefore, deep-sea ecologists and the ISA divided the CCZ in three East – West strata and three North – South strata to account for the gradients in the environmental management (ISA, 2019; Smith and Demopoulos, 2003; Wedding *et al.*, 2013). The nine sub-regions obtained are expected to be more homogeneous in their respective ecosystems compared to those of the entire CCZ (delimited by black lines and green in Figure 3- 4). For each sub-region, the environmental management plan sets up one strictly protected area of 400 km x 400 km, called the Area of Particular Environmental Interest (APEI, green areas in Figure 3- 4). Each APEI is assumed to be representative for its sub-region but also, for mining areas (ISA, 2012). However, this representativity is controversial because of their positioning outside the central and nodules-rich parts of the CCZ (Glover *et al.*, 2016; Jones *et al.*, 2019).

So far, no classification framework of deep seafloor ecosystems has been proposed and validated for the CCZ. Consequently, this case study relies on the spatial approach developed by the ISA for its environmental management plan. Each subregion includes various habitats, from soft sediment abyssal plains to seamounts (Wedding *et al.*, 2013) and hence, might include multiple ecosystems. However, this study considers each subregion as a dissociated and unique ecosystem due to the lack of information at lower scale level, *i.e.* regarding communities' assemblages on the entire CCZ. The subregion n°6 (delimited by red lines in Figure 3- 4) is selected for this case study because it is highly being subject to mining activities in the next decades, *i.e.* it contains the highest abundance of polymetallic nodules (Morgan *et al.*, 2010; Wedding *et al.*, 2013). The region extends on 1,017,411 km² including exploration and reserved areas that represent respectively 48.7 and 14.1% of the total area (Figure 3- 4). In addition, 5.0% of the area is considered as strictly protected because of the overlap with the APEI n°6. In addition to the APEI, preservation reference zones (additional protected areas) will be implemented for monitoring purposes of the mining impact. However, the extension and the distribution of these zones are still under discussion (ISA, 2017). Therefore, the present case study considers the APEI as the only protected area of the subregion.

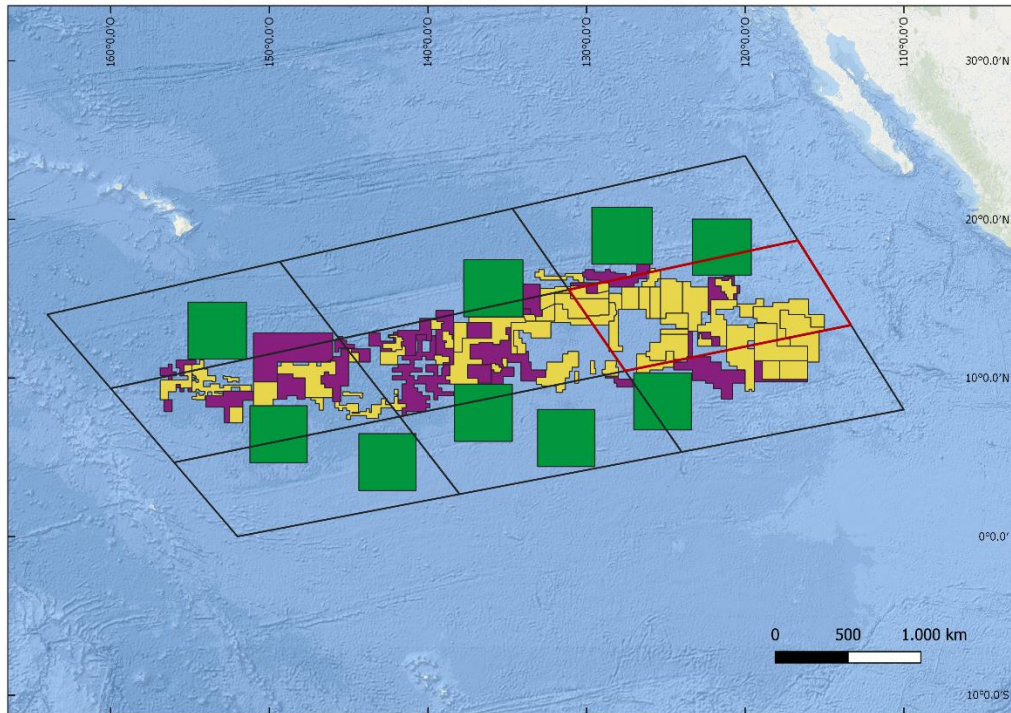


Figure 3- 4 Illustration of the nine subregions of the Clarion Clipperton Fracture Zone (black boxes) compared to the distribution of the potential exploration and reserved areas for mining (exploration areas in yellow; reserved areas in purple) and the areas of particular environmental interest (green), red lines define the boundaries for the subregion under study.

(B) Intervention description

Deep-sea nodules will be harvested by a remotely operated collector and transported to a vessel through a hydraulic system (Cuyvers *et al.*, 2018). Nodules are separated from the sediment fraction on the vessel and sediments are returned to the water column (*i.e.* return plumes). The evaluation of the impacts is relying on experimental conditions because no commercial exploitation has taken place so far. The mining operations are expected to impact deep-sea biodiversity through seafloor alteration (nodules removal, soil compaction, plumes deposits) but also, noise, light and vibrations are potential drivers of biodiversity loss (Cuyvers *et al.*, 2018; IUCN, 2018). The framework, focusing on seafloor intervention includes biodiversity impacts caused by nodules removal and from soil compaction in mining tracks, and by plumes deposition outside mining tracks. The last is caused by the visit of the collector that suspends sediments from the seafloor, but it may also be caused by the release of the return plumes from the vessel after the separation of the nodules and sediments fractions. However, the fate of the return plumes is uncertain with technical challenges to release them at the seafloor level (Heffernan, 2019). The sediments suspension and deposition is expected to induce changes in ecosystem functioning and to impact suspension and filter feeders (Gollner *et al.*, 2017). However, the magnitude of the impact and the area disturbed are highly uncertain because of the lack of information regarding benthic communities' response to plumes deposits. Indeed, the area disturbed and the depth of the settling sediment layer

depend on specific parameters such as the seafloor topography, the water currents but also the water depth at which return plumes are released (Aleynik *et al.*, 2017; Wedding *et al.*, 2013). Moreover, the pattern of the plumes deposition around the mining site is highly directional according to the water column flows and this directionality changes with time (Weaver, 2016).

(C) Plumes deposition scenarios to assess k

Three different scenarios are modelled according to plumes dispersion in order to estimate a range of values for habitat loss caused by similar LCI (Table 3- 3). Scenario A assumes that plumes deposition is limited to mining tracks and, mining tracks are spread on the entire concession. A second scenario B considers a plumes dispersal range of 20 km around the mining operations that are restricted to a single square-shaped location for each concession. The dispersal distance being highly sensitive to the hydrodynamic conditions, the value selected (20 km) is comprised in the range of what has been reported for the deposition of few millimetres of settling sediment depth (Aleynik *et al.*, 2017; Cuyvers *et al.*, 2018; Weaver, 2016). A third scenario C assumes that each concession is entirely impacted by either plumes deposition or nodules removal because mining operations are spread across each concession and plumes deposition from different mining sites are overlapping. This scenario does not consider the presence of buffer zones at the border of each concession. Scenarios B and C consider a plumes dispersal range of 20 km with homogeneous deposition and rely on the simplifying hypothesis that plumes are affecting the diversity of benthic megafauna similarly as for nodules harvesting. Therefore, the local biodiversity impact from plumes deposition is comparable to mining tracks. These scenarios may overestimate the impact of plumes deposition, but the absence of reliable information justifies this precautionary approach.

Table 3- 3 Deep-sea mining scenarios according to plumes deposition, mining patterns and area impact factor of direct intervention on the seabed to habitat loss.

	Scenarios		
	(A)	(B)	(C)
Mining sites	Spread on the entire concession	One single location, square-shaped	Spread on the entire concession
Plumes dispersal	Restricted to mining tracks	20 km	20 km
Impact of plumes	Similar as for nodules harvesting	Similar as for nodules harvesting	Similar as for nodules harvesting
Area impact factor (k)	1	6.12	20

For each plumes deposition scenario, the LCI flows (occupied and transformed area) are converted to habitat loss according to k (Figure 3- 3) considering the magnitude of the area impacted by plumes deposition compared to the area mined. The quantification of k considers that the mineable area represents 5% of each exploration in the subregion n°6 according to resource estimates and nodule abundance (similar assumption in 3.4.2.3) (Morgan *et al.*, 2010; Volkmann and Lehnen, 2018). While k is constant for scenarios A ($k = 1$) and C ($k = 20$, *i.e.* = 0.05^{-1}), scenario B presents changing k values depending of the concession. In this scenario, the area impacted by plumes is approximated by a square with edges being 40 km larger than the square where mining operation take place. To obtain k , the area of each concession is divided by the area of the square impacted by plumes deposition and nodules harvesting. Therefore, the average value of all concessions belonging to the subregion n°6 is selected ($k = 6.12$).

This first subsection provides specific information related to the ecosystem (total area, exploration areas, protected areas) that are necessary for the quantification of the CFs. In a second step, three plumes deposition scenarios have been described in order to estimate k , required to assess habitat loss from the LCI flows.

3.4.2.2. Data of megafauna recovery to develop regional CFs

The LCIA for the regional biodiversity impact relies on the megafauna species diversity for two horizons: straight after disturbance and, after regeneration has occurred to reach a stable secondary state. However, most of the experiments carried out in the CCZ do not necessary rely on megafauna and assess density recovery rather than diversity (Gollner *et al.*, 2017; Jones *et al.*, 2017). In addition, the benthic disturbance experiments are too recent to understand the secondary succession patterns of the communities on long time perspective. The first disturbance experiment in the CCZ has been carried out in 1978 by *Ocean Minerals*

Company (OMCO) and was sampled 37 years after disturbance (Vanreusel *et al.*, 2016). Two publications report the recovery levels of megafauna diversity after benthic disturbance in mining tracks (Bluhm, 2001; Vanreusel *et al.*, 2016) and their results are considered for the development of the CFs (Table 3- 4).

Table 3- 4 Disturbance experiments selected for the study and recovery level of deep-sea benthic megafauna diversity.

Parameter	Experiment	Disturbance type	Time after disturbance	Benchmark	Diversity recovery	Method	Reference
$\frac{SR_{i,j}}{SR_{i,o}}$	DISCOL	Plough-arrow, in tracks	1 day	Pre-impact	17.5%	Video transect	Bluhm, (2001)
$\frac{SR_{i,p}}{SR_{i,o}}$	OMCO	Mining, in tracks	37 years	Nearby undisturbed	44.4%	Video transect	Vanreusel <i>et al.</i> (2016)

Therefore, the LCIA is built on the results from Bluhm (2001) for the biodiversity level straight after benthic disturbance. These report 17.5% of initial megafauna diversity (sessile and mobile, measured in *animal category*) for one day after disturbance. The sampling has been performed in the context of the *Disturbance and Recolonisation* experiment (DISCOL). However, the DISCOL experiment has occurred in nodules fields located outside the CCZ, in the South East Pacific Ocean of Peru (Figure 3- 5). Furthermore, no nodules were removed during this experiment in which a plough-like trawl, without collector, was deployed in the area. The DISCOL study consisted of monitoring the recovery (density and diversity) of megafauna within mining tracks (0, 0.5, 3, 7 and 26 years after disturbance). Because no other benthic disturbance experiment reports the recovery of megafauna diversity directly after disturbance, the results from DISCOL are selected as a proxy for the development of the CFs.

The observations from Vanreusel *et al.* (2016) are considered for megafauna diversity (sessile and mobile, measured at the class taxonomic level) for 37 years after disturbance in the OMCO experiment. This benthic disturbance experiment has taken place in the Eastern part of CCZ and more precisely, in a nodule-rich zone of the IFREMER exploration area. The spatial extension of OMCO crosses the border of the subregion n°6 (Figure 3- 5). The study reports the presence of 4 taxonomic groups (at the class level) in mining tracks visited 37 years after disturbance while 9 groups are observed in nearby undisturbed areas. This corresponds to a recovery level of 44.4% in terms of class diversity.

The deep-sea benthic megafauna recovery from mining disturbance experiments remains relatively low (*i.e.* 44.4% after 37 years in the OMCO experiments). CFs for regional

biodiversity impacts are quantified based on these results that are inserted in equations 3-2, 3-5 and 3-6.

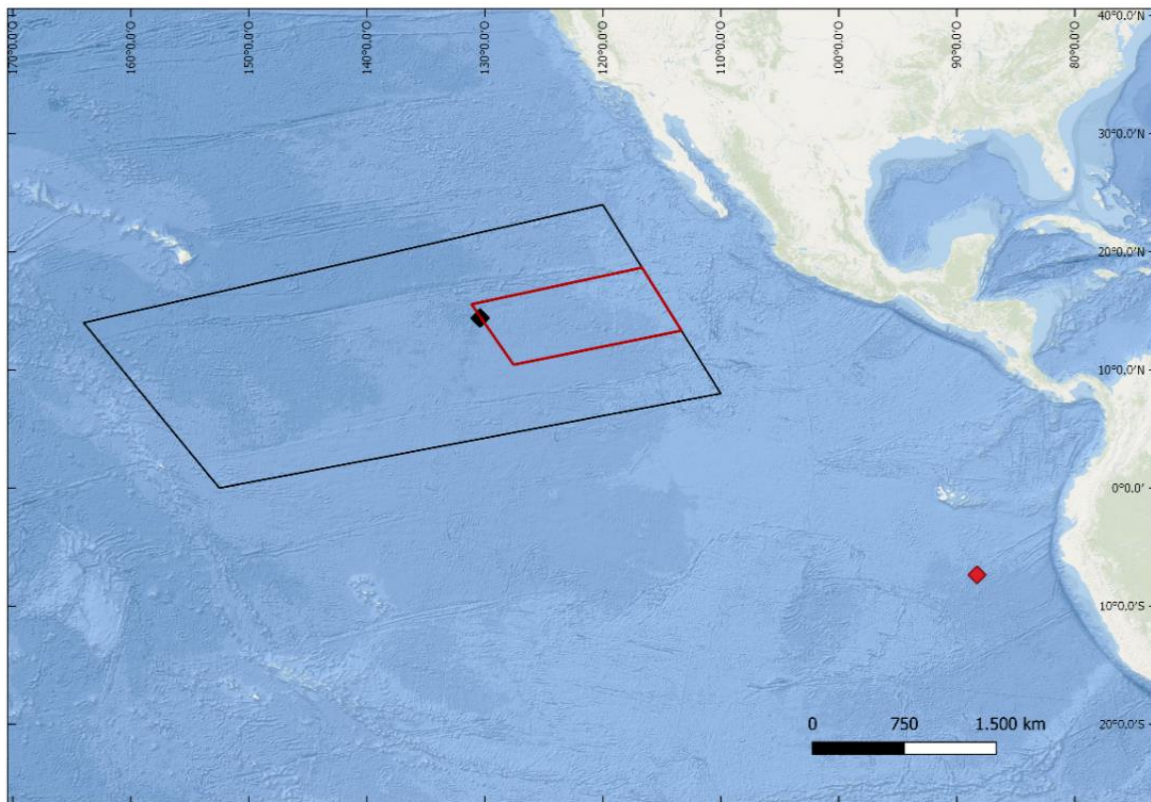


Figure 3- 5 Location of the OMCO (black diamond) and the DISCOL (red diamond) disturbance experiments. The CCZ area and the subregion n°6 are respectively outlined in black and red lines.

3.4.2.3. Data to assess ecosystem vulnerability (required to quantify CF_{glo})

The evaluation of CF_{glo} requires to perform a vulnerability assessment (EV) according to the WWF method to assess the final conservation status (Dinerstein *et al.*, 1995). Specific parameters for the application of the WWF method are assessed and described in Table 3- 5.

The first set of parameters refers to the present situation to assess the snapshot conservation status. The subregion is assumed to occur in a single location, despite the occurrence of deep-sea nodules fields in all oceans (McKelvey *et al.*, 1983). However, the communities are expected to differ because of variable abiotic conditions (Agostini *et al.*, 2009) and the fundamental uncertainties on communities' distribution, species' endemism or habitats' connectivity do not allow statements on the worldwide distribution of the regional patterns (Cuyvers *et al.*, 2018; Gollner *et al.*, 2017; Miller and Gunasekera 2017). The subregion is considered as undisturbed (pristine) because no single commercial activity has taken place so far and anthropogenic disturbance is limited to few disturbance experiments (Jones *et al.*,

2017). Therefore, the present situation assumes that no habitat fragmentation or degradation has occurred so far.

The threat status considers that 5.0% of each exploration and reserved area is mined within 20 years. However, the conversion of the initial habitat relies on plumes dispersion scenarios. If plumes dispersion is limited to mining tracks, the prospective habitat conversion within 20 years is estimated to 3.1% (*i.e.* 5.0% each concession is mined and the total area of the concessions represents 62.9% of the total area of the sub-region n°6, *c.f.* 3.4.2.1-(A)). If plumes deposition extends on each entire concession, the prospective habitat conversion is thus equal to the proportion of concessions belonging to the sub-region n°6 (*i.e.* 62.9%). The evaluation of the prospective habitat conversion in the plumes deposition scenario (B) is less straightforward. For each concession from the sub-region n°6, the area impacted by plumes deposits is assessed considering that mining takes place in a single square extending on 5.0% of the total area of the concession. It is assumed that plumes extent around the square with a maximum dispersal distance of 20 km (*c.f.* 3.4.2.1-(C)). Therefore, the total area of the habitat impacted is estimated to 150,156 km², representing 14.8% of sub-region n°6 area (*i.e.* 1,017,411 km²). The degradation threat is considered as maximal, assuming that mining activities induce high mortality to native species that are located within the influence zone. The criterion wildlife exploitation is arguable due to the uncertainty on the exploitation of marine genetic resources from deep-sea beyond national jurisdiction. Although there is an evident economic potential for deep seafloor biota, its current exploitation is limited to scientific research but the commercial exploitation for marine genetic resources in the future is highly probable (Harden-Davies, 2017; Leary *et al.*, 2009). Therefore, this criterion receives an intermediate status that corresponds to low levels of wildlife exploitation (Dinerstein *et al.*, 1995).

Table 3- 5 Specific parameters for the subregion under study to assess the snapshot conservation status and the threat status according to the WWF method. (1) This choice is justified by the precautionary principle: in the context of existing knowledge gaps for deep-sea biodiversity (Smith et al., 2006; Wedding et al., 2013), deep-sea nodules ecosystems are expected to differ because of variable abiotic conditions (Agostini et al., 2009). (2) The vastness of the water column preserve deep-sea ecosystems from major anthropogenic disturbances (fishing, ocean acidification, temperature increase, pollution) (Smith et al., 2020). (3) Assuming that 5% of each concession is mined and the three plumes deposition scenarios defined in Table 3- 3.

Status	Criteria	Assessment	Reference
Snapshot conservation status	Geographic distribution	Single location	Assumption ¹
	Proportion of habitat loss	0.0%, intact ecosystem	Assumption ²
	Habitat block size	One single block of 1.02 E+06 km ²	ISA (2011)
	Habitat fragmentation	No fragmentation	Assumption ²
	Habitat degradation	No degradation	Assumption ²
	Annual habitat conversion rate	0.0%	Assumption ²
	Proportion of protected areas	5.0% (APEI)	ISA (2011)
Threat status	Prospective habitat conversion	3.1% (A), 14.8% (B), 62.9% (C)	Assumption ³
	Prospective degradation threat	Many populations of native species experiencing high mortality	Smith et al. (2020)
	Prospective wildlife exploitation	Low exploitation	Harden-Davies (2017)

This paragraph provides key elements (Table 3- 5) to assess the final conservation status of the subregion n°6 according to the WWF method (Table 3- 8). It highlights that the ecosystem represented by the subregion is unique and has not been disturbed so far. However, mining is expected in the near future and the pressure on the ecosystem will vary according to plumes deposition scenarios.

3.4.3. Operationalisation of the LCIA framework for deep-sea mining in the CCZ subregion n°6

3.4.3.1. From deep nodules mining to habitat loss

The two LCI flows: occupied area [m² x yr] and transformed area [m²] are converted to habitat loss according to *k* identified for each plumes deposition scenario (*c.f.* 3.4.2.1). Table 3- 6 represents the total habitat loss caused by plumes deposition and by nodules harvesting for the three scenarios under study. The on-site occupied area is assumed neglectable (Table 3- 6) because the collector does not revisit the mining tracks (*c.f.* 3.4.1).

Table 3- 6 Habitat loss (occupation and transformation) caused by the harvesting of 1 ton of nodules dry weight in mining sites with an average nodule abundance of $12 \text{ kg} \times \text{m}^{-2}$.

	Habitat occupation [$\text{m}^2 \times \text{yr}$]	Habitat transformation [m^2]
Scenario A	0	83.33
Scenario B	0	509.67
Scenario C	0	1666.67

3.4.3.2. Specific CFs for regional and global biodiversity impacts

(A) Regional CFs

The CFs are obtained from equations 3-2, 3-5 and 3-6 with inputs parameters from the disturbance experiments monitoring the recovery level of deep-sea benthic megafauna diversity (*c.f.* 3.4.2.2) and the area of the ecosystem. Therefore, CF for the transformation impact considers a relative recovery of megafauna diversity from 17.5 to 44.4% occurring after 37 years. CF for the permanent impact considers a constant relative diversity level of 44.4% that is integrated over 500 years (Table 3- 6).

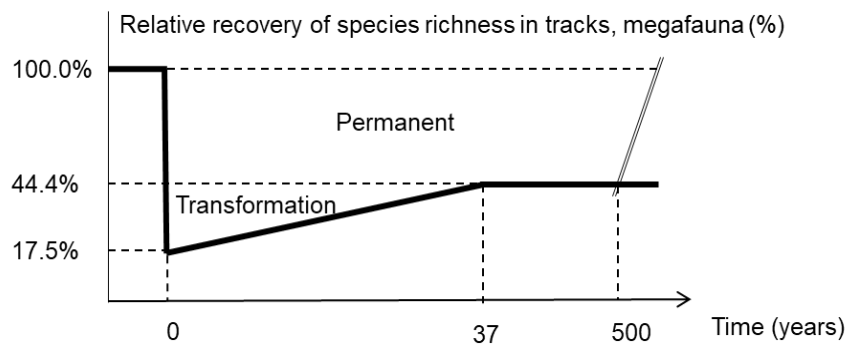


Figure 3- 6 Two-dimensional representation of the impacts from deep-sea mining in the CCZ according to the observations from the disturbance experiments and the time horizons selected.

The three CFs obtained (Table 3- 7) represent the proportion of biodiversity loss for the benthic megafauna caused by the disturbance of one square meter of deep seafloor. The CF for permanent impact is larger than for transformation impact because of its integration on 500 years and the constant gap of 66.6% of relative diversity between the undisturbed ecosystem) and secondary state communities.

Table 3- 7 CFs for the regional biodiversity impacts from deep-sea mining in the subregion n°6 of the CCZ. The quantification of the CFs is performed according to equations 3-2, 3-5 and 3-6.

CFs	Value
$CF_{occ,i,j}$ [PDF x m ⁻²]	8.11E-13
$CF_{trans,i,j}$ [PDF x yr x m ⁻²]	4.91E-12
$CF_{per,i,j}$ [PDF x yr x m ⁻²]	2.73E-10

(B) Global CF

The global biodiversity impact relies on two additional aspects according to equation 3-11: ecosystem vulnerability and ecosystem scarcity. The first requires the implementation of the WWF method (Dinerstein *et al.*, 1995) to assess the final conservation status. The evaluation is performed according ecosystem-specific parameters described in Table 3- 5. The snapshot conservation status receives the minimal score (3/100) because the subregion is intact while most of the criteria are referring to past anthropogenic disturbance. Therefore, the three scenarios are presenting a similar snapshot conservation status (*i.e. relatively intact*). The evaluation of the threat status within 20 years is contrasted amongst scenarios because of the effect of plumes dispersion on the first criterion, the conversion threat (Table 3- 5). Consequently, the three scenarios present varying threat scores but scenarios A and B present a similar threat status because their respective threat scores fall in the same interval for the evaluation of the threat status. The status *medium threat* is assigned for scenarios A (40/100) and B (60/100), whilst scenario C is considered as *high threat* (90/100). The final conservation status is then *relatively stable* for scenario C because it consists to the snapshot conservation status increased by one level when the threat status is *high threat*. On the other hand, the evaluation of the final conservation status for scenarios A and B is less straightforward because of their intermediate threat statuses (*i.e. in case of medium threat*, the final conservation status is similar or one level higher than the snapshot conservation status). The status *relatively stable* is assigned for scenario B because of the uncertainty regarding plumes impact on ecosystem functioning. In this scenario, 14.8% of the subregion is impacted by plumes deposition (Table 3- 5) but the threshold value for ecosystem collapsing is unknown. On the other hand, the scenario A is considered as *relatively intact* because the area impacted is far reduced (3.1%) and plumes are not dispersing outside mining tracks. The final conservation score is converted into a vulnerability status according to Chaudhary *et al.* (2015), scenarios A, B and C receive vulnerability scores of 0.2, 0.4 and 0.4, respectively. These values remain low compared to the maximum value of 1.0 when the final conservation status is *critical* (Table 3- 1). This is explained by the higher importance of the snapshot conservation status than the threat status for the evaluation of the final conservation status.

Table 3- 8 Evaluation of the final conservation status according to the WWF method in order to estimate the vulnerability score.

	Scenarios		
	A	B	C
<i>Evaluation of the snapshot conservation status</i>			
Loss of original habitat	0	0	0
Habitat blocks size	2	2	2
Habitat fragmentation	0	0	0
Habitat degradation	0	0	0
Conversion rate	0	0	0
Degree of protection	1	1	1
Conservation score	3	3	3
Snapshot conservation status	<i>Relatively intact</i>	<i>Relatively intact</i>	<i>Relatively intact</i>
<i>Evaluation of the threat status</i>			
Conversion threat	0	20	50
Degradation threat	30	30	30
Wildlife exploitation	10	10	10
Threat score	40	60	90
Threat status	<i>Medium threat</i>	<i>Medium threat</i>	<i>High threat</i>
Final conservation status	<i>Relatively intact</i>	<i>Relatively stable</i>	<i>Relatively stable</i>

An ecosystem scarcity score of 0.310 is obtained according to equation 3-13 for the subregion n°6. The score is compared with those from the terrestrial ecoregions defined in the WWF Wildfinder. The largest (Sahara Desert), the smallest (Lord Howe Island subtropical forests) and the average size of terrestrial ecoregions have respectively a scarcity score of 0.234, 0.770 and 0.401 according to equation 3-13. ES and EV are multiplied to obtain CF_{glo} ; while ES depends on the area of the ecosystem under study, EV focuses on anthropogenic disturbance. None of the input parameters for EV relies directly on the area of the ecosystem (*i.e.* proportion of disturbed area compared to total area). Therefore, the multiplication of these two indicators in the evaluation of CF_{glo} does not induce any double counting.

Table 3- 9 Evaluation of CF_{glo} for global biodiversity impacts according to equation 3-11. EV is estimated by converting the final conservation status into score according to Chaudhary et al. (2015). ES is estimated according to equation 3-13.

	Scenarios		
	A	B	C
EV	0.2	0.4	0.4
ES	0.310	0.310	0.310
CF_{glo}	0.062	0.124	0.124

The CF for scenario A is half of those for scenarios B and C because of varying final conservation status (Table 3- 9). This means that less weight is given to biodiversity impacts under scenario A because the ecosystem is considered as less endangered than for other scenarios.

3.4.3.3. Results: regional and global biodiversity impacts assessment

Following equations 3-1, 3-3 and 3-4, the LCI for the harvesting of one ton of nodules DW (Table 3- 6) are multiplied with k and the CFs (Table 3- 7) to assess the impacts on regional biodiversity (Figure 3- 7). While the LCI and the CFs are constant amongst scenarios, k is varies and explains the difference in regional biodiversity impacts (*i.e.* it determines the variability in habitat loss amongst scenarios - Table 3- 3, Table 3- 6). The last is sensitive to the three plumes deposition scenarios under study, showing the importance of controlling the plumes to limit the biodiversity impact. No occupation impact is observed because of the occupation time that is considered as null for the activities on mining site. The permanent impact contributes to most of the total impact because of its higher CF compared to the transformation impact, explained by the larger integration time (500 years). On the other hand, this impact is highly uncertain because it relies on the assumption that biodiversity remains constant after a recovery period of 37 years. However, this is the best information available so far for megafauna recovery on long time horizons.

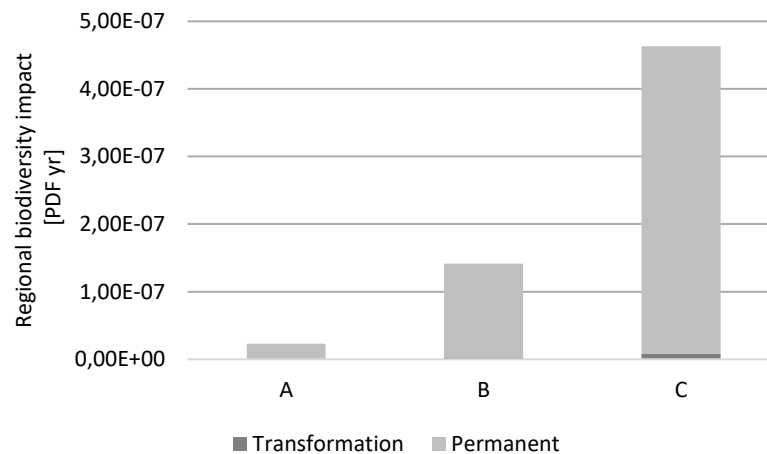


Figure 3- 7 Regional biodiversity impact on deep-sea benthic megafauna for the harvesting of one ton of nodules (DW) in the subregion n°6 according to plumes deposition scenarios: (A) plumes limited to mining tracks, (B) dispersal range of 20 km – a single and square-shaped location is mined in the contract area, (C) plumes are dispersing on the entire concession because of patchy disposition of mining areas.

The difference in global biodiversity impacts between scenario A and the two other scenarios (Figure 3- 8) becomes greater than for local biodiversity impacts (Figure 3- 7) because of the varying EV (Table 3- 9). This does not mean that less species are lost with a lower global CF but the regional biodiversity impact is expected to be less harmful to world biodiversity because of the lower vulnerability level of the ecosystem. Instead of relying on exact numbers for the comparison of global biodiversity impacts, the present LCIA sets benchmarks that are quantified in order to compare biodiversity impacts occurring in different ecosystems.

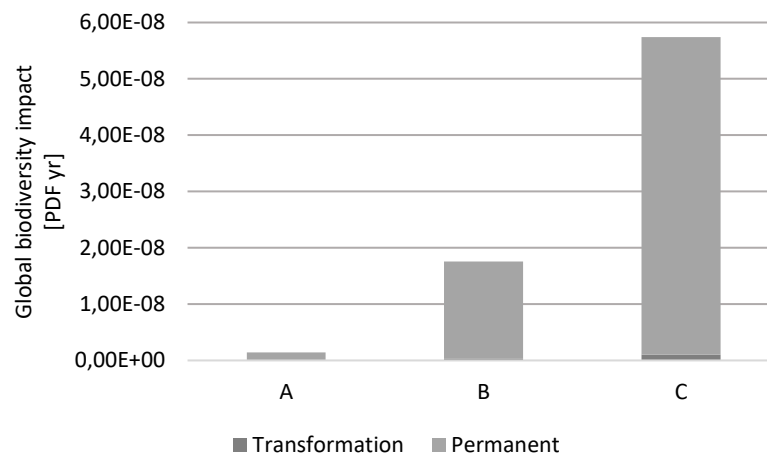


Figure 3- 8 Global biodiversity impact on deep-sea benthic megafauna for the harvesting of one ton of nodules (DW) in the subregion n°6 according to plumes deposition scenarios: (A) plumes limited to mining tracks, (B) dispersal range of 20 km – a single and square-shaped location is mined in the contract area, (C) plumes are dispersing on the entire concession because of patchy disposition of mining areas.

3.5. Discussion

This framework is a first attempt to extend the scope of LCA methods to biodiversity impacts in deep seafloor ecosystems. However, it builds on existing frameworks for coastal marine and terrestrial ecosystems. The framework starts from Woods and Verones (2019) to assess the regional biodiversity impacts because it follows the state of the art for land use impact accounting in LCA (Milà i Canals *et al.*, 2007) and transpose it to marine ecosystems. According to the Life Cycle Initiative (Jolliet *et al.*, 2018), the cause effect chain is extended to global biodiversity in order to bring an additional perspective in the results and to allow the comparison of impacts occurring in different ecosystems. While most methods for terrestrial ecosystem focuses on species attributes for the development of CF_{glo} , this new framework focuses on ecosystem attributes due to data availability reasons. It requires to perform a vulnerability assessment using the WWF method (Dinerstein *et al.*, 1995) while the results for terrestrial ecoregion vulnerability are available in the Wildfinder database (WWF, 2006).

3.5.1. Applicability of the framework: highlights from the case study

3.5.1.1. Definition of the ecosystem under study

In the case study considered, the definition of the ecosystem follows the partitioning of the CCZ in nine subregions by the ISA that relies on particulate organic matter fluxes (ISA, 2011; Wedding *et al.*, 2013). However, each subregion from the CCZ is too heterogeneous in terms of bathymetry and habitat composition (McQuaid *et al.*, 2018; Wedding *et al.*, 2013) to argue that they consist of a single ecosystem. A detailed habitat classification and mapping for the CCZ has been recently proposed by McQuaid *et al.* (2018). These authors developed a framework that identifies 42 habitats from statistical inference using the cluster analysis method for the following variables: salinity, temperature, nodule abundance, topography and POC fluxes. This work is the first attempt to characterize the habitats occurring in the CCZ and the extension of this work to other abyssal regions would lead to a global mapping of deep seafloor ecosystems. However, the validation of the results through sampling is still required and the definition of the deep seafloor ecosystems from the habitats identified calls for additional studies. The LCIA for global biodiversity being strongly influenced by the identification of the ecosystem under study, the impacts may change significantly if deep seafloor ecosystems are defined at finer scale than for this case study. Prior to start the mining operations, it becomes urgent to develop a standard mapping of the deep-sea ecosystems to understand their distribution and connectivity, and to implement conservation strategies according to their vulnerability.. In addition, the evaluation of ES_i might be implemented if an exhaustive classification of marine ecosystems becomes available. This will provide consensus on the spatial extent of the ecosystem under study. Moreover, the equation 3-13

could consider the area of the largest ecosystem as the reference area (instead of earth's area), avoiding the use of a logarithmic scale.

3.5.1.2. Uncertainty on the benthic response

The operationalisation of the method for deep-sea mining is limited by the data availability requiring several assumptions and thus, increasing the uncertainty on the results. The recovery of deep seafloor communities from anthropogenic disturbance is poorly documented in terms of diversity and disturbance events are too recent to understand recoveries in the long term. The case study illustrates this issue with the oldest observation of deep-sea megafauna diversity recovery in the CCZ being 37 years after benthic disturbance. Because no data was available for longer time horizons, the assumption is made that no further recovery occurred after this period. This simplification hypothesis has a significant effect on the results because it implies to integrate the low recovery observed (44.4%) on 500 years to compute the CF for permanent impact. For this reason, this impact is by far the largest contributor to the total impact and is uncertain because of the absence of information for longer times after disturbance. On the other hand, the time selected for the permanent impact does not correspond to the geological time for nodule formation. The nodule formation is extremely slow with a growth of one millimetre to one centimeter each million year, depending on the local conditions (Hein *et al.*, 2013). Some species requiring the presence of nodules for recolonization (belonging to epibenthic sessile epifauna), those will remain absent after 500 years. The high uncertainty regarding the total area impacted by plumes deposits and regarding the recolonization after 37 years motivate our choice of integrating the permanent impacts on 500 years. However, the choice of applying such conservative approach instead of assumptions on continuous recovery after 37 years is based on the precautionary principle, a worst-case evaluation is proposed rather than scenarios on hypothetical recovery. Furthermore, the model relies on data for biodiversity loss at higher taxa level and does not consider lower levels such as species or even population diversity for which the impact can differ (Gollner *et al.*, 2017). The impact assessment is performed for megafauna only, but biodiversity response to disturbance can differ for other size groups (e.g. meiofauna) (Gollner *et al.*, 2017). Megafauna diversity is used in this study as proxy to address the complexity of deep seafloor ecosystems, presenting uncertain species composition. However, it may give a distorted view of the biodiversity response of the entire community.

In addition, the CFs for regional biodiversity impacts are relying on two different disturbance experiments that differ in their experimental set-ups. The diversity level of benthic megafauna just after disturbance is modelled according to the results from DISCOL experiment (1989) comparing communities on a same site before and after disturbance caused by a “plough-

harrow” benthic disturber. The disturber from DISCOL did not harvest nodules but only few remained at the sediment surface (Bluhm, 2001) and tracks were still observed after 26 years (JPI, 2017). The author measures diversity in terms of *animal categories* rather than specific taxonomic level. Each *animal category* encompasses a variety of organisms identified at different taxonomic levels because it was not possible to identify organisms at species level using video imagery. On the other hand, diversity level 37 years after disturbance relies on the OMCO experiment (1977) with diversity being reported at the class taxonomic level. Therefore, the LCIA for the subregion n°6 relies on two different metrics for the biodiversity impact assessment. A standard method to monitor and report the recovery of deep seafloor communities from anthropogenic disturbance is crucial to combine the results from various experiments in a single LCIA. In addition, the two benthic disturbance experiments occur in distinct areas with DISCOL being outside the CCZ, in the Peru Basin (Figure 3- 5). This zone experiences different productivities and communities might differ despite being both settled in nodules fields area (Glover *et al.*, 2016). However, the DISCOL results are only considered for the biodiversity level right after disturbance to limit the effect of varying recoveries between the Peru Basin and the subregion n°6.

The LCIA might overlook the recovery of deep-sea benthic megafauna in case of commercial mining operations because it relies on observations from experimental disturbance. The connectivity amongst communities under commercial mining will differ from experimental disturbance consisting to few tracks spread in extensive nodule areas (Glover *et al.*, 2016). Therefore, biodiversity impacts may be underestimated for large-scale mining operations leading to high levels of habitat fragmentation and thus, to reduced recolonization rate (Weaver, 2017). In addition to habitat fragmentation, plumes deposition is highly uncertain in terms of dispersion distance and impact on benthic megafauna communities (Gollner *et al.*, 2017). Dissolved materials and resuspended particles settle on the seafloor at various distance from their source and change the sediment density of the seafloor upper layer for at least 20 years (Becker *et al.*, 2001). For this reason, the case study considers three scenarios dealing with different plumes dispersion assumptions. The current uncertainty regarding biodiversity impact such as the spatial extent, plume dilution patterns and overall benthic recovery caused by plumes deposition has resulted in conservative assumptions within this paper. So far, scenarios B and C assume similar biodiversity impacts in areas affected by plumes deposition and in areas affected by nodules harvesting and this, because of the lack of information on the recovery of benthic megafauna diversity in area affected by plumes deposition and the lack of information on the dilution profile of plumes over dispersal distances. Hence, it is assumed that by plumes deposition is homogeneous on the impacted area. Although, the DISCOL experiment has shown that biodiversity impacts are not similar in areas affected by plumes

deposition and in areas affected by nodules harvesting (Bluhm, 2001). To further improve our understanding of benthic recovery in mining tracks and in areas impacted by plumes deposition, large-scale benthic disturbance experiments using a nodule collector prototype are required to monitor biodiversity changes within the area of economic interest.

3.5.2. Perspectives

The development of a framework for terrestrial biodiversity impacts in Life Cycle Assessment is facilitated by the identification of terrestrial ecosystems by the WWF and their intrinsic attributes (size, conservation status, species composition) (Chaudhary *et al.*, 2015; De Baan *et al.*, 2013a; Michelsen, 2008; Winter *et al.*, 2018). For data availability reasons the existing frameworks are limited to terrestrial ecosystems. Therefore, the framework developed in this study is a first attempt of a LCIA applicable for biodiversity impact in deep seafloor ecosystems considering their current knowledge status. While the regional biodiversity impacts assessment follows the method from Woods and Verones (2019), the results are converted to global biodiversity impacts considering ecosystem-specific attributes. This approach does not aim to provide precise numbers for biodiversity loss but rather additional information to compare regional biodiversity impacts occurring in different ecosystems. For example, this approach is also applicable to terrestrial ecosystems for comparative assessments amongst multiple alternatives such as the comparison of biodiversity impacts between deep-sea and terrestrial mining. Existing databases for terrestrial ecosystems such as the WWF Wildfinder facilitate the evaluation for ES and EV compared to deep-sea ecosystems. However, the development of CF_{reg} still requires the identification of suitable terrestrial ecosystem components and data on their actual biodiversity and recovery rates. However, its application to various case studies is limited by knowledge gaps on deep-sea environments. On the other hand, it provides an objective view to compare impacts occurring in different deep-sea communities through converting regional into global biodiversity impacts. Additional aspects might need some more research to implement the method in the long term. First, no information is provided regarding the PDF level at which the ecosystem collapse. The regional and global biodiversity impacts follow a linear trend with the seafloor area impacted and this, regardless of the maximum area that can be disturbed without affecting ecosystem continuity. Acknowledging that biodiversity response is not linear to its stressor (e.g. area disturbed), Winter *et al.* (2018) assess biodiversity loss through a specific impact function of the stressor. This requires experts' judgements to understand how the community will behave for different levels of stressor intensity. However, the LCIA method recommended by the Life Cycle Initiative for regional biodiversity loss (Chaudhary *et al.*, 2015) makes use of species area relationship to establish their CFs for land use. The CFs are not re-evaluated according to the magnitude of land use and thus, the regional biodiversity impact varies linearly with the area impacted. While the case

study is illustrated for the mining of 1 ton of nodules DW in a pristine ecosystem, the CF should be reviewed in case of advanced stages of large-scale commercial mining that are affecting the continuity of ecosystem processes.

In addition, CF_{glo} relies on the WWF method for the vulnerability score. The method has been originally developed for ecosystems that have been/or being under anthropogenic disturbance because it gives higher importance to the snapshot conservation state compared to the threat status. Consequently, CF_{glo} is lower for pristine ecosystems (such as in deep-sea environments) than those for ecosystems under disturbance regime. Similar to other LCIA methods for terrestrial biodiversity impact, the present method gives more weight to biodiversity impacts occurring in ecosystems with higher levels of anthropogenic pressure. This rises the concern on which ecosystems should be more protected amongst intact and disturbed ecosystems e.g. what is worst between mining in pristine deep-sea or in vulnerable environments such as the Atacama Desert? The argumentation for preserving disturbed ecosystems at the expense of intact ecosystems goes beyond the scope of this study. However, this question must be considered when interpreting the results on global biodiversity impact. Moreover, the WWF method has been established to assess the conservation status for terrestrial ecosystems and therefore, the thresholds for the evaluation of each criteria must be re-evaluated to ensure their appropriateness to deep-sea environments.

Some LCIA models for biodiversity impacts make use of other metrics such as species endemism, total species richness or species threat level in the evaluation of the global biodiversity impact (Chaudhary *et al.*, 2015; Verones *et al.*, 2013; Winter *et al.*, 2018). Such additional aspects focusing on species' attributes could be integrated with the present method to sharpen CF_{glo} . However, unknown species are still being discovered with sampling campaigns in the deep-sea (Gollner *et al.*, 2017) and the geographic distribution of identified deep-sea species is not fully understood. Further developments that are not considered by any LCIA method for biodiversity impacts consist to the integration of organisms densities (Paulikas *et al.*, 2019) or to the consideration of other aspects such as functional diversity. In case of sufficient data availability, this method can be extended to additional interventions occurring in deep-sea environments but also to case studies related to terrestrial mining in order to benchmark both solutions for the recovery of base metals.

CHAPTER 4

Identification of microalgae biorefinery scenarios and development of mass and energy balance flowsheets

Redrafted from

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Abstract

The notion of bioeconomy is at the basis of recent European strategies aiming at conciliating economic growth and sustainability. Consequently, extensive research has been conducted on biobased solutions such as microalgae products. Numerous initiatives to commercialize microalgae have been launched but few of them were successful. Algae biofuel is the most obvious illustration with its promises as energy supply but faces many challenges to become economically competitive. Consequently, it was recently proposed to develop microalgae biorefineries for an optimal biomass valorisation, to dilute the overall costs within a wide range of products. Herein, the energy demand for different microalgae biorefinery scenarios is investigated and critical steps identified. Each scenario is modelled using information from literature and process engineering principles. The production of lipids, proteins, methane, fertilizers and dried biomass are considered. Once defined, the scenarios are modelled and their energy inputs are discussed. We also investigate the impact of using a biobased solvent for lipid extraction instead of a conventional one. On top of that, each scenario is assessed for two cells disruption methods. In both cases, the study starts with dewatering the growth medium of the microalgae *Chlorella vulgaris* (240 kg DW h⁻¹) and ends with the recovery of the products. The results vary from 20.07 to 66.53 MJ kg⁻¹ input DW and highlight the importance of the cell disruption method in the total energy demand. While lipid extraction presents adverse impacts on proteins extraction due to solvent recovery, proteins extraction has beneficial effects on further methane production step. Our study concludes with the comparison of microalgae biomass with soy, for proteins and lipids production, and demonstrates quantitatively that microalgae-based technologies are still inefficient compared to present alternatives. This work provides quantitative numbers for further evaluation of microalgae projects considering the current stage of the technology.

4.1. Introduction

The global context of natural resources depletion, worldwide pollution issues or fossil-based energy dependency has raised new concerns to our society. A key aspect being the renewability of resources, public authorities have launched many research programs to reform progressively important pillars of our economies (e.g. Horizon 2020 in Europe). The energy sector is a good illustration of this trend with recent advances in renewable energy such as biobased energy, still consisting in a relatively small contribution to the world energy production (IEA, 2019a). In this context, microalgae were proposed for energy production with promising forecasts, mainly through the production of third generation biofuels (*i.e.* mostly for biodiesel production from their lipids) (Chisti, 2007). Despite intensive research, it is not yet possible to decrease the production costs sufficiently for viable production of microalgae-based biofuels. Moreover, many authors have warned on the net negative energy contribution of microalgae-based biofuels: at the current technology development, the production requires more energy than it releases (Sorguven and Özilgen, 2010; Mercer and Armenta, 2011; Razon and Tan, 2011; Delrue *et al.*, 2012; Martin, 2016). To a larger extent, the bottlenecks from the downstream processes significantly affect the processing costs, limiting the economic viability of microalgae products to niche markets targeting added value applications such as nutraceuticals and food/feed ingredients, using microalgae as a whole (Enzing *et al.*, 2014). Coming back to microalgae-based biodiesel production, most of the studies do not further valorise the residual biomass, considered as waste material and this, despite representing from 60 to 85% DW of the initial biomass. Attempts are made in recent studies to integrate the recovery of microalgae by-products in biorefinery systems (*i.e.* integration of downstream processes in cascade framework) with the aim to produce a range of products instead of a single product, in a way to increase the profitability (Wijffels *et al.*, 2010; Hariskos and Posten, 2014; Chew *et al.*, 2017; Gifuni *et al.*, 2019). Before envisioning a golden age for microalgae, it is essential to better understand the global efficiency of different biorefinery scenarios at the current technology readiness level. This is done by considering the quantity of inputs required for each product and the compatibility among processes in a cascade framework. We select energy demand as key input affecting the economic viability of microalgae biorefineries and discuss different designs accordingly. Nevertheless, productions costs (wages, maintenance, investments), sales revenues, but also the fiscal policy, constitute a set of parameters to be included for further economic evaluations of microalgae projects (Thomassen *et al.*, 2018). The literature on quantitative assessments of microalgae biorefineries efficiency is rather limited and relies on many ambiguous assumptions (Lardon *et al.*, 2009; Roux *et al.*, 2017; Thomassen *et al.*, 2017). This chapter highlights opportunities and trade-offs in microalgae biorefineries designs to extract lipids and additional products from the residual biomass. We

identify and model multiple processes for the recovery of microalgae products at the present stage of the technology. Instead of studying each process separately, we combine them in cascade design with the aim to extract lipids and additional products from the residual biomass. The novelty from this study lies in the association of the processes as building blocks for biorefinery designs, requiring a deep understanding of each process specifications. Moreover, the composition of the algae biomass is modelled throughout the cascade processes to understand the influence among the single processes. The study provides a transparent comparison of the opportunities to valorise microalgae biomass and identifies essential elements to improve their global energy efficiency.

4.2. Methodology

4.2.1. Scope of the analysis

With the aim to investigate microalgae valorisation pathways in a biorefinery context, our research focuses on downstream processes of raw biomass, including the dewatering of the growth medium until the recovery of the target (by-)products (*i.e.* gate-to-gate analysis). Each of the biorefinery scenarios analysed in this study relies on the processing of an identical growth medium of *Chlorella vulgaris* produced under nitrogen replete conditions. Referring to scientific literature (Tokusolgu and Unal, 2003; Becker, 2007; Stephenson *et al.*, 2010; Griffiths *et al.*, 2014; Safi *et al.*, 2014a), the composition of the biomass grown under these conditions is assumed to be 50% DW of proteins, 20% DW of carbohydrates, 15% DW of lipids and 15% DW of other components (pigments, vitamins, minerals). The cell wall resistance is lower than strains grown under nitrogen depletion conditions.

4.2.2. Overview of the scenarios and common characteristics

Nowadays, a large fraction of literature on microalgae focuses on its potential for the production of third generation biofuels. Therefore, all scenarios investigate lipid extraction prior to additional downstream processes, aiming at maximising the overall performance of the biorefinery. The lipid extraction step is performed using conventional or biobased solvents. The scientific literature on microalgae biomass processing was screened to design feasible valorisation pathways for the residual fraction resulting from lipid extraction. In addition to lipids fraction, we identified the recovery of proteins, the production of dried biomass, or the production of methane and fertilizers from the microalgae-defatted biomass. According to the setup compatibility of the processes (section 2.3.), four biorefinery scenarios are developed and identified according to the range of products delivered: Lh.P.E., Lh.E., Lh.D. and Lm.E. (Figure 4- 1). On the top of this comparison, and prior to lipid extraction, all scenarios are tested for two methods of cell disruption: bead milling (BM) and high-pressure homogenisation (HPH) (*c.f.* 4.2.3.2).

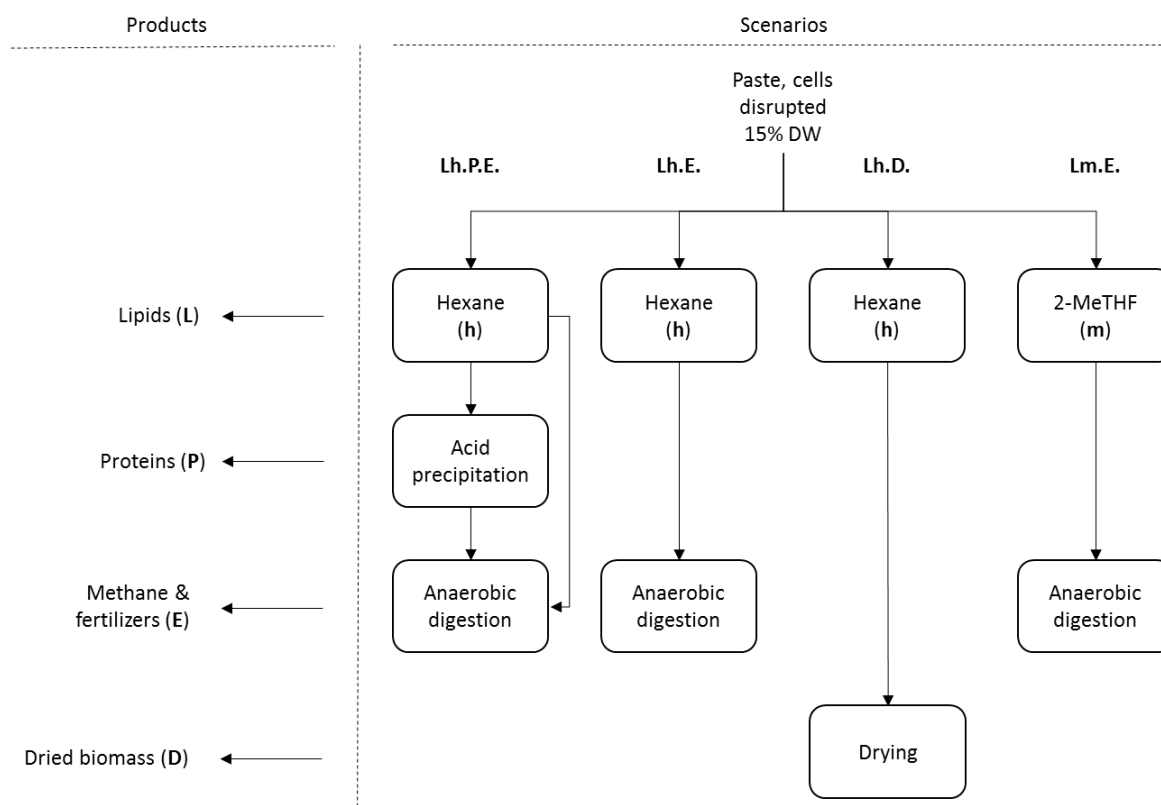


Figure 4- 1 Biorefinery scenarios for microalgae wet biomass valorisation. Lh.P.E.: hexane lipid extraction, proteins extraction, energy production; Lh.E.: hexane lipid extraction, energy production; Lh.D.: hexane lipid extraction, dried biomass production; Lm.E. 2-Methyltetrahydrofuran lipid extraction, energy production; DW: dry weight. For each scenario, two methods for cells disruption are tested: bead mill (BM) and high-pressure homogenisation (HPH) (c.f. 4.2.3.2).

4.2.3. Processes description

This section describes the downstream processes that are included in the four biorefinery scenarios, all set up based on a processing capacity of $240 \text{ kg} \times \text{h}^{-1}$ of microalgae dry weight (DW), corresponding to commercial scale applications. For each unit process, information is provided on the key parameters and the compatibilities with other processes is discussed. The scenarios are defined according to the compatibility of the downstream processes in a cascade approach. The calculation of the microalgae paste density, and its thermodynamic properties such as heat capacities, are similar for each process. The equations from Schneider et al. (2016) were used to estimate these parameters at various concentrations. Although these equations have been developed for the green microalgae *Nannochloropsis salina*, these were assumed applicable for *Chlorella vulgaris* due to the lack of similar information for this species. The data and equations used for the modelling of the processes described below are available in supporting information (Table B- 1 and equations B-1 – B-3).

4.2.3.1. Dewatering

The algae growth medium is pumped from the cultivation system to a centrifuge for a single-step dewatering. An initial algae concentration of 0.2% DW is assumed, corresponding to the

average concentration obtained in flat panel and tubular photo bioreactors (Fasaei *et al.*, 2018). A disc stack centrifuge is considered with a capacity of $120 \text{ m}^3 \times \text{h}^{-1}$ and energy consumption of $1.3 \text{ kWh} \times \text{m}^{-3}_{\text{feed}}$ (13,000 g) to concentrate the solution from 0.2 to 15.0% DW. The cell recovery efficiency is 95% (GEA Mechanical Equipment; Milledge and Heaven, 2013; Fasaei *et al.*, 2018).

4.2.3.2. Cell disruption

Releasing the intracellular compounds is fundamental to improve extraction efficiencies from microalgae. Therefore, microalgae process designs often include a cell-disruption process. Although this step is mostly known for its beneficial effect on lipid extraction yield (Jun *et al.*, 2009; Olmstead *et al.*, 2013; Angles *et al.*, 2017), it also increases proteins extraction yield (Ursu *et al.*, 2014) while improving the methane production potential in anaerobic digestion (Keymer *et al.*, 2013). Several cell disruption methods have been developed and they can be distributed in two main groups: mechanical and non-mechanical methods (Günerken *et al.*, 2015). Cell disruption methods are evaluated according to their scalability, their operating costs and their cell disruption efficiency representing the proportion of disrupted cells compared to initial cells. For these reasons, mechanical methods are often preferred as they can be applied continuously and use the algae solution as the only material input. Among them, HPH and BM are the most feasible at large-scale due to equipment availability from other commercial applications (Halim *et al.*, 2012a; Günerken *et al.*, 2015).

HPH creates liquid shear stress under high pressure (100 to 1500 bar) to disrupt the cells. The cell disruption efficiency is positively correlated to the number of passes and the working pressure (Spiden *et al.*, 2013; Yap *et al.*, 2015). On the other hand, these parameters affect the specific energy demand and increase the temperature. Nevertheless, the energy demand can be limited if HPH is performed for concentrated solutions (up to 25% DW) with strains having moderate-to-low cell wall resistance (Spiden *et al.*, 2013; Yap *et al.*, 2015). The combination of these parameters and the presence of upstream processes to weaken the cell explain the large variability in specific energy demand for HPH reported in literature (Günerken *et al.*, 2015). In this study, the energy demand is modelled according to the results from Yap *et al.* (2015) (Yap *et al.*, 2015) who reported $0.58 \text{ MJ} \times \text{MJ}^{-1}_{\text{TAG}}$ to reach 92% of cell disruption efficiency for *Chlorella vulgaris* paste at 25% DW with 4 passes at 1000 bar. The energy units are converted from $\text{MJ} \times \text{MJ}^{-1}_{\text{TAG}}$ back to $\text{kWh} \times \text{kg}^{-1}_{\text{DW}}$. This is done by considering the assumptions made by the authors (Yap *et al.*, 2015) while reporting the energy consumption per MJ of TAG (a TAG content of 20% and a TAG energy density of $41.7 \text{ MJ}_{\text{TAG}} \text{ kg}^{-1}_{\text{TAG}}$). Cell disruption efficiency being insensitive to feed concentrations up to 25% DW in HPH (Yap *et al.*, 2015), we report the energy consumption for a feed concentration of 15% DW assuming a

linear correlation between specific energy consumption and feed concentration from 0 to 25% DW.

BM is a grinding technique commonly used for paints, pharmaceutical, agrochemical or food industries to reduce particles size (WAB, no date). It consists in accelerating beads in a chamber filled with the product and collisions induce solid shear stress. This process has been optimised for the disintegration of *Chlorella* cells (Doucha and Lívanský, 2008). Among the set of processing parameters, the stirring intensity and the biomass concentration are the most influential on the energy demand and the cell disruption efficiency (Doucha and Lívanský, 2008; Postma *et al.*, 2015). However, the stirring intensity significantly affects the energy demand and increases the temperature (Halim *et al.*, 2012a). Since temperature control is crucial to avoid damages to intracellular compounds, the milling chamber is jacketed with a cooling system. We consider the results from Doucha and Lívanský (2008) (Doucha and Lívanský, 2008) to evaluate the energy requirements for disrupting 90% of *Chlorella* cells in 15% DW paste.

4.2.3.3. Lipid extraction

Among microalgae components, lipids are the most studied as fatty acids can be readily transformed into biodiesel. Lipids are defined as components of living organisms being insoluble into water, soluble into organic solvents and containing long chains of hydrocarbon groups, consisting out of fatty acids/glycerides in most cases (Gadella, 1972). They are divided in two categories according to their polarity: the neutral lipids and the polar lipids (Kates, 1986). Microalgae produce neutral lipids for energy storage while the polar lipids (phospholipids and glycolipids) are the main constituents of the bilayer membrane. Neutral lipids are the most abundant of microalgae lipids. However, the concentration of lipids into microalgae biomass, and the proportions in neutral and polar lipids, depend on the strain selection and the culture conditions (Harun *et al.*, 2010). Under nitrogen depletion conditions, some strains can reach lipids concentrations exceeding 60% of the biomass DW as shown by Rodolfi *et al.* (2009) (Rodolfi *et al.*, 2009) for *Nannochloropsis* sp. Nevertheless, the increase in lipids productivity results in the reduction of biomass productivity and also affects the cell disruption efficiency (Rodolfi *et al.*, 2009). Lipids are typically extracted by organic solvents, by supercritical fluid extraction or by pressing (Mercer and Armenta, 2011). Most of the solvent extraction methods consists of a mixture of non-polar and polar organic solvents to release and recover the major fraction of neutral and polar lipids. These techniques can be combined with microwaves or ultrasounds in order to maximise the extraction yield (Halim *et al.*, 2012b). Solvent extraction requires centrifugation and distillation steps to recover the lipids from the organic layer. Besides its lipid extraction efficiency and its price, a solvent must be easily recoverable. Suitable solvents exhibit low densities compared to water, are not miscible in water, and/or

require low energy for distillation (*i.e.* low boiling point, no azeotrope) (Mercer and Armenta, 2011). For these reasons, hexane is often cited as an efficient organic solvent for neutral lipids extraction and is widely used in plant oil industry (Harun *et al.*, 2010). Nevertheless, petroleum-based solvents, such as hexane, are being contested by public authorities for their effect on both human health and the environment (*i.e.* European Reach regulation) (Wan Mahmood *et al.*, 2017). In most of the cases, microalgae wet biomass is dried prior to solvent extraction in order to perform the dry route. Compared to wet extraction, this step reduces solvent requirements but the extra energy demand for drying makes the process not efficient if lipids are considered solely for energy production (Lardon *et al.*, 2009; Razon and Tan, 2011; Posada *et al.*, 2016). On the other hand, the wet route requires more solvent and energy compared to the dry route during the lipid extraction stage (Xu *et al.*, 2011).

Starting from the recent findings from microalgae lipids literature, two different extraction processes are modelled, both in a wet route to avoid drying costs (Figure B- 1). We select hexane as conventional solvent for its general acceptance as an efficient solvent for lipids extraction. On the other hand, we model a second lipids extraction route using 2-methyltetrahydrofuran (2-MeTHF), a biobased solvent obtained from sugarcane and proven to be efficient for wet lipid extraction from microalgae (Sicaire *et al.*, 2015; Angles *et al.*, 2017; Wan Mahmood *et al.*, 2017; de Jesus *et al.*, 2018). The extraction procedure is similar for the two solvents: the solvent-biomass contact is performed at 40 °C in a double-jacketed stirred tank operated in batch mode for 2 hours with a stirring intensity of 60 rpm to avoid excessive micelles formation (Olmstead *et al.*, 2013; Martin, 2016; Angles *et al.*, 2017). Extraction is followed by centrifugation (15 min, 9000 rpm) to recover the lipids in the solvent fraction (Halim *et al.*, 2016). The products obtained after centrifugation differ in their compositions according to the solvent used. Therefore, the solvent chosen will affect the results in the downstream processes and thus, the biorefinery scenarios.

(A) Lipid extraction using hexane

The centrifugation step after extraction results in four distinct layers: the organic layer, the emulsion layer, the aqueous layer and the cell debris layer (Halim *et al.*, 2016). The organic layer contains the lipids which are further recovered from the hexane by vacuum distillation heated with steam. This step is modelled using specific rules of thumb and heat transfer coefficients (Woods, 2007). A lipid extraction yield of 25% DW of total lipids in the initial biomass is considered and neutral lipids represent most of the extracted lipids (Lu *et al.*, 2015). This conservative value is slightly higher than the results from Lu *et al.* (2015), obtained without a cell disruption step; but slightly lower than the results from Halim *et al.* (2016) for a similar procedure with *Nannochloropsis* sp. The emulsion layer is made of micelles resulting from the interactions between hexane, water, intact cells and the polar compounds released (proteins,

polar lipids). The hexane is recovered from the wet biomass using the desolventising-toasting process, similarly to the soybean industry. The emulsion layer is injected at the top of a staged column heated with direct (injected) and indirect (in jacket) steam. The hexane – water vapour is condensed and hexane is dried by vacuum distillation. The solvent-free wet biomass is recovered at the bottom of the desolventiser (Paraíso *et al.*, 2008). We adapt the mathematical model from Martin (2016) to estimate the steam requirements and mass flows based on the relative volatilisation of water to solvent [$\text{kg}_{\text{water}} \times \text{kg}^{-1}_{\text{solvent}}$], the mass fraction in the mixture, and the latent heat of vaporisation of solvent and water. It was assumed that no energy could be recovered from the evaporated solvent and water in the first cycle, and that hexane could be recovered completely. The proteins-rich aqueous and cell debris layers do not contain hexane and are recovered for further valorisation (Halim *et al.*, 2016). We estimate the compositions of the 4 layers by adapting the results from Halim *et al.* (2016) for hexane lipids wet extraction on *Nannochloropsis* sp. according to *Chlorella vulgaris*' composition.

(B) Lipid extraction using 2-MeTHF

The centrifugation step provides two layers: the organic layer and the water layer. The latter can be further divided into the aqueous layer and the cell debris layer, which are not differentiated in this study. The higher solubility of 2-MeTHF in water (9.4% weight at 39.6 °C) and of water in 2-MeTHF (4.3% weight at 39.6 °C) explains why the emulsion layer is not observed in the experimental results for similar temperature and mixing intensity (Angles *et al.*, 2017; Wan Mahmood *et al.*, 2017). The solubility also affects the separation of the layers in centrifugation: some water remains in the organic layer and some solvent remains in the water layer. Moreover, 2-MeTHF creates an azeotrope with water, requiring azeotropic distillation for its recovery (PENN Specialty Chemicals, 2005). This process is illustrated in Figure B- 2. The azeotropic distillation of the organic layer removes completely the water, the latter being the smaller fraction of the layer. The dried organic layer is distilled under vacuum to isolate the lipids and recover the solvent. A second azeotropic distillation for the water layer is performed in parallel to extract the solvent. The two azeotropes (from the organic and water layers) are decanted and the layers obtained are re-injected in the azeotropic distillation columns. This system is operated continuously to recover the initial amount of dried solvent, steam is used for heating and specific heat transfer coefficients are obtained from rules of thumb in process engineering (Branan, 2002; Woods, 2007). Mass and energy flows were modelled using the Excel solver function and the data from Aycock (2007) and a technical fiche from PENN Specialty Chemicals Inc. (2005). Excel solver determines the total quantities of refluxes in the azeotropic distillation columns to achieve the total recovery of solvent. The objective cell is fixed to the amount of 2-MeTHF that needs to be recovered All other mass and energy flows from the azeotropic distillations are set to variable cells. Then, the solver function adjusts the

variable flows until the objective cell has met its selected value. We inferred the lipid extraction yield of 2-MeTHF for *Chlorella vulgaris* due to a lack of published data. 2-MeTHF presents a similar lipid extraction yield as chloroform (40% DW of total lipids) for wet biomass at 29% cell disruption rate (Angles *et al.*, 2017), it was assumed that both solvents perform similarly for higher cell disruption. Therefore, we consider a lipid extraction yield of 50% for 2-MeTHF because this value has been reported for similar wet extraction performed with chloroform for a cell disruption rate of 84% (Angles *et al.*, 2017).

4.2.3.4. Proteins extraction

Proteins are poorly valorised despite their abundance (from 40 to 60% DW of algae dried biomass) (Becker, 2007). The commercial interest for whole microalgae as food and feed ingredients is explained by high nutritional value of proteins (*i.e.* amino acid composition and digestibility) although these are not often extracted for this purpose (Spolaore *et al.*, 2006; Becker, 2007). Indeed, proteins are rather extracted for value-added applications such as the cosmetic industry due to similar or higher emulsifying properties compared to plant-derived proteins (Stolz and Obermayer, 2005; Ursu *et al.*, 2014). The extraction procedure starts with the cell disruption in an aqueous medium to solubilise the proteins. The cell disruption efficiency is a key parameter in the proteins solubilisation yield and, therefore, the cell wall resistance and the disruption method are crucial for the proteins recovery (Safi *et al.*, 2014b). Two options are commonly used for soluble proteins recovery: precipitation based on the isoelectric point or ultrafiltration (Safi *et al.*, 2014a; 2014b). Precipitation is often preferred for its lower operational cost (Ursu *et al.*, 2014), therefore we consider this option for this study.

The extraction of proteins is performed from the residual biomass resulting from hexane lipid extraction. We assume that the recovery of 2-MeTHF affects the structure of proteins due to temperature in the azeotropic distillation and therefore, the residual biomass from 2-MeTHF is not used for proteins extraction. The aqueous and cell debris layers obtained after centrifugation in hexane extraction are mixed at room temperature for 10 minutes using an impellor mixer set-up at an intensity of 60 rpm. A proteins solubilisation rate of 52.8% DW is assumed according to the results from Safi *et al.* (2014b) for proteins extraction from *Chlorella vulgaris* at pH 7 and using HPH for cells disruption. The soluble proteins are recovered by centrifugation for 10 minutes at 10,000 g and the bottom layer is transferred to the anaerobic digester. Proteins are precipitated by pH decrease (pH 4 obtained by addition of 1 M HCl in the solution stirred at 60 rpm for 10 minutes). The precipitate is dried to 50.0% DW by centrifugation and needs to be solubilised for further drying. The solubilisation is performed by mixing for 10 minutes at 60 rpm with 1M NaOH. Protein powder is obtained by spray drying under atmospheric pressure and using saturated steam, a thermal efficiency of 50.0% is assumed (Woods, 2007). The data used for the modelling are detailed in Table B- 4. The

effects of the recovery steps (pH precipitation and spray drying) on the 3-dimensional structure of the proteins are not discussed in this manuscript. It is necessary to understand their impacts on the market value of proteins if the results are used for further economic feasibility studies of microalgae proteins valorisation.

4.2.3.5. Anaerobic digestion of residual algal biomass: methane and fertilizer production

This technique is implemented at large scale to produce biogas (mostly methane and carbon dioxide) from sludge from wastewater treatment, crop residues, food waste or manure. Anaerobic digestion is performed under specific conditions for its biochemical steps: hydrolysis, fermentation and methanogenesis. Although anaerobic digestion of microalgae biomass is still at its infancy (Ward *et al.*, 2014), it is worthy to evaluate its potential for the final valorisation of the residual biomass from the extraction processes (*c.f.* sections 4.2.3.3 and 4.2.3.4). Anaerobic digestion has been proposed to cover a fraction of the on-site heating requirements if coupled with the cultivation or lipid extraction stages (Lardon *et al.*, 2009; Sialve *et al.*, 2009; Collet *et al.*, 2010; Ras *et al.*, 2011). The experimental results are benchmarked with the theoretical methane potential (TMP). This value is calculated by the Buswell equation converting lipids, proteins and carbohydrates contained in the biomass into methane based on the stoichiometry of the reactions (Buswell and Muellepi, 1952; Sialve *et al.*, 2009; Heaven *et al.*, 2011). The effective methane production is compared to this value to evaluate the methane to biomass conversion efficiency. The latter is highly sensitive to the intrinsic composition of the biomass such as the cellulose fraction, reducing the biodegradability, or the proteins fraction inhibiting fermentation through conversion to ammonia/ammonium (acidosis) (Sialve *et al.*, 2009; Ras *et al.*, 2011; Klassen *et al.*, 2017). These aspects are partially considered by the carbon to nitrogen ratio (DW basis) which should be between 15 and 30 for an optimal fermentation (Klassen *et al.*, 2017). In parallel to biomass intrinsic factors, the digester parameters are influencing the methane to biomass conversion efficiency. The critical parameters are the temperature, moisture level, mixing intensity or the hydraulic retention time (HRT). Therefore, experimental results for *Chlorella vulgaris* biomass report methane-to-biomass conversion efficiencies varying from 15 to 84% of TMP depending of the intrinsic biomass characteristics (influenced by N-supply during cultivation) or the digester set-up (Lakaniemi *et al.*, 2011; Ras *et al.*, 2011; Mahdy *et al.*, 2016; Klassen *et al.*, 2017). The biogas produced is used for heating the digester and the excess fraction is upgraded by water scrubbing to isolate CH₄ fraction based on its lower solubility compared to CO₂. Table B- 5 enumerates the data used and their references. This process is included in each scenario for the final valorisation of the biomass except in the scenario drying the residual biomass (*c.f.* 4.2.3.6).

4.2.3.6. Drying for the production of dietary supplement for animal feed

Instead of anaerobic digestion, a second process considers the valorisation of the defatted biomass as dietary supplement for animal feedstock and consists to dry the algae paste to 90% DW. However, the European directive 2009/32/EC does not allow the use of 2-MeTHF-defatted biomass for animal feedstock production (European Commission, 2009), limiting the drying step to the hexane-defatted biomass. Different methods have been tested for further microalgae drying after an initial dewatering step. The most energy efficient technique is solar drying, but its application is limited due to time requirements and climate conditions. On the other hand, freeze drying is one of the most commonly used method at lab or pilot scales (Chen *et al.*, 2015) but this method is energy intensive (Rentería Gámiz *et al.*, 2019). In addition to freeze drying, spray drying and conveyor drying are popular methods for drying value added microalgae compounds (Chen *et al.*, 2015). The economic viability of feedstock supplement production from microalgae requires to consider energy efficient techniques to compensate the expected low value of the product. Therefore, the drying is performed with a conveyor belt dryer, this technique being common for the treatment of low-value biomass such as sludge drying in wastewater treatment (WWT). The drying system is composed of (1) a furnace combusting methane in order to heat a thermofluid (ethylene-glycol) to 200 °C, (2) a heat exchanger where the thermal energy is transferred from the thermofluid to heat dry air to 120 °C, and (3) a conveyor belt with fans ensuring the circulation of dry heated air above the thin biomass layer. A 90% DW biomass film is produced out of the conveyor belt. The dried biomass obtained is added to animal feedstock to increase its dietary value (Madeira *et al.*, 2017). The conveyor belt design from Hosseinizand *et al.* (2017) (Figure B- 5) is used and adapted to the moisture content and to the heat capacity from this study (the data are available in Table B- 6).

4.3. Results and discussion

The results are described at the process level (c.f. 4.3.1) prior to the global comparison among the scenarios (c.f. 4.3.2).

4.3.1. Process level

4.3.1.1. Dewatering and cell disruption

Table 4- 1 reports the mass and energy flows for the pre-extraction steps. All scenarios present similar flows for the dewatering step: they assume a single centrifugation of the microalgae growth medium to increase the concentration from 0.2% to 15.0% DW. The electricity demand for dewatering is limited by the lower concentration required in the wet route. This reduces significantly the energy consumption compared to further drying processes aiming to obtain dried microalgae (Xu *et al.*, 2011). The scenarios start to differ once considering the cell disruption method due to significant effects in the electricity requirements. The results (Table 4- 1) illustrate the difference in the electricity consumption between HPH and BM to obtain similar cell disruption levels. The energy demand is partially explained by the degree of cell disintegration achieved (90%) and by the intrinsic cell wall resistance of *Chlorella vulgaris* (Doucha and Lívanský, 2008; Spiden *et al.*, 2013). Nevertheless, this value is reduced 6-fold if HPH is used instead of BM due to progress in setting the optimal parameters (number of passes and working pressure) for disrupting *Chlorella vulgaris* (Yap *et al.*, 2015). HPH is commonly used in the pharmaceutical and food sector and is an effective cell disruption method for biodiesel production from microalgae. Yap *et al.* (2015) investigated the potential of using HPH on concentrated biomass to reduce the energy needs for biodiesel production. In their study, the authors showed that the cell disruption efficiency and the total energy requirements are not dependent to the feed concentration up to 25% DW for *Nannochloropsis* sp. They suggest working at high concentrations to decrease the specific energy consumption of this step. On the other hand, the specific energy demand for the BM method is highly sensitive to the cell disruption efficiency, showing excessive energy requirements to achieve cell disruption efficiencies above 80% (Doucha and Lívanský, 2008; Postma *et al.*, 2015). A specific energy consumption of 10 kWh x kg⁻¹_{DW} (cooling system not included) is required to achieve 90% of cell disruption efficiency (feed rate, agitator speed, processing time). Doucha and Lívanský (2008) (Doucha and Lívanský, 2008) highlight the effect of cell disruption levels on the specific energy consumption. The authors increased the feed flow from 35 to 120 kg_{DW} x h⁻¹ and observed a significant drop in the specific energy consumption from 10.03 to 2.82 kWh x kg⁻¹_{DW}. Despite this significant drop, the cell disruption efficiency decreases to a lesser extent, from 90.6 to 77.7%. The degree of cell disintegration being essential for the efficiencies of the downstream processes considered in this study (Halim *et al.*, 2012a; Safi *et al.*, 2014b;

Ward *et al.*, 2014), it was decided to model the following processes for 90% of cells disrupted. Therefore, our study does not investigate the trade-offs between cell disruption efficiency (*i.e.* downstream processes yields) and global energy demand. We decided to focus on mechanical cell disruption methods that are applicable at large scale while upscaling is limited for biological and chemical methods. Recent procedures aim to reduce the energy consumption of this step by coupling mechanical with non-mechanical methods. Despite their potential to increase the overall efficiency of the biorefinery in the long term, these procedures are not considered in this study due to their limited applications at large-scale. It is also necessary to investigate properly how the cultivation parameters influence the cell wall resistance. Similarly to cell disruption, dewatering by centrifugation is preferred for its application at large-scale in a single step operation but the energy demand can be reduced by pre-treatments such as flocculation or pressure filtration (Fasaei *et al.*, 2018). It is important to mention that the wet biomass concentrations are sufficiently high to induce a non-Newtonian fluid behaviour: this situations requires extra energy to pump microalgae slurries due to higher viscosity and shear stress (Wileman *et al.*, 2012). However, due to the lack of qualitative data, and because it is not expected to affect significantly the comparisons between the scenarios, the pumping system is not considered in this study.

Table 4- 1 Mass flows [kg, kilogram], dry weight percentage (%DW) and energy inputs [kWh, kilowatt-hour] for the dewatering and cell disruption steps reported per kilogram of input biomass dry weight at the biorefinery gate. BM: bead mill, HPH: high pressure homogenisation. The flows refer to the overview of the process available in Figure B- 1.

Dewatering		
<i>In</i>		
Electricity [kWh]		0.65
Medium [kg] (%DW)		500.00 (0.2)
<i>Out</i>		
Medium [kg]		493.67
Wet biomass [kg] (%DW)		6.33 (15.0)
Cell disruption		
<i>In</i>		
Electricity [kWh]	11.41 (BM)	2.12 (HPH)
Wet biomass [kg]		6.33
<i>Out</i>		
Wet biomass [kg] (%DW)		6.33 (15.0)

4.3.1.2. Lipid extraction

The energy demand for heating varies slightly according to solvents heating capacities, latent heats and minor differences in solvent dosages (*Table 4- 2*). The aqueous layer flowing out of the phase separation is the largest fraction of the total output due to the large volume of water in wet extractions. Compared to dry extractions, the wet route requires larger volumes of solvents, additional energy requirements at the extraction and presents lower lipid extraction yields due to limited mass transfer (Balasubramanian *et al.*, 2013; Dong *et al.*, 2016; Roux *et al.*, 2017). Nevertheless, the additional thermal drying step required to achieve concentrations up to 85% DW limits the applications of dry extractions at (semi-)industrial scale (Lardon *et al.*, 2009; Sander and Murthy, 2010). Hexane and 2-MeTHF extractions differ in their outputs from the layers isolation. The organic layer from hexane extraction contains lipids and solvent, while the organic layer from 2-MeTHF extraction contains 3.9% (weight) of water due to the higher solubility of water in this solvent. Moreover, the biomass content of the aqueous layer is higher for 2-MeTHF compared to hexane due to the emulsion layer in hexane extraction, containing a fraction of the initial biomass. The steam requirements for 2-MeTHF recovery is about twice higher than that for hexane due to its azeotropic distillations. On the other hand, hexane recovery from the emulsion layer requires a desolventising process but its energy demand remains limited compared to the azeotropic distillations. Using 2-MeTHF instead of hexane in wet extraction doubles the lipid extraction yields according to our hypothesis (25 and 50% of total lipids for hexane and 2-MeTHF respectively (Lu *et al.*, 2015; Angles *et al.*, 2017; Wan Mahmood *et al.*, 2017) and thus, the energy inputs per kg of lipids extracted by 2-MeTHF are about two thirds compared to hexane (0.22 and $0.36 \text{ MJ} \times \text{g}^{-1}_{\text{lipids}}$ respectively). On the other hand, we did not considered the use of chlorinated solvents (*e.g.* Bligh and Dyer method) despite being known to increase the lipid extraction yield in wet and dry routes (and this, even without any cell disruption step) (Balasubramanian, Yen Doan and Obbard, 2013; Angles *et al.*, 2017). Notwithstanding, these solvents are controversial because of their potential environmental impact (Lardon *et al.*, 2009; ECHA, 2019). The use of an additional polar solvent (alcohol) to a mixture with a non-polar solvent is an option to improve the extraction yield due to higher affinity for polar and neutral lipids (Halim *et al.*, 2012b; Angles *et al.*, 2017). Multiple studies have tested the potential of lipids extraction from microalgae using hexane and an alcohol such as methanol, ethanol or (iso)propanol). If performed with an optimal hexane to alcohol ratio (usually between 1:1 and 3:1 vv), the extraction yield is significantly increased compared to single solvent extraction with the recovery of glycolipids, phospholipids, cholesterol and other pigments. The higher yields are explained by the lipids transfer from alcohol to hexane, the disruption of the polar lipids/membrane proteins interactions and the reduction of micelles formation (Halim *et al.*, 2011; Chen *et al.*, 2012; Li *et al.*, 2014). Therefore,

the addition of alcohol in the extraction mixture can increase the lipids extraction efficiency but the effect on energy demand for solvents recovery has to be investigated.

Table 4- 2 Mass flows [kg, kilogram], dry weight percentage (%DW) and energy inputs [kWh, kilowatt-hour; MJ, megajoule] for lipid extraction processes reported per kilogram of input biomass dry weight at the biorefinery gate. The flows refer to the overview of the process provided in Figure B- 1. (1) Proportion of biomass in water and/or solvent solution. 2-MeTHF, 2-methyltetrahydrofuran.

	Hexane lipid extraction	2-MeTHF lipid extraction
Solvent extraction		
<i>In</i>		
Electricity [kWh]	0.03	0.03
Steam [MJ]	0.57	0.50
Solvent [kg]	3.17	2.65
Wet biomass [kg]	6.33	6.33
<i>Out</i>		
Mixture [kg] (%DW)	9.50 (10.0)	8.98 (10.6)
Phases separation		
<i>In</i>		
Electricity [kWh]	2.77	2.77
Mixture [kg]	9.50	8.98
<i>Out</i>		
Aqueous layer [kg] (%DW) ¹	4.87 (11.7)	6.70 (13.1)
Emulsion layer [kg] (%DW) ¹	1.90 (21.0)	0.00
Organic layer [kg] (%DW) ¹	2.73 (1.3)	2.28 (3.1)
Lipids and solvent recovery		
<i>In</i>		
Aqueous layer [kg]	0.00	6.70
Emulsion layer [kg]	1.90	0.00
Organic layer [kg]	2.73	2.28
Electricity [kWh]	0.05	0.22
<i>Out</i>		
Lipids [g]	35.71	71.42
Solvent [kg]	3.17	2.65
Wet biomass [kg] (%DW)	1.42 (24.3)	6.11 (14.4)
Water [kg]	0.00	0.15

4.3.1.3. Proteins extraction

The mass and energy flows related to proteins extraction (Lh.P.E. scenario) from the hexane-defatted biomass are detailed in Figure B- 3. The spray-drying step represents about half of the total energy demand of the process, the other fraction consisting in the electricity for the subsequent mixing and centrifuge steps. The total energy demand ($5.2 \text{ MJ} \times \text{kg}^{-1}_{\text{proteins}}$) is contrasted with the results from Berardy *et al.*, (2015) reporting $2.5 \text{ MJ} \times \text{kg}^{-1}_{\text{proteins}}$ for soy proteins isolate (SPI) production using a similar processes except for drying where freeze-drying was used. The difference between these results is explained by the allocation to co-

products in the SPI case. The valorisation of microalgae with proteins extraction is still at its infancy compared to the research performed for energy production from microalgae. To our knowledge, only one study reports the specific energy requirements for proteins extraction from microalgae (Gnansounou and Raman, 2016) and considers a recent patent based on ethanol extraction (Aniket, 2014). Nevertheless, the relatively low energy requirement reported ($1.33 \text{ MJ} \times \text{kg}^{-1}_{\text{proteins}}$) is not a fair comparison with our results since this study uses lipids hexane-extraction as a proxy for the ethanol-extraction of proteins and do not include the drying of proteins. The proteins extraction yield is highly sensitive to the cell disruption level and thus to the method and the cell wall resistance of the microalgae (Safi *et al.*, 2014b). Cell disruption being critical in the energy demand of the process, low energy demanding methods, such as enzymatic or chemical hydrolysis, are investigated but are not efficient once applied without any other cell disruption method due to low proteins solubilisation rates (Safi *et al.*, 2017). Moreover, proteins diffusion behaviour is also a key aspect in the extraction yield but its relation with cell disruption method is still poorly understood (Safi *et al.*, 2015). Nevertheless, both HPH and BM are the most efficient methods when considering the amount of solubilised proteins resulting from higher proteins diffusivity than other methods (Safi *et al.*, 2014b, 2015, 2017).

4.3.1.4. Anaerobic digestion of residual biomass: methane and fertilizers production

Both lipids and proteins extractions affect the methane production in the anaerobic digestion step (Table 4- 3). The difference in methane production between Lh.E. and Lm.E. illustrates the effect of lipid extraction yield on anaerobic digestion: on dry weight basis, the 2-MeTHF-defatted biomass contains 8.1% DW of lipids while this value is 11.7% for hexane scenarios. This slight difference in lipids content consequently affects methane production because lipids present the biggest TMP (Sialve *et al.*, 2009). The comparison between Lh.P.E. and Lh.E. illustrates the effect of proteins extraction on methane production yields resulting from difference in the biomass to methane conversion efficiencies (60% of TMP in Lh.P.E. and 40% of TMP in Lh.E). Klassen *et al.* (2017) measured a conversion efficiency of 84% of TMP from *Chlamydomonas reinhardtii* in continuous fermentation. Similarly, Lakaniemi *et al.* (2011) performed the anaerobic digestion of *Chlorella vulgaris* consisting to a HRT of 49 days in batch-wise fermentation. Based on the biomass composition reported in Lakaniemi *et al.* (2011), we calculated the TMP using the coefficients from Sialve *et al.* (2009) and corrected by Heaven *et al.*, (2011); and found a conversion yield of 88% of TMP. Considering these results, the biomass to methane conversion efficiency assumed in this study seems to be underestimated but justified by higher proteins content of the biomass that can reduce the conversion efficiency. For Lh.P.E., the protein content of the biomass is 43% DW, while this value is 28

and 36% DW for Klassen *et al.* (2017) and Lakaniemi *et al.* (2011), respectively. This higher concentration leads to more nitrogen release ($\text{NH}_3/\text{NH}_4^+$) and causes inhibition by acidosis to methanogenic bacteria (Ward *et al.*, 2014; Klassen *et al.*, 2017). This also explains our choice to limit the biomass to methane conversion efficiency to 40% in the case of Lh.E. scenario considering the proteins content of the biomass (52% DW). The co-digestion of microalgae biomass with other substrates is worth to investigate to reduce nitrogen inhibition and thus to improve the biodegradability of the biomass (Wang *et al.*, 2013). The fraction of biogas not consumed for heating the digester is upgraded using water scrubbing technique, based on the difference in solubility between methane and carbon dioxide. If the cultivation and biogas production facilities are too far, alternative biogas upgrading methods - such as chemical absorption, pressure swing absorption or membrane separation - are proven to be efficient for large-scale applications (Awe *et al.*, 2018). This step requires a large volume of freshwater which can be recycled as carbon feedstock for algae growth in the cultivation (Collet *et al.*, 2010). In addition to affect methane production, proteins extraction also influences the nitrogen content of the digestate and thus, the composition of the fertilizers. Considering a mineralisation efficiency of 90% (Ras *et al.*, 2011), the nitrogen concentration in the organic fertilizer is 3.6 and 4.0 g x kg⁻¹_{fertilizer} (wet weight) for Lh.P.E. and Lh.E., respectively, and the same trend is observed for the mineral fraction.

Table 4- 3 Mass flows [kg, kilogram], dry weight percentage (%DW) and energy inputs [kWh, kilowatt-hour, MJ, megajoule] for the anaerobic digestion processes reported per kilogram of input biomass dry weight at the biorefinery gate. Lh.P.E., hexane lipid extraction, protein extraction, anaerobic digestion; Lh.E., hexane lipid extraction, anaerobic digestion; Lm.E., 2-methyltetrahydrofuran lipid extraction, anaerobic digestion.

	Lh.P.E.	Lh.E.	Lm.E.
Anaerobic digestion			
<i>In</i>			
Electricity [kWh]	0.09	0.09	0.09
Steam [MJ]	1.88	2.07	2.15
Water [kg]	10.48	12.59	11.46
Wet biomass [kg] (%DW)	5.64 (13.6)	5.31 (16.0)	6.11 (14.4)
<i>Out</i>			
Raw biogas [kg]	0.37	0.30	0.28
Digestate [kg] (%DW)	15.75 (2.5)	17.60 (3.4)	17.30 (3.4)
Steam generation			
<i>In</i>			
Raw biogas [kg]	0.10	0.12	0.13
Oxygen [kg]	0.15	0.16	0.17
<i>Out</i>			
Water [kg]	0.08	0.09	0.10
Carbon dioxide [kg]	0.17	0.19	0.20
Steam [MJ]	1.88	2.07	2.15
Biogas upgrading			
<i>In</i>			
Electricity [kWh]	0.04	0.03	0.02
Water [kg]	67.68	45.74	38.07
Raw biogas [kg]	0.27	0.18	0.15
<i>Out</i>			
Methane [kg]	0.10	0.06	0.05
Water [kg]	67.85	45.86	38.17
Digestate purification			
<i>In</i>			
Electricity [kWh]	0.02	0.02	0.02
Digestate [kg]	15.75	17.60	17.30
<i>Out</i>			
Mineral fertiliser [kg] (%DW)	14.63 (0.4)	15.84 (0.5)	15.63 (0.5)
Organic fertiliser [kg] (%DW)	1.12 (30.0)	1.76 (30)	1.67 (30.0)

4.3.1.5. Drying for the production of dietary supplement for animal feed

This process is applied in the BM/HPH-Lh.D. scenarios: 6.31 kg of algal paste is dried from 24.3 to 90.0% DW (Figure B- 5). Only 2% of the total energy consumption is used for the fans (air circulation) and pumps, most of the energy demand serves the heating purpose. Moreover, a considerable amount of heat is lost in moist air (80 °C) that leaves the conveyor belt. A fraction of this energy can be recovered to reduce the total energy demand by the means of heat exchangers to pre-heat the dry air such as for industrial belt dryers used in WWT plants (Stela Laxhuber GmbH, 2019). The implementation of this method requires to consider the dew point of moisture, as it affects the energy transfer efficiency. Hosseinizand *et al.* (2017)

explored a second possibility for pre-heating air consisting in coupling the drying process with a power plant to recover a fraction of residual heat.

4.3.2. Global evaluation of microalgae biorefinery scenarios

A large variability is observed among the scenarios. For example, the BM-Lh.D. requires about three times more energy than HPH-Lh.P.E. (Table 4- 4). The difference is mainly explained by the choice of the cell disruption method (BM being the largest contributor to the total energy demand in each scenario) but also by the higher energy requirements for drying while anaerobic digestion has a positive net energy balance. The energy demand for lipid extraction includes the extraction itself (mixing and heating the reactor) but also the solvent recovery. This last step explains the difference in the total energy demands of Lh.E. and Lm.E. scenarios, consisting to hexane and 2-MeTHF extractions, respectively. Besides the heating capacity and the specific heat of vaporisation, the variation in the energy demand is mostly explained by the azeotrope in 2-MeTHF extraction, requiring additional distillation and decantation steps to recover the dry solvent. 2-MeTHF is an effective solvent for lipids wet-extraction from *Chlorella vulgaris* but the higher heating requirements for its recovery generates additional heat losses compared to hexane. Nevertheless, 2-MeTHF wet-extraction is more interesting than hexane in wet conditions (*c.f.* 4.3.1.2). Finally, the profile of the lipids extracted is expected to vary with a higher proportion of polar lipids in the extract from 2-MeTHF compared to hexane due to higher polarity.

Table 4- 4 Total energy demand [MJ, megajoule], products recovered (g, gram) and energy losses from heating steps [MJ] reported per kilogram of input biomass dry weight [kg^{-1} input] at the biorefinery gate. The cell disruption is performed by bead milling technique (BM) or high-pressure homogenisation method (HPH). (-) indicates a net energy production being discounted to the total energy demand. (1) Includes 10% of water (weight). Lh.P.E., hexane lipid extraction, protein extraction, anaerobic digestion; Lh.E., hexane lipid extraction, anaerobic digestion, Lh.D., hexane lipid extraction, drying; Lm.E., 2-methyltetrahydrofuran lipid extraction, anaerobic digestion.

	Lh.P.E.		Lh.E.		Lh.D.		Lm.E.	
	BM	HPH	BM	HPH	BM	HPH	BM	HPH
Energy demand [$\text{MJ} \times \text{kg}^{-1}$ input]								
Dewatering		2.34		2.34		2.34		2.34
Cell disruption	40.98	7.65	40.98	7.65	40.98	7.65	40.98	7.65
Lipids extraction		12.80		12.80		12.80		15.62
Proteins extraction		1.45		-		-		-
Anaerobic digestion	(-) 4.17		(-) 2.44			-	(-) 2.17	
Dietary supplement		-		-		10.41		-
Total energy demand	53.40	20.07	53.68	20.35	66.53	33.20	56.77	23.44
Products [$\text{g} \times \text{kg}^{-1}$ input]								
Lipids		35.71		35.71		35.71		71.42
Proteins		146.43		-		-		-
Methane		93.93		59.64		-		53.57
Animal feed		-		-		1018.51 ¹		-
Waste heat [$\text{MJ} \times \text{kg}^{-1}$ input]								
Losses from heating		0.62		0.65		5.60		0.84

The energy demand for proteins extraction is relatively low due to the absence of specific heating system. While proteins account for 50% DW of the initial biomass, the amount available for extraction is limited to 29% of the initial proteins because the extraction can only be performed on the aqueous layer recovered from the hexane extraction (*c.f.* (A) and Figure 4-2). The comparison between Lh.P.E. and Lh.E. scenarios illustrates the benefit from extracting proteins from the substrate for the anaerobic digestion process. Despite its additional protein extraction step, the Lh.P.E. scenario requires less energy than the Lh.E. scenario due to higher methane conversion efficiency. Therefore, protein extraction is beneficial from an on-site

energetic aspect if the waste biomass is further valorised by anaerobic digestion. Considering the difference in methane production among Lh.P.E. and Lh.E. scenarios (93.9 and 59.6 g), we estimate a marginal gain of methane production of 0.2 g per gram of proteins extracted, however this value is highly sensitive to the range of proteins concentrations. The comparison of these two scenarios being determined by proteins extraction yields, the cell disruption efficiency is decisive for proteins solubilisation (Suarez Garcia *et al.*, 2018). In both scenarios, the anaerobic digestion presents a net positive energy balance which is discounted in the total energy demand for each scenario. The energy produced is estimated considering the lower heating value (LHV) of methane to account for inefficiencies occurring at methane combustion for heating. The second way for the valorisation of the residual biomass is drying. This process produces 1.02 kg of biomass (90% DW) per kg of dry biomass entering the biorefinery.

4.3.3. Fate of the components

The understanding of the fate of each algal constituent within the biorefinery is crucial to improve the recovery of the different products. This modelling was made for the Lh.P.E. scenario (Figure 4- 2) presenting the highest valorisation targets. It illustrates the evolution of the biomass composition in DW related to the production of 1 kg of lipids from *Chlorella vulgaris*. The results show the impact of the lipid extraction process on the subsequent protein extraction step: a fraction of proteins is damaged by solvent recovery and is directly transferred to anaerobic digestion. For data availability reasons, we assume that no biomass losses occur in the different processes except during dewatering. Therefore, our study is probably overestimating the real efficiency of the process due to the lack of specific results for biomass losses in microalgae biorefineries. The assumption of pure products recovery from microalgae biomass is also visible in Figure 4- 2 (*i.e.* pure lipids and pure proteins fraction). Nevertheless, studies on lipid extraction using hexane or 2-MeTHF do not report any co-extraction of other biomass components, the extraction mixture being mostly composed of triglycerols. As 2-MeTHF is more polar than hexane, the lipid extraction mixtures will differ in their respective compositions, and the hexane extract will contain a higher proportion of neutral lipids than for 2-MeTHF (Ehimen *et al.*, 2010; Halim *et al.*, 2016; Angles *et al.*, 2017; Wan Mahmood *et al.*, 2017; de Jesus *et al.*, 2018). The proteins solubilisation step prior to precipitation is expected to solubilise carbohydrates, polar lipids or pigments. The method for precipitation based on the isoelectric point limits the co-extraction, the other compounds remain in the acidic solution after the recovery of the precipitate. The Lh.P.E. scenario assumes that only proteins are solubilised and thus does not consider the potential losses of additional polar components dissolved in water. Such simplifications can contribute to overrate the downstream methane production yield and thus to overestimate the difference in the energy production between Lh.P.E. and Lh.E. scenarios (Table 4- 1, Table 4- 4).

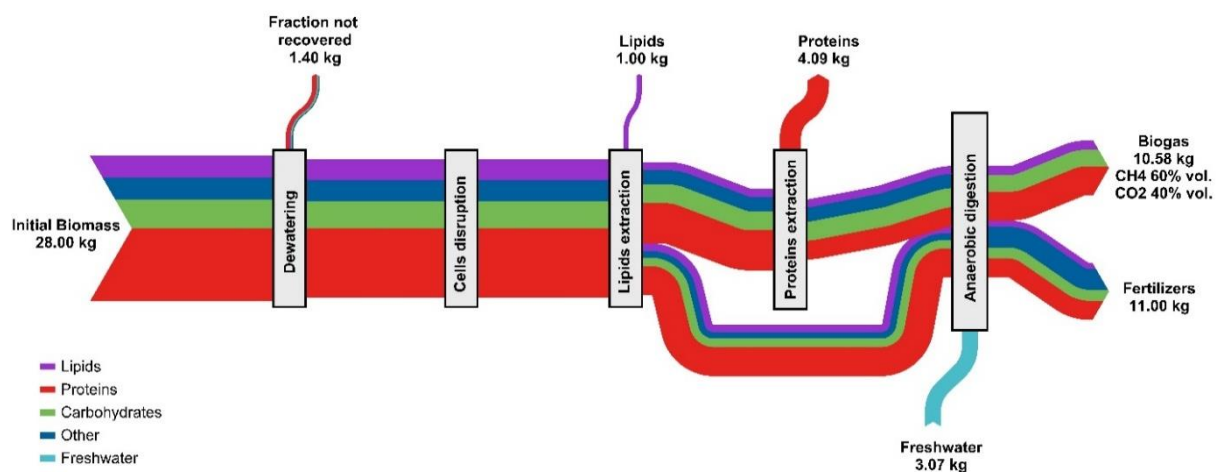


Figure 4- 2 Fate of the constituents of *Chlorella vulgaris* within the hexane lipid extraction, proteins extraction, anaerobic digestion scenario (Lh.P.E.) to extract 1 kilogram of lipids. Biomass composition is expressed in kilogram [kg] of dried weight.

4.4. Conclusions

Microalgae-based technologies are extensively discussed in literature, but their applications remain limited to few niche markets. Their potential for creating value for investors depends on a complex set of factors including their energy efficiency as a key driver in the production costs. Our study evaluates the energy efficiencies for diverse options in microalgae biomass valorisation and identifies their critical steps. Different alternatives were modelled using process engineering practices and discussed in function of energy demand. First, we showed the importance of the cell disruption method and its potential to improve the energy demand by using HPH instead of BM. Moreover, we tested two solvents for lipids extraction and we demonstrated that the higher energy demand for the recovery of the green solvent is compensated by its increased lipid extraction yield. These results are nevertheless highly sensitive to the microalgae strain and the extraction conditions. While lipids extraction has a negative effect on proteins recovery, we showed that proteins extraction is particularly interesting if the residual biomass is further valorised through anaerobic digestion. Alternatively, it may be interesting to perform similar study for scenarios with proteins recovery prior to lipids extraction. We also investigated the drying of the residual biomass for dietary supplement instead of anaerobic digestion. Nevertheless, the comparisons among the different options are limited by poor insights on the market value of the products. Therefore, our results provide part of the information required for economic models to evaluate microalgae biorefinery projects. The scientific literature reports varying results for the feasibility of microalgae biorefineries at (semi-)industrial scale (e.g. the lack of consensus on third generation biodiesel). As the production costs of microalgae biomass are relatively high compared to alternatives (Chew *et al.*, 2017), it is essential to tailor the outputs to high value added products (e.g. cosmetics, pharmaceuticals, etc.) and extract multiple components instead of targeting an individual extraction. By detailing each single process unit that operates for a specific product, our study provides key numbers to balance with the expected market value of the products for a first insight on the feasibility of each scenario. A preliminary comparison has been done between the HPH-Lh.P.E. scenario with lipids and proteins isolated production from soy. Despite the discounting of methane production in the total energy demand, the microalgae scenario is far less energy efficient than in the case of soy (50 times less energy efficient for lipids and proteins production). The products recovered being not identical between microalgae and soy (lipids and amino acids profiles), it is important to look for specific applications of microalgae components, requiring a deep understanding of their properties. These are still underexplored despite their potential as perceived by the scientific community and the growing interest for the biobased economy. Our work clearly illustrates the inefficiencies in the processing of microalgae biomass due to the novelty of the methods (e.g.

28 kg of algae biomass are required to produce 1kg of lipids and 4 kg of proteins in the Lh.P.E. scenario) and therefore, the importance of targeting value-added applications in addition to further research to increase the efficiency of the processes. Besides the considerations among processes, it is necessary to design the value chain of microalgae biomass in its entirety to enhance resources efficiency. The integration of multiple solutions in business models for microalgae products is worth to investigate, for example by combining the cultivation stage with wastewater treatment and/or industrial fumes abatement. We see the opportunity to integrate our results in the entire value chain of microalgae and compare them with prospective scenarios assuming a similar design but at industrial scale with higher technology readiness level. Combined with environmental impact assessment (*e.g.* Life Cycle Assessment) and costs evaluation (*e.g.* Life Cycle Costing), the results are helpful to design early stage microalgae-based technologies.

CHAPTER 5

Conclusions and perspectives

5.1. Main results

The first two research chapters offer new insights related to the environmental sustainability assessment of marine production systems. Site-specific marine LCIA characterization factors are developed to better address impacts on regional ecosystem quality through the integration of pathways including ecosystem services (**Chapter 2**) and biodiversity (**Chapter 3**), that are intrinsically related to ecosystem health and natural resources. However, to establish environmental sustainability assessment of marine products, there is a need to look at the entire value chain, and thus also to the downstream processing and valorisation of marine biomass. Therefore, **Chapter 4** provides a LCI of different valorisation routes for microalgae biomass and highlights which downstream processes can be applied to wet biomass in particular.

In **Chapter 2**, a first attempt is made to account for the reduction of fishery yields due to seaweed aquaculture. In this chapter, a LCIA pathway is developed for a specific ecosystem service (*i.e.* fish provision) and can be easily included in the LCA methodology of seaweed farming using data on fish landings and on the NPP of the zone under study. The concept of Human Appropriation of Net Primary Production (HANPP) (Haberl *et al.*, 2007) is applied considering nutrients availability as limiting factor for phytoplankton growth (Breton *et al.*, 2006; Chassot *et al.*, 2007; Conley *et al.*, 2009), and it is combined with the marine trophic food web approach (Pauly and Christensen, 1995). From these two different concepts, a single cause-effect chain linking seaweed harvesting to fisheries, is modelled. Three types of LPY indicators are proposed for the ten most caught fish species in the North Sea. They only differ in their unit of measurement, depending on the perspective considered: biomass, economic (converting fish biomass loss according to averaged market value of each fish) and eco-exergy (converting the loss of fish biomass according to the genome size of each fish species). While the biomass and monetary value aspects depart from an economical production perspective, the eco-exergy LPY indicator is a way of weighting natural resources according to their ecological complexity. Considering the cascade framework for ecosystem services (De Groot *et al.*, 2002), it is clear that LPY expressed in monetary value is the most consistent indicator to assess ecosystem services loss. The LPY results obtained for the three indicators dealing with the ten most caught fish in the North Sea are always negligible compared to the production of seaweed reported in their equivalent unit. This shows a net positive effect in terms of natural resources availability: the loss of ecosystem service (*i.e.* fish provisioning) is largely compensated by the gain in the other ecosystem provisioning service (*i.e.* seaweed biomass). Therefore, seaweed production in the North Sea is beneficial from an ecosystem provisioning service point of view as far as it does not induce major changes in the global functioning of the North Sea (*c.f.* 5.2). The consideration of effects on ecosystem services in the LCA

methodology requires the inclusion of a new set of LCIA pathways to cover different ecosystem services. However, the implementation of such exhaustive modelling is limited by data availability/accessibility regarding ecosystem functioning and knowledge gaps for the quantification of ecosystem services in some marine ecosystems. To avoid such complex modelling, changes in the supply of total ecosystem services can be evaluated through proxy indicators rather than in monetary terms. Therefore, **Chapter 3** focuses on biodiversity as an indicator for ecosystem health and total ecosystem services (Worm *et al.*, 2006). Unlike **Chapter 2** where methodological developments were based on non-LCA literature, LCIA developments of **Chapter 3** are built on existing frameworks for land-use and sea-use impact assessment in LCA (Milà i Canals *et al.*, 2007; Langlois *et al.*, 2014a; Woods and Verones, 2019) and on recommendations from the UNEP SETAC Life Cycle Initiative (Jolliet *et al.*, 2018). The regional biodiversity impact does not rely on general models fitted to ecosystem parameters such as in de Baan *et al.*, (2013a) and Chaudhary *et al.*, (2015) because this would limit its application in marine environments. The framework developed in this chapter is conceived to be applicable for any type of ecosystem: from deep-sea ecosystems presenting limited information regarding their biodiversity (Costello *et al.*, 2010) to intensively studied terrestrial ecosystems. Therefore, the comparison of biodiversity impacts caused by two alternatives taking place in varying ecosystems can use this framework for weighting regional impacts according to the ecosystem in which they occur (*i.e.* conversion to global impacts). For example, including *in situ* biodiversity in a comparative LCA of deep-sea and terrestrial mining brings a new but meaningful dimension to the total environmental impact. A comparative LCA of deep-sea and terrestrial mining to produce battery materials concluded that deep-sea mining is the best option when considering total greenhouse gases emissions only (*i.e.* mining and downstream processing) (Paulikas *et al.*, 2020). However, other impact categories must be considered to fully understand the environmental sustainability performance of deep-sea mining. Because of the complexity regarding *in-situ* impacts (Levin *et al.*, 2020), the total environmental sustainability assessment of deep-sea mining requires far more aspects than those included in the scope of traditional LCA methods, but the development of specific LCIA CFs strongly relies on the availability of various data. This is illustrated for the LCIA step of deep-seabed disturbance on biodiversity: to develop CF_{reg} , an exhaustive literature review needs to be performed to understand and model benthic recovery. Similarly, CF_{glo} requires preliminary work to identify the ecosystem boundaries and to perform a vulnerability assessment if the conservation score is not available in the Wildfinder database. Moreover, the values obtained for CF_{reg} and CF_{glo} rely on the total area of the ecosystem under study and hence their evaluation requires a proper assessment of ecosystem extension. The operation of the framework to the CCZ shows that regional and global impacts are mostly driven by the permanent loss of biodiversity because of the absence of recovery observed after

37 years for some sessile benthic species. The method provides CF_{reg} that are consistent with CF values observed in *ad-hoc* methods for biodiversity impacts expressed in similar units (PDF x yr) (De Baan *et al.*, 2013a; Chaudhary *et al.*, 2015; Woods and Verones, 2019). The first two research chapters (**Chapters 2** and **Chapter 3**) emphasize the development of new LCIA pathways related to upstream processes (*i.e.* raw material production) in the value chain of marine products. However, the total environmental impact of marine products must be assessed and benchmarked for their entire value chain in order to understand their sustainability compared to alternatives.

In a second step, this PhD research focuses on expanding LCI knowledge related to processing of aquatic wet biomass (**Chapter 4**). The study builds on existing literature to model mass and energy flows for a microalgal biorefinery. As a main result, an exhaustive LCI for various valorisation routes is developed, considering the current technology readiness level. Compared to **Chapter 2** and **Chapter 3** dealing with marine natural resources, **Chapter 4** differs because it considers microalgae grown artificially in photobioreactors under fully controlled conditions. However, the technology for downstream processes considered in the study is also applicable to marine biomass (*e.g.* seaweed) in biorefinery systems. The study investigates two cell disruption routes (HPH and BM) and highlights the potential of HPH to reduce significantly the total energy consumption of the process. Two trade-offs amongst the extraction of multiple products from microalgae are highlighted: (1) lipids extraction reduces the biomass fraction available for proteinic extraction because heat is required to recover solvent and (2) anaerobic digestion produces higher methane yields if performed from biomass with lower protein content because of more favourable carbon to nitrogen ratios. Moreover, the use of an alternative and bio-based solvent (2-MeTHF) compared to hexane for lipids extraction has potential to increase the total energy efficiency, but extraction and solvent recovery yields should be validated at an industrial scale. Drying defatted microalgal paste is an alternative to methane production but, from an economic viability perspective, it needs to target high value end-applications to compensate its energy requirements. Compared to soy biomass, lipidic and proteinic extraction from microalgae is far less energy efficient but the properties of the final products are not similar. The LCI provides reliable information to compare the environmental and economic sustainability of downstream processes for wet biomass.

5.2. Perspectives: assessing marine ecosystem health

This section discusses the further implementation of site-specific LCIA models to account for marine ecosystems quality. In a first step, the work achieved in the PhD is compared to additional site-specific LCIA models that are relevant to assess the environmental sustainability performance of marine products. The major challenges of implementing further marine site-specific LCIA models are described. While most of LCIA models for ecosystem quality make use of species-richness related metrics, the different types of biodiversity are reviewed and alternative approaches to species richness biodiversity are discussed. In a second step, the development of further site-specific marine LCIA models is reviewed in the context of the conservation criteria developed by the United Nations to report seas and oceans' health status. LCA methods being used as policy decision supporting tool, it is crucial to ensure the relevance of the results regarding policy concerns. Considering the outcomes from the previous section, the integration of marine ecosystem services in LCA and its link with regional biodiversity are discussed in order to develop further LCIA models accounting for both biodiversity and ecosystem services. Finally, the last subsection emphasizes on the non-consideration of cumulative ecological impacts and ecosystem collapsing in LCA methods. It proposes a schematic framework to account for cumulative impacts on regional ecosystems and highlights the major challenges of its implementation.

5.2.1. Challenges of implementing LCIA models for the marine environments

The development of site-specific LCIA indicators has always been a challenge in environmental sustainability assessment because of high data requirements to account for local ecological conditions. This becomes even more challenging when it comes to the marine environment (Woods *et al.*, 2016). The PhD focused on the development of two site-specific LCIA pathways and illustrated them for two case studies (**Chapter 2** and **Chapter 3**). Within the scope of natural resources provisioning, the first LCIA considers biotic resources uptake (*i.e.* seaweed) to account for lost potential yields (LPY) in fisheries and the second LCIA focuses on the effect of deep seafloor destruction on benthic biodiversity. When put in perspective of sea-use impact categories (Langlois *et al.*, 2014b), these two LCIA models analyse site-specific impacts belonging to ecosystem services damage potential (ESDP) and biodiversity damage potential (BDP) (Figure 5- 1). However, the framework for sea-use impact categories offers fertile ground for the development of many complementary LCIA models, following the framework for land-use impact assessment in LCA (Lindeijer *et al.*, 2002; Koellner *et al.*, 2013a). Compared to impacts caused by land-use, sea-use impacts are not necessarily located in the area where the intervention takes place because of water circulation. Therefore,

in the two LCIA models developed in this PhD research the marine ecological processes are necessarily simplified.

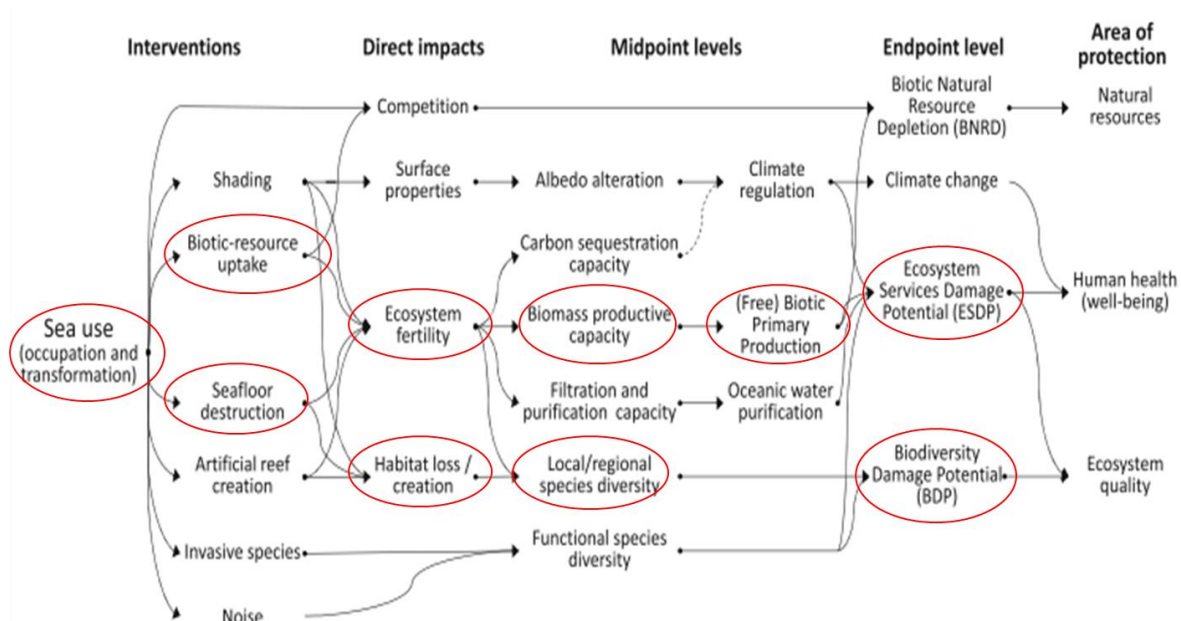


Figure 5- 1 LCIA approach for sea-use impact pathways (Langlois *et al.*, 2014b) and scope of **Chapter 2** and **Chapter 3** (in red).

Chapter 2 simplifies the ecological complexity in a linear model with a limited number of parameters (site-specific daily NPP, fish landings seaweed growth and carbon content). Consequently, it presents uncertainties regarding its results. Additional aspects related to seaweed farming impact on commercial fish landings (e.g. habitat provision, toxic algae bloom regulation) should be considered through further LCIA modelling. However, the cumulative effect on fish landings is complex: the LCIA's results should be considered separately and summed to estimate the final impact on fish landings. In other words, each LCIA pathway represents a different mechanism caused by a single pressure (*i.e.* seaweed farming) but the evaluation of the final effect (*i.e.* fish landings reduction) requires dynamic models (Borja *et al.*, 2016; Smith *et al.*, 2016) (*c.f.* 5.2.7). Moreover, the accuracy of the LCIA results could be improved by developing fate factors¹⁸ based on water circulation models in order to understand which parts of the North Sea are more impacted by HANPP due to nutrients levels reduction. The impact on fisheries yields not necessarily occurs in the same area impacted by HANPP and thus, a fully spatially differentiated LCIA must consider geographical aspects all along the trophic food web.

Similarly to **Chapter 2**, **Chapter 3** relies on linear modelling to account for deep-sea biodiversity loss from benthic disturbance. Due to the lack of information regarding benthic

¹⁸ Fate factors are used in the development of CFs. The CFs are obtained from the multiplication of FF with effect factors and exposure factors.

recovery in the long run, the implementation of the framework has faced data gaps regarding the evaluation of the permanent biodiversity impact. **Chapter 3** also faces uncertainties regarding cumulative impacts caused by different stressors (e.g. deep-sea mining generates artificial light and vibrations / noise in addition to seafloor disturbance). However, the major challenge for further development of site-specific marine LCIA indicators consists of the development of an integrated classification system for marine ecosystems. Most of LCIA models accounting for impacts on regional ecosystems still rely on the classification and the database for terrestrial ecoregions from the WWF (WWF, 2006). For example, the CFs recommended by UNEP-SETAC for land-use impacts on biodiversity are differentiated according to land-use intervention and according to the WWF ecoregion in which they take place (Chaudhary *et al.*, 2015). Such databases for terrestrial ecosystems have facilitated the development of site-specific LCIA CFs through providing key information regarding ecosystem characteristics (species composition, threat level, total area). The terrestrial environment has been classified into 8 realms, 14 biomes / major habitat types and 825 ecoregions (Dinerstein *et al.*, 1995; Olson and Dinerstein, 1998; Olson *et al.*, 2001). More recently, a similar initiative has been implemented for coastal and shelves areas, identifying 12 realms, 62 provinces and 232 ecoregions / ecosystems (Spalding *et al.*, 2007). However, oceans and open seas do not present any unified classification at the ecosystem level (Figure 5- 2). So far, a global classification for marine provinces has been proposed and differentiates amongst provinces located in pelagic (>800 m depth), bathyal (800 – 3000 m depth), abyssal (3500 – 6500 m depth), hadal (> 6500 m depth) and hydrothermal vent environments (Agostini *et al.*, 2009). However, no global classification at the ecosystem level is available for marine environments.

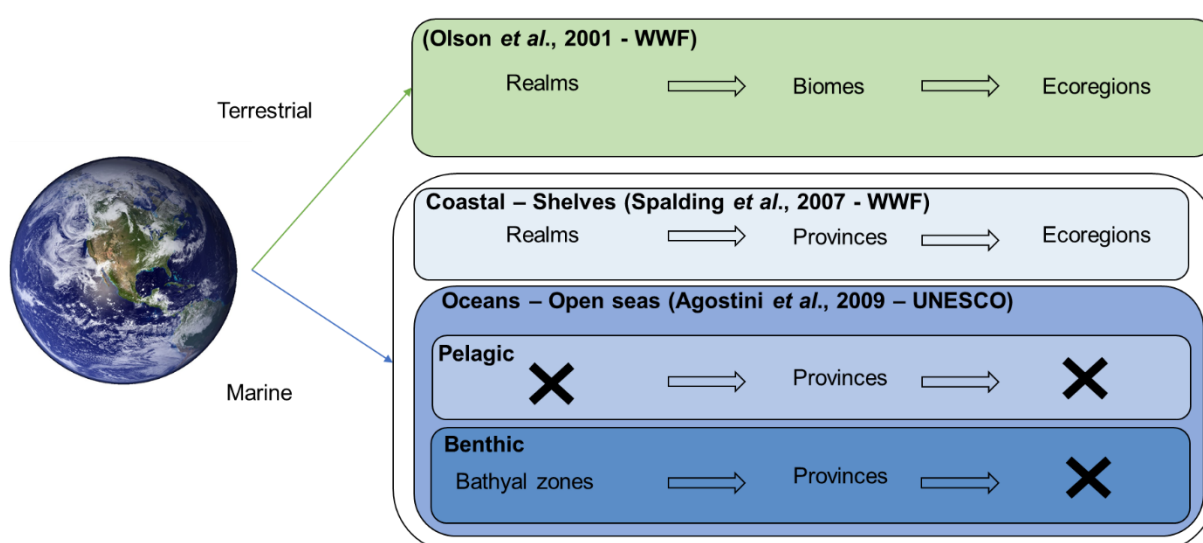


Figure 5- 2 Comparison of the classifications for terrestrial and marine environments, “X” indicates that no global classification is available for the corresponding level, the classification (authors and corresponding institution) for each entity is indicated between brackets.

Next to the development of a classification system for marine ecosystems, another challenge consists of monitoring changes in ecosystem quality through relevant indicators. The following section focuses on indicators for ecosystem quality in LCA methods.

5.2.2. Indicators for (marine) ecosystems quality: drawbacks of species richness related metrics

Existing LCIA methods make use of species richness related indicators¹⁹ as endpoints for the AoP ecosystem quality (De Baan *et al.*, 2013b; Jolliet *et al.*, 2014; Chaudhary *et al.*, 2015; Huijbregts *et al.*, 2016). The availability of species area models in ecology has facilitated the implementation of characterization models for ecosystem quality based on species richness. Globally, the evaluation of changes in environmental conditions using species richness indicators is a common practice in ecology (Magurran, 2005) and ecological conservation goals implicitly include the maximization of species richness or other species-related indicators such as the Shannon-Wiener index (Gotelli and Colwell, 2001). On the other hand, the reliance on species richness-related indicators for ecological conservation purposes overlook information regarding species identity (*i.e.* vulnerability, functional role, food web level). For these reasons, the identification of changes in key species occurrence is relevant to assess perturbations in ecological conditions; these include (1) ecological indicators, *i.e.* proxy for other species sharing a similar habitat, (2) keystone, *i.e.* crucial species underpinning the survival of other species, (3) umbrella, *i.e.* species requiring large habitat needs, covering the habitats of multiple other species, and (4) vulnerable, *i.e.* rare species, variable in population density, prone to extinction (Simberloff, 1998; Tim, 2010). Except for some LCIA methods considering species vulnerability in biodiversity impact assessment (Verones *et al.*, 2013; Chaudhary *et al.*, 2015), other methods consider implicitly all species as equally important in their evaluations of changes in ecological conditions (*i.e.* ecosystem quality). Moreover, biodiversity impacts are expressed in relative metrics (*e.g.* PDF) which do not provide information regarding absolute species numbers of the ecosystem impacted. Inversely, biodiversity impacts are expressed in absolute metrics (*e.g.* species lost), overlooking the relative loss of species. The total number of organisms impacted, or species density, is also neglected in species richness related indicators reported in LCIA. Figure 5- 3 illustrates in a simplified way two main issues related to single and relative indicator use for monitoring changes in ecological conditions. The biodiversity impact caused by a similar punctual disturbance occurring in two ecosystems with varying ecological patterns is calculated in PDF. Both PDF results are similar (1/3) because ecosystem B, hosting higher number of species, contains an additional species that is not affected by disturbance. Therefore, PDF results are

¹⁹ *E.g.* potentially affected fraction of species (PAF) or potentially disappeared fraction of species (PDF).

equal because proportions are identical amongst species lost and total number of species for the two ecosystems. The final biodiversity impact would not differ amongst the two ecosystems without further consideration of the results into a PDF metric calibrated in function of species endemism, species vulnerability or total species number (absolute terms). In addition, the PDF metric or its equivalent metric in absolute terms (e.g. total species lost) does not consider organisms density and ecological importance in impacted ecosystems. Therefore, there is potential to consider other metrics than species richness related indicators to assess changes in ecological conditions / ecosystem quality in LCIA (De Souza *et al.*, 2013).

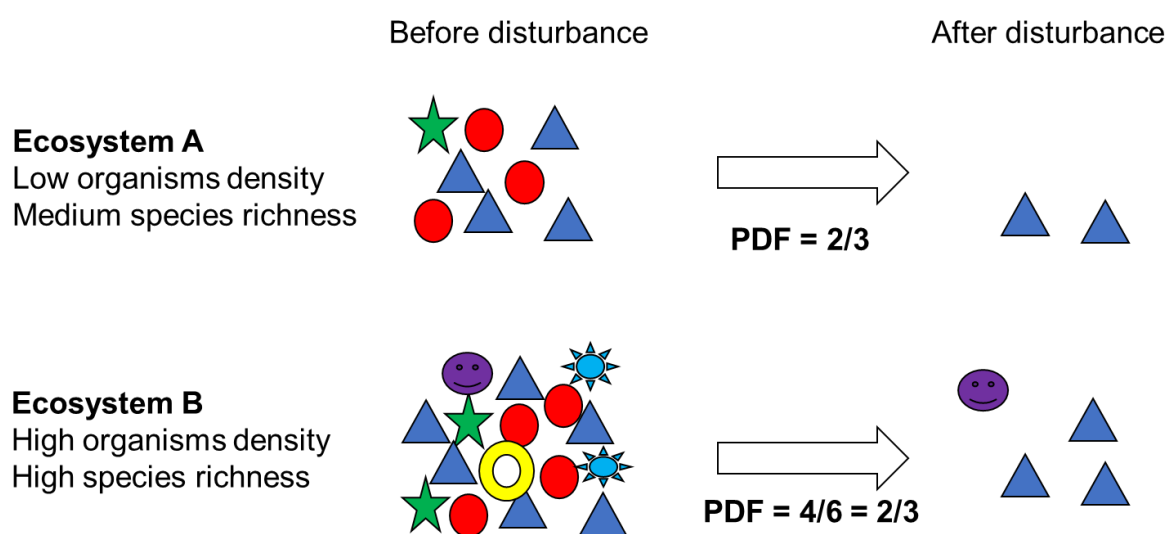


Figure 5- 3 Evaluation of potentially disappeared fraction (PDF) of species in two ecosystems with varying species richness and species density. Each distinct geometric pattern represents the occurrence of a species.

5.2.3. Potential of complementary indicators for (marine) ecosystem quality

Species-related metrics are only one possibility amongst others to measure changes in ecological conditions. Ecosystems consist of various elements that are interdependent and arranged in definite patterns (Margalef, 1963), thus assessing changes in ecosystem quality consists of measuring modifications within the organization of ecosystem constituting elements. Globally, biodiversity can be defined as “the variety and variability among living organisms and the ecological complexes in which they occur” (OTA, 1987). Therefore, ecological conditions can be captured from different perspectives, depending on the indicator selected. Following this broader perspective, the hierarchical approach has been proposed to monitor biodiversity and considers four levels of organization nested in three ecological attributes (Noss, 1990). The levels of organization consist of genetic diversity (e.g. variety of crops), species diversity, communities diversity²⁰ and ecosystems / landscapes diversity (e.g.

²⁰ This level was merged with species diversity in the initial definition of ecosystem levels proposed in OTA (1987).

spatial complexity of regions) (OTA, 1987). Therefore, biodiversity is measured at each level of organization, depending on the objective of the study. However, each level can be measured through three different perspectives, *i.e.* composition, structure and function attributes (Franklin, 1981). Composition refers to the identity and variety of elements from the collection, structure refers to the physical patterns observed in the collection, and function refers to aspects related to ecosystem functioning. Therefore, multiple indicators are proposed for each combination of organization level and attribute level in order to measure specific aspects of ecosystem patterns (Noss, 1990) (Table 5- 1). Initially, biodiversity monitoring has mainly focused on compositional communities' diversity, changes in ecological conditions being reported in terms of species richness related metrics (Franklin, 1988). Such a simplified vision of biodiversity is being criticized because it overlooks other biodiversity types and thus leads to incomplete monitoring and fuzzy conservation goals for biodiversity (Mcgill *et al.*, 2015; Pollock *et al.*, 2020). In addition, biodiversity monitoring through species richness related metrics is often relying on a proxy taxon for the entire community (*e.g.* vascular plant species in ReCiPe LCIA for land use) but a variation in species richness in one group does not necessarily involves a variation at the same magnitude in the species richness of other groups (Lawton, 1998).

Table 5- 1 Relevant indicators for the different types of biodiversity defined by each combination of attributes and levels, adapted from Noss (1990). The scope of LCIA methods considering biodiversity is appears in green.

	Composition	Structure	Function
Genetic	Allelic diversity	Genetic distance	Mutation rate
Species	Abundance	Distribution	Demographic processes
Communities	Species richness	Structural complexity	Biomass production
Landscape	Number of ecosystems	Patch distribution	Erosion potential

So far, the evaluation of impacts caused to the AoP ecosystem quality in LCA methods is focusing on measuring biodiversity in terms of composition of the community (Table 5- 1, green cell). Curran *et al.* (2011) recommend making use of complementary metrics to achieve a more complete understanding of damage to the AoP ecosystem quality. This way, ecosystem quality is split in two perspectives with distinct characterization models and metrics. First, changes in the composition of the community are measured through species richness related indicators in a similar way as existing LCIA for biodiversity. Functional diversity of the community through NPP is proposed as potential second and complementary information to monitor changes in

ecosystem quality²¹. Ecosystem NPP has potential for being used as a second indicator for ecosystem quality because of data availability from satellite imagery. However, other indicators such as species functional diversity related metrics are interesting for complementary use with species richness-related metrics (Gallardo *et al.*, 2011; Santini *et al.*, 2017). Functional diversity related metrics are based on functional traits observed in the ecosystem to monitor changes in its functional attributes (e.g. nutrient cycling, productivity, resilience). In contrast to species richness related indicators, functional diversity related metrics detect differences between losing functionally redundant species and losing keystone species (*i.e.* high consequences on ecological conditions). Gallardo *et al.* (2011) conclude that species functional trait diversity is the most informative metric for biodiversity and has potential to be combined with a taxonomic distinctness metric (e.g. species richness related metrics) to provide complementary information. Meanwhile, it is worth to investigate other set ups of complementary metrics with indicators that do not necessarily belong to biodiversity at the community level while ecological studies have initially emphasized on monitoring this type of biodiversity. All types of biodiversity cannot be captured through two metrics and thus, their interpretation might overlook impacts to ecosystem health (Mcgill *et al.*, 2015). It is also important to keep in mind that complementary metrics for biodiversity are correlated to some extent because the different biodiversity types influence each other (Naeem *et al.*, 1996; Haberl, 1997; Catovski, *et al.*, 2002). To avoid potential double counting, these two complementary indicators should not be combined into an aggregated single metric. These might be interpreted as complementary information and are expected to be correlated to a given extent. Their redundancy can be quantified through establishing correlation matrices for the values observed in different ecosystems.

Making use of complementary metrics to broaden the scope of the AoP ecosystem quality does not only present obvious advantages in terms of understanding of biodiversity impacts, it also includes major challenges. The development of LCIA assessing biodiversity changes through species richness related indicators (*i.e.* community composition) is challenging itself when it comes to site-specific characterization models. Expanding impact assessment modelling for the species richness endpoint with a second impact pathway for the complementary endpoint indicator (e.g. NPP or functional traits diversity) requires developing a new set of site-generic and site-specific LCIA models²² (Figure 5- 4). Consequently, the development of such an additional impact pathway requires a thorough understanding of ecological processes underpinning the value of the second indicator. Figure 5- 4 illustrates the

²¹ The authors do not exclude assessing other types of biodiversity (e.g. functional diversity of landscapes) instead for functional diversity of communities.

²² However, two indicators can be quantified through a common LCIA model that is differentiated at the end of the impact pathway.

doubling of indicators to monitor impacts on the AoP ecosystem quality through NPP (addressing community functionality)²³ and PDF (addressing community composition) indicators, two impact pathways may be considered: seafloor disturbance (*c.f.* **Chapter 3**) and marine ecotoxicity (*e.g.* in ReCiPe). The evaluation of impact caused by changes in marine ecotoxicity on the global marine ecosystem requires to develop site-generic CFs for each biodiversity indicator. On the other hand, impacts from seafloor disturbance on regional biodiversity are based on site-specific CFs and hence are very challenging to develop. Globally, the implementation of the AoP ecosystem quality with two complementary metrics brings additional complexity in the evaluation of biodiversity impacts while current LCIA models are still at the level of trying to better understand site-specific effects on species richness biodiversity. This is especially the case for sea-use impacts on regional marine biodiversity (*c.f.* **Chapter 3**). The development of LCIA pathways for a second and complementary metric to monitor biodiversity changes in marine ecosystems seems too early as LCIA models of species richness related metrics are still lacking. In addition, assessing changes in species functional traits diversity such as suggested by Gallardo *et al.* (2011) is challenged by data requirements regarding functional traits associated to each marine species observed in the ecosystem.

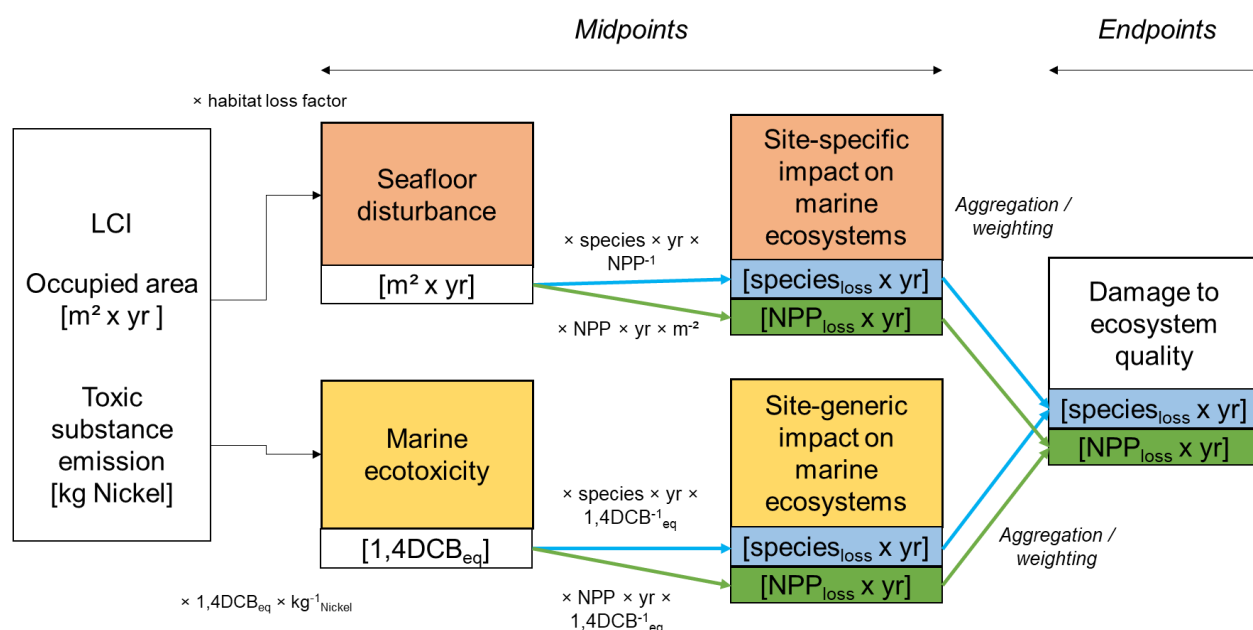


Figure 5- 4 Illustration of using two complementary impact pathways and indicators for the marine damage to ecosystem quality endpoint indicator. The impact pathways are simplified for the sake of clarity. Site-specific and site-generic damage pathways appears respectively light red and orange. *in ReCiPe 2016, hazard-weighted increase in marine waters.

²³ NPP has been proposed as an indicator to monitor ecosystem quality (*e.g.* Taelman *et al.*, 2016) but also, as an indicator to monitor ecosystem services (*e.g.* Zhang *et al.*, 2010).

5.2.4. A step back from species richness related metrics to monitor changes in (marine) ecosystem quality

Applying complementary metrics to monitor changes in ecosystems' conditions is challenging in LCIA. Meanwhile, monitoring changes in biodiversity through single species richness metrics encompasses major knowledge gaps when it comes to marine environments (*c.f.* **Chapter 3**). Hence, there is potential to investigate alternative metrics than species richness to include changes in marine ecosystem quality in environmental sustainability assessment methods such as LCA. This means looking for indicators that are available for the marine environment and relevant to detect changes in ecological conditions.

A first possibility consists of making use of taxonomic distinctness metrics for higher taxa than the species level (*e.g.* family richness) (Williams and Gaston, 1994). Because of data gaps regarding the recovery of deep-sea species assemblages, **Chapter 3** follows this approach of monitoring biodiversity for higher taxonomic levels than species. The application of such a method is relevant to overcome existing information constraints associated with species richness metrics. On the other hand, monitoring biodiversity at higher taxonomic level (in terms of richness) is expected to present a lower sensitivity than for species level.

Besides considering higher taxonomic levels, monitoring biodiversity at higher ecosystem levels (*i.e.* genes, species, communities, landscape) presents advantages in terms of data requirements and relies on the hierarchy theory for ecosystems. The hierarchy theory emphasizes on relationships amongst ecosystem components; it considers that ecosystems are made of different hierarchical levels of organisation (called *holons*) that are interconnected. Consequently, changes at one level of the organization (*e.g.* genes composition) influences patterns observed at another level (*e.g.* species composition) (O'Neill *et al.*, 1986). On the other hand, higher levels of organization change slower than lower levels because of higher structural complexity (*i.e.* compensation mechanisms) (Müller, 1992). Mapping higher levels such as habitats is common practice in biodiversity conservation programs, but one should acknowledge its reduced sensitivity compared to lower levels of organization. Even if it deals with structural patterns of landscape level (Table 5- 1), habitat diversity appears as a relevant proxy for monitoring changes in other types of biodiversity; it has been successfully applied to both terrestrial (Benton *et al.*, 2003) and marine ecosystems (Messmer *et al.*, 2011). A greater diversity in habitats provides more opportunities for differentiated ecological niches and hence, allows more species to coexist (Figure 5- 5). Different habitat diversity metrics are proposed. For example, habitat diversity is measured by summing the Euclidian distances between each pair of habitat patches composing the ecosystem. Changes in habitat diversity are assessed relatively to the initial distance and scored between 0 and 1 (Alsterberg *et al.*, 2017). Other habitat diversity metrics include habitat dominance in their evaluation (Loehle and Wein, 1994).

Monitoring changes in habitat diversity has potential for the development of site-specific LCIA pathways for land-use and sea-use, especially when it comes to land or seafloor transformation and occupation. For example, by applying this metric for the LCIA of deep-sea mining in the CCZ (c.f. **Chapter 3**) it would consider the different benthic habitats observed in the sub-region study. However, a detailed mapping of habitats located in the CCZ is under development (McQuaid *et al.*, 2018). Once finalized, its results might be useful to develop habitat diversity metrics.

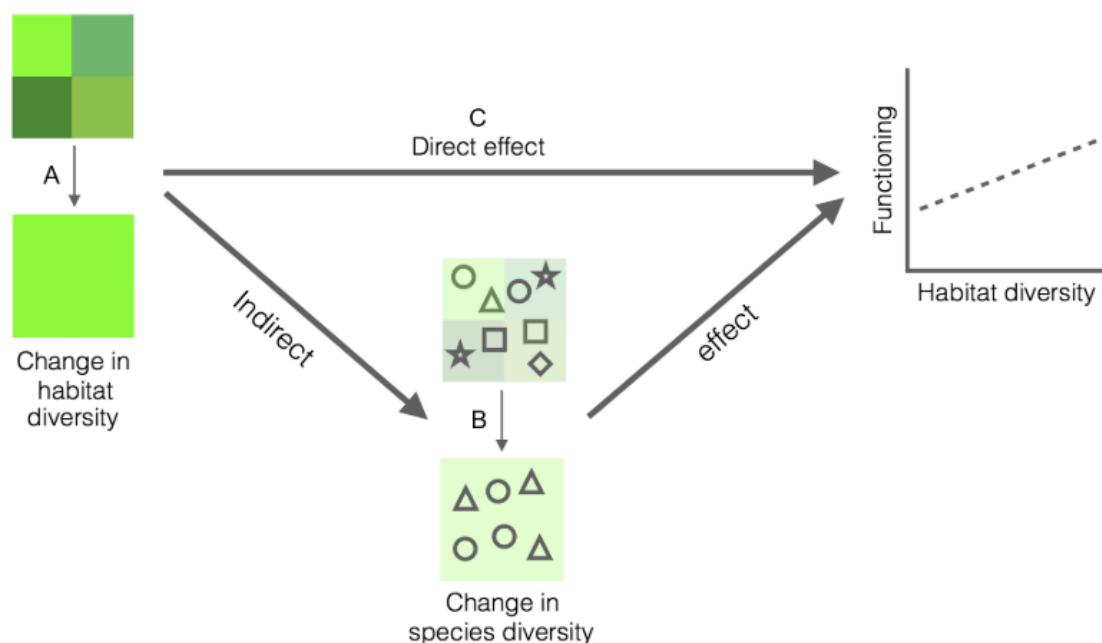


Figure 5- 5 Effects of habitat diversity reduction on species diversity and ecosystem functioning, Alsterberg *et al.*, (2017). Each habitat is represented by a different shade of green, each species occurrence is represented by a distinct geometric pattern. In case habitat diversity is reduced to a single habitat (light green), species richness decreases (two species are remaining).

Another potential metric to develop LCIA for biodiversity consists of relying on key factors for biodiversity, which are identified and described for each type of ecosystem (Larsson, 2001). Key factors influence the diversity of ecological niches available in the ecosystem and hence, create conditions for maintaining biodiversity (Selig *et al.*, 2013). A methodology using key factors has been developed for LCIA; it considers relative changes in the sum of key factor values compared to the initial level, weighted by ecosystem scarcity and vulnerability to assess damages to ecosystem quality [ΔQ , unitless] (Michelsen, 2008). The method follows the framework for land-use impacts on ecosystem quality (Milà i Canals *et al.*, 2007): it multiplies ΔQ with the area impacted and integrated over time to estimate the final impacts [$\Delta Q \times \text{ha} \times \text{yr}$] (Michelsen, 2008). This LCIA method has only been applied for the WWF ecoregions “Scandinavian and Russian taiga” and “Scandinavian coastal coniferous forests” and relied on three key factors for biodiversity monitoring: amount of decaying wood, proportion of alien species and proportion of areas set aside. It presents certain advantages for site-specific LCIA

assessing biodiversity impacts caused by land-use/sea-use activities, especially in marine environments, because limited information is required regarding species composition and recovery from disturbance. However, the LCIA method has not been applied to other case studies, as it is constrained by the absence of a unified classification for marine ecosystems (c.f. 5.2.1).

The hemeroby concept is also used in some LCIA to monitor impacts from land-use on ecosystem quality (Brenttrup *et al.*, 2002; Taelman *et al.*, 2016; Lindner *et al.*, 2019). In ecology, hemeroby (or naturalness) is defined as the distance to nature and is used to measure disturbance in ecosystems. Land-use classes are ranked according to their naturalness degradation potential (NDP), a unitless score ranking from 0 (“*ahemerobic*”, *i.e.* no human influence) to 1.0 (“*metahemerobic*”, *i.e.* purely artificial). This way, high hemeroby of a land-use class corresponds to low ecosystem quality. A naturalness degradation indicator (NDI) [$\text{m}^2 \times \text{yr}$] is calculated through multiplying the area under use [$\text{m}^2 \times \text{yr}$] with the respective NDP. In order to compare land-use impacts occurring in different biogeographical regions, the NDI results are divided by the total NDI of the region (*i.e.* normalization value) and further weighted with regional-specific factors (Brenttrup *et al.*, 2002). Criteria for ranking land-use classes and normalization values are mostly limited to European regions (Brenttrup *et al.*, 2002; Fehrenbach *et al.*, 2015). Recently, the concept has been integrated in the framework for land-use impact assessment (Lindner *et al.*, 2019). Compared to the monitoring of biodiversity through modelling changes in species richness metrics, the hemeroby concept is more intuitive and its scope is broader than biodiversity *per se*. Furthermore, it relies on observations at macroscale level (*i.e.* ecosystem description) and hence, it does not require extensive datasets to deal with the complexity of land-use effects on ecosystem quality (Fehrenbach *et al.*, 2015). Particularly, this method has potential for application in environments with lower data availability regarding species composition and response to disturbance such as deep-sea ecosystems. However, the evaluation of NDP classes for disturbed deep-sea environments requires experts’ consultations and global consensus.

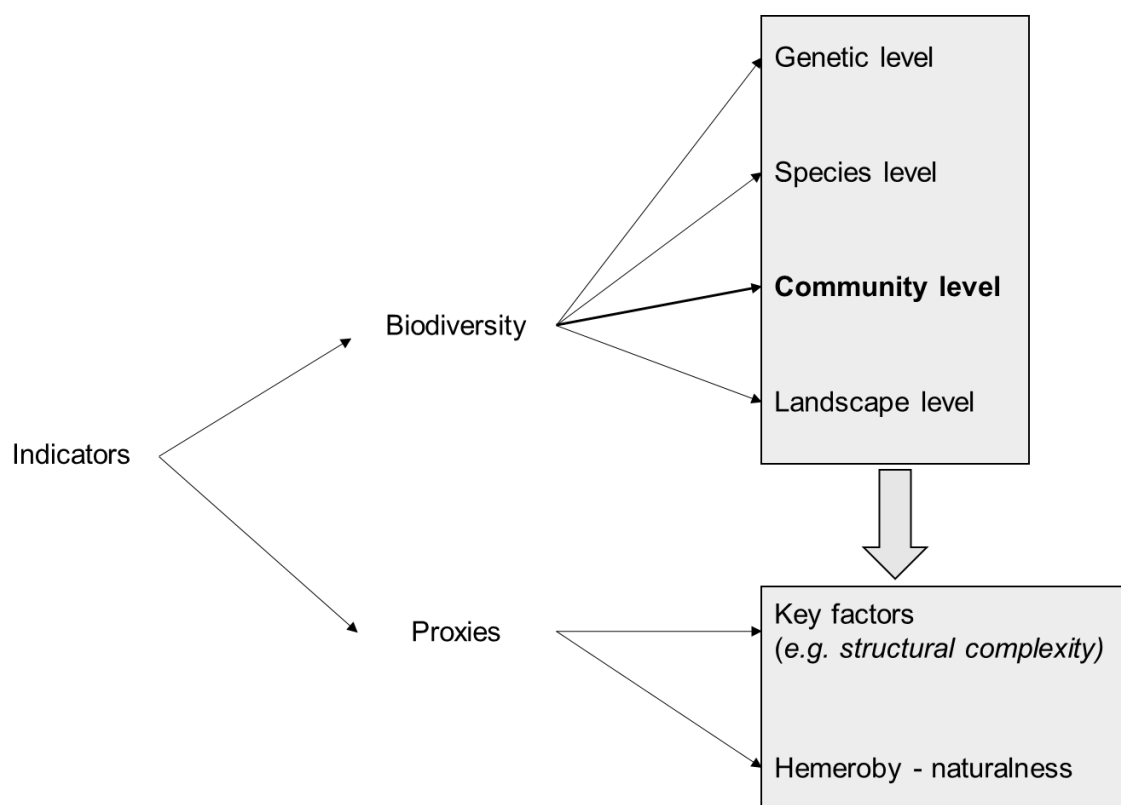


Figure 5- 6 Scope of the indicators for biodiversity considering the four levels of biodiversity. Indicators for biodiversity at the community level (bold characters) are mostly studied in ecology and hence implemented in LCIA. Key factors and hemeroby indicators include the three levels of biodiversity.

Figure 5- 6 summarizes the previous paragraphs on indicators for biodiversity with the four biodiversity levels being represented. Most of indicators consist of direct evaluation of biodiversity and focus on biodiversity at the community level (e.g. species richness related metrics, functional traits diversity). On the other hand, the use of proxy indicators to monitor changes in biodiversity includes the four levels of biodiversity in the assessment.

The ReCiPe methodology (Figure 1- 10) assesses impacts in freshwater, marine and terrestrial species separately at the midpoint level, measured in PDF x yr x m⁻² (terrestrial) or x m⁻³ (freshwater, marine) (*i.e.* at the community level). Each of these midpoint impacts is further multiplied with the world average species density of its corresponding environment (terrestrial, freshwater, marine) to assess its contribution to the AoP ecosystem quality (species x yr), at the endpoint level. Therefore, the evaluation of impacts to the AoP ecosystem quality in ReCiPe is facilitated by the consistency in the indicators used at the midpoint level (PDF). On the other hand, the development of *ad hoc* and site-specific LCIA CFs for ecosystem quality might use different biodiversity indicators (Figure 5- 6) depending on the impact pathway. The evaluation of the endpoint impact on ecosystem quality relying on multiple LCIA pathways and the use different biodiversity indicators amongst impact pathways raises major challenges in terms of aggregation to a single score for ecosystem quality (e.g. aggregation of key factors and habitat diversity indexes).

5.2.5. LCIA for marine ecosystem quality in the context of the SDG 14

The development of LCIA for marine ecosystem quality must take into consideration how scientists and policy makers define and measure it. LCA being used as a decision-support tool for policy makers, its impact categories must be relevant for existing protection goals. In this context, it is important to understand how LCA methods are linked to the implementation and the monitoring of the United Nations' sustainable development goals (SDG)²⁴. Amongst them, the SDG 14 “*Conserve and sustainably use of the oceans, seas and marine resources for sustainable development*” sets protection goals and action plans for marine ecosystems. The European Union relies on its marine strategy framework directive (MSFD)²⁵ for setting guidelines to achieve the SDG 14 (EC, 2008). The MSFD aims to achieve “*good environmental status*” (GES) in European marine ecosystems, defined as “*the environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are intrinsically clean, healthy and productive, and the use of the marine environment is at a level that is sustainable, thus safeguarding the potential for uses and activities by current and future generations*”. The GES is defined by a list of 11 descriptors including 29 criteria measured through 56 indicators that are benchmarked with reference values (EC, 2008, 2010; Borja *et al.*, 2013). Compared to LCA methods assessing ecosystems quality through a single metric, the evaluation of the environmental status for marine ecosystems under the MSFD relies on an exhaustive list of indicators to grasp the complexity of ecosystem functioning. On the other hand, the continuous evaluation of each of the 56 indicators and for each marine area requires a substantial effort to fulfil data requirements. In addition, it is not clear yet how the different indicators will be aggregated into a single score in order to evaluate the environmental status of (particular) marine ecosystems.

While LCA methods are more mature in the modelling of impacts from a global perspective, the MSFD focuses entirely on regional impacts observed in marine environments. Consequently, the latter does not rely on the quantification of cause and effect chains but rather on observations and on the benchmarking of each indicator. LCA methods and the MSFD are thus intrinsically different in their scope and evaluation. Without extending LCA methods to include all descriptors of the MSFD in their scope, it is necessary to understand which descriptors of the MSFD overlap with existing site-specific LCIA indicators for marine ecosystems (Figure 5- 7). This allows one to clarify whether LCA studies of marine products

²⁴ The 2030 Agenda for Sustainable Development has been adopted by the United Nations Member States (2015) and relies on 17 Sustainable Development Goals (SDG) in order to prioritize their actions towards sustainable development. Each SDG focuses on an overarching thematic that is further divided in multiple targets calling for specific actions (UN, 2020).

²⁵ The marine strategy framework directive (MSFD, 2008/56/EC) is a legal framework for European Member States to protect, conserve and enhance marine ecosystems located in their exclusive economic zone.

provide useful information to ensure their alignment with the SDG 14. Different LCIA methods have been developed to assess impacts of non-indigenous species (Hanafiah *et al.*, 2013 for freshwater species), fisheries / food-webs (Emanuelsson *et al.*, 2014; Langlois *et al.*, 2014a), human-induced eutrophication (Cosme and Hauschild, 2017), seafloor integrity (Woods and Verones, 2019), contaminants (Huijbregts *et al.*, 2016), marine litter (Civancik-Uslu *et al.*, 2019; Saling *et al.*, 2020) and noise (Middel and Verones, 2017) at midpoint level except for contaminants and human-induced eutrophication that are aggregated into the AoP ecosystem quality (Figure 5- 7, orange boxes). In addition, this PhD focuses on two descriptors identified by the MSFD: fisheries / food-webs in **Chapter 2** and seafloor integrity in **Chapter 3** (Figure 5- 7, bold characters).

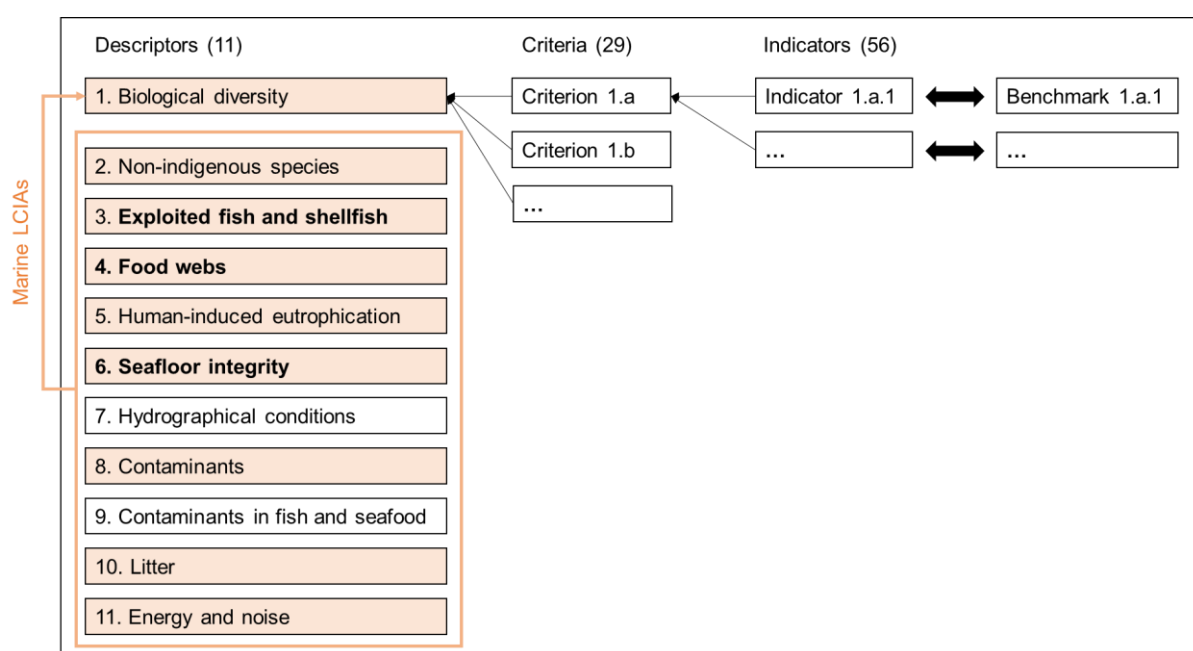


Figure 5- 7 Overlaps between the marine strategy framework directive and ad-hoc methods for life cycle impact assessment. The black outer box defines the scope of ecosystem environmental status assessment according to the MSFD. The orange descriptors are considered in ad-hoc LCIA methods at the midpoint level for marine ecosystem quality and marine natural resources (and thus, overlap in some extent with the MSFD). LCIA categories in bold characters (biological diversity, food webs) have been developed in this PhD thesis.

The MSFD and site-specific marine LCIA methods rely on common aspects: the MSFD descriptors are overlapping with some LCIA midpoint impact categories (Figure 5- 6). These LCIA methods might be used as complementary assessment methods to understand the sustainability of marine products regarding the SDG 14. In addition, some LCIA methods assess the impact of the MSFD descriptor on biological diversity (*i.e.* biodiversity) and measure it in regional or global species richness (Hanafiah *et al.*, 2013; Huijbregts *et al.*, 2016; Cosme and Hauschild, 2017; Woods and Verones, 2019, **Chapter 3**). With respect to the status of biological diversity relying on the other MSFD descriptors (Figure 5- 7, orange arrow), there is potential to aggregate marine LCIA methods including different descriptors into a single score for regional or global biodiversity.

5.2.6. Towards a comprehensive impact category for ecosystem services and global biodiversity

The integration of ecosystem services in LCA requires site-specific LCIA pathways linking changes in ecosystem properties to changes in the flow of ecosystem services, expressed in monetary value at endpoint level (*c.f.* 1.1). A LCIA pathway linking NPP loss (ecosystem property) to the reduction of fisheries landings (ecosystem services) is developed in **Chapter 2**. The understanding of key ecosystem properties and their quantitative relation with ecosystem services is crucial to improve ecosystem services assessment in LCIA. Meanwhile, ecosystem properties are indicators for the different types of regional biodiversity (e.g. NPP being an indicator for functional biodiversity at the community level, Table 5- 1). The evaluation of regional biodiversity in LCIA is thus central to integrate ecosystem services assessment, using regional properties to initiate the cascade framework for ecosystem services (Figure 1- 3, Figure 5- 8). This way, regional biodiversity is a midpoint indicator in the impact pathway of ecosystem services but also, for global biodiversity.

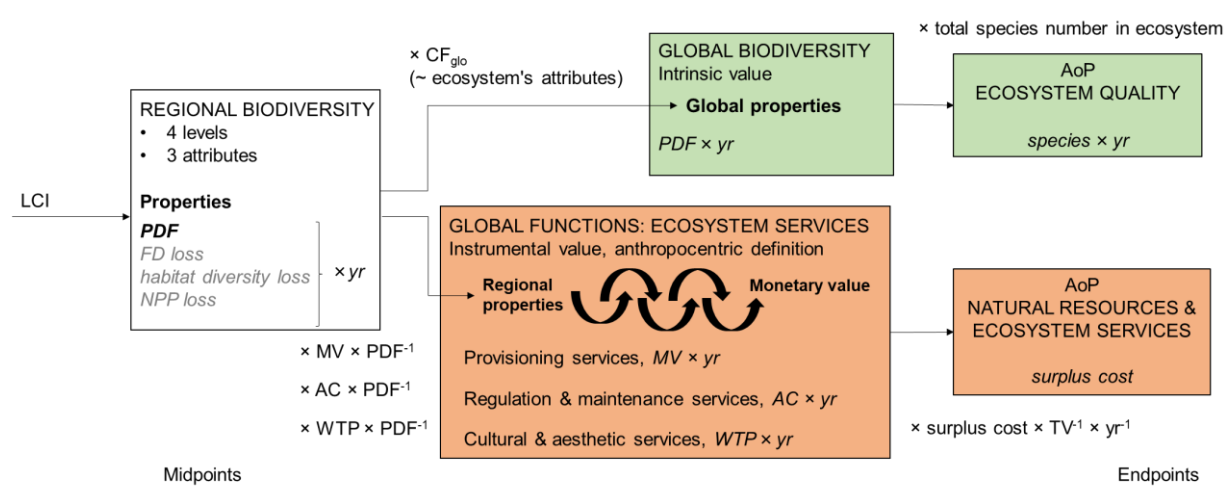


Figure 5- 8 Regional biodiversity as midpoint impact indicator for global biodiversity and global ecosystem functions in Life Cycle Assessment perspective. Potential indicators appear in *italic*, PDF potentially disappeared fraction of species, FD functional diversity, NPP net primary production, yr year, MV market value, AC avoided cost, WTP willingness to pay, TV total value. The impact pathways are illustrated for changes in regional biodiversity measured in PDF.

Figure 5- 8 proposes to aggregate ecosystem services into a new AoP including natural resources and ecosystem services according to UNEP-SETAC recommendations, considering ecosystem services as part of natural resources because of their instrumental values (Verones *et al.*, 2017). However, this requires assessing ecosystem services in monetary values and aggregating them into a single indicator for natural resources such as surplus cost (Alvarenga *et al.*, 2015). In addition to the difficulty of identifying key ecological processes behind ecosystem services, their monetary valuation is still a challenge when it comes to non-marketable ecosystem services (regulation & maintenance, cultural & aesthetic services) (De Groot *et al.*, 2002). While provisioning services are evaluated based on their market value

(MV), regulation & maintenance services might be assessed through avoided costs (AC) and cultural & aesthetic valorisation might rely on the willingness to pay (WTP) principle (Figure 5-8)²⁶.

For example, some deep-sea ecosystem services are crucial because of their uniqueness (e.g. carbon storage in sediment for million years) and the vastness of deep-sea areas²⁷. With the growing interest for the deep-sea-sourced commodities, exhaustive reviews of deep-sea ecosystem services have been carried out and they identify provisioning (e.g. fisheries and marine genetic resources), regulating (carbon and nutrients cycling, contaminants absorption) and cultural services (educational and aesthetic/artistic) (Armstrong *et al.*, 2012; Thurber *et al.*, 2014). It has been shown that maintenance of global ocean productivity strongly relies on key ecological processes from the deep-sea such as nutrients regeneration from dissolved organic material. Those are returned at the surface in upwelling zones and fuel phytoplankton net primary production, stimulating the entire food-web (Thurber *et al.*, 2014). However, evaluating the total value will require to perform detailed cascade modelling, from properties to monetary value, for each of the ecosystem service identified and might thus inevitably lead to high uncertainty.

Rather than evaluating ecosystem services at the endpoint level (e.g. by monetary valuation), it is worth to investigate further the potential of using metrics related to ecosystem properties as proxy for ecosystem services such as biodiversity (Worm *et al.*, 2006; Mace *et al.*, 2012) (Figure 5- 8). While species richness metrics (e.g. PDF) have limited advantages to understand ecosystem services, the use of functional biodiversity indicators of functional diversity or/and net primary production (respectively FD and NPP in Figure 5- 8) has potential for monitoring impacts on ecosystem services at midpoint level. Considering section 5.2.3, there is potential to introduce a midpoint impact category “regional biodiversity” in site-specific LCIA for ecosystem quality and ecosystem services, measured through functional biodiversity indicators (*i.e.* FD / NPP rather than PDF). Instead of developing site-specific LCIA CFs to assess impacts on global biodiversity and ecosystem services separately, it is relevant to return at midpoint level and focus on the evaluation of regional biodiversity through complementary functional metrics. Although these metrics do not provide precise numbers on ecosystem services flows, they allow one to appraise potential changes in their supply. Consequently, the framework proposed in **Chapter 3** could be adapted to such biodiversity

²⁶ Contrastingly, Rugani *et al.* (2019) consider that ecosystem services are belonging to the three AoP similarly to previous recommendations from UNEP-SETAC (Koellner, Baan, *et al.*, 2013). The authors propose alternative endpoint indicators for ecosystem services according to their corresponding AoP (e.g. disability-adjusted life years for regulation and maintenance services belonging to both ecosystem quality and human health).

²⁷ Deep-sea environments are representing 97% of total ocean volume (Le and Sato, 2017)

indicators in order to use regional biodiversity loss as indicator for global ecosystem services provided by the deep sea.

5.2.7. Accounting for ecosystem collapsing: non-linear impacts and cumulative impacts

A main challenge in LCIA is the identification and assessment of the stressor-response relationships, which is nowadays mainly assumed to be a linear relation. This has been briefly discussed for biodiversity in **Chapter 3**. This linearity is inherent to the fundamentals of LCIA models: the elementary flows (LCI) are multiplied with constant CF values (and with fate factors) to estimate their impact to a given category. CFs values are fitted for marginal contribution and are obtained from general models (e.g. species area relationship) or observations (e.g. **Chapter 3**). This does not represent any issue for LCIA models scoping global-scale aspects (e.g. mineral depletion) because the elementary flow is marginal compared to total resources (e.g. the production 1 metric ton of copper cathode will not influence the global function of the surplus cost response). On the other hand, this becomes invalid for site-specific LCIA models accounting for large-scale impacts on regional ecosystems. For example, biodiversity response to land use does not behave linearly when interventions are no longer marginal and affect the resilience of the ecosystem. Therefore, accounting for such situations in LCIA requires making use of dynamic CFs that are adjusted to the level of stressor intensity.

The previous sections focus on the improvement of site-specific LCIA models to account for regional ecosystems' properties in the global environmental sustainability assessment of marine-sourced products. However, it is necessary to understand how resilient a regional ecosystem is when it becomes impacted by different stressors (*i.e.* different damage pathways / LCIA models). For example, the total effect of marine acidification and seabed disturbance on regional biodiversity is not necessarily the sum of their impacts on regional biodiversity taken separately because of potential feedback loops. The final response of an ecosystem under different disturbances is site- and disturbance-specific and thus, its modelling might require additional CFs (Figure 5- 9). This way, site-specific LCIA models first provide information on the contribution of each stressor (α_j , γ_k , β_l) to regional ecosystem biodiversity through a first set of CFs ($CF_{i,j}$, $CF_{i,k}$, $CF_{i,l}$). The contributions are then summed and multiplied with a weighting factor accounting for the cumulative impact on regional biodiversity ($WF_{i,j,k,l}$). Combined with dynamic CFs to account for nonlinear responses of regional biodiversity to each stressor (*c.f.* previous paragraph), the WF integrates contributions of each stressor through a cumulative effect perspective. This issue has already been pointed by Curran *et al.* (2011), highlighting that the LCA methodology does not consider interactions amongst impact pathways.

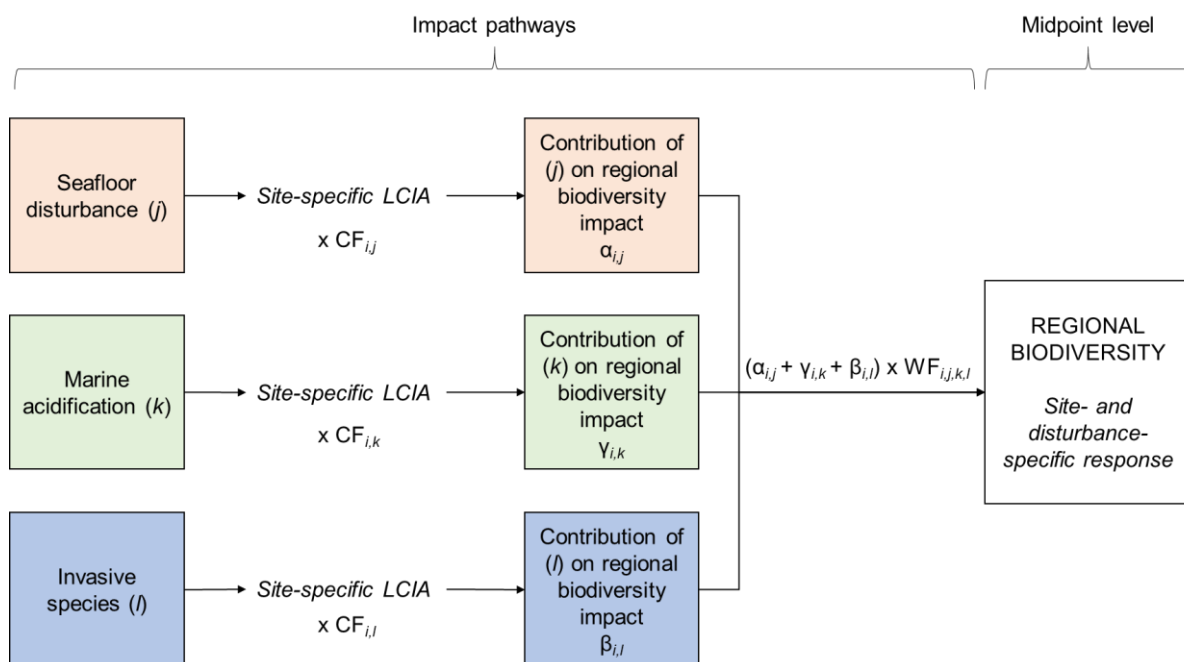


Figure 5- 9 Accounting for cumulative impact in LCA, an illustration for regional marine biodiversity. Impact pathways of three disturbance types (j, k, l) occurring in ecosystem i and further aggregation of each contribution ($\alpha_{i,j}, \gamma_{i,k}, \beta_{i,l}$) to regional biodiversity considering additional characterization factors accounting for the cumulative impact $WF_{i,j,k,l}$.

Meanwhile, the development of such an additional site-specific set of CFs that also specifically relies on the combination of each disturbance type is a main challenge. Globally, the impacts dealing with ecosystem functioning are problematic to squeeze into the LCA modelling framework (De Haes, 2006; Dewulf *et al.*, 2015; Taelman *et al.*, 2016; Boone *et al.*, 2018). For this reason, it is worth to investigate the potential integration of ecosystem models (e.g. Ecopath / Ecosim) in LCIA or other tools for cumulative effects (Quemmerais-Amice *et al.*, 2020) to consider changes in ecosystem response to disturbance according to the combination of stressors, their intensity, the ecosystem type and even, the temporal aspects. Alternatively, the integration of LCA methods with ecological risk assessment models such as InVEST Habitat Risk Assessment has potential to understand the final effect of multiple human pressures on regional ecological conditions (Chaplin-Kramer *et al.*, 2017; Willaert *et al.*, 2019). However, this requires detailed dataset on the spatial extent and intensity of human pressures occurring in the ecosystem under study. Combined with such tools to model ecological conditions, LCA studies could provide meaningful results for the global sustainability assessment of marine products.

5.3. Concluding remarks: towards global environmental sustainability assessments of marine products

This PhD dissertation has provided building blocks to improve the LCA methods for the environmental sustainability assessment of marine products. In a first step, the manuscript has focused on the development of site-specific LCIA pathways to include new aspects in the global environmental sustainability assessments of marine products. Two frameworks have been developed and illustrated in case studies to account for ecological processes in the offshore production stages. These have potential for other case studies, but knowledge gaps and marine ecosystems identification remain a major challenge for the development of exhaustive datasets for site-specific CFs. However, it is worth to investigate the potential of alternative metrics to assess ecosystem health in a comprehensive way when data availability is reduced (*e.g.* making use of habitat diversity as proxy for ecosystem health). With increasing uncertainty towards endpoint indicators, there is room to address ecosystem health and ecosystem services in a single midpoint impact category (*e.g.* regional biodiversity) that can be assessed through multiple metrics. Rather than focusing on evaluating impacts at the endpoint level, it is worth to develop new impact pathways and hence, to align LCIA models with the MSFD descriptors for marine environments. In a first step, these impact pathways can be combined at the midpoint level to assess changes in regional biodiversity as a proxy for ecosystem health and ecosystem services. However, the evaluation of the cumulative impact from different stressors on regional biodiversity should consider the interactions amongst the different impact pathways.

In a second step, this PhD dissertation has emphasized the processing stage of wet biomass. A dataset including multiple valorisation routes for microalgal biomass has been developed and provides crucial information for further LCI of wet biomass processing. Data availability on the entire value chain of marine products is major challenge for their environmental sustainability assessment. On the one hand, the site-specific LCIA models developed in the PhD require datasets on the local ecological conditions but, on the other hand, it is also necessary to obtain data related to the post-harvest processes of marine raw materials. While databases are available for agriculture production / food processing (*i.e.* Agri-footprint) or for metal production (*i.e.* GaBi databases or datasets released by metal commodity associations), databases regarding the processing of marine biomass such as seaweed or marine minerals such as polymetallic nodules are lacking. Therefore, there is potential to develop global databases gathering crucial information on the processes associated to the production of marine commodities. Such material will allow the benchmarking with terrestrial alternatives and understand the potential of sourcing marine raw materials. For example, developing such datasets for the downstream processes associated to metal refining from polymetallic nodules

is crucial to understand their environmental sustainability compared to terrestrial supply. In a similar way than for the processing of wet biomass, it is necessary to quantify materials and energy flows for varying downstream processing scenarios. In the long term, the integration of site-specific information on regional marine ecological conditions (to feed site-specific LCIA models) with information on post-harvest processes will provide significant improvement for the environmental sustainability assessments of marine products.

SUPPLEMENTARY MATERIAL

Appendix A, supplementary material of Chapter 2

A1. Methodology for the development of equation (2-3)

$$PPR_a = \frac{C_a}{M_a} \times \left(\frac{1}{TE}\right)^{TL_a-1} \text{ adapted from Pauly and Christensen (1995)}$$

Assuming a carbon to biomass ratio of 1:10 and a trophic transfer efficiency of 10% (suggested by Pauly and Christensen, 1995), the equation becomes:

$$PPR_a = \frac{FL_a}{9} \times 10^{TL_a-1}$$

$$RPPR_a = \frac{PPR_a}{NPP_{NS}}$$

$$PLNPP_a = RPPR_a \times HANPP$$

The estimation of the PLNPP relies on the hypothesis that the impact on fish landings is proportional to the relative NPP appropriation of the landings compared to the ecosystem total NPP

$$LPY_a = \frac{PLNPP_a \times 9}{10^{TL_a-1}} = \frac{FL_a}{NPP_{NS}} \times HANPP$$

PPR_a [kg C_{eq} x yr^{-1}]: primary production required to sustain the fish landings of the species a

C_a [kg_{FW}]: mass of the species a catches

M_a : ratio biomass to carbon content of the species a

TE: averaged trophic transfer efficiency of the ecosystem

TL_a : trophic level of species a

FL_a [kg_{FW}]: fish landings for species a

$RPPR_a$: relative primary production required to sustain the fish landings of species a

NPP_{NS} [kg C_{eq} x yr^{-1}]: net primary production of the North Sea

$PLNPP_a$ [kg C_{eq} x yr^{-1}]: potential lost net primary production to sustain the fisheries of species a

$HANPP$ [kg C_{eq} x yr^{-1}]: human appropriated net primary production

LPY_a [kg_{FW}]: lost potential yield for fisheries of species a

A2. Net Primary Production of *Saccharina latissima* farmed in France and in Ireland

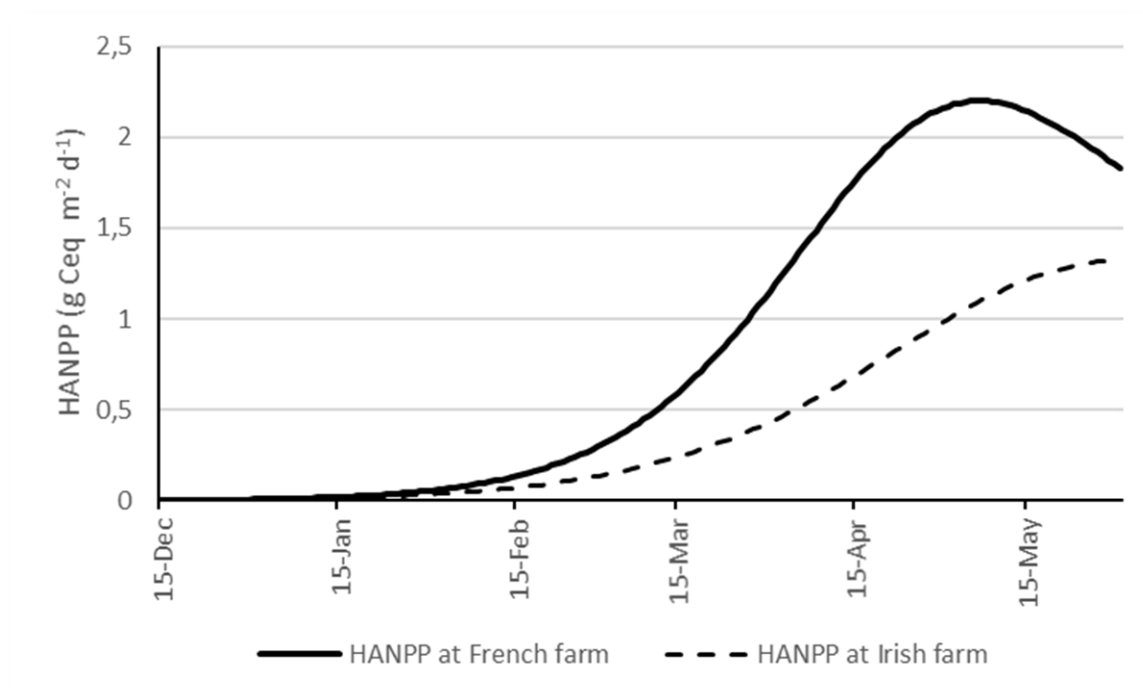


Figure A- 1 Seaweed Net Primary Production (HANPP) at the French and Irish farms. Seaweeds are deployed on December 15th and harvested May 31st of May. The time spent in hatchery is respectively 36 days and 23 days for France and for Ireland. The cultivation ropes are spaced from 2 meters in France and 9 meters in Ireland.

A3. Weighting factors for eco-exergy calculation

Table A- 1 Weighting factors (β) estimated for the 10 most caught species in the North Sea. The calculations are based on Table 2-2 and equations (2-5) and (2-6).

Fish landings	β
Atlantic herring	324
Atlantic mackerel	321
Sand eels	311
Sprat	324
European plaice	232
Saithe (Pollok)	308
Norway pout	308
Haddock	308
Cod	308
Blue whiting	308
Sugar kelp	215

Appendix B, supplementary material of Chapter 4

B1. Mass and energy flows diagrams

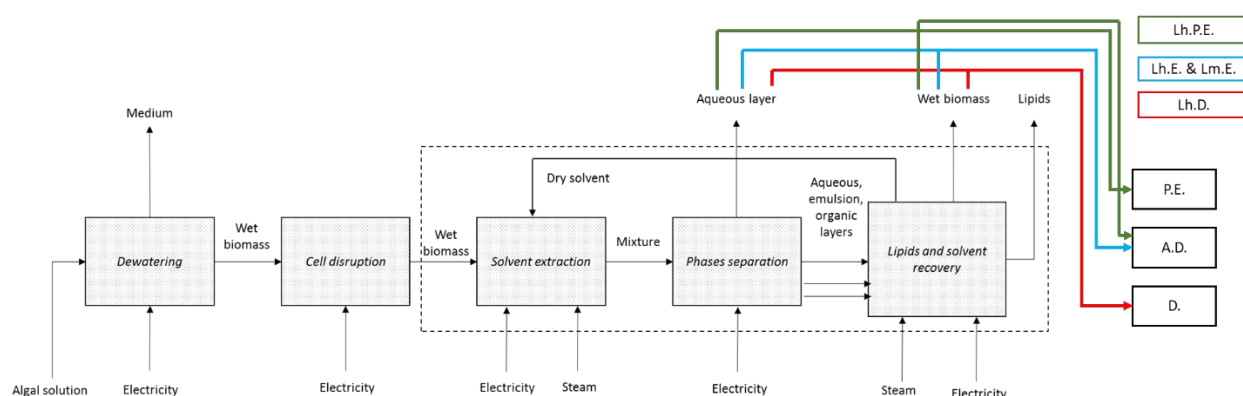


Figure B- 1 Generic flow diagram for pre-extraction steps and lipid extraction applicable for all scenarios. Detailed values for mass and energy flows are provided in Table 1 and Table 2. The lipid extraction step is delimited by dotted lines. The coloured arrows represent the fate of the streams to further valorisation steps according to the scenario (P.E. Proteins Extraction, A.D. Anaerobic Digestion, D. Drying biomass).

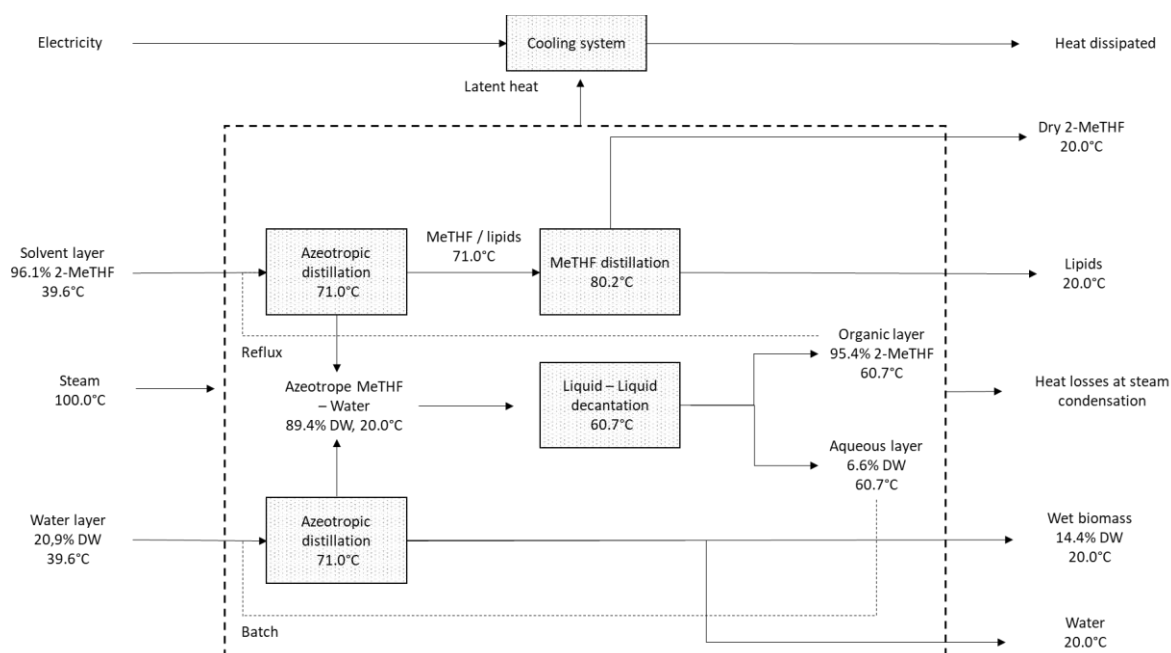


Figure B- 2 Generic flow diagram for the recovery of dry 2-MeTHF via continuous azeotropic distillation and the recovery of lipids via 2-MeTHF distillation.

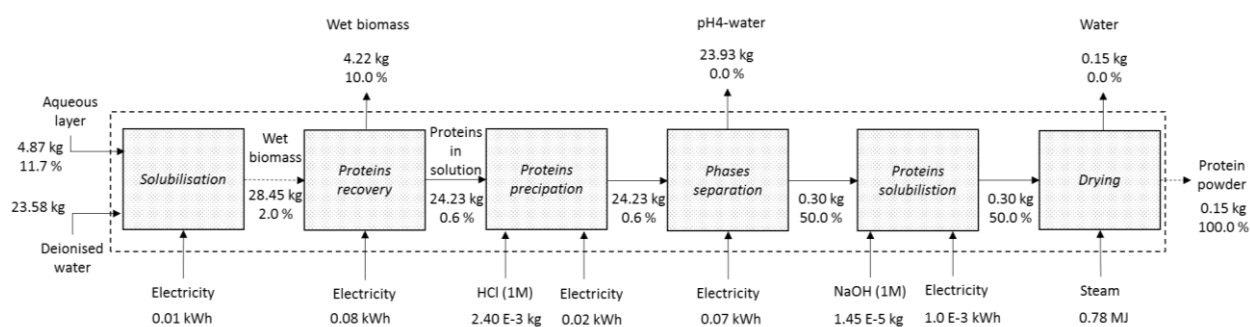


Figure B- 3 Mass and energy flows diagram for proteins extraction from waste biomass resulting from hexane lipid extraction. Values are reported per kg dry weight at the biorefinery gate, heat losses are not shown.

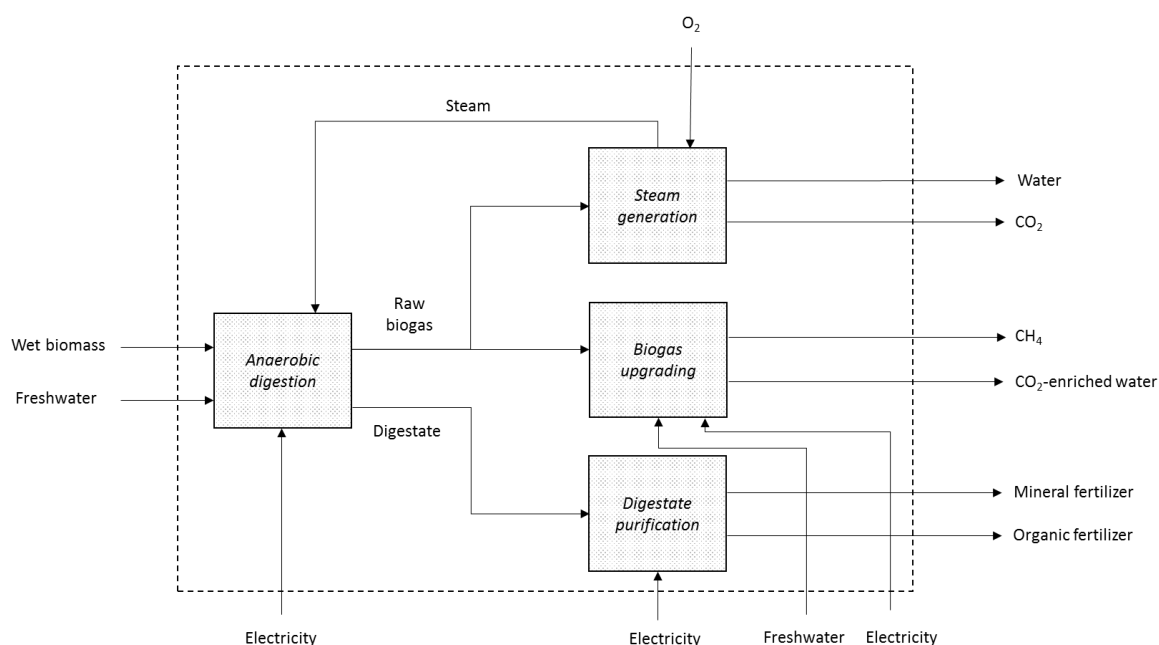


Figure B- 4 Generic flow diagram for anaerobic digestion step. Detailed values for mass and energy flows are provided in Table 4.

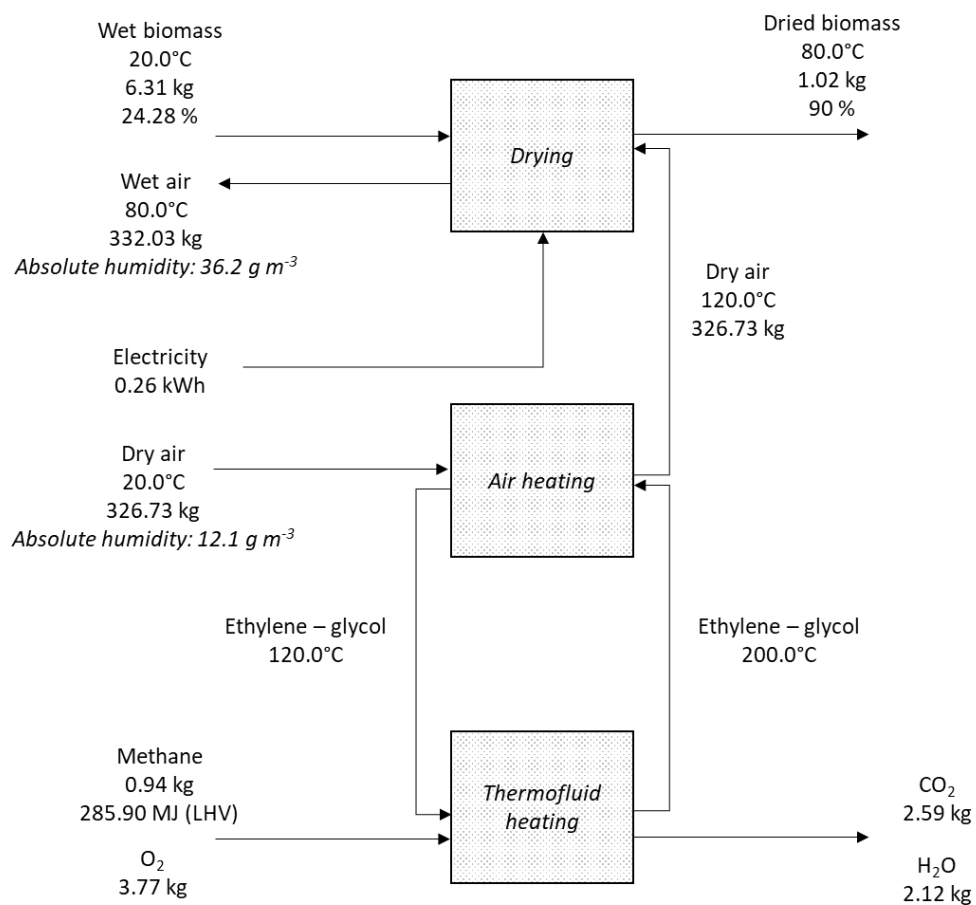


Figure B- 5 Mass and energy flows diagram for drying algae wet biomass resulting from hexane lipid extraction. Values are reported per kg dry weight at the biorefinery gate, heat losses are not shown.

B2. Evaluation of the density and heat capacity of the algal paste

Equations and coefficients values are obtained from Schneider et al., (2016)

$$\rho(T, TS) = \rho_{H_2O}(T) + a_{\rho} \times T^{b_{\rho}} \times \left[c_{\rho} \times \left(\frac{TS}{100} \right)^{d_{\rho}} + e_{\rho} \times \left(\frac{TS}{100} \right)^{f_{\rho}} \right] \quad (B-1)$$

$$Cp(T, TS) = Cp_{H_2O}(T) + f_{Cp}(TS) \times T^{e_{Cp}} \quad (B-2)$$

$$f_{Cp}(TS) = a_{Cp} \times \left(\frac{TS}{100} \right) + b_{Cp} \times \left(\frac{TS}{100} \right)^2 + c_{Cp} \times \left(\frac{TS}{100} \right)^3 + d_{Cp} \times \left(\frac{TS}{100} \right)^4 \quad (B-3)$$

Table B- 1 Symbols and coefficients for the evaluation of algal paste density and heat capacity, developed by Schneider et al. 2016

Symbols	Parameters
ρ	Algal paste density [kg x m ⁻³]
Cp	Isobaric heat capacity [J x g ⁻¹ x K ⁻¹]
T	Temperature [K]
TS	Total solid content [%]
ρ_{H_2O}	Density of water [kg x m ⁻³]
Coefficients	Values
a_{ρ}	6939.24
b_{ρ}	-0.61026
c_{ρ}	-35.7589
d_{ρ}	4.8912
e_{ρ}	1.13287
f_{ρ}	1.01803
a_{Cp}	-10937.47
b_{Cp}	100317.1
c_{Cp}	-408167
d_{Cp}	312572.3
e_{Cp}	-1.29592

B3. Data used for the modelling of mass and energy flows of the processes

Table B- 2 Main data for the composition of *Chlorella vulgaris* biomass, the dewatering and cell disruption.

Initial composition of microalgae biomass			
Proteins	50	% DW	2-5
Lipids	15	% DW	2-5
Carbohydrates	20	% DW	2-5
Other	15	% DW	2-5
Dewatering			
Electricity	1.3	kWh x m ⁻³ feed	6-8
Algae recovery	95	%	6-8
Initial concentration	0.2	% DW	6-8
Final concentration	15.0	% DW	6-8
Cell disruption – Bead mill			
Electricity	10.0	kWh x kg ⁻¹ dry microalgae	9
Disruption efficiency	90	% of initial cells	9
COP cooling	5		10
Cell disruption - High pressure homogenisation			
Electricity	2.2	kWh x kg ⁻¹ DW	11-12
Disruption efficiency	90	% of initial cells	11

Table B- 3 Main data for lipid wet extraction using hexane or 2-MeTHF. (a) Volume at 25 °C; (b) Integrated over 40°C and 68.8°C; (c) Coefficient of performance; (d) No data are available to integrate heat capacity of 2-MeTHF between 40 °C and boiling temperature.

Global parameters of extractions			
Temperature	40	°C	13-15
Solvent contact time	120	min	13-15
Rotational speed, stirring	60	rpm	13-15
Electricity	1.5	kWh x m ⁻³	10, 13, 16
Heat transfer coefficient	3E-03	kJ x m ⁻² x s ⁻¹ x K ⁻¹	10
Heat transfer efficiency	90	% of initial heat	10
Hexane extraction			
Ratio solvent : paste	0.5	weight:weight	14, 17
Heat capacity, mixture (40°C)	3.3	kJ x kg ⁻¹ x K ⁻¹	1, 18
Density, mixture (40°C)	2676	kg x m ⁻³	1
Lipid extraction yield	25	% of total lipids	19
2-MeTHF extraction			
Ratio solvent : paste	0.5	vol.:vol. ^a	15
Heat capacity, mixture (40°C)	3.2	kJ x kg ⁻¹ x K ⁻¹	1, 20
Density, mixture (40°C)	977	kg x m ⁻³	1
Lipid extraction yield	50	% of total lipids	15, 21
Phases separation			
Centrifuge	15	min	17
Rotation speed	9000	rpm	17
Electricity	11	kWh x m ⁻³	13
Hexane recovery			
Heat capacity, integrated ^b	2.40	kJ x kg ⁻¹ x K ⁻¹	22
Vapour pressure, saturated	759.8	mm Hg	23
Boiling temperature	68.8	°C	22
Heat of vaporisation	340	kJ x kg ⁻¹	22
COP ^c , cooling	5		10
2-MeTHF recovery			
Heat capacity, 2-MeTHF (40°C) ^d	1.8	kJ x kg ⁻¹ x K ⁻¹	20
Boiling temperature, 2-MeTHF	80.2	°C	24, 26
Heat of vaporisation, 2-MeTHF	34	kJ x mol ⁻¹	24, 26
Boiling temperature, azeotrope	71.0	°C	24, 26
Water in azeotrope	10.6	% wt	26
Heat of vaporisation, azeotrope	572.2	kJ x kg ⁻¹	26
Heat capacity, azeotrope, 40°C ^d	2.1	kJ x kg ⁻¹ x K ⁻¹	26
Boiling temperature, azeotrope	71.4	°C	26
Temperature in decanter	60.0	°C	25
COP cooling	5		10

Table B- 4 Main data for proteins extraction using isoelectric precipitation method. (a) The parameters for the centrifuge in precipitate recovery are assumed identical as the parameters for the extraction of proteins solution.

Proteins solubilisation and recovery of aqueous fraction			
Temperature	20	°C	27-29
Concentration of the solution	2	% DW	27
Time, stirring	10	min	27
Rotational speed, stirring	60	rpm	27
Protein solubilisation yield	52	% of initial proteins	27
pH	7		27
Electricity, stirring	1	kWh x m ⁻³	10
Rotational speed, centrifuge ^a	9000	rpm	10, 16
Time, centrifuge ^a	10	min	27
Electricity, centrifuge ^a	10	kW x s ⁻¹ x L ⁻¹	10
Proteins precipitation, recovery and re-solubilisation			
pH, precipitation	4		27
pH, re-solubilisation	7		27
Rotational speed, stirring	60	rpm	10
Electricity for stirring	1	kWh x m ⁻³	10
Precipitate concentration	50	% DW	30
Centrifuge parameters ^a			
Precipitate spray drying			
Thermal efficiency	50	% heat transferred	10

Table B- 5 Main data for anaerobic digestion of microalgae residual biomass, biogas upgrading and fertilisers production. (a) Potential methane production from substrate, TS total solid; (b) Methane conversion efficiency (MCE); (c) Lower heating value; (d) Solubility in water; (e) Reported per kg of wet paste.

Digester parameters			
Hydraulic retention time	46	days	31
Temperature	35	°C	31
Sludge concentration	5	%	31
Electricity, stirring	0.11	kWh x kg ⁻¹ algae	10, 31
Biogas production and upgrading aspects			
Methane production, proteins ^a	0.446	L CH ₄ x g TS ⁻¹	32
Methane production, lipids ^a	1.014	L CH ₄ x g TS ⁻¹	33
Methane production, carbohydrates ^a	0.415	L CH ₄ x g TS ⁻¹	33
MCE ^b , proteins extracted	60	% of TMP	34-36
MCE ^b , proteins not extracted	40	% of TMP	34-36
Carbon dioxide in biogas	40	% vol.	37
Methane in biogas	60	% vol.	37
LHV ^c methane	13.90	kWh x kg ⁻¹ CH ₄	38
Electricity, biogas upgrading	0.3	kWh x m ⁻³ biogas	31
Carbon dioxide solubility (20°C) ^d	1.72	g x L ⁻¹ water	39
Fertilizers production			
Mineralisation rate	90	%	36
Electricity, centrifuge ^e	0.03	kWh x kg ⁻¹ paste	10, 31

Table B- 6 Main data for drying for animal feed dietary supplement production, conveyor belt dryer. (a) Entering the dryer (air and paste are circulating in counter current); (b) Under atmospheric pressure conditions; (c) Integrated between 20°C and 80°C; (d) Heat transferred from calorific fluid to air in shell and tube heat exchanger.

Conveyor belt parameters			
Temperature, air ^a	120	°C	40
Temperature, algae paste ^a	20	°C	40
Absolute humidity, air (20°C) ^b	12.1 E-03	kg _{water} x m ⁻³	41
Heat capacity, air (100°C) ^b	1.01	kJ x kg ⁻¹ x K ⁻¹	42
Enthalpy of vaporisation, water ^b	2256	kJ x kg ⁻¹ _{water}	43
Heat capacity, algae paste ^c	3.34	kJ x kg ⁻¹ x K ⁻¹	1
Electricity, belt and fans	0.05	kWh x kg ⁻¹ H ₂ O evaporated	44
Furnace and air-calorific fluid energy transfers			
Heat capacity, ethylene - glycol (160 °C)	0.64	kJ x kg ⁻¹ x K ⁻¹	45
Transfer efficiency, thermal energy ^d	60	%	10, 40
Temperature, heated calorific fluid	200	°C	40
Thermal efficiency, furnace	70	%	10

Table B- 7 Corresponding references to numbers in Table B-1 – Table B-6.

1	Schneider <i>et al.</i> (2016)
2	Safi <i>et al.</i> (2014a)
3	Stephenson <i>et al.</i> (2010)
4	Griffiths <i>et al.</i> (2014)
5	Becker (2007)
6	Milledge and Heaven (2013)
7	GEA (no date)
8	Fasaei <i>et al.</i> (2018)
9	Doucha and Livansky (2008)
10	Woods (2007)
11	Spiden <i>et al.</i> (2013)
12	Yap <i>et al.</i> (2015)
13	Martin (2016)
14	Olmstead <i>et al.</i> (2013)
15	Angles <i>et al.</i> (2017)
16	Branan (2002)
17	Halim <i>et al.</i> (2016)
18	Pruzman, (1991)
19	Lu <i>et al.</i> (2015)
20	Francesconi <i>et al.</i> (2007)
21	Wan Mahmood <i>et al.</i> (2017)
22	Dortmund Data Bank (2019a)
23	Dortmund Data Bank (2019b)
24	Aycock (2007)
25	PENN Specialty Chemicals (2005)
26	Aspen Technology Inc. (2013)
27	Safi <i>et al.</i> (2014b)
28	Gerde <i>et al.</i> , (2013)
29	Ursu <i>et al.</i> (2014)
30	Kandasamy <i>et al.</i> (2012)
31	Collet <i>et al.</i> (2010)
32	Heaven <i>et al.</i> (2011)
33	Sialve <i>et al.</i> (2009)
34	Klassen <i>et al.</i> (2017)
35	Lakaniemi <i>et al.</i> (2011)
36	Ras <i>et al.</i> (2011)
37	Converti <i>et al.</i> (2009)
38	Friend <i>et al.</i> (1989)
39	Dodds <i>et al.</i> (1956)

40	Hosseinizand <i>et al.</i> (2017)
41	Shallcross (1997)
42	Hilsenrath <i>et al.</i> (1955)
43	Osborne <i>et al.</i> (1939)
44	Stela Laxhuber GmbH (2019)
45	CAMEO Chemicals (1999)

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Resume of the Author

General information

Belgian
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Nils.Preat@UGent.be
Born 23rd of October, 1992 in Etterbeek

Education

- 2017 – 2021 **PhD in biosciences engineering**
Faculteit Bioingenieur Wetenschappen, Universiteit Ghent, Belgium
PhD thesis: Development of environmental impact assessment methods for marine sourced products.
- 2018 – 2020 **Master degree in Business Administration and Management**
(specialisation in finance) - *Magna cum laude*
Solvay Brussels School, Université Libre de Bruxelles, Belgium
Master thesis: Sustainable and green finance, language misuse or proven consistency? A case study for green bonds. - *Summa cum laude*
- 2013 – 2015 **Master degree in Agronomy Engineering** - *Magna cum laude*
Ecole Interfacultaire de Bioingénieurs, Université Libre de Bruxelles, Belgium
Master thesis: Assessment of economic potential of *Holothuria lessona* and bring out the holothurian hybridization phenomenon (Madagascar) – *Summa cum laude*, rewarded by Duvigneaud's award
- Jan. - Jun. 2014 **Exchange program in Agronomy Engineering** Master degree –
Magna cum laude
Supagro School, Montpellier, France
- 2010 – 2013 **Bachelor degree in Bioengineering** – Cum laude
Université Libre de Bruxelles, Belgium
- 2004 – 2010 **Secondary School – Sciences-Mathematics**
Athénée Royal Jean Absil, Brussels, Belgium

Experience

- Jan. 2017 - Doctoral researcher in biosciences engineering
Universiteit Ghent, Belgium
- 2016 – 2017 Students coaching
Arboretum College – Tervuren, Belgium

Nov. – Dec. 2014	Development Cooperation: implementation of sea cucumbers farming systems for local communities Indian Ocean Trepang, Toilara, Madagascar
May - Jun. 2014	Internship in statistics: development and implementation of experimental design to assess the population size of the vector of the pinewood disease Biogeco's UMR, Bordeaux, France
Jan. – Aug. 2012	Student job: stock controller Wonderfood, Nivelles, Belgium
Aug. 2010 – Mar. 2012	Student job: assistant store manager Fixerati Concept Cyclery, Ixelles, Belgium.

Teaching experience

UGent, faculty of biosciences engineering

2017 – 2020	Clean Technology (Lecturer Prof. Jo. Dewulf): responsible of the organisation and evaluation of the task consisting to a literature research (report and presentation).
2017 – 2019	Analysis and abatement of air pollution (Lecturer Prof. Christophe Walgraeve): responsible of the organisation and evaluation of practicum IV: Henry's law constant.

Arboretum college

2016 – 2017	Responsible of following and coaching secondary school and higher education students for sections mathematics, chemistry and physics.
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Other

2016 -	Particular lessons in scientific and economic courses for students in secondary school and higher education.
2020 -	Mountain bike instructor.

Languages

French	Native
English	Fluent
Dutch	Good knowledge

Computer skills

Adobe Photoshop, Microsoft Office, QGIS, SAP, Promethée, R	Very good knowledge
Octave (Matlab), Python, Simapro	Good knowledge

List of publications in peer reviewed international journals (A1)

Quesada-Salas, M. C., Willig, G., Pr  at, N., Allais, F., & Ioannou, I. (2021). Optimization and Comparison of Three Cell Disruption Processes on Lipid Extraction from Microalgae. *Processes*, 9(2), 369.

Pr  at, N., Lefaible, N., Alvarenga, R., Taelman, S. E. & Dewulf, J. (2021). Development of a life cycle impact assessment framework accounting for biodiversity in deep seafloor ecosystems: a case study on the Clarion Clipperton Fracture Zone. *Science of the Total Environment*. 770, 144747.

Alvarenga, R. A. F., Huysveld, S., Taelman, S. E., Sfez, S., Pr  at, N., Cooreman-Algoed, M., Sanjuan Delmas D. & Dewulf, J. (2020). A framework for using the handprint concept in attributional life cycle (sustainability) assessment. *Journal of Cleaner Production*, 121743.

Pr  at, N., Taelman, S. E., De Meester, S., Allais, F., & Dewulf, J. (2020). Identification of microalgae biorefinery scenarios and development of mass and energy balance flowsheets. *Algal Research*, 45, 101737.

Pr  at, N., De Troch, M., van Leeuwen, S., Taelman, S. E., De Meester, S., Allais, F., & Dewulf, J. (2018). Development of potential yield loss indicators to assess the effect of seaweed farming on fish landings. *Algal research*, 35, 194-205.

Contributions to international conferences, workshops and symposia

Pr  at N. Taelman, S. E., De Meester, S., Allais, F., & Dewulf, J. (2018). Analyse de Cycle de Vie du bioraffinage des microalgues. Presented at the conference organized by the "Centre Europ  en de Biotechnologie et de Bio  conomie", October 2018: "microalgues, de l'optimisation    la valorisation", Pomacle, France.

Pr  at, N., De Troch, M., Taelman, S. E., De Meester, S. & Dewulf, J. (2018). Seaweed farming: the impact on local biodiversity and potential cause-effect chain with fisheries landings. Presented at the 16th Marine Biology Section Symposium, May 2018, Ghent, Belgium.

Poster presentations

Pr  at N., Taelman S. E., De Meester S., Allais F. & Dewulf J. (2018). Process engineering for microalgal biorefineries: environmental and economic assessments. Poster presented at the Workshop - Summer School of ALPO INTERREG FWVL project: "nouveaux mat  riaux polym  res issus de la biomasse micro-algale", September 2018, Mons Belgium.

Hobbies

Triathlon, mountain sports, bicycle mechanics, student coaching