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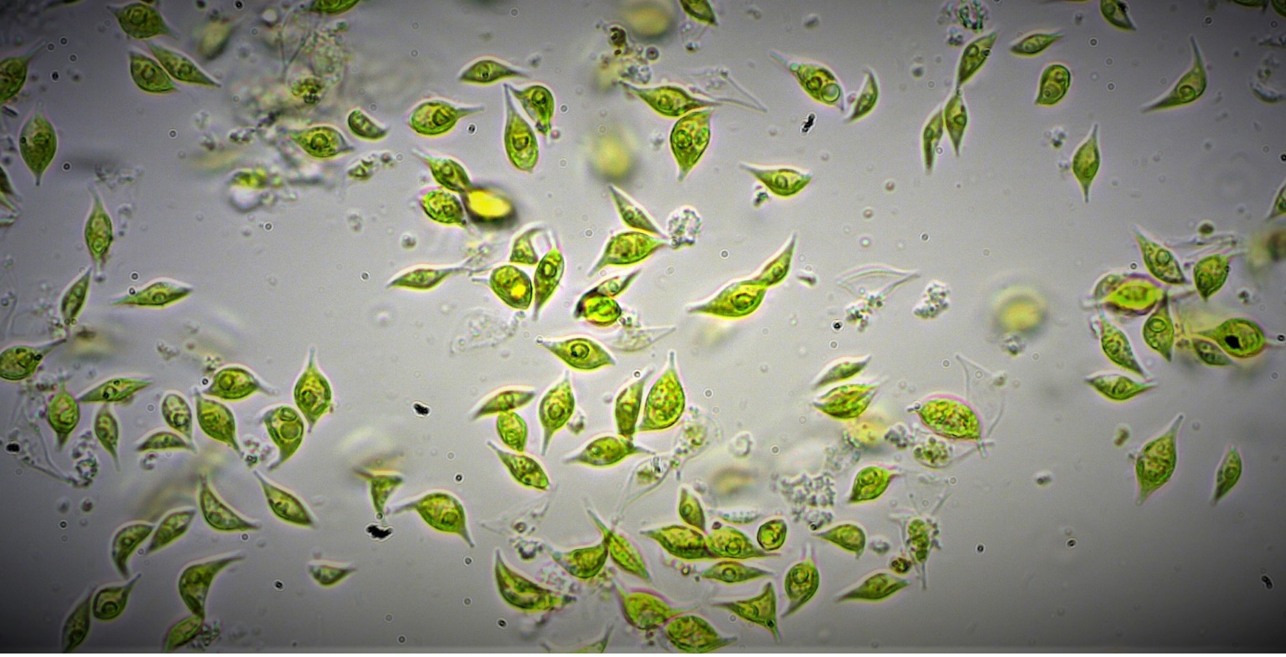

NAVIGATING INDETERMINACY IN EX-ANTE LCA

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BY
PIERRE JOUANNAIS

DISSERTATION SUBMITTED 2023

AALBORG UNIVERSITY
DENMARK

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”Det er svært at spå – især om fremtiden.”

“Prediction is very difficult, especially if it's about the future.”

Niels Bohr (uncertain)

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In 2020, Pierre Jouannais earned an engineering degree from AgroParisTech in France, where he chose to specialize in environmental sciences and ecosystem management. He combined this education with a master's degree at Sorbonne University, Paris, France, in integrative biology focused on marine organisms.

During this education, he carried out research as an intern for the French National Center for Scientific Research (CNRS), studying metal hyperaccumulators among plants. He also worked in northern Norway on seaweed harvesting before carrying out a master's thesis with the French National Institute for Agriculture, Food and Environment (INRAE), where he investigated and bioprospected microalgae for coupling with biohydrogen production.

Since August 2020, Pierre Jouannais has been a PhD Fellow in the Danish Center for Environment Assessment (DCEA), Department for Sustainability and Planning, Aalborg University, Denmark. His PhD project was part of the AquaHealth European project, within which his work constituted the systemic environmental assessment dimension while partners focused on the biotechnological aspects.

Overall, Pierre has developed a profile combining classic engineering and system-thinking skills with biological and ecological knowledge to tackle complex issues through modeling. In parallel to his research, he dedicates most of his time to music, guitar, politics, fishing, and trekking.

ENGLISH SUMMARY

In the face of ecological emergencies, various technological concepts are emerging to address the systemic issues confronting human societies and production systems. Although these concepts may appear promising at first look, it is crucial to anticipate the future environmental impacts associated with the market-scale deployment of these technologies. One of the emerging technological concepts is the use of new microalgal compounds to enhance health management in fish farms. This synergy presents an opportunity to create a market for high-value microalgal compounds, thereby alleviating the economic constraints faced by the microalgal sector and its diverse co-products, while simultaneously addressing the disease crisis prevalent in finfish farming. These compounds are currently at the center of bioprospecting efforts and thus remain undiscovered. They could be found in any microalgal strain and provide a vast range of potential bioactivities from therapeutic antiviral applications to prophylactic immunostimulation and growth enhancement. The final technological configuration emerging from this concept, which is still within the bioprospecting stage, therefore remains indeterminate.

This PhD research aims to assess quantitatively the environmental impacts associated with this technological concept by performing an ex-ante Life Cycle Assessment (LCA) of the use of undiscovered compounds for finfish farming across Europe. The primary objective is to provide decision-making support regarding the development of this concept. To do so, a stepwise approach is implemented to progressively acknowledge and quantitatively assess all the indeterminacies in the system and their associated levels of incertitude. The research begins by developing a novel parameterized LCA model to simulate the cultivation and production of any microalgal strain and compound in any location and season. Multiple forms of uncertainty are assessed regarding the European production of a yet undiscovered microalgal compound. This model is eventually combined with a new parameterized model for trout farming to assess the environmental impacts of producing fish using microalgal compounds that modulate the production characteristics of the fish farm. Uncertainty and global sensitivity analysis are performed thanks to stochastic propagations. The last part of this work acknowledges the deep uncertainty that lies upon the assessment and that hinders the definition of probabilities regarding the parameters' values. As decision-making is still needed under such conditions, an

algorithmic procedure is proposed and demonstrated to evaluate and approximate the probability of successful technological development.

The results unravel the concept's environmental signal which consists of probability distributions of impacts combined with a thorough understanding of how the different types of uncertainty interact and contribute to the impact uncertainty. The median global warming impact score across all potential European production mixes, strains, compounds, and photobioreactor setups amounted to 96 kg CO_2-eq. per kilo of compound, which can be interpreted as a reasonable expectation of impact when the compound and its production locations remain unknown.

The stepwise approach divided the work into standalone tasks that provided results and models for the microalgal and finfish farming sector. Thus, assessing the indeterminacies as stake led to performing an LCA of all microalgal products over Europe. This showed how the same microalgae productions environmentally perform once deployed over large geographic zones with distinct energy mixes, climates, and solar resources, instead of in single locations as previously assessed. In addition, the current environmental opportunity cost of mortality in the studied trout farm ranged from 3% to 5.5% across impact categories. Losses happening at the end of the production cycle were shown to greatly increase the overall life cycle impacts for trout production.

Finally, this work presents theoretical and methodological advances for ex-ante LCA when applied to deeply uncertain technological concepts. In particular, this work highlighted the difference between risk and uncertainty as two forms of incertitude and proposed a procedure to make ex-ante LCA comply with this key distinction within post-normal science. This procedure informs on what decision-makers should be sure about to ensure a certain probability of successful technological development from an environmental point of view.

Overall, this PhD study constitutes an ex-ante LCA of a very early-stage technological concept with a focus on decision-making in the face of deep uncertainty. The research places significant emphasis on recognizing the chaotic nature of technological development and deployment which are influenced by many factors that are difficult to control once the exploration of a concept is initiated. Examples of such uncontrollable factors include the outcomes of bioprospecting efforts and the integration of the concept into the market. The approach illustrates the use of ex-ante LCA to decide whether concepts should be further explored or if the limited available time and resources should be assigned to other concepts, especially from the perspective of performant technological planning under ecological emergency.

DANSK RESUME

I lyset af økologiske nødsituationer opstår forskellige teknologiske koncepter for at imødegå de systemiske udfordringer, som menneskesamfund og produktionssystemer står over for. Selvom disse koncepter ved første øjekast kan virke lovende, er det afgørende at forudse de fremtidige miljømæssige konsekvenser ved markedsmæssig implementering af disse teknologier. Et af de fremadstormende teknologiske koncepter er brugen af nye mikroalgeforbindelser til forbedring af sundhedsstyring i fiskeopdræt. Denne synergi giver en mulighed for at skabe et marked for mikroalgeforbindelser med høj værdi og dermed afhjælpe de økonomiske begrænsninger, som mikroalgeindustrien og dens forskellige medprodukter står over for, samtidig med at man adresserer sygdomskrisen, der plager opdræt af finfisk. Disse forbindelser er i øjeblikket genstand for bioprospektering og forbliver derfor uopdagede. De kan findes i hvilken som helst mikroalgeart og giver et bredt udvalg af potentielle bioaktiviteter, lige fra terapeutisk antiviral anvendelse til profylaktiske immunstimulerende midler og vækstfremmere. Den endelige teknologiske konfiguration, der opstår ud fra dette koncept, som stadig er i bioprospekteringsfasen, er derfor uafklaret.

Dette ph.d.-projekt sigter mod kvantitativt at vurdere de miljømæssige konsekvenser forbundet med dette teknologiske koncept ved at udføre en ex-ante Life Cycle Assessment (LCA) af brugen af uopdagede forbindelser i fiskeopdræt på tværs af Europa. Det primære formål er at levere beslutningsstøtte vedrørende udviklingen af dette koncept. For at opnå dette anvendes en trinvis tilgang til gradvist anerkende og kvantitativt vurdere alle usikkerheder i systemet og de tilknyttede niveauer af usikkerhed. Forskningen begynder med at udvikle en ny parameteriseret LCA-model til at simulere dyrkning og produktion af enhver mikroalgeart og forbindelse på enhver placering og i enhver sæson. Forskellige former for usikkerhed vurderes i forbindelse med europæisk produktion af en endnu uopdaget mikroalgeforbindelse. Denne model kombineres til sidst med en ny parameteriseret model for ørredopdræt for at vurdere de miljømæssige konsekvenser ved produktion af fisk ved brug af mikroalgeforbindelser, der påvirker produktionsegenskaberne for fiskeopdræt. Usikkerheds- og global følsomhedsanalyse udføres ved hjælp af stokastisk fremdrift. Den sidste del af dette arbejde anerkender den dybe usikkerhed, der hviler over vurderingen og som forhindrer definitionen af sandsynligheder for parameterværdierne. Da der stadig er behov for beslutningstagning under sådanne forhold, foreslås og demonstreres en algoritmisk procedure til at vurdere og tilnærme sandsynligheden for en succesfuld teknologisk udvikling.

Resultaterne afslører konceptets miljømæssige signal, der består af sandsynlighedsfordelinger af påvirkninger kombineret med en grundig forståelse af, hvordan de forskellige typer usikkerhed interagerer og bidrager til usikkerheden om påvirkningerne. Den gennemsnitlige påvirkning af global opvarmning på tværs af alle

potentielle europæiske produktionsblandinger, arter, forbindelser og fotobioreaktoropsætninger beløber sig til 96 kg CO_2-ækv. pr. kilo forbindelse, hvilket kan tolkes som en rimelig forventning til påvirkningen, når forbindelsen og dens produktionssteder forbliver ukendte.

Den trinvise tilgang opdelte arbejdet i selvstændige opgaver, der gav resultater og modeller for mikroalge- og fiskeopdrætssektoren. Dermed blev usikkerhederne undersøgt som individuelle aspekter og førte til udførelse af en LCA af alle mikroalgeprodukter i Europa. Dette viste, hvordan de samme miljømæssige præstationer af mikroalgeproduktioner er, når de implementeres over store geografiske områder med forskellige energiblandinger, klimaforhold og solressourcer, i modsætning til tidligere undersøgelser, der fokuserede på enkeltstående placeringer. Derudover var den nuværende miljømæssige omkostning ved dødelighed i den undersøgte ørredfarm mellem 3% og 5,5% på tværs af påvirkningskategorierne. Tab, der opstår ved afslutningen af produktionscyklussen, viste sig at have stor indflydelse på de samlede livscykluspåvirkninger for ørredproduktionen.

Endelig præsenterer dette arbejde teoretiske og metodologiske fremskridt for ex-ante LCA, når det anvendes på dybt usikre teknologiske koncepter. Især fremhæver dette arbejde forskellen mellem risiko og usikkerhed som to former for usikkerhed og foreslår en procedure til at sikre, at ex-ante LCA overholder denne afgørende forskel inden for post-normal videnskab. Denne procedure informerer om, hvad beslutningstagere skal være sikre på for at sikre en vis sandsynlighed for en succesfuld teknologisk udvikling set ud fra et miljømæssigt perspektiv.

Alt i alt udgør dette ph.d.-studie en ex-ante LCA af et meget tidligt stadie for et teknologisk koncept med fokus på beslutningstagning under dyb usikkerhed. Forskningen lægger vægt på anerkendelsen af den kaotiske karakter af teknologisk udvikling og implementering, som påvirkes af mange faktorer, der er vanskelige at kontrollere, når udforskningen af et koncept påbegyndes. Eksempler på sådanne ukontrollerbare faktorer inkluderer resultaterne af bioprospekteringsindsatsen og integrationen af konceptet på markedet. Tilgangen illustrerer brugen af ex-ante LCA til at beslutte, om koncepter bør undersøges yderligere, eller om de begrænsede tilgængelige tid og ressourcer skal anvendes til andre koncepter med henblik på en effektiv teknologisk planlægning i en økologisk nødsituation.

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I am also grateful to the members of the AquaHealth project for the insightful discussions and their well-intended curiosity toward my ideas.

I need to thank my family and friends for supporting me despite the distance and having nurtured the curiosity and passion that led me to the scientific world. My grandfathers should know that they played a particular role in this orientation. *Je dois aussi remercier toute ma famille et mes amis pour m'avoir soutenu malgré la distance et avoir nourri la curiosité et passion qui m'ont mené au monde scientifique. Mes grands-pères doivent savoir qu'ils ont joué un rôle particulier dans cette orientation.*

Thank you, Camilla, for making these years much more loveable than I was expecting them to be.

Finally, I want to thank all those that will never read this because they are too busy fighting for a better world, for environmental and social justice for humans and non-humans. It is because I know that some are standing that I can bear sitting at a desk with the same goals in mind.

LIST OF ACADEMIC PUBLICATIONS

The following publications have been completed as part of this PhD dissertation:

I. Jouannais, P., Hindersin, S., Löhn, S., Pizzol, M., 2022. Stochastic LCA Model of Upscaling the Production of Microalgal Compounds. Environ. Sci. Technol. 56, 10454–10464. https://doi.org/10.1021/acs.est.2c00372

II. Jouannais, P., Pizzol, M., 2022. Stochastic Ex-Ante LCA under Multidimensional Uncertainty: Anticipating the Production of Undiscovered Microalgal Compounds in Europe. Environ. Sci. Technol. 56, 16382–16393. https://doi.org/10.1021/acs.est.2c04849

III. Jouannais P, Gibertoni PP, Bartoli M, Pizzol M (2023) LCA to evaluate the environmental opportunity cost of biological performances in finfish farming, Int J LCA (**Re-submitted after revison July 2023**)

IV. Jouannais, P., Blanco, CF., Pizzol, M., ENvironmental Success under Uncertainty and Risk (ENSURe): A procedure for probability evaluation in ex-ante LCA (**under review**)

DISSEMINATION IN ACADEMIC CONFERENCES

Platform presentations were given during three conferences, short abstracts are available at https://vbn.aau.dk/en/persons/pierre-antoine-jouannais.

I. SETAC Europe, 31st Annual meeting, Sevilla, Virtual conference
May 2021
Ex-Ante LCA for Microalgae-Based Veterinary Molecules in Finfish Aquaculture: How to Assess the Environmental Performance of Unknown Molecules?

II. SETAC Europe, 32nd Annual meeting, Copenhagen,
May 2022
Modeling biological and techno-operational uncertainty in Ex-Ante LCA of microalgal molecule productions

III. SETAC Europe, 33st Annual meeting, Dublin
May 2023
Combining Ex-ante LCA with Scenario-Discovery and conditional probabilities to assist decision-making under distinct degrees of Incertitude

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1. INTRODUCTION

1.1. CONTEXT

After five previous reports already documenting the progress, and the current and expected damages of climate change, the 2022 IPCC report highlighted that climate action and adaptation are "*more urgent than previously assessed*" (Ipcc, 2022). With the current climate being 1.09°C warmer than at the beginning of industrial times, the window of opportunity to limit climate change and its effects is shrinking (Ipcc, 2022). Air and water pollution, ecosystem and biodiversity loss resulting from climate change or direct human pollution and expansion are pushing the Earth's system beyond the planetary boundaries which ensure a safe-operating space for human societies (Bastianoni et al., 2019; Steffen et al., 2015).

In this adverse context, the population increase and diet changes are driving an increase in demand for food of around 50 % between 2010 and 2050 (Keating et al., 2014) while agriculture already impacts humans and-non humans substantially. Major changes are expected from the food system to meet this challenge, and the development of aquaculture could play a big role in the supply of nutritional value to the global population (FAO, 2022; Keating et al., 2014).

With a production steadily increasing with a 3% annual growth rate over the last 60 years, the total farmed aquatic animal production including finfish production is expected to reach 106 million tons in 2030 to supply a 21.4 kg aquatic product consumption per capita (FAO 2022). This would comfort finfish farming as a major contributor to proteins and omega-3 supply to the global population. However, these projections depend among other factors on the capacity of finfish farming to handle the disease crisis plaguing the sector (Abolofia et al., 2017; Chamberlain, 2015; Ferreira et al., 2014; Jansen et al., 2012; Jonkers et al., 2010; Leung and Bates, 2013; Merino et al., 2012; Murray and Peeler, 2005).

The estimates for the associated losses vary across the wide diversity of farming systems over the world. In Europe, statistics for Danish trout indicate 5 to 7 % (Danmarks statistik, 2021) mortality and Norwegian salmon aquaculture undergoes 15 to 20% loss rate, including mortality and escapes (Bang-Jensen et al., 2019). While global trade and ecosystem disruption bypass biological borders and spread pathogens across farms and countries (IPBES, 2020; The Lancet Planetary Health, 2021), climate change increases disease occurrence and seriousness worldwide (Reverter et al., 2020). The gravity of the issue goes beyond the associated reduced economic and environmental efficiency, as the reinforcement of antimicrobial resistance due to the use of therapeutics increases the chances of epidemics and fuels a never-ending race toward new biocontrol substances and practices (Done et al., 2015; Miranda et al., 2018; Reverter et al., 2020; Rico and Henriksson, 2021; Van Boeckel et al., 2019).

In this context, "*new paradigms to help solve the global aquaculture disease crisis*" (Stentiford et al. 2017) are searched, including new farming practices, treatments, and above all prophylactic approaches to biosecurity and health issues.

On the other hand, at the very beginning of the aquatic trophic chain, microalgae have engaged the attention of the scientific and industrial communities for many years and for many reasons. A strong asset of microalgae for biotechnological purposes lies in the very definition of this group and its evolutionary history; microalgae do not belong to a unique phylogenetic clade with a common ancestor. Instead, they constitute a widely diverse group of more than 60 phyla and 70 000 known species of unicellular organisms, either eukaryotic or prokaryotic (cyanobacteria), which all integrated photosynthetic chloroplasts through their distinct evolutionary histories (Barra et al., 2014; del Campo et al., 2014; Metting, 1996; Sánchez-Baracaldo and Cardona, 2020). This phylogenetic trait makes microalgae a group of organisms featuring a wide range of biochemical properties and products, all fueled by similar microscopic solar plants with high photosynthetic efficiencies (Williams and Laurens, 2010). While the cyanobacteria *Arthrospira platensis* (also known as Spirulina) was cultivated and harvested for centuries by the Aztecs and by populations in Chad, industrial productions of microalgal nutraceuticals can be traced back to the 1950s (García et al., 2017; Williams and Laurens, 2010). Motivated by the oil crisis in 1973, the production of microalgal lipids as the substrate for third-generation biofuels then gained interest but the comparatively low crude oil barrel price has since then prevented the sector from developing extensively (Prokop et al., 2015).

Efforts were then made to valorize the vast diversity of high-value compounds that the microalgal biodiversity can produce (Da Silva et al., 2014; Patel et al., 2021). These compounds include proteins, vitamins, and antioxidant pigments (Ananthi et al. 2020; Williams and Laurens 2010), but also antibacterial, antiviral and antitumoral molecules (Falaise et al., 2016). Recent discoveries on the bioactivities of compounds found in some microalgal strains suggest that they could be used in finish farming to enhance health management (Falaise et al., 2016; Liu et al., 2016; Yaakob et al., 2014). Such a synergy with finfish farming would tackle the aforementioned disease crisis and allow the economic viability of the associated microalgal production. It would thus enable the co-production of microalgal biomass for other purposes such as feed or energy and foster the growth of the European Blue Bioeconomy (European Union, 2018).

This promising potential synergy is what motivated the AquaHealth project(*Aquahealth : Microalgae Microbiomes — A natural source for the prevention and treatment of aquaculture diseases*[1]) which gathers several research institutions from Denmark, Norway, and Germany intending to find new microalgal compounds and develop their production. The exact nature of the technology that will emerge from

[1] *https://aquahealth-project.com/*

this initiative remains highly uncertain at this stage and will highly depend on what is discovered during bioprospecting, i.e., during the exploration of microalgal biodiversity and their microbiomes. This PhD work was developed within the AquaHealth project and started when the biologists from the consortium were still bioprospecting for these compounds.

1.2. PROBLEM SCOPE

At the time of this PhD work, the technology that the AquaHealth project could lead to is a mere concept. AquaHealth is fundamentally explorative and its outcomes will depend on which compounds are found and how the market integrates them. In fact, the only definition of the final technology that can be given at this stage is

"Some microalgae produce certain bioactive compounds supplied to finfish farms to enhance fish health and performance in Europe."

This concept appears at first look as a promising and potentially environmentally beneficial synergy. Multiple claims and studies have been made on the environmental performance of microalgal productions while the environmental and economic burden of health-related issues in finfish farming is acknowledged (cf. 2.3). Yet, the path from a mere idea to a market-scale functioning technology is highly uncertain due to the chaotic nature of technological development (Hung and Tu, 2011; Pizzol and Andersen, 2022). It is not possible to deterministically anticipate the final configuration of the commercial-scale technology that will emerge once the concept starts being explored, and the associated environmental impacts therefore remain unknown. The Research and Development (R&D) process and the integration of the technology in the market will be shaped by a large number of internal and external causes. These causes will eventually affect the final configuration for the commercial-scale technology, and its environmental impact. The very first cause determining the outcome of the R&D process associated with the technological concept will be the discovery of a bioactive compound in a microalgal strain. Depending on what is found, the technology could take various forms ranging from the production of microalgal antibiotics in a few German locations for specific fish diseases to the production of fish prophylactic nutraceuticals over southern Europe. The final technology and its environmental performance are therefore "indeterminate" in the broadest meaning of indeterminacy: "*not clearly known or measured"* (Cambridge University Press, n.d.).

In times of absolute ecological emergency, while new environmentally performant technologies are recognized as part of the requirements for human societies to mitigate and adapt to ecological threats (Ipcc, 2022), the assessments of these technologies cannot wait for their market-scale configurations to become determinate. As Collingridge highlighted in his eponym dilemma (Collingridge, 1980; Genus and Stirling, 2018), the correctability of a technology toward better environmental performance is maximum in its very early development stages, where uncertainty is high, and minimum when the technology is deployed and the impacts are measurable. The "Social control of Technology" (Collingridge, 1980) and the Responsible Research and Innovation (RRI) paradigm (Owen et al., 2013; Wender et al., 2014) thus require considering technological concepts at a very early stage. These concepts become objects of study in themselves by performing ex-ante (i.e., "before the event") quantitative environmental assessments. The AquaHealth technological concept should therefore be assessed from its very beginning, to be able to guide it and to understand what can be expected in terms of environmental impacts.

1.3. OBJECTIVES

This PhD project takes up the challenge of modeling and quantitatively assessing the future environmental impact associated with the indeterminate technology that will emerge from the search for new microalgal compounds for health management in European finfish farming. The assessment should be as comprehensive as possible by using a Life Cycle Assessment (LCA) framework. The resulting insights should assist decision-making regarding the development and deployment of this technology.

While the new synergy between microalgae and finfish farming that may arise from the current research within Aquahealth is unknown, both components of the synergy, namely microalgal production and fish farming, are known. This work therefore aims at using the knowledge and the state of the art regarding the two components of the synergy to be able to anticipate the environmental impacts and performance of the future potential synergy. The numerous indeterminacies regarding the final technological configuration should be thoroughly acknowledged and handled according to the level of knowledge that can assist the proposition of values, probabilities, and scenarios. The assessment will provide insights on the scope of possibilities for the environmental impact associated with the technological concept but also inform on the most reasonable expectation one could have regarding these impacts by informing about *probabilities of impacts*.

Overall, anticipating the impact of the technology will help decision-makers at different levels. At the industry and R&D level, it will provide insights on which type of microalgae and bioactive effects should be targeted in priority, together with information on the most efficient ways to decrease the future impact while still in the R&D phase. Above all, this work will work mainly aims at informing decision-makers at the political level regarding the planning of technological development via incentives, subsidies, and funding of research projects. Hence, an objective of this work is to use the case study as an archetype of early-stage technological concept to advance theoretical and methodological tools for decision-making. In a state of environmental emergency, technological planning is necessary to invest time and resources into research projects that are likely to lead to technologies that will improve the status quo. The complex innovation ecosystem (Hojnik and Ruzzier, 2016; Pizzol and Andersen, 2022; Schot and Steinmueller, 2018) in which concepts emerge should thus be informed as soon as possible about the probability of a technological concept eventually performing over a certain threshold. In a context of incomplete knowledge (ex-ante perspective), but also time (emergency for action) and resource scarcity (finite amount of money and researchers), it is crucial to be able to "navigate indeterminacy" to decide if a mere concept is worth further exploring, or if it should be amended or dismissed from the beginning.

1.4. STRUCTURE

The structure of this thesis consists of eight sections. Section 1 introduces the general context of this PhD work together with the specific problem scope and overall objectives of this work. Section 2 provides an overview of the state of the art regarding the existing knowledge and theory required to anticipate the environmental impacts of the technological concept. Section 3 explores the research design of this work by defining the questions and hypothesis that motivated it, and explains how the different articles associated with this PhD study cover these questions. Section 4 presents an overview of the theories and methods mobilized along the PhD study. Section 5 summarizes the main results of the articles. Section 6 takes hindsight on the obtained results and discusses them, proposing additional perspectives. Finally, Section 7 concludes by answering the research questions, highlighting the main contributions of this work to technological development and to the field, and proposes directions for future research.

The articles published or submitted during this PhD work are available at the end of the manuscript. Important Supplementary information explaining models in detail and providing additional results were provided with each article. They were not added to this manuscript but I encourage the reader to retrieve them if needed from the original online sources.

2. STATE OF THE ART

This section starts by exploring the different definitions and concepts behind the complex terminology associated with "uncertainty" and describes how I used the terms in this manuscript and the research articles. An overview of the scientific literature and knowledge that constitutes a basis to be able to anticipate the environmental impacts of the technology is then provided. This knowledge covers industrial ecology with LCA models and results, but also biology and ecology.

2.1. DEFINITIONS OF UNCERTAINTY, INDETERMINACY, INCERTITUDE, RISK AND PROBABILITY

It's a general consensus that uncertainties must be acknowledged when using models to support decision-making regarding the real world's complex systems (Saltelli et al., 2020). However, the terminology and different concepts behind the broad notion of uncertainty vary within and across disciplines. In order to propose a consensual and cross-disciplinary definition, Walker et al. (2003) describe uncertainty as "*any departure from the unachievable ideal of complete determinism*". The authors define " *determinism*" as "*the ideal situation in which we know everything precisely*".

This broad definition could correspond to the one used within the LCA field which almost exclusively uses the term uncertainty to qualify anything that is "not clearly known". Since the first direct mention of uncertainty in a LCA paper in 1995 (Chen, 1995), considerations for uncertainty in LCA have remained arguably too scarce (Lo Piano and Benini, 2022) with 5 to 10 % of all published LCA studies mentioning uncertainty in the title, abstract or keywords (Mahmood et al., 2022). The number of LCA case studies directly tackling uncertainty in a case study or proposing methodological advances has however increased over the last years, thus showing a rising interest in the matter (Blanco et al., 2020; Cucurachi et al., 2021; Hauck et al., 2014; Mahmood et al., 2022; Mendoza Beltran et al., 2018; Pérez-López et al., 2018; Pizzol, 2019; Qin and Suh, 2021).

Walker et al. (2023) identify three dimensions of uncertainty, namely location, nature, and level. The LCA literature usually distinguishes uncertainty based on two of these three dimensions. The uncertainty "location" is defined by differentiating the uncertainty lying on the model structure and the drawn causal relationships, defined as "model uncertainty" (Huijbregts, 1998) or "model structure and context uncertainty" (Igos et al., 2019), from the uncertainty regarding the values of the model's parameters, described as "parameter uncertainty" (Huijbregts, 1998) or "quantity uncertainty" (Igos et al., 2019).

A distinction is also made in the LCA literature between two main types of uncertainty "nature". Uncertainty can be "epistemic" (Clavreul et al., 2012), also designated as "epistemological" (Björklund, 2002; Mendoza Beltran et al., 2020), and stemming from a lack of knowledge regarding the studied system. This implies that epistemic uncertainty can potentially be reduced by additional knowledge. The other main nature of uncertainty is "aleatory"(Helton et al., 2010) also described as "stochastic" (Clavreul et al., 2012), "ontic" (Mendoza Beltran et al., 2020), or "irreducible" (Helton et al., 2010). Aleatory uncertainty is sometimes conceptually equivalent to "variability" in LCA (Mahmood et al., 2022), which Walker et al. (2003) present as "variability uncertainty" thus highlighting the proximity between variability and uncertainty. Aleatory uncertainty is not due to a lack of knowledge but to the inherent variability and heterogeneity in the real-world such as the uncertainty regarding the specific weather at a certain time and location or the consumer choices and behaviors during the use phase of a product (Hauck et al., 2014; Michiels and Geeraerd, 2022). In their procedure for uncertainty assessment in LCA, Michiels and Geeraerd (2022) differentiate epistemic uncertainty from variability and their combination is defined as "overall uncertainty". Adding nuance to the discussion, Frey (1992) rightly emphasizes that the difference between variability and uncertainty is above all a matter of perspective regarding the question that the analyst is posing. He argues that variability is a mere description of the heterogeneity within a sample of individuals (factories, people, points in time etc.) and can be described with descriptive statistical tools such as frequency distributions, while uncertainty (aleatory) can apply when a question is posed regarding the characteristics of one random individual within this sample.

Among the LCA studies that can be defined as ex-ante, prospective or anticipatory, uncertainty generally becomes a key topic due to the impossibility of deterministically predicting the future (Bergerson et al., 2020; Cucurachi et al., 2018; Douziech et al., 2021).

In a recent critical review, Lo Piano and Benini (2022) identify two issues associated with the appraisal of uncertainty in LCA: the common downplay of uncertainty and the lack of consideration for the quality of the knowledge underlying it. These issues are directly related to the third dimension of uncertainty proposed by Walker et al. (2003) which is the "level" of uncertainty. The use of the pedigree matrix in LCA partly considers this dimension by converting ratings of the data quality and representativity into probability distributions for parameters' values (Ciroth et al., 2016), but other disciplines related to model-supported decision-making propose a further exploration of the level of uncertainty.

Hence, in 1921 already, Knight (1921) differentiates "uncertainty" from "risk" which is a measurable uncertainty that is "*so far different from an unmeasurable one that it is not in effect an uncertainty at all*". Later, Wynne (Wynne, 1992) and Stirling (Stirling, 2010) elaborate and propose a categorization of "uncertainty" based on two dimensions/axis: the level of knowledge one has regarding the nature of the possible events at stake, and the level of knowledge about the probabilities associated to the events. For instance, a decision could lead to different futures that cannot be consensually described with accuracy (what do these futures exactly consist of?), but the probabilities of reaching each future could be well-known and based on sufficient information. This hypothetical decision-making exercise would therefore be done under conditions of "ambiguity". Ambiguity constitutes one of the four categories of "Incertitude" (Scoones, 2019), illustrated in Figure 1.

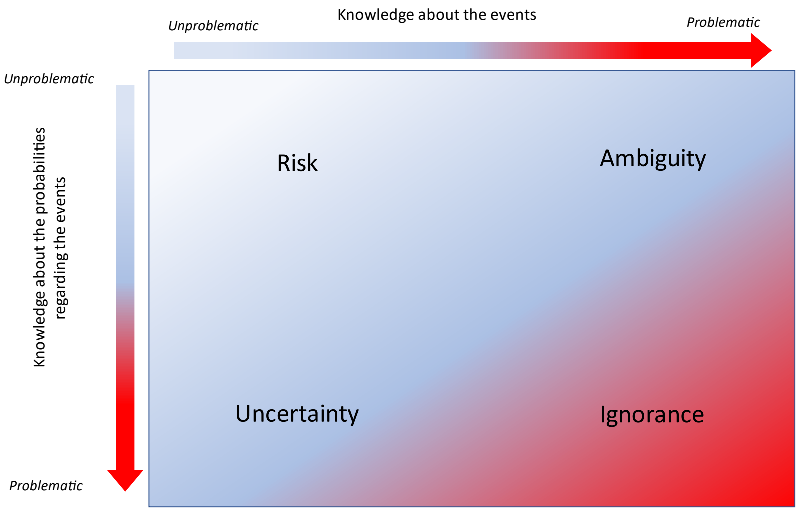

Figure 1: Incertitude matrix. Adapted from (Stirling, 2010)

"Uncertainty" therefore constitutes only one type of incertitude, when the level of knowledge about the probabilities is "problematic", i.e., low, compared to the level of knowledge associated with "risk". The presence of uncertainty in Stirling's terms has also been described as "deep-uncertainty" (Kwakkel and Jaxa-Rozen, 2016; van der Giesen et al., 2020), when the establishment of probabilities faces limitations. The importance of distinguishing between various forms of incertitude in their handling and communication has been emphasized as crucial to "*keep it complex*" (Stirling, 2010) and avoid conveying a misleading sense of confidence with a one-dimensional measure of risk (Saltelli and Ravetz, 2021; van der Giesen et al., 2020).

The notion of "probability" as commonly employed in model-based sciences for decision-making mostly corresponds to its Bayesian interpretation, which is a measure of the degree of belief in an event or statement (von der Linden et al., 2014). This notion also corresponds to on the definitions proposed by R.T Cox (Cox, 1946) which describes probabilities as a measure of what constitutes a "*reasonable expectation*". The other interpretation of probability is frequentist and defines probabilities as resulting from average performances observed in the repetition of a hypothetically infinite sample. The following example briefly illustrates the philosophical differences without entering into the mathematical details (Hoekstra et al., 2014; Samaniego, 2012; von der Linden et al., 2014): a member of the frequentist school will refuse to give the probability that a freshly tossed coin landed on heads, but will say that if this experiment was performed ad infinitum, the proportion of heads would tend to 50%. On the other hand, the Bayesian may state that, from his perspective, there is a 50% chance that this specific coin landed on heads.

In this PhD thesis, it was necessary to find a compromise between the different and sometimes ambiguous terminologies that have been used in the literature. For instance, Wynne describes "uncertainty" as a type of "uncertainty", together with "indeterminacy" (Wynne, 1992), while Stirling and Scoones describe "uncertainty" as being a type of "incertitude" and do not use the term "indeterminacy" (Scoones, 2019; Scoones and Stirling, 2020).

Article I, II, and III used "uncertainty" in its broad sense in the LCA community while this manuscript and Article IV adapt the incertitude terminology when not specified otherwise. In my terminology, an indeterminacy is simply an element being "*not clearly known or measured*"(Cambridge University Press, n.d.), for which a unique deterministic value cannot be proposed. This indeterminacy can be associated with different categories of incertitude, according to Stirling's classification.

2.2. MICROALGAL PRODUCTIONS AND THEIR ENVIRONMENTAL IMPACTS

2.2.1. CURRENT PRODUCTIONS AND DEMONSTRATED BIOACTIVITIES ON FISH HEALTH-RELATED ISSUES

Despite a very large biodiversity, only few species are cultivated at commercial scale for food, nutritional supplements and feed purpose. In 2016, the global production of microalgae amounted to at least 16.7 Kt dry biomass production including *Arthrospira sp.* (for food and pigments), *Chlorella sp.* (food supplements), *Dunaliella salina* (carotene), *Aphanizomenon flos-aquae* (food supplements), *Haematococcus pluvialis* (astaxanthin), *Crypthecodiinium cohnii* and *Shizochytrium sp.* (European Union, 2018; García et al., 2017). The European production amounts to 182 tons dry weight for eukaryotic microalgae and 142 tons dry weight for *Arthrospira sp.* (Araújo et al., 2021). These production estimates come with high uncertainty as production numbers for microalgae are practically inexistent (Araújo et al., 2021). *Chlorella sp.* and *Haematococcus pluvialis* correspond to more than 80% of the European production of eukaryotic microalgae (excluding *Arthrospira sp*.) and are cultivated for their high-value bioactive molecules. Overall, the European microalgal production including *Arthrospira sp*. production consists of around 450 productions units in 23 countries, and 70% of the eukaryotic microalgae productions use closed Photobioreactors (PBR) (Araújo et al., 2021).

Microalgae are already used in aquaculture with the astaxanthin pigment from *Haematococcus pluvialis* being fed to salmon or sea breams (Yaakob et al., 2014). More than providing pigmentation, astaxanthin was shown to improve resistance to diseases and acute stress in yellow catfish (Liu et al., 2016). Microalgae also already constitute a source of omega 3 for juvenile farmed fish and can be fed to zooplankton such as rotifers which will eventually be fed to carnivorous fish (Benemann, 1992). While antibacterial, antifungal, and antiviral activities against aquaculture diseases have been demonstrated in vitro for some microalgal compounds, a few in vivo tests also shown beneficial effects (Falaise et al., 2016). These bioactive compounds are very diverse and range from antiviral polysaccharides to antifungal lipids (Falaise et al., 2016).

A major issue with the use of microalgae in fish feed is the presence of anti-nutritional factors contained in the microalgal biomass which can negatively affect the nutrition of carnivorous fish including tannins, lectins, and digestive enzymes inhibitors (Norambuena et al., 2015). If the whole algal biomass is fed to the fish, these anti-nutritional effects can counterbalance the targeted positive bioactivities of other compounds. Studies have shown that the algal biomass share in fish feed could be raised up to 10% with a positive, or at least no effect on fish growth performance (Norambuena et al., 2015). Norambuena et al. (2015) could include 5% of a diatom-derived product in the Atlantic Salmon feed without observing any positive or negative effect on fish growth.

Overall, the microalgal biodiversity is considered an underexploited and promising reservoir of bioactive compounds for aquaculture (Falaise et al., 2016; Krohn et al., 2022).

2.2.2. CHALLENGES OF MICROALGAE CULTIVATION

Microalgae can be cultivated in open systems such as open-pond raceways (OPR). This cultivation system generally presents a suboptimal use of photons because a large part of the water column is in the dark due to light absorption by the biomass in the first centimeters (Prokop et al., 2015). The productivity of these systems is therefore limited compared to closed systems which seek to better control conditions, prevent contamination, and optimize the use of photons (Carvalho et al., 2011; Prokop et al., 2015; Pruvost et al., 2020; Williams and Laurens, 2010).

Closed systems also called photobioreactors (PBR) consists of tubes or panels of different geometries in which the microalgal culture containing the cells and the nutritive medium circulates, either propelled by pumps or agitated with bubbles. The main types of reactors include outdoor flat panels and horizontal and vertical tubular

PBR (Wang et al., 2012). Vertical tubular PBRs are among the most used types of reactors for their performance and could likely accommodate most strains (De Vree et al., 2015; Norsker et al., 2011; Prokop et al., 2015).

Microalgae present a general theoretical maximum photosynthetic efficiency of around 12% on the total solar radiation (Tredici, 2010; Williams and Laurens, 2010). This maximum efficiency is almost three times as high as the one for higher plants with C3 metabolism (e.g., wheat, oat, rice) and twice as much as the one for plants in C4 (e.g., maize, sugarcane)(Williams and Laurens, 2010). In practice, however, between one-tenth and one-third of this maximum efficiency is measured in large-scale PBRs (FAO, 2010; Williams and Laurens, 2010) as the optimal conditions cannot be maintained over time in an outdoor reactor. These conditions are the ones allowing to reach and maintain a luminostat, which is the state under which growth is only limited by the available light and all photons are absorbed by the culture while no cell is in the dark (Pruvost et al., 2020; Takache et al., 2010). The perfect conditions should also minimize photosaturation which is the decrease of photosynthetic efficiency under high irradiance which can quickly lead to photoinhibition, involving damage to the photosynthetic system and death of the cell (Carvalho et al., 2011).

In addition to these photobiological aspects, stable and efficient microalgal production should provide the right temperature, nutrients, and gas gradients within the culture, and fight potential contamination by maintaining high biomass concentration (Molina et al., 2019). Detrimental shearing stress on the cells due to the water flow implemented in the reactor should also be avoided (Wang and Lan, 2018; Yatirajula et al., 2019). The distinct requirements and sensitivities of different microalgal strains have been reported (Barra et al., 2014; Butterwick et al., 2005; Richardson et al., 1983; Wang and Lan, 2018) and modeled (Slegers et al., 2013, 2011). The combination of these challenges, both photonic and biological, entails several trade-offs regarding the techno-operational setup of a reactor for a specific location, season, and strain. Mata et al. (2010) designate the strain and location-specific solution to these trade-offs as the “photobiological formula” that must be found by the bioengineers developing the PBR.

2.2.3. MODELING AND ENVIRONMENTAL IMPACTS OF MICROALGAL PRODUCTIONS

2.2.3.1. Modeling microalgal growth

Due to little primary data from real-world microalgal productions at a commercial scale but continuous interest from the scientific and industrial worlds, important efforts have been made to be able to predict microalgal growth and anticipate achievable yields. In their reviews, Béchet et al. (2013) and Darvehei et al. (2018) list at least forty different mathematical models aiming at predicting microalgal growth under different conditions. These models are mainly kinetic, which means that they aim at describing the temporal evolution of the biomass concentration with differential equations. A large share of these models applies to indoor controlled conditions and the reviews' authors highlight that most models are not validated with outdoor data on large-scale reactors. The simplest models only consider the influence of the average light irradiance on the growth rate (Bordel et al., 2009; Molina Grima et al., 1996, 1994). Others consider local light intensities to predict the growth rate at every point within the culture (Bosma et al., 2007) and need to model the light travel and absorption which adds complexity (Fernández et al., 1998; Pruvost, Cornet, Goetz, 2012; Slegers et al., 2013, 2011). The most comprehensive and complex models account for the joint effect of light, nutrient, and gas gradients, pH and temperature (Béchet et al., 2013; Butterwick et al., 2005).

The other type of modeling to predict microalgal growth is a thermodynamic approach which does not predict the growth rate at the cell level but considers the energy balance and the productivity at the cultivation system level. It can be considered as a top-down approach as it starts by assuming a composition of the biomass and its associated energetic content, accounts for the biological losses happening during photosynthesis, and estimates productivity based on the energy input from the incident solar irradiance. This approach, which requires assuming which percentage of the theoretical maximum productivity will be achieved, is commonly used for estimating the productivity of large-scale systems over specific periods and locations (Skarka, 2012; Williams and Laurens, 2010).

2.2.3.2. Modeling microalgal productions and their associated environmental impacts

Among the studies which cannot rely on primary data to study the cost and/or the environmental impacts of microalgal productions, many make assumptions regarding the productivity without necessarily specifying the strain composition, the location or the season (Brentner et al., 2011; Lardon et al., 2009; Smetana et al., 2017; Stephenson et al., 2010). Schade and Meier (2020) use a simplified version of the previously exposed thermodynamic approach to estimate the productivity of *Nannochloropsis sp.* in a vertical tubular PBR.

Only two LCA studies using a kinetic model for microalgal productivity were found in the literature. Powers and Baliga (2010) use a Monod model considering light as the growth substrate and estimate the average light intensity in the reactor according to the reactor geometry, location, weather, and time of the day. Recently, Duran Quintero et al. (2021) use in their LCA the model for maximal productivity developed by Cornet and Dussap (2009) and Pruvost et al. (2012) consisting of an analytical solution of growth kinetic equations. They also corrected this model by including the influence of the culture's temperature.

In the LCA studies relying on models, the energy and material consumptions are estimated using thermodynamic and fluid dynamic equations (Duran Quintero et al., 2021; Onorato and Rösch, 2020; Powers and Baliga, 2010). In particular, Schade and Meier (2020) propose a detailed parameterized LCA model which estimates the amounts of materials and energy needed for microalgae cultivation.

The LCA studies consider different strains, cultivations systems, functional units and locations. Studies based on primary data at large or pilot scale exist for *Haematococcus pluvialis* (Jawahar et al., 2016; Pérez-López et al., 2014a), *Nannochloropsis sp.* (Pérez-López et al., 2017) or *Tetraselmis suecica* (Pérez-López et al., 2014b). Other works model strains such as *Arthrospira platensis* (Duran Quintero et al., 2021; Smetana et al., 2017), *Phaedactylum tricornutum* (Porcelli et al., 2020), Chlorella vulgaris (Collet et al., 2011; Lardon et al., 2009; Stephenson et al., 2010; Zaimes.G and Khanna.V, 2014) or consider generic strains (Baliga and Powers, 2010; Brentner et al., 2011; Clarens et al., 2011; Kadam, 2001).

Results vary largely depending on the functional unit, the cultivation system and the modeling assumptions. The studied scenarios can be substantially different, ranging from indoor thermoregulated reactors for specific compounds such as astaxanthin (Onorato and Rösch, 2020) to open PBR for bioenergy purposes (Collet et al., 2011). Grierson et al. (2013) report results as low as -222 kg CO_2-eq. per ton dry weight for the PBR cultivation of *Tetraslemis chui* by assuming permanent biogenic carbon sequestration after pyrolysis of the microalgal biomass. Without assuming this permanent sequestration, a study relying on primary data (Porcelli et al., 2020) estimates the global warming impacts at 300 kg CO_2-eq per kg dry weight for *Phaeodactylum tricornutum* in an indoor bubble column. Pérez-López et al. (2017) report 214 and 514 kg CO_2-eq. per kg dry weight of *Nannochloropsis sp.* In a thermoregulated outdoor tubular reactor respectively during summer and winter in the Netherlands. Schade and Meier (2020) report an impact score of 1.7 kg CO_2-eq. per kg of dried biomass while relying on models and assumptions for an upscaled version of the same technology but without considering the need for continuous thermoregulation.

Overall, the diversity of scenarios makes comparisons between the impact scores across studies mostly irrelevant without previous harmonization such as proposed by (Tu et al., 2018) for microalgal biofuels. A common feature found across studies is the high energy consumption associated with PBR and water pumping, mixing, and thermoregulation compared to ORP.

2.3. FINFISH FARMING HEALTH-RELATED ISSUES AND ENVIRONMENTAL IMPACTS

2.3.1. NATURE AND EFFECT OF FINFISH HEALTH-RELATED ISSUES

Health issues in finfish farming are very diverse and range from endoparasites and ectoparasites (e.g., sea lice) to bacterial or viral disease outbreaks (Bang-Jensen et al., 2019; Jansen et al., 2012; Rigos et al., 2020). This diversity of causes leads to two main outcomes which are fish mortality and increased Feed Conversion Ratio (FCR) and growth rate. The increase of the FCR can be due to mortality if the FCR is calculated as *feed input/live fish output* but the biological FCR defined as *feed input/(live +dead fish output)* is also affected by diseases and reflects the metabolic and anabolic efficiency of the fish. Viral outbreaks in finfish farms are particularly catastrophic and often require the slaughtering of the whole stock and impose a break in production (Bang-Jensen et al., 2019). In 2021, Denmark witnessed its first outbreak of viral infectious hematopoietic necrosis (IHN), leading to halted production in ten Danish trout farms (EURL, 2022). Limiting viral threats to finfish farming relies on vaccine research and effective hygiene management (Miccoli et al., 2021; Muktar and Tesfaye, 2016).

Although viral outbreaks are highly lethal, the majority of production losses are often attributed to chronic biological FCR increase and routine mortality. For instance, in Indian carp production in Bangladesh, it was estimated that 78% of economic losses associated with parasitic diseases were due to fish growth reduction, with only 11% attributed to mortality (Monir et al., 2015). While these findings may not directly translate to European finfish farming, they emphasize that diseases pose a continuous pressure on production, requiring comprehensive health management and incurring additional costs for treatments and hygiene.

On a global scale, it must be stressed that global and European aquaculture production trends and markets are different and adopt different health management systems. Europe banned the non-therapeutic use of antibiotics in the early 2000s and limited its main use to hatcheries, thus promoting a prophylactic use of vaccines and biosecurity measures (Done et al., 2015). For instance, Norway has reduced its antibiotic use to 0.39g per ton of salmon while Chile's use still amounted to 500g per ton in 2016 (Brun 2016). Brun (2016) therefore considers that "D*ue to the low sales, it is unlikely that any new antibacterial veterinary medical products will be marketed for farmed fish in Norway in a foreseeable future."* This statement further illustrates a new paradigm for health management in finfish farming based on vaccines, immunostimulants, vitamins, plants secondary metabolites, humic substances, prebiotics, probiotics, postbiotics, parabiotics and symbiotics (Assefa and Abunna 2018; Lieke et al. 2020)**.**

2.3.2. ENVIRONMENTAL IMPACTS OF FINFISH FARMING AND ASSOCIATED HEALTH-RELATED ISSUES

The environmental impacts caused by finfish farming have been studied in more than 65 LCAs reviewed by three recent studies (Bohnes et al., 2019; Bohnes and Laurent, 2019; Philis et al., 2019) which cover various fish farming systems. Special interest is commonly given to global warming impacts and eutrophication and acidification impacts due to the release of nutritive elements in the surrounding water bodies, either in freshwater or in the sea. For salmonid productions, which constitute most of the European finfish production in current volumes and trends (European Environment Agency, 2021) global warming impacts were shown to range (Philis et al., 2019) from 1157 kg CO_2-eq (d'Orbcastel et al., 2009) to 13622 kg CO_2-eq. per ton of fish (Dekamin et al., 2015). Other impact categories typically show the same variability of results, which can be mostly explained by different cultivation systems divided into open and closed land-based or sea-based systems. These systems feature different environmental profiles and hotspots. Closed land-based systems offer lower direct emissions of nitrogen and phosphorus to the surrounding water bodies thanks to filters but will often feature higher electricity consumption due to pumps and other pieces of machinery. Except for highly recirculating and energy consuming land-based closed systems (RAS) (Ayer and Tyedmers, 2009; Philis et al., 2019; Samuel-Fitwi et al., 2013), the feed consumption remains the main environmental hotspot which can reach up to more than 90% of the impact for impact categories (d'Orbcastel et al., 2009; Samuel-Fitwi et al., 2013). A low, i.e., performant FCR is therefore tendentially associated with lower impact values (d'Orbcastel et al., 2009) for a specific production system but this observation does not hold across systems and studies (Philis et al., 2019).

The reviews (Bohnes et al., 2019; Bohnes and Laurent, 2019; Philis et al., 2019) highlight the lack of consideration for health-related issues in the finfish LCA studies, which for instance can rarely rely on detailed LCA data for chemotherapeutics. Recent studies start addressing this gap. Philis et al. (2021) assess the environmental impacts of different biological delousing treatments for salmon production. The contributions of these treatments, involving the use of cleaner fish, were shown insignificant for the studied impact categories (<1%). The study did not consider the different treatments efficiencies and their effect on salmon mortality which would have enabled a comprehensive comparison of different health management options. Cristiano et al.(2022) assess different options to valorize sludge and dead fish in a modern Norwegian smolt farm from a Life Cycle perspective. The technical and biologicals aspects of the valorization of finfish farm waste via anaerobic digestion or drying for direct field application are being increasingly studied (Brod et al., 2017; Estevez et al., 2022; Kafle et al., 2013; Mirzoyan et al., 2010; Wu and Song, 2021). Besson et al. (2014, 2016) provide a parametric model to study how the life cycle impacts are affected by the genetic improvement of the biological FCR and growth rate in an African catfish farm. These studies precisely identify how modified growth rates and

FCRs differentially affect the farm operation and the associated impacts depending on the factors limiting the current production. If the fish density in the tanks is the limiting factor, decreasing the FCR only helps to decrease the amount of feed required per kilogram of fish. However, it does not reduce the amount of other production inputs needed. On the other hand, if the nutrient discharge in the environment is the limiting factor, improving the FCR also reduces the requirement for all other inputs.

The report (Just Economics 2021) assesses the economic opportunity cost that can be associated with mortality in salmon farms, i.e., which cost could be avoided if mortality was tackled. They also assess the cost of lice treatment and the environmental impact of the wild stock depletion due to lice spreading from fish farms. This economic opportunity cost of mortality is calculated by multiplying the lost biomass by the salmon price and amounts to 15 billion US dollars across Canada, Norway, Chile and Scotland over the period 2013-2019. Following the same logic, the environmental opportunity cost of mortality could be calculated by multiplying the amount of losses by a single emission factor associated with the production of 1 kg of commercial-size salmon. Such methodology would fall short in thoroughly examining these impacts as a more comprehensive life cycle assessment (LCA) approach could accomplish. In particular, it is problematic to use a single emission factor, calculated for the commercial fish size, to multiply the lost biomass consisting of fish of various sizes.

3. RESEARCH DESIGN

3.1. RESEARCH GAPS

The general research gap that this work will address is the ex-ante assessment of an explorative technological concept that has not been assessed before. While scientific knowledge and models exist regarding the environmental impacts associated with microalgal production and finfish farming, a potential synergy between both has not been studied yet. Assessing the environmental impacts of such future potential synergy requires addressing multiple additional research gaps that mainly stem from the ex-ante nature of the assessment.

First, the early stage of R&D imposes that a large number of configurations must be modeled, including different potential strains, reactor setups, locations, bioactive effects of the compound on the fish etc. Estimating the impacts of very diverse configurations requires highly parameterized LCA models. These models should also be comprehensive and include most of the processes, even the ones that remain rarely included in current LCA studies because they only consider specific configurations in which some processes can be neglected or assumed unnecessary. For instance, thermoregulation of microalgal photobioreactors has rarely been assessed in LCA studies, while it is needed for many strains, particularly when targeting the stable production of specific compounds (Barra et al., 2014; Beardall and Raven, 2012; Ras et al., 2013). Similarly, diverse strains imply diverse microalgal biomass compositions which imply diverse functional properties and uses for the co-produced biomass. The fate of microalgal products must therefore be modeled more precisely and with more options than in the existing studies proposing substitutions or allocation methods for co-produced microalgal biomass (Onorato and Rösch, 2020; Pérez-López et al., 2014b).

Microalgal production in general can still be considered an emerging technology because the production volumes remain low (European Union, 2018; García et al., 2017) and the cultivation means and processes are still being studied, improved and developed. Existing LCA studies generally focus on one specific strain in one or a few locations with primary data or modeling assumptions. While several studies consider upscaled, commercial-size production (Brentner et al., 2011; Schade and Meier, 2020), none model the next step of technological development which is the integration of the technology into a market and its deployment over a potentially large territory. To achieve a comprehensive assessment of the anticipated environmental impacts, it is essential to model production mixes comprising multiple microalgae production plants that supply the same products from diverse locations, as it is already the case in the existing European microalgal sector (Araújo et al., 2021). This is particularly needed as microalgae are photosynthetic organisms that are highly dependent on thermal and irradiance conditions.

While uncertainty has been assessed in microalgae LCA studies (Brentner et al., 2011; Pérez-López et al., 2018; Tu et al., 2018), no work includes an ex-ante perspective that would consider the uncertainty associated with the long and uncertain upscaling process that brings a specific microalgal strain discovered in a laboratory to commercial production in outdoor photobioreactors. The knowledge of this uncertainty could be useful for any project to anticipate the impact of microalgal products before the techno-operational setup to cultivate a strain is developed.

Regarding the methodological and theoretical aspects, although the ex-ante LCA literature partially recognizes various types of uncertainty, previous studies have seldom addressed the level of incertitude that this project aims to encompass. Specifically, accounting for all the possible configurations resulting from a mere technological concept based on bioprospecting has never been done. This requires new theoretical and methodological tools to consider different levels of incertitude. The current literature usually focuses on future technologies for which the functions, and locations are assumed as defined and not included as part of the indeterminacies when modeling the environmental impacts. Discrete scenarios for the future are commonly used in prospective/ex-ante LCA studies (Langkau and Erdmann, 2021; Thonemann et al., 2020; Vandepaer et al., 2020), but there is a pressing need to transition from considering mere possibilities to probabilities in order to provide valuable insights for decision-making. This shift has been gaining attention, as exemplified by the work of Blanco et al. (2020) who assign probabilities to scenarios in the ex-ante life cycle assessment of photovoltaic systems. In line with this shift, the existing literature does not directly address pertinent questions such as "should we even attempt to develop this technology?" despite their significance in relation to the precautionary principle (Owen et al., 2013; Van Asselt and Vos, 2006). These questions are particularly relevant in the context of fast, purpose-driven technological planning under emergency.

Overall, there is a remaining potential for building bridges between ex-ante LCA research and tools and concepts for decision-making under deep uncertainty. This requires further expanding the object of study for LCA from specific technologies in the future to technological concepts including their chaotic journey toward a final commercial-size configuration in the future. A specific research gap lies when uncertainty applies and prevents the definition of probabilities for the models' parameters while there is still a need for making educated decisions based on quantitative indicators such as probabilities of impact.

Regarding health issues in finfish farming, a few LCA studies included aspects related to health and growth performance such as additional impact of cleaner fish in salmon farms (Philis et al., 2021), modulation of the FCR and growth rates (Besson et al., 2016), or valorization of dead fish via anaerobic digestion (Cristiano et al., 2022b). However, to our knowledge, no study could provide detailed and disaggregated inventories of fish farms to be able to assess the effect of losses happening at distinct timings along the rearing process. This detailed modeling is necessary to anticipate the effect of new bioactive microalgal compounds on the farm's impact and will also propose an LCA approach to calculate the environmental opportunity cost of mortality in fish farms. So far, this opportunity cost was only assessed economically and without life cycle modeling (Just Economics, 2021) which hinders the capacity to decide on how to prioritize actions regarding health issues in finfish farming.

Finally, LCA studies both on finfish and microalgae production are largely dominated by attributional LCA modeling with only one consequential LCA study for finfish production (cf. Methods 4.1 for consequential LCA and attributional LCA) (Philis et al., 2019; Samuel-Fitwi et al., 2013). No consequential LCA was found for microalgal products even though substitution is sometimes performed for co-products exclusively in the foreground (Onorato and Rösch, 2020). There is therefore a pressing need for a more consequential perspective on these systems as consequential LCA specifically aims at assessing the consequences of actions (cf. 4.1), which should eventually support decision-making.

3.2. RESEARCH QUESTIONS AND HYPOTHESES

Based on the objectives and the identified research gaps, this PhD work was driven by the following **main research question**

Which environmental performance and impacts can be expected regarding the use of yet undiscovered microalgal compounds for health management in finfish farming?

This broad research question is motivated by the following **main hypothesis**:

Handling of incertitude combined with stochastic ex-ante LCA approach can provide relevant estimates for the environmental impacts of the technological concept.

The main research question is fundamentally explorative and no hypothesis a priori is formulated regarding the expected impacts.

Addressing this research question can only be done by considering each component of the technological concept and its associated incertitude separately in a step-wise approach before combining models and results. The main research question can therefore be decomposed into three questions with associated hypotheses.

The first research question is focused on the microalgal production part and considers the impacts of an increase in demand for any yet-undiscovered microbial compound, without considering its use in finfish farming.

Research Question 1

What will be the environmental impact associated with the European production of a currently bioprospected microalgal compound?

Hypothesis: *The impacts associated with the future production of a yet-undiscovered microalgal compound in Europe can be anticipated via probability distributions. These distributions can be obtained with a stochastic LCA approach based on process simulation which accounts for geographic, biological and techno-operational dimensions.*

The second research question addresses the finfish farming component of the future synergy and aims at understanding how a bioactive microalgal compound could improve the environmental performance of a finfish farm.

Research Question 2

How does suboptimal biological performances in fish farms affect the life cycle environmental impacts of fish production?

Hypothesis: *Mortality and poor feed conversion ratios have distinct influences on the life cycle impacts regarding the timing of their occurrence during the fish production cycle.*

The third and last research question arises when all models have been built and all indeterminacies in the two different systems have been acknowledged and assessed. The combination of the distinct models, on microalgal production and finfish farms, adds a new layer of incertitude that requires a novel approach to make decisions under conditions of deep uncertainty, i.e., under Stirling's uncertainty.

Research Question 3

How can ex-ante LCA be used for decision-making regarding deeply uncertain technological concepts?

Hypothesis: *A distinction between risk and uncertainty is necessary to define meaningful probabilities in the output of ex-ante LCA models and provide assistance to decision-making*

3.3. A STEP-WISE APPROACH TO EMBRACE ALL INDETERMINACIES

As previously introduced, this PhD work followed a step-wise approach to progressively encompass the different indeterminacies regarding the future technology that will emerge from the exploration of the technological concept.

The scientific articles produced during this work follow this step-wise approach and each article focuses on additional indeterminacies while addressing specific research gaps and providing models and methods for other uses. The succession of articles can thus first be represented from a perspective focused on the indeterminacies and associated types of incertitude, as shown in Figure 2. The succession of articles can also be considered from a product system and modeling perspective, where each article focuses on a part of the product system, as shown in Figure 3.

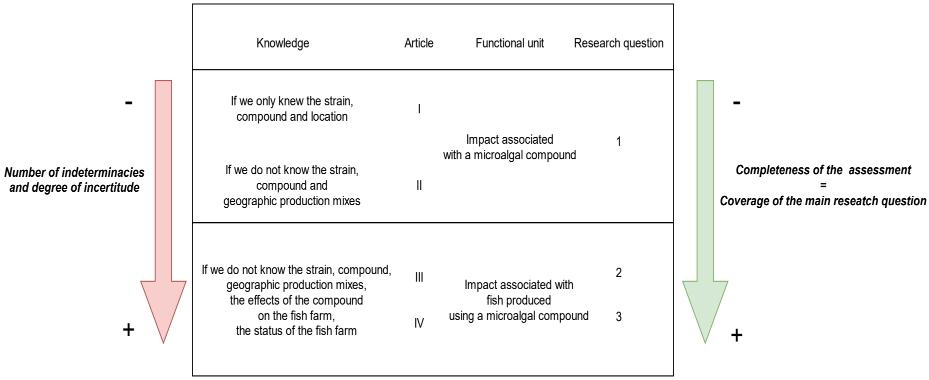

Figure 2: Incertitude perspective on the research project

Article I starts by proposing a parameterized cultivation simulation and LCA model for microalgal cultivation which can accommodate different microalgal strains and compounds in any chosen location. We here study the indeterminacies associated with the techno-operational setup of a commercial scale vertical tubular PBR if we knew some biological characteristics about the strain and the compound that were to be produced. Article II builds on top of this first model to study the environmental impact of a whole European production mix supplying a microalgal compound, acknowledging that the production mix and the microalgal strain and compound remain unknown. Article III focuses on evaluating the environmental opportunity cost of suboptimal biological performances in finfish farms to be able to model the consequences of new health-management solutions on the environmental impacts of fish production. This is done with a new parameterized model that can be used to simulate the use of bioactive compounds affecting the farm's biological performance.

Finally, article IV considers the whole product system (cf. Figure 3) and combines the previously built models to assess the future impact of fish produced with or without the yet undiscovered microalgal compound. The level of incertitude here considered becomes very high and the article proposes a new algorithmic and computation procedure for decision-making assistance under the joint presence of risk and uncertainty. In this article, we advocate that decisions in technological planning should be grounded in the anticipation of a successful technological development from an environmental standpoint. Such success can be defined as achieving an impact lower than a certain threshold or surpassing the environmental performance of an alternative projected into the same future. Although uncertainty hampers the calculation of success probabilities, the procedure presented in Article IV enables the comparison of this probability against a predetermined threshold, which serves as the minimum requirement for investing time and resources in further exploring a technological concept. At the same time, the procedure allows finding conditions regarding what should be predicted about the most uncertain parameters to ensure a certain probability of success. In this article, we apply the procedure on the AquaHealth technological concept which concludes the ex-ante assessment.

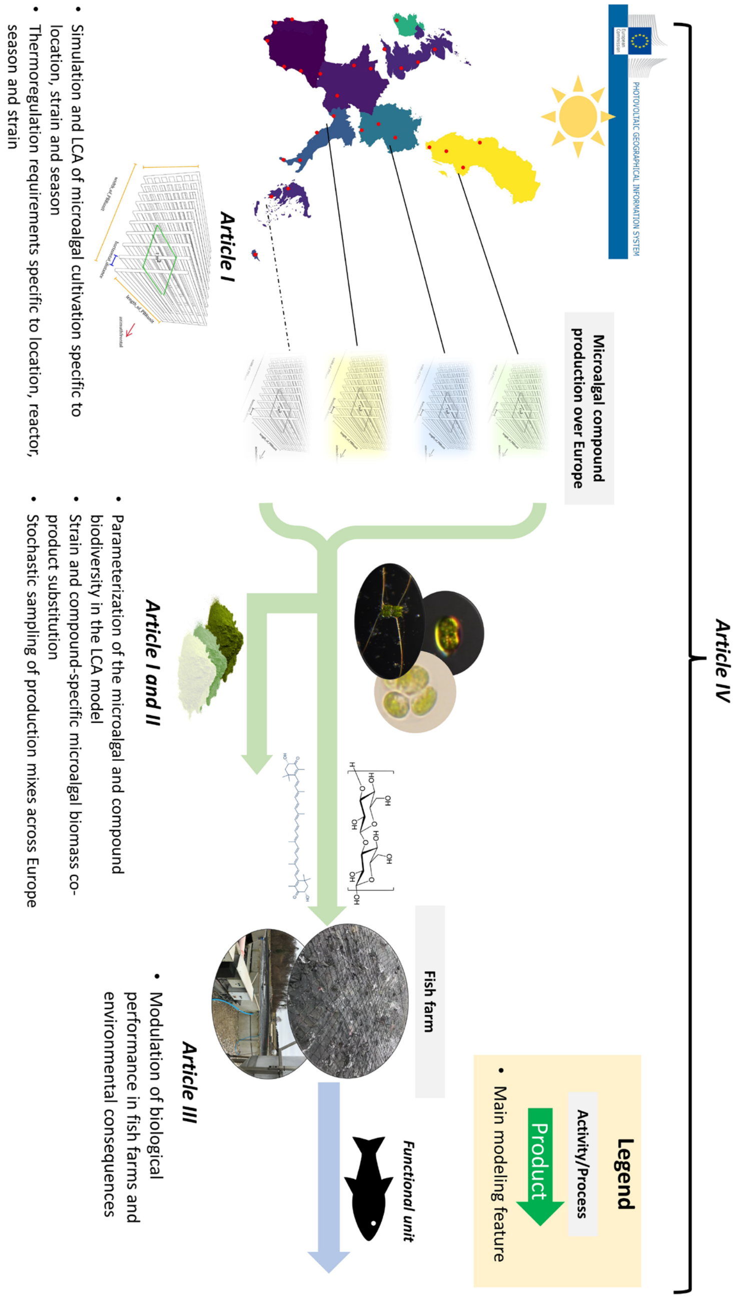

Figure 3: Product system and modeling perspective on the research project. (personal pictures)

4. THEORIES AND METHODS

This section presents the main theories and methods mobilized within this PhD work. Table 2, at the end of the section, summarizes the methods and tools used in each article.

4.1. INDUSTRIAL ECOLOGY AND LIFE CYCLE ASSESSMENT FRAMEWORK

Industrial Ecology (IE) is a multidisciplinary scientific domain examining the interactions between human activities and the biophysical world, with the aim of understanding how to limit anthropic impacts on humans and non-humans (Allenby, 2006). The notion of "Ecology" stems from a direct analogy with this field as IE illuminates how anthropic/industrial and ecological/biological worlds share common traits. Both worlds feature a high system complexity involving a form of metabolism with flows of products, energy and wastes sustaining the stability of ecosystems, homeostasis in living beings, or the development and well-being of human societies (Allenby, 2006). Due to its broad and polymorphic object of study, IE is an evolving field whose boundaries are not strictly defined. IE includes qualitative and quantitative modeling methodologies such as Mass Flow Analysis (MFA), System Dynamics (SD), input-output analysis (IOA), Environmental Impact Assessment (EIA) and Life-Cycle Assessment (LCA).

Among these tools, this PhD work uses exclusively LCA as a standardized (ISO 14040 to 14044) and systematized modeling method to quantify the environmental impacts of products and services over their life cycle. LCA has been acknowledged for its comprehensiveness and its ability to compare product options, and inform decision-making for policies, consumers'purchases, or R&D choices during the development of new products. The main methodological steps of an LCA, thoroughly described in the existing literature (Ekvall and Weidema, 2004; Hauschild et al., 2017; Heijungs and Suh, 2002) include goal and scope definition, inventory analysis (LCI), impact assessment (LCIA) and interpretation of the results.

The LCI phase and the identification of the relevant processes and emissions to be linked to the functional unit, for which the impacts are assessed, are what separates two main types of LCA: attributional LCA (ALCA) and consequential LCA (CLCA). The fundamental differences between the two approaches lie in the questions they aim at answering and in the way they allocate social responsibility for impacts (Weidema et al., 2018). ALCA aims at allocating a share of the total emissions of human activities to a product or service (Schaubroeck et al., 2021; UNEP, 2011; Weidema et al., 2018). Determining this share is done according to normative choices as in the modern highly-connected economy, almost all activities are linked by products.

ALCA therefore uses factors based on mass, energy or monetary values to allocate the economic and environmental flows to specific products when activities have different co-products. In an ALCA model, the activities linked to the functional unit are those which are physically or economically linked in the current studied system (Schaubroeck et al., 2021; UNEP, 2011).

On the other hand, CLCA assesses the consequences of decisions such as the purchase of the functional unit and the resulting increase in demand (Weidema et al., 2018). The activities are linked to the functional unit to the extent that they change as a result of the decision which sends a signal of increase in demand to the economic system (Ekvall and Weidema, 2004; Schaubroeck et al., 2021). This implies that only activities and technologies that can increase their production as a result of the increase in demand are modeled in the marginal mix supplying products to provide the functional unit (Pizzol and Scotti, 2017). Regarding the modeling of co-products, the impacts of a multifunctional activity and all its economic inputs (products from other activities) are allocated to the main product for which the activity would increase production to answer an increase in demand. The dependent co-products of this activity are consequently produced and will avoid the production of functionally equivalent products from marginal suppliers elsewhere. The impacts of this avoided production are thus subtracted from the total impact associated with the main product. The dependent co-products are “constrained” and an increase in demand for these products will not result in increased production. The CLCA product system instead includes the marginal supplier of a functionally equivalent product. Additional details on CLCA methodology and specific cases, are available in the literature (Ekvall and Weidema, 2004; Schmidt and De Rosa, 2020; Thonemann and Pizzol, 2019).

In this PhD work, CLCA was preferred over ALCA for its focus on consequences and chains of physical and economic causalities rather than normative choices. This focus was deemed more suitable to inform decision-making and deal with the multifunctionality of microalgal production. In all simulated configurations, the high-value bioactive microalgal compound was always considered as a main product, while the co-produced biomass substituted products based on its parameterized composition.

All the LCA models built in this work are parameterized which means that they are not representing one specific configuration of a product system with fixed amounts of the different flows. Instead, the studied product system and the flows change according to the mathematical dependencies drawn between the input parameters.

The fish farm parameterized LCA model presented in Article III was built thanks to primary data collection in Italian and Danish trout farms.

4.1. EX-ANTE LCA AND POST-NORMAL SCIENCE

Ex-ante LCA constitutes a specific application of LCA, attributional or consequential, focusing on technologies and products in the future. While the precise terminology still varies across studies, a recent homogenization attempt (Arvidsson et al., 2023) proposed that prospective LCA should designate any study for which the studied product system is in the future, including currently mature technologies. Ex-ante LCAs are prospective and focus exclusively on technologies that are currently at a low maturity level, which is the case of the technological concept studied within this work.

Ex-ante LCA has to deal with challenges such as primary data scarcity, anticipating the technical performances once upscaled and deployed (Faber et al., 2022; Tsoy et al., 2020), and projecting the technology into future scenarios for the surrounding market, alternatives, and biophysical world (Thonemann et al., 2020). Ex-ante LCA typically aims at informing decision-making at an early stage, comparing a future technology to alternatives, and guiding R&D toward better eventual environmental performance (Thonemann et al., 2020). While it has been stressed that ex-ante LCA does not predict the future (Cucurachi et al., 2018) but explores different possible scenarios, it must be acknowledged that when probabilities are assigned to parameters and scenarios, ex-ante LCA does constitute a projection into the future that could be defined as a "forecasting exercise". More precisely, this forecasting exercise can be interpreted as such in the light of the definition of probability related to reasonable expectations one has regarding the realization of an uncertain event (cf. State of the art 2.1).

Due to this "forecasting exercise", or at least to the exploration of futures according to limited knowledge, ex-ante LCA falls within the scope of post-normal science, defined by Funtowicz and Ravetz (1993) as the domain of science for policy when stakes and uncertainty (in its broad definition) are high and debated and decisions are made under emergency (Funtowicz and Ravetz, 1993). The distinction between different types of incertitude previously described (cf. State of the art 2.1) constitutes a conceptual pillar of post-normal science and its "*ethics of quantification*"(Saltelli and Ravetz, 2021). Overall, these ethics promote intellectually honest model-based support for decision-making. They aim at avoiding the reduction of a complex problem involving complex socio-economic interactions, subjective values, and relations of power into a mystifying mathematical exercise to please technocratic interests (Saltelli and Ravetz, 2021; Stirling, 2010). The ethics of quantification were summarized in a collaborative manifesto (Saltelli et al., 2020) and include the thorough and honest appraisal of uncertainty and sensitivity in the models, the reporting of the scope and frame of the model to avoid risky extrapolation beyond the model's purpose, the consideration for the potential consequences of communicating results in a certain way and the acceptance and humble reporting of ignorance. These rules should naturally apply to ex-ante LCA.

4.2. TREATMENT OF INCERTITUDE IN MODELS

4.2.1. "UNCERTAINTY" ANALYSIS

The term "uncertainty analysis" is commonly used to designate the methods presented in the following paragraph and refers to the broad acceptance of "uncertainty". Using Stirling's framework, these methods could be more accurately described as "risk and uncertainty analysis".

Two main types of approaches to incertitude in modeling can be singled out. The first type of approach focuses on identifying and studying the "possibles" by typically running a deterministic model in a set of discrete scenarios (Langkau and Erdmann, 2021; Thonemann et al., 2020; Vandepaer et al., 2020). These scenarios are defined a priori by the analyst, who will for instance model worst and best-case scenarios. A more advanced and sound approach to scenarios will involve multiple stakeholders and experts in a participatory scenario development exercise to generate a set of relevant and/or likely scenarios with associated narratives (Ash et al., 2010; Franco and Greiffenhagen, 2018; Sedlacko et al., 2012).

This scenario approach alone is limited when the number of indeterminate parameters grows which is typically the case in a background LCA database containing hundreds of thousands of flows with imperfectly known amounts. It also fails at supporting decision-making with the rare exception of cases where the conclusion regarding the original question that can be drawn from the results is the same in all scenarios. In other cases, this mere assessment of the possibilities does not inform about the most rational decision to make (Blanco et al., 2020).

The second type of approach assists decision-making by studying the "probabilities" instead of mere "possibilities" (Helton et al., 2010). Instead of single deterministic values, indeterminate parameters are assigned probability distributions that can be shaped thanks to collected samples of values for this parameter (Heijungs, 2020), or expert elicitation (Morgan, 2014; O'Hagan, 2019). Note that probabilities can be applied to any type of parameters and not exclusively to "quantity parameters". For instance, parameters could determine model structures or scenarios (e.g., indeterminate marginal supplier choice, indeterminate downstream processes) and switch between options stochastically according to probability functions (Blanco et al., 2020). The pedigree matrix approach was proposed and implemented in LCA databases such as the ecoinvent one to convert qualitative measures of data quality regarding the values of parameters into quantitative probability distributions (Ciroth et al., 2016; Weidema and Wesnæs, 1996).

Once probability distributions are established in the input, they are propagated to the output, typically to obtain probability distributions of impact scores in LCA. This propagation can be performed analytically (Groen et al., 2014; Heijungs, 2020; Von Pfingsten et al., 2017) using the mathematics of probability theory but this exercise quickly gets constrained when the input probability functions differ from classic normal, lognormal or uniform functions. Analytical propagation becomes practically impossible when the indeterminate parameters are not exclusively LCA flows but process simulation parameters that could for instance be used in differential equations. On the grounds of these shortfalls, stochastic propagation of uncertainty via Monte Carlo iterations is by far the most used propagation technique in LCA (Groen et al., 2014; Mahmood et al., 2022). This technique consists of computing a large number of random draws within the input probability distributions and calculating the model output for each iteration. The obtained frequencies of output values constitute the output probability distribution and allow estimating statistical information for decision-making such as the mean and standard deviation of the value of interest.

It is important to note that, although aleatory uncertainty, sometimes described as variability, is also designated as “stochastic uncertainty”, both epistemic and aleatory uncertainty can be represented with probability distributions and propagated with stochastic computing approaches.

While Monte Carlo simulations rely on pseudo-random sampling, specific sampling schemes such as Latin hypercube sampling (Groen et al., 2014) and quasi Monte Carlo sampling including Sobol sampling allow faster convergence of the estimators and can save computational time (Kim et al., 2021). These alternative methods are commonly used for sensitivity analysis.

Monte Carlo and quasi Monte Carlo methods were used in all articles to propagate the different types of “uncertainty” (in its broad meaning).

4.2.2. SENSITIVITY ANALYSIS

Following the ethics of quantification previously exposed, uncertainty analysis should ideally be supported by sensitivity analysis to identify the indeterminate parameters responsible for most of the uncertainty lying upon the output of interest. A sensitivity analysis will thus help prioritize the efforts for knowledge refinement and data collection to reduce the uncertainty in the output of interest. In most cases, most of the uncertainty in the output is due to the propagation of a few parameters’ uncertainties only, while the uncertainty on some input parameters does not substantially affect the shape and spread of the output distribution (Douziech et al., 2021; Kim et al., 2021). On top of guiding research efforts to reduce uncertainty, sensitivity analysis will inform about the most influencing assumptions made in the model and thus participate in sound and nuanced decision-making assistance.

The simplest sensitivity analysis are local (LSA) one-at-a-time (OAT) sensitivity analysis, where local changes are performed for one input parameter at a time while the others remain fixed. The ratio between the change in the output value and the studied parameter indicates the sensitivity of the model to this parameter. OAT-LSA cannot account for the interactions between the parameters, assume model linearity, and study only local portions of the whole ranges of values that the parameters can take (Lo Piano and Benini, 2022; Mahmood et al., 2022). To palliate these weaknesses, Global Sensitivity Analysis (GSA) (Borgonovo and Iooss, 2016; Saltelli, 2005) studies the response of the model to a parameter over its whole possible value range while the other parameters' values are also changing. In this sense, calculating a Spearman correlation between the output of interest and an input parameter over the whole sampled space with all parameters being stochastically sampled (e.g., Monte Carlo simulations) is a simple form of GSA (Kim et al., 2021). A low Spearman correlation will indicate a low sensitivity of the output to the studied parameter.

Variance-based GSA is more powerful and provides additional insights into the role of interactions between parameters. Variance-based GSA relies on a decomposition of the variance observed in the output into a sum of terms involving the variance of each input and of the interactions between them (Saltelli, 2005). The first-order sensitivity index in a variance-based GSA measures the contribution to the output variance of an input parameter's variance, without accounting for its interactions with other parameters. The total order sensitivity index indicates the contribution of a parameter to the output variance while accounting for all its interactions with the other parameters. This total order sensitivity index estimated for a parameter Xi thus reflects the share of output variance that would remain if all parameters but Xi were fixed (Saltelli, 2002). Methods such as Fourier Amplitude Sensitivity Test (FAST) (Saltelli et al., 1999) or Sobol are widely used Variance-based GSA methods which can use quasi-Monte Carlo sampling schemes to speed up the convergence to the targeted estimators of sensitivity.

Variance-based GSA methods are "moment-dependent" as they focus on variance, which in statistical terminology is a "moment" of a distribution. Borgonovo introduced a moment-independent GSA method that considers the shift of the whole output distribution when the studied parameter is fixed to a certain value (Borgonovo and Iooss, 2016). To make the sensitivity analysis "global", the δ index measures the expectation of this shift over all possible values in a parameter's distribution. Borgonovo's δ index constitutes an intuitive measure of sensitivity which is equal to 0 if the output is completely independent of the studied input and 1 if it only depends on this input.

4.2.3. SCENARIO DISCOVERY

Decision-making exercises can often be simplified by searching conditions that would facilitate a certain outcome. Instead of defining scenarios a priori and running a model within their boundaries to study the value of the output parameters, scenario discovery uses algorithms to browse a purposely large input space looking for “boxes”, i.e., sets of intervals of values for the input parameters, associated with specific outputs of interest. For instance, scenario discovery was used to find under which conditions, i.e., in which boxes for the input parameters, a policy for energy planning in the US would lead to an unacceptably high economic cost (Bryant and Lempert, 2010). Scenario logics and narratives are built for the identified boxes post-analysis and the reflection and decision-making exercise are simplified. The search for boxes of interest within the input space can be performed with hill climbing algorithms such as the Patient Rule Induction Method - PRIM (Friedman and Fisher, 1999; Kwakkel and Jaxa-Rozen, 2016) which browses the space to find boxes with a high density of outputs of interest. PRIM iteratively discards parts of the output space to maximize a function which considers the density of outputs of interest in the remaining sample. To some extent, scenario discovery could be pictured as a multi-dimensional, model-independent, and stochastic version of a break-even analysis (Gear et al., 2018), which finds the threshold value on one parameter to achieve a certain output value.

By computationally exploring a very large scope of possibilities, scenario discovery avoids the drawbacks and challenges of expert elicitation when attempting to define scenarios or parameter probability distributions *a priori* (Bryant and Lempert, 2010; Morgan, 2014). These drawbacks mainly stem from the difficulty for a group of stakeholders, for example within a Participatory Scenario Development workshop (Ash et al., 2010; Franco and Greiffenhagen, 2018; Sedlacko et al., 2012), to summarize a complex problem and large scope of possibilities into three or four main storylines. Humans in general also feature poor skills in navigating between “probabilities” and “possibilities” and tend to overlook surprising but potentially impactful “Black Swan” events.

In fact, a Participatory Scenario Development Workshop gathering 8 experts and stakeholders in microalgal biotechnologies and fish health was organized and held as part of this PhD work. During three afternoons, the participants were guided according to established Participatory Scenario Development methodologies to co-construct plausible and insightful scenarios regarding the use of microalgal compounds for health management in European finfish farming. Four scenarios were identified based on combinations of high and low poles for the two drivers that were collectively chosen as the most important ones. We attempted to use these scenarios and run the parameterized models within these four main storylines. This exercise was quickly deemed hardly feasible and scientifically weak, because leaving too much freedom to the analysts (mainly myself) in the conversion of mostly qualitative scenarios into quantitative parameters and mathematic dependencies. From this observation emerged the idea of resorting to scenario discovery approaches instead.

A major asset of scenario discovery is that it alleviates the burden of having to define all dependencies between parameters, which can be hard to draw when considering all parameters a priori. Typically, one could model two problematic parameters A and B as independent, stochastically simulate a large output space and search for boxes associated with a successful outcome of any kind. If these boxes are all associated with high values for A and B, predicting a success would require assuming that there is a dependency between A and B and that a high value entails a high value for B and vice versa. If this dependency is considered unlikely, the probability of success will be low. If the boxes of success can be found in the same proportions for any combination of values for A and B, there is no need to know if A and B are dependent to evaluate the probability of success.

Scenario discovery and PRIM were integrated and adapted within the ENSURe procedure proposed in Article IV.

4.3. MODELING AND SIMULATION OF MICROALGAE PRODUCTIONS

Ex-ante LCA commonly faces the scarcity of primary data for industrial-scale productions. Process simulation is one of the most used methods to anticipate LCIs for the upscaled versions of the studied technology (Corona et al., 2018; Piccinno et al., 2016; Thonemann et al., 2020; Tsoy et al., 2020). Process simulation uses software and computing tools combined with equations from established physical and biological laws such as stoichiometry (Langhorst et al., 2022), thermodynamics for energy balances in industrial and biological systems or fluid physics for energy requirements associated with pumps and stirring (Piccinno et al., 2016). Specific software such as Aspen exist to simulate chemical processes and are used in ex-ante LCA to obtain upscaled LCIs for emerging technologies (Thonemann and Schulte, 2019).

In this PhD work, it was necessary to simulate microalgal cultivation to account for all potential locations and possible strains and compounds that may be discovered and successfully deployed at a commercial scale. While I aimed at exploring most of the scope of possibilities, I limited the assessment to outdoor vertical tubular PBRs considered most likely to be able to host any microalgal strain and maintain performant and stable cultivation conditions (Sforza et al., 2014). Open systems were dismissed due to their incapacity to provide stable contamination-free conditions necessary to ensure the stability and purity high-value microalgal compounds (Barra et al., 2014; Liu, 2017; Sforza et al., 2014). The extraction of the hypothetical compound was not modeled as the method and technology are too dependent on the exact nature of this compound. This nature and the potential extraction method arguably fall within the scope of “ignorance” within the Stirling’s framework.

Simulating microalgae cultivation typically requires considering the interactions between three main components : the biology of the strain, the location and associated climate and the techno-operational setup of the reactor. These interactions can be explicitly modeled with a clear distinction between what depends on the strain, the location, and the technology (Slegers et al., 2013). They are most often implicitly considered but hidden into aggregated parameters, for instance when assuming the energetic yield of a cultivation (Schade and Meier, 2020).

My research questions imposed to distinguish as much as possible the three main components of microalgal production. In Article I, we thus combined the parameterized LCA model from Schade and Meier (2020) with the more detailed decomposition of the theoretical maximum productivity from Williams and Laurens (2010). We added specific calculation modules to increase the detail level in the modeling of some processes (e.g. pumping, harvesting), adapted the parameterization to accommodate any microalgal compound and added a new simulation module that dynamically estimates the energy requirements associated with thermoregulation. This new model was directly connected to the geolocalized real-world climatic and irradiance data from the Photovoltaic Geographic Information System of the European commission (Huld and Mu, 2012).

As previously addressed in the state of the art (cf. 2.2) most LCA studies that resort to modeling of the process instead of primary data make assumptions regarding the achievable yields in specific determinate PBR setups. When considering the microalgal biodiversity and the ex-ante perspective, this assumption cannot hold anymore as the desired photobiological formula implemented in the reactor remains unknown. We thus used insights from more detailed kinetic models and real-world observations on actual productivities and challenges of microalgae cultivation to estimate the uncertainties, i.e., the probability distributions, regarding the techno-operational setup of the PBR that will yield a certain percentage of the strain-specific maximum productivity in a location.

Overall, our model for microalgal cultivation was conceived in Article I by anticipating the requirements and compromises associated with the research questions of the whole PhD work. The main requirements that guided the choice and building of the model are listed below:

- *Distinction between the influence of the strain and compound, the location, the reactor and the capacity of bioengineers to find the right photobiological formula.*
- *Granularity level allowing to accommodate most of the microalgal biodiversity and their compounds.*
- *Granularity level allowing to state about uncertainties on each parameter.*
- *Detail level allowing relatively "fast" computation of the simulation.*
- *Granularity level allowing to simulate the same strain in any location.*

- *Explicit dependence between biomass composition and productivity.*

The parameterized microalgae LCA model includes 70 parameters describing the strain and compound, the PBR setup, the location, and the physical constants. These parameters and their interactions are listed in the Supplementary Information of Articles I and II and include for instance the strain's optimal temperature and its thermal plateau, its diameter and density which will influence centrifugation energy needs, its lipid content and ash content, which will determine protein, carbohydrate contents. Some parameters are also associated with "scenario changes" such as the probability that the strain requires night thermoregulation or that the co-produced biomass has a certain fate. Distributions for the parameters specific to the strain and compounds were chosen according to available data on microalgal biodiversity.

4.4. MAIN ASSUMPTIONS

While the approach was explorative and aimed at considering a maximum of configurations, a few general assumptions were made and kept consistent across the articles. The assumptions underlying the findings and delimiting the scope of their application are outlined in Table 1. Furthermore, each article provides explicit descriptions of any additional minor assumptions about specific modules within the different models.

Table 1: Main assumptions made in this work

Assumption	Justification
The compound is produced in an outdoor thermoregulated vertical tubular PBR.	Widely used, performant system, could accommodate most strains. Open systems do not provide stable conditions for high-value compounds (Barra et al., 2014; Sforza et al., 2014; Williams and Laurens, 2010).
Photo-autotrophic microalgal cultivation, i.e., the source of energy is light, and the source of carbon is CO_2	Most common trophic mode. Common to all microalgae with the exception of depigmented strains and some taxa such as *Euglena* (del Campo et al., 2014; Neilson and Lewin, 1974; Ogbonna et al., 1998). No need for additional organic carbon source.
The bioactive effect on the fish is carried by a compound that constitutes a certain share of the microalgal biomass	This allows encompassing a multitude of scenarios from the ones where the compound is a powerful antibiotic that constitutes 0.5% of the biomass to those where the "bioactive compound" is a group of lipids representing 20% of the biomass.
The bioengineers in charge of developing the cultivation of the compound will find a photobiological formula delivering 30% of the theoretical strain-specific maximum energetical yield. Microalgal productions are optimized for areal productivity.	30 % is what is commonly achieved in existing productions (Hindersin et al., 2014; Williams and Laurens, 2010). Refer to discussion section 6.1 for further justification.
The compound is of high-value and is the main product of the microalgal production. The co-produced biomass is always the dependent co-product.	The economic viability of microalgal productions for bioenergy or feed is currently poor. New bioactive compounds are expected to drive the increase in production. If the microalgal compound was a dependent co-product, an increase in demand for this product would not result in the production of this compound.
Microalgal cultivation is assumed from April to September regardless of the location	Limiting thermoregulation needs and optimizing productivity. Same assumption as (Skarka, 2012) and (Schade and Meier, 2020).

4.5. COMPUTING TOOLS

This work extensively relied on the use of computational power to simulate a vast range of possibilities and study the sensitivity of models with tens or hundreds of variable parameters. The number of stochastic simulations of microalgal productions ranged from 9000 for uncertainty analysis and FAST GSA in Article I to more than 350 000 000 in Article IV. These millions of simulations were necessary within the ENSURe procedure to cover the entire uncertain space with 500 000 LCA data points. One point was associated with a compound production mix made of 5 to 35 locations for which microalgae cultivation was dynamically simulated over six months in tens of different techno-operational PBR setups. To perform such simulations, we used the flexibility, modulability and computational speed of Python combined with the LCA package Brightway 2 (Mutel, 2017). R was used for specific data manipulations. Most simulations were performed in parallel on remote instances with numerous CPUs. The consequential version of ecoivent 3.6 was used as background database in Article I and II and ecoinvent 3.8 was used in Article III and IV. ReCiPe 2016 midpoint impact categories were mostly used across the articles.

The codes allowing reproduction of the results and use of the models were shared in Github repositories whose links and DOI were displayed at the end of each article.

Table 2: Methods, tools and theories mobilized across the articles

Article	Specific methods and tools	Theories and scientific domains
I	• Parameterized dynamic process simulation • Parameterized CLCA • Optimization algorithm to determine the substitution triggered by the microalgal biomass • Uncertainty analysis • GSA (FAST)	Industrial ecology, Post-normal Science, LCA
II	• Parameterized dynamic process simulation • Parameterized CLCA • Uncertainty analysis • GSA (Sobol)	Industrial ecology, Post-normal Science, LCA
III	• Parameterized CLCA • Primary data collection in trout farms • Mass balance model for fish excretion and waste recovery • Uncertainty analysis • OAT LSA	Industrial ecology, LCA
IV	• Newly developed ENSURe procedure combining previous models, scenario discovery, and GSA (Borgonovo)	Industrial ecology, Post-normal Science, LCA

5. RESULTS

This section provides an overview of the main insights and methodological advances associated with this PhD work and the research articles. The results are presented in the same order as the articles, which follow the progressive encompassing of all indeterminacies in the assessment (cf. Figure 2 and 3).

5.1. ENVIRONMENTAL IMPACTS ASSOCIATED WITH THE PRODUCTION OF A YET UNDISCOVERED MICROALGAL COMPOUND

5.1.1. IMPACTS ESTIMATES IF THE COMPOUND, STRAIN AND LOCATION WERE KNOWN

Article I (Jouannais et al., 2022) introduces a new parameterized LCA model for the production of microalgal compounds and demonstrates its use in a scenario where the targeted compound is a lipid constituting 2.6% of the microalgal biomass and the cultivation takes place in Aalborg, Denmark or Granada, Spain. The strain is modeled as a generic one, with among other parameters, a thermal range from 20°C to 30°C in order to maintain a stable production at 30 % of the maximum energetic yield.

A large spread of the impact scores was observed regarding the impact scores due to the propagation of the uncertainty regarding the techno-operational setup of the PBR that will enable the production. We designated this uncertainty as “Techno-operational”. The uncertainty regarding the location and weather-dependent thermal coefficient ruling heat exchanges between the pipes and the air was also propagated. The impact scores were substantially lower when assuming cultivation in Granada compared to Aalborg. While cultivation in Granada without thermoregulation at night resulted in an average global warming impact of 537 kg CO_2-eq per kilo of compound, the worst scenario, in Aalborg with thermoregulation at night, reached an average of 3436 kg CO_2-eq (cf. Figure 4). This reflected the difference in productivity due to a lower irradiance in Aalborg, but also the high heating requirements in Aalborg. The energy needed for thermoregulation was indeed an environmental hotspot reaching a mean contribution to the global warming impact across stochastic simulations of 45 % in Granada and 80% in Aalborg due to high heating requirements. The high contribution of heating compared to cooling had been highlighted by other studies (Onorato and Rösch, 2020; Pérez-López et al., 2017).

The possibility to compare the obtained mean or median impact scores with the ones from the existing literature was limited as all known studies used attributional LCAs, with different cultivation systems and functional units. However, the scores were similar to the ones obtained by (Pérez-López et al., 2017) in a pilot scale version of a similar cultivation system after rescaling our results to the same functional unit and accounting for the efficiency differences between the pilot scale operation and the large scale production assumed in the model. The module estimating thermoregulation requirements in the model had been validated using the primary data published in this study.

The negative score for Terrestrial Ecotoxicity (cf. Figure 4), and therefore positive impact on the environment, is mostly due to the assumed substitution of fish feed by the co-produced biomass (97,4% of the total biomass). The agricultural productions used in fish feed (soy, wheat etc.) involve the use of fertilizers and pesticides in open environments, unlike for closed PBRs used in microalgal productions. The substitution of fish feed was modeled with an optimization algorithm that calculates the quantity of each fish feed ingredients displaced by a given microalgal biomass to provide the same nutritional profile (lipid, carbohydrate, protein, ashes) as in the reference feed. This iso-nutritional profile was considered as the obligatory property to ensure equivalent function and substitution (Weidema, 2017).

Interestingly and of particular importance for our research questions, the spread of the impact scores was lower when assuming cultivation in Granada instead of Aalborg (cf. Figure 4). Similarly, assuming that the culture did not require night thermoregulation yielded narrower impact score distributions. This result was further explored with the GSA which demonstrated the high contribution of the uncertainty on the PBR volume overall, may this uncertainty lie upon the pipe diameter, the horizontal distance between the pipes or the horizontal distance between the stacks. As the thermoregulation requirements are highly dependent on the PBR volume, the uncertainty on the PBR to achieve a certain percentage of the maximum productivity entails a larger uncertainty on the impact scores when the combination of the strain thermal range and the local climate involve high thermoregulation needs. In other words, accurately predicting the impact of microalgal production is harder when the strain thermal range and the location’s climate do not match, especially if the climate is relatively cold.

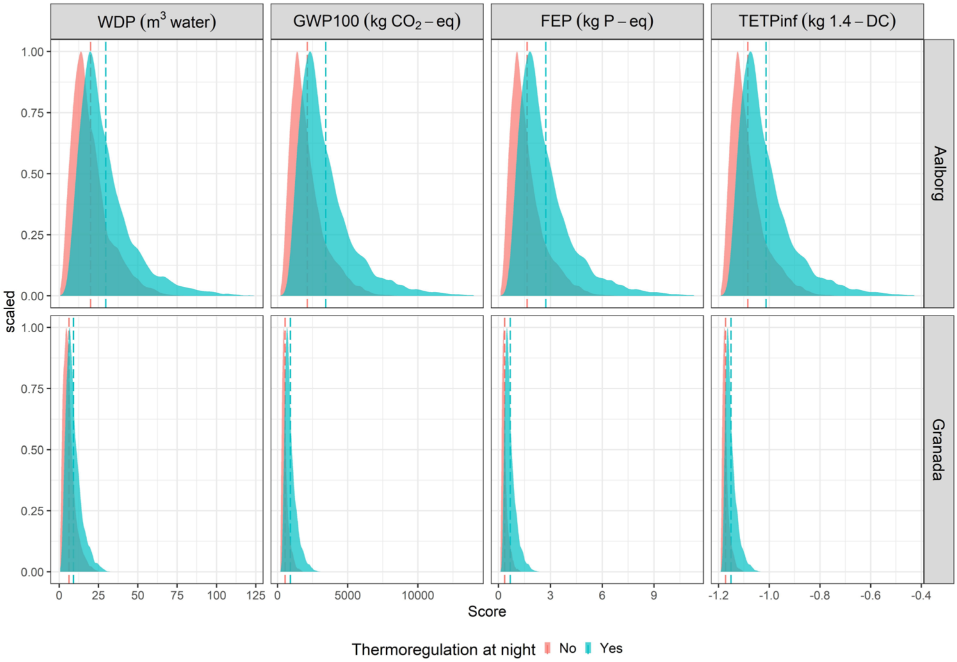

Figure 4: Impact score density curves for the cultivation of 1 kg of compound in Aalborg and Granada, with or without thermoregulation at night. The dashed lines indicate the means. Each curve is the result of 9000 simulations and the densities are scaled to 1 for readability. WDP: water depletion, GWP100: 100-year time horizon global warming potential, FEP: freshwater eutrophication, TETPinf: terrestrial ecotoxicity. Reproduced from (Jouannais et al., 2022).

5.1.2. IMPACTS ESTIMATES IF THE COMPOUND AND THE LOCATIONS ARE NOT KNOWN

The parameterized LCA model for the production of microalgal compound was used in Article II (Jouannais and Pizzol, 2022). This article aimed at assessing the environmental impact of a microalgal compound produced in Europe before knowing what the compound and the strain will be and where they will be produced. Using two different stochastic propagation strategies, thousands of potential strains and compounds (i.e., strain-compound pairs) were simulated in hundreds of potential PBRs all over Europe. This allowed assessing the distinct propagations of three main forms of uncertainty categories. The techno-operational uncertainty (cf. 5.1.1) is associated with the PBR setup that will enable a strain to grow with a certain energetic yield. The bioprospecting uncertainty stems from the fact that the strain-compound pair and their characteristics are not discovered yet. Finally, the production mix uncertainty is due to the indeterminate locations constituting the future mix producing the compound in Europe. We confined the technology deployment over Europe to the 10 countries identified by Skarka (2012) as the most suitable ones regarding microalgal biomass production potential based on productivity, temperature, and available land, namely Spain (ES), Sweden (SE), Italy (IT), Portugal (PT), United Kingdom (UK), France (FR), Greece (EL), Cyprus (CfY), Ireland (IE), and Germany (DE).

To be able to simulate as many microalgal strains as possible, the model was extended to include two additional fates for the co-produced microalgal biomass which depending on the strain could then be incorporated into fish feed, enter the generic animal feed market or be anaerobically digested to produce heat and recover nutrients. Data on microalgal biodiversity was used to define distributions for the biological parameters. Due to the potential high thermoregulation needs observed in Article I, we assumed a heat pump instead of electric heating for the deployment of the production over Europe.

A first insight was gained regarding the environmental impact of microalgal productions within each country by observing the distributions of the impact scores within the national boundaries (cf. Figure 5). While the average and median impact scores tendentially decreased with the latitude due to higher irradiance and lower thermoregulation needs, some countries featured scores that did not match the trend. For instance, Portugal, in the south, featured the highest average score for water depletion due to its specific electricity mix. This result highlights how the impacts of microalgal production depend on its location both due to climate and local energy mixes.

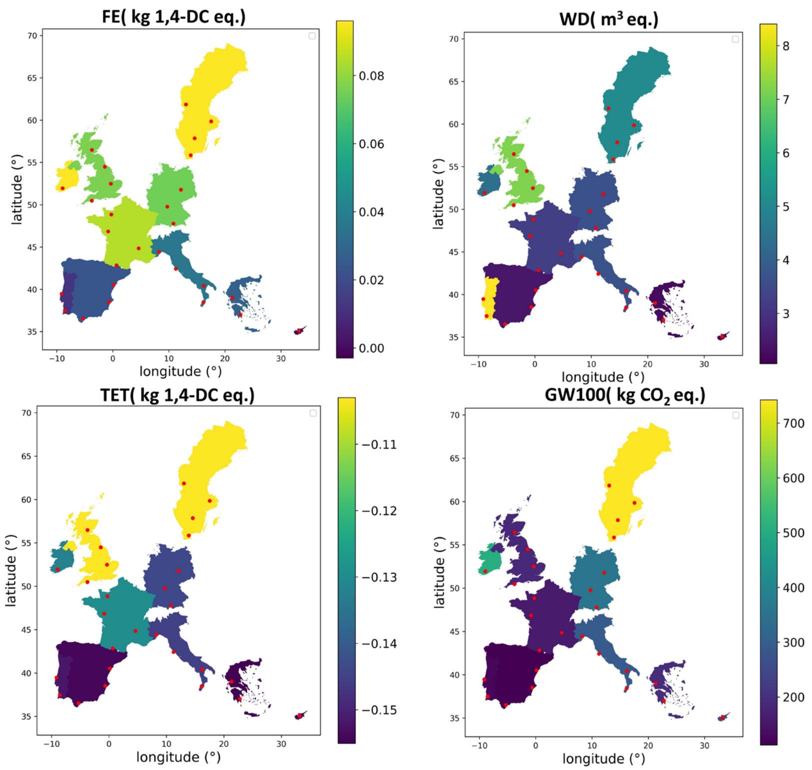

Figure 5: Average national impact scores per kg of compound across simulations for different strain-compound pairs and PBRs techno-operational setups. For each country, the cultivations are simulated on the red dots. FE: Freshwater Eutrophication, WD: Water Depletion, TET: Terrestrial Ecotoxicity, GW100: Global Warming with a 100-year time horizon. Adapted and extended from (Jouannais and Pizzol, 2022).

Among the various simulations of strain-compounds in different PBRs and locations, the impact scores for Global Warming (GW) exhibited a nearly lognormal distribution spanning from -100 to +89,000 kg CO_2-eq. per kg of compound. The vast range can be attributed to the significant diversity of configurations considered, stemming from the nature of the research question. The most extreme worst-case scenarios entail compounds constituting a mere 0.2% share of the dried biomass for strains with high thermal ranges cultivated in large-volume PBRs located in Sweden. Conversely, the best-case scenarios feature compounds accounting for up to 60% of the biomass, produced in PBRs with smaller volumes situated in southern latitudes.

These exceptional impact cases lie at the extremes of the distributions and are highly improbable. It is important to note that the tails of the distributions are particularly narrow for the high-impact values due to the approaching lognormal distribution curve. To gain a comprehensive understanding of the environmental profile of microalgal productions in Europe, one must consider the entirety of the distributions as well as the median values.

Regardless of the stochastic propagation strategy, the indeterminate content of the compound in the biomass was responsible for a very large share of the uncertainty on the impact scores. This was expected as a variation of content implies a rescaling of the production impacts. It should be stressed that, by considering bioprospecting uncertainty, the obtained distributions of impacts correspond to an LCA of the whole biodiversity of microalgal products. In addition to informing about probabilities of future impacts for a project based on a specific bioprospected compound, the distributions could be considered as the environmental profile of microalgal productions in general over Europe. The important contribution of thermoregulation to the impacts, and heating in particular, was confirmed for the whole microalgal biodiversity in Europe. Thus, high strain-specific optimal temperatures were tendentially associated with higher impacts across all simulations as observed in Figure 6. The regression coefficient α of the linear regression $\log(GW) = \alpha * Topt + b$ was larger for countries of northern latitudes than for southern countries.

Interestingly, the results confirmed and extended the observation made in Article I on the techno-operational uncertainty propagating more substantially in scenarios where the thermoregulation requirements are inherently high due to the strain and the location. Article II further illuminates the interactions between techno-operational, bioprospecting, and production mix uncertainties as illustrated in Figure 7. This figure shows how the coefficient of variation (Standard deviation /Mean) of the global warming impact scores across all simulations increases when these simulations are performed for strains with high optimal temperatures. Accurately predicting production impacts across Europe is therefore more difficult if a strain with a high thermal range is anticipated.

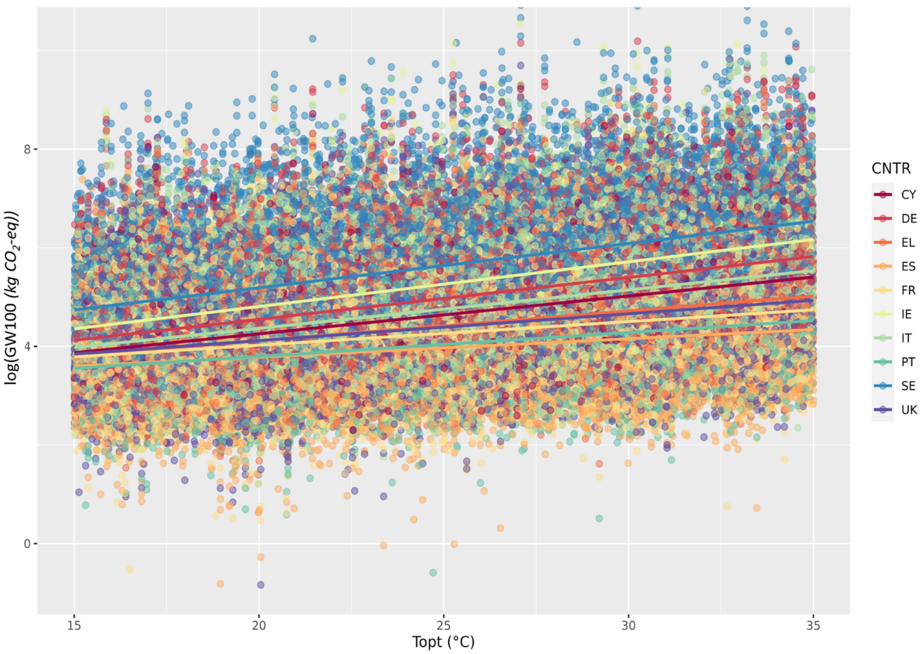

Figure 6: Pairplot log(GW100) = f(Topt). Topt is the optimal temperature for a strain-compound pair and is the temperature at the middle of the thermal range the culture should kept within. Each point represents one LCA calculated with a specific strain-compound pair, PBR techno-operational setup and location. The colors indicate the countries where the cultivation take place. The displayed lines are linear regression lines whose equations are shown in SI I from (Jouannais and Pizzol, 2022). Reproduced from the supplementary information SI I of (Jouannais and Pizzol, 2022).

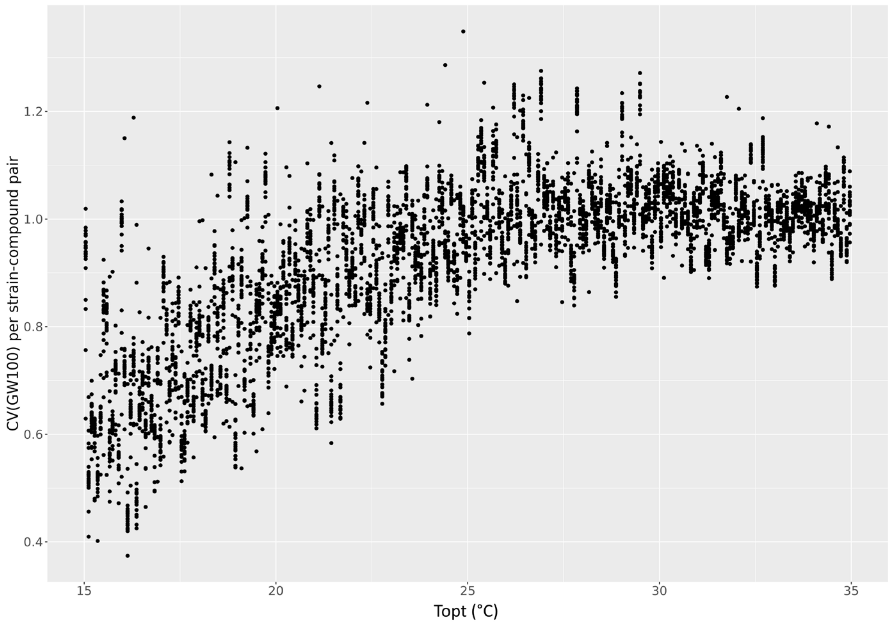

Figure 7: Pairplot Coefficient of Variation(GW100) = f(Topt) per strain-compound pair. 1 point is 1 of the 5376 strain-compound pairs simulated within one of the sampling strategies. The coefficient of variation (Standard deviation /Mean) describes all the LCAs calculated for this pair (in all locations, all PBR geometries and setups). Reproduced from the supplementary information SI I of (Jouannais and Pizzol, 2022), which further explores this behavior.

When considering that the compound will be produced by an indeterminate production mix whose locations are supplying an equal share of the increase in demand, the median scores across strain-compounds and production mixes amounted to 1.5 m^3 for water depletion (WD); 96 kg CO_2- eq. for global warming (GW); 0.017 kg P-eq. for freshwater eutrophication (FE), and 0.007 kg 1.4-DC-eq. for terrestrial ecotoxicity (TET) (Figure 8). These scores could be considered as the reasonable expectation one could have regarding the impacts associated with the deployment over Europe of a microalgal production whose exact target compound is not known yet. The distributions visible for GW in Figure 8 resemble lognormal distribution shapes and the mean scores (250 kg CO_2-eq) could also be used for more cautious decision-making. The distribution of results for GW overlaps with impacts reported for different drugs produced by the chemical industry and which could represent functionally equivalent products (Parvatker et al., 2019).

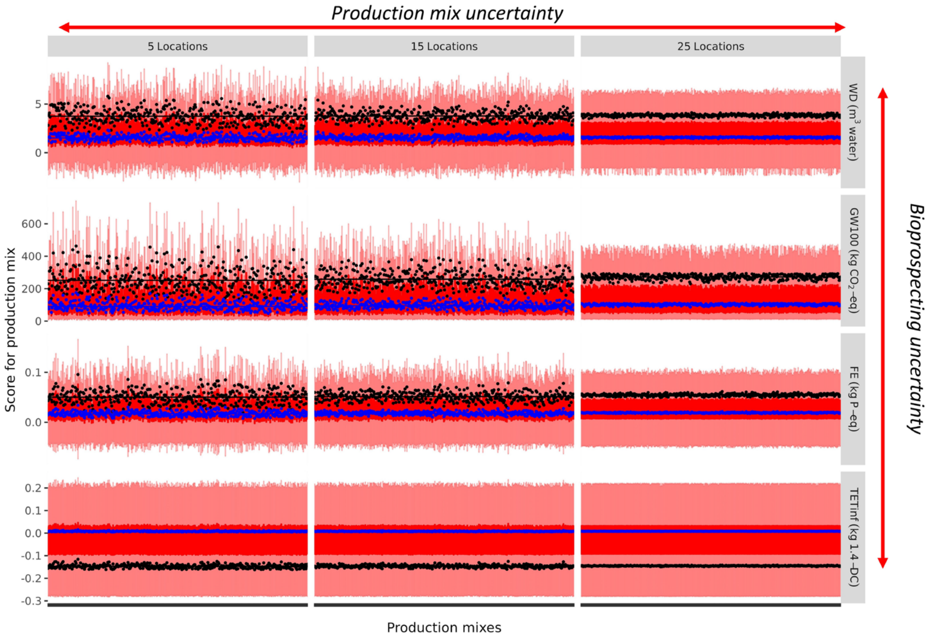

Figure 8: Production mix and bioprospecting uncertainties in aligned boxplots. The red area consists of a succession of narrow boxplots along the horizontal axis corresponding to 400 randomly generated production mixes over Europe. The darker red area corresponds to the ranges between the first and third quantiles. The range of each boxplot on the vertical axis corresponds to the dispersion of impact score per strain-compound pair produced in the production mix. Techno-operational uncertainty is aggregated intro median scores as the impact score for a strain-compound i in the production mix p is calculated as follows: $SMimp_{p,i} = \frac{\sum_{L=0}^{N_p} Median([imp_{i,L,0}, \ldots, imp_{i,L,a}, imp_{i,L,150}])}{N_p}$*, with Np the number of locations in the production mix p, L the identifier of a location being part of p and* $imp_{i,L,a}$ *the impact score calculated for the production of strain-compound pair i, in location L and PBR a. The black and blue dots respectively indicate the means and medians of each boxplot. The horizontal black and blues lines respectively represent the mean across boxplots and the means of the medians across boxplots. Adapted and extended from (Jouannais and Pizzol, 2022).*

As observed in Figure 8, the variability of the scores across production mixes decreased when the production mix was constituted of a larger number of random producing locations (e.g., 25 instead of 5 locations). In other words, the production mix uncertainty has a lower contribution to the total uncertainty on the impact scores if a large geographic deployment of the production can be assumed. It means that anticipating the impact of a microalgae-based technology deployment over Europe is easier when predicting that the geographic spread will be substantial. Indeed, the larger production mixes and their geographic coverages get, the closer they all get to the same overall European mix. This has direct implications for technological planning and anticipation of environmental impacts.

Article II further explores the interactions between techno-operational, bioprospecting and production mix uncertainties. This understanding illuminates how new knowledge would change the reasonable expectations of impacts.

5.2. WILL MICROALGAE-BASED SOLUTIONS FOR HEALTH ISSUES IN FISH FARMING BE ENVIRONMENTALLY PERFORMANT?

5.2.1. THE EFFECT OF IMPROVING BIOLOGICAL PERFORMANCES IN TROUT FARMS

Article III explores the current environmental opportunity cost of mortality and suboptimal biological FCR in a European trout farm based on new primary data from Italy and Denmark. The model is parameterized so that the biological FCR and the mortality can be modified separately in each of the six growth stages constituting the trout growth process. This process begins in inland hatcheries and ends in seafarms.

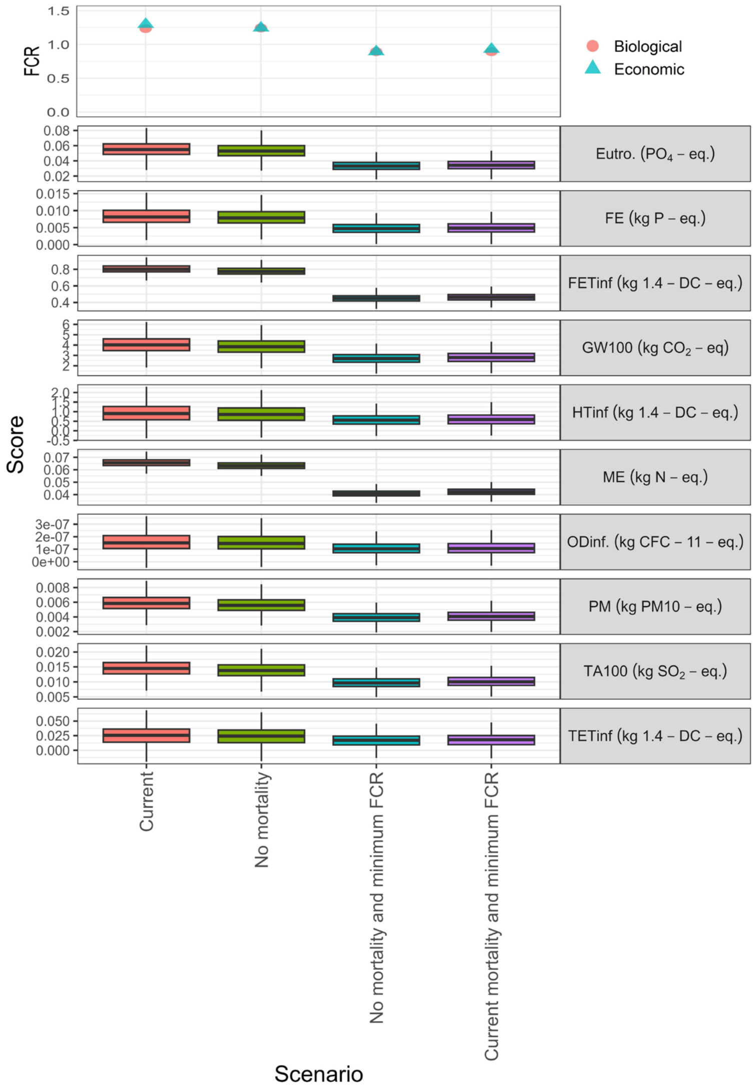

*Figure 9: Boxplots for the environmental impacts associated with an increase in demand for 1 kg of commercial-size sea trout under different scenarios. The boxes' limits represent the 1st and the 3rd quartile of the scores from the stochastic simulations on the background and fish waste treatment uncertainties. Current: Unmodified primary data. GW100: global warming with 100-year time horizon, TA100: terrestrial acidification, TETinf: terrestrial ecotoxicity, HTinf: human ecotoxicity, FETinf: freshwater ecotoxicity, PM: particulate matter emissions, ODinf = ozone depletion and eutrophication, both for freshwater and marine ecosystems, FE: Freshwater eutrophication, ME: marine eutrophication. Reproduced from (Jouannais et al. 2023, **under review**)*

The current mortality rate in the farm was 4.5 % (dead fish/(dead + live fish)), with losses happening during different growth stages. Avoiding these losses and reducing the mortality to 0 % would decrease the environmental impacts from a minimum of 3.2% (marine eutrophication, ozone depletion) to a maximum of 5.2% (human toxicity) (cf. Figure 9). These potential impact reductions constitute the current environmental opportunity cost associated with mortality. On the other hand, reducing the biological FCR by 30% to reach its minimum theoretical value, which constitutes a theoretical optimum under which liquid phosphorus excretion would be brought to zero, also decreased the environmental impacts by around 30 % for all impact categories. The proportionality between the FCR reduction and the environmental impact reduction was also observed by Paptryphon et al. (2004) and d'Orbcastel et al. (2009). These results suggest that the currently low mortality in the studied farm is associated with a low opportunity cost, but that substantial environmental impact reduction can be achieved by reducing the biological FCR. Note that the solutions to tackle mortality and biological FCR can often be the same, as the biological FCR can be hindered by long-term infections or gut inflammation that statistically increase the chances of the fish eventually dying (cf. State of the art 2.3.1).

The results clearly showed that losses occurring in the first growth stages of fish production do not have the same influence on the environmental impacts as losses happening at the end, which entail the loss of all the production investments into this fish. Thus, assuming 15 % additional mortality in the hatchery had no visible effect on the environmental impact while 15 % mortality in the last stage of the seafarm increased the impact by 15%. These results question the notion of opportunity cost as calculated in the literature (Just Economics, 2021). Using an LCA approach, the environmental opportunity cost is the difference of impact between an increase in demand for fish produced with a given biological performance and an increase in demand for the same fish but produced with a better performance. The opportunity cost as calculated following the economic opportuny cost logic (Just Economics, 2021), consisting of a mere multiplication of the lost biomass by a single factor, only constitutes a "virtual" shortfall that considers indistinct fish biomass as equivalent to the final commercialized fish. Note that the LCA approach does not assign an environmental opportunity "cost" to a specific mortality event, but can only assess the additional fish production impacts if the farm was consistently subjected to this mortality over the next production cycles.

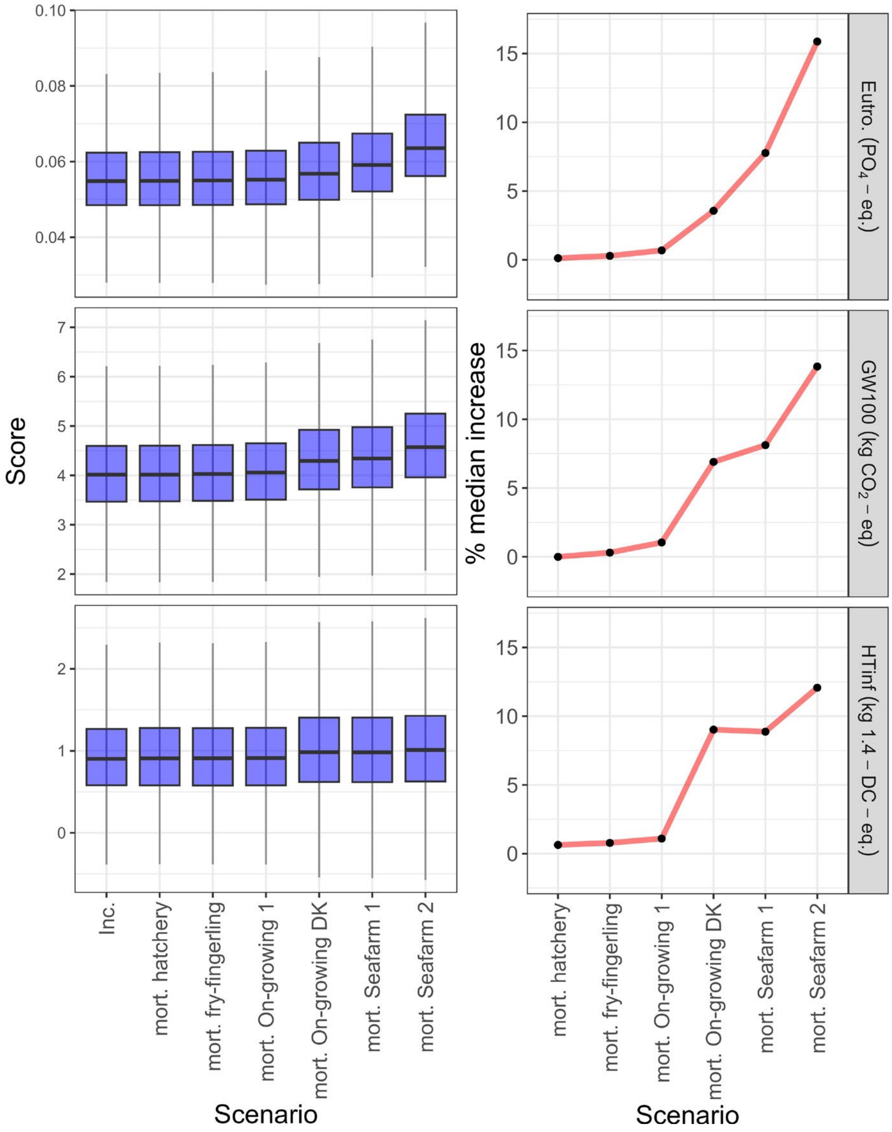

*Figure 10: Boxplots for the environmental impacts associated with an increase in demand for 1 kg of commercial-size sea trout with 15 % additional mortality occurring at different timings. The left panel shows the impact score expressed in the corresponding impact category unit. The box limits represent the 1st and the 3rd quartile of the scores from the stochastic simulations. The edges of the vertical lines correspond to the minimums and maximums. The right panel shows the percentage of median increase by setting the current median score as a baseline (0). The results for the other impact categories are presented in the Online Resource I.5 (Jouannais et al. 2023, **under review**). Legend of the horizontal axis: Current=Unmodified primary data. Mort. Growth stage X = Scenario with modified mortality in the growth stage X. The growth stages are ordered by fish individual weights from the left to the right. Reproduced from (Jouannais et al. 2023, **under review**).*

The anaerobic digestion of the dead fish and sludge for heat production and nutrient recovery for fertilization did not show a substantial minimization of the fish impact under the current mortality. However, simulating higher mortality rates showed that the role of dead fish valorization could be important to minimize the impacts under future more severe mortality regimes.

5.2.2. A NEW PROCEDURE FOR EX-ANTE LCA UNDER DEEP-UNCERTAINTY

5.2.2.1. Description of ENSURe

The results associated with Article IV are essentially methodological and theoretical advances applied to the technological concept under study. While the incertitude associated with all the previously addressed indeterminacies was designated as "uncertainty", combining the microalgal model with the fish farm model made it clear that at least two types of incertitude, namely risk, and uncertainty, were present when trying to anticipate the environmental performance of fish produced with an undiscovered microalgal compound. For instance, data about microalgal biodiversity could help establish probability distributions regarding the biomass composition or the quantic yield of the photosynthetic system. In comparison, the level of knowledge regarding the performance of the undiscovered compound on the fish and the dependencies between bioactivities, treatment doses, and compound content in the microalgal biomass was highly problematic. We thus differentiated "risk parameters", also called "risk factors" in Article IV, from "uncertain parameters" ("uncertain factors" in Article IV).

Abiding by the ethics of quantification (cf. State of the art 4.1) and following Knight and Stirling's definition of risk and uncertainty, we argued that the distributions observed in the output of an LCA model after stochastic propagation of input distributions should not be used a such to derive *probabilities of impacts* if the level of knowledge regarding some input distributions is comparatively too low (uncertain parameters). In other words, the probabilities of impacts presented to decision-makers should only stem from risk parameters. Semantically, this fully aligns with Cox's definition of probability being a "reasonable expectation" which suggests that it should stem from a reasonable level of knowledge only.

Yet, even under conditions of uncertainty, decisions must be made according to these quantified reasonable expectations, i.e., probabilities, and the procedure assists in evaluating them.

The new procedure named ENSURe, which stands for ENvironmental Success under Uncertainty and Risk, essentially relies on four main ideas:

- Making decisions does not necessarily require having access to the full probability distribution of impacts, but can be informed by estimating the probability of a *success* P(S) which is a desired outcome for the decision-makers. For instance, a success can simply be defined as a future in which the technology emerging from the exploration of the technological concept environmentally outperforms a baseline or an alternative projected into the same future. Furthermore, decisions can be made by setting a minimum probability of success to invest time and resources in the exploration of the technological concept. We call this minimum probability a "decision threshold". We further discuss the definition of a "success" in the article and section 7.2.1.

- It is usually easier to reflect on the probability of a specific scenario characterized by a few parameters than to define *a priori* the probability distributions of all parameters, including some for which dependencies are suspected but cannot be modeled.

- For a specific combination of values for the uncertain parameters in a model (e.g., U1=a, U2=b, U3=c), the exclusive propagation of risk parameters allows computing a probability of success which is *conditional* to this combination. We can note this conditional probability P(S|U1=a, U2=b, U3=c).

- The law of total probabilities states that P(S) =P(S|A)*P(A) + P(S|B)*P(B) if the union of the events A and B constitutes the full scope of possibilities.

In essence, ENSURe aims at unraveling different sets of conditions on the uncertain parameters that would need to be predicted to ensure a conditional probability of success superior to the decision threshold. In complex models with many uncertain parameters, many sets of conditions could be found to ensure this conditional probability of success. By finding and returning these distinct sets of conditions (called "boxes") across the whole uncertain space, ENSURe simplifies the decision-making exercise by focusing the reflection on these portions of the space only.

Using the law of total probability, the total probability of success P(S) can be compared to the decision threshold by estimating the probability P(Boxes) of the final technology featuring a parameter configuration that is at least within one of the boxes' limits. If this probability P(Boxes) can be estimated to 1, i.e., it can be anticipated that the technology will fulfill a certain set of requirements regarding the uncertain parameters, the total probability of success can be considered superior or equal to the decision threshold. This constitutes the ideal solution which greatly eases decision-making. If P(Boxes) can be estimated to any value, the conclusion is generalized as *P(S) ≥ P(Boxes)* Decision threshold* (cf. Eq. 1).

$$P(S) = P(S|Boxes) * P(Boxes) + P(S|Outside\ the\ boxes) * P(Outside\ the\ boxes) \geq P(S|Boxes) * P(Boxes) \geq Decision\ threshold * P(Boxes) \quad \textit{Eq. 1}$$

Practically, calculating all the conditional probabilities in the continuous uncertain space is not possible due to excessively high computational requirements. ENSURe thus involves a regionalization step assisted by GSA to approximate the calculation of risk-exclusive conditional probabilities in the uncertain space. Once the space has been divided into small regions, the conditional probability of success in each region is assessed as the proportion of successes in the region. ENSURe then uses the PRIM algorithm (cf. Methods 4.2.3) to find boxes, i.e., groups of regions associated with a conditional probability of success superior to the decision threshold (cf. Figure 11).

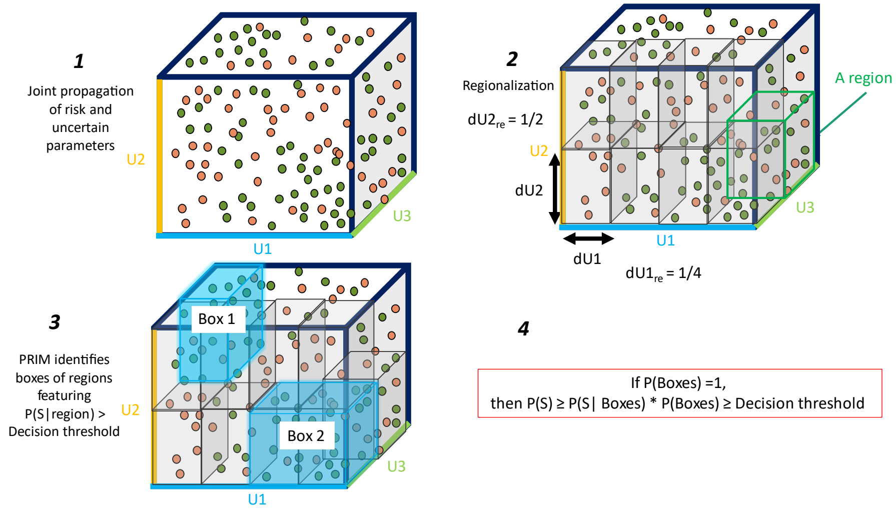

Figure 11: Illustration of the ENSURe procedure for an LCA model with three uncertain parameters U1, U2 and U3 and some risk parameters. Green and red dots respectively indicate data points constituting successes and failures. dUx is the resolution for the parameter Ux, i.e., the length of the interval used to divide the total range for Ux. dUx_{re} is the relative resolution assigned to parameter Ux. A relative resolution of ½ means that the total range for Ux is divided in two parts.

A particular feature of the procedure is that it begins by treating both risk and uncertain parameters similarly as both types are sampled together in Monte Carlo iterations. The uncertain parameters are assigned arbitrarily large uniform ranges and are only used to explore and populate a large uncertain space with data points (cf. 1 in Figure 11). By simultaneously propagating both types of parameters, it becomes possible to conduct Global Sensitivity Analysis (GSA) through the calculation of Borgonovo's δ indices for the uncertain parameters. These indices help determine the appropriate number of intervals to divide each uncertain parameter's range. The underlying principle is that a region can have a significant extent along the dimension of a non-sensitive uncertain parameter because the variation of the parameter within the region will not substantially influence the assessed probability. On the other hand, a highly sensitive uncertain parameter requires a smaller resolution, implying that its range should be divided into smaller intervals. The choice of a small resolution ensures that the parameter's variation within these intervals does not substantially affect the calculated conditional probabilities, which should ideally stem from the propagation of the risk parameters exclusively.

A strong asset for ENSURe is that the analyst can modulate the different settings for PRIM, the regionalization step, the decision threshold, and the definition of a success according to their objectives and computing needs and resources. Looking for boxes where the conditional probability is higher than the threshold of success constitutes a canonical use, but other approaches can be adopted with the same procedure to navigate the uncertain space and refine the evaluations. Satisfying results can be evaluated based on different criteria further detailed in Article IV.

5.2.2.2. Application of ENSURe to the AquaHealth technological concept

We defined a success as a possible future in which producing trout with the undiscovered microalgal compound has a lower impact than without. In the simulations, the farm with or without the compound are submitted to the same level of losses before the application of the compound. This level can be different from the current one. Background parameters and parameters associated with the production of the microalgal compound were considered “risk parameters“. The parameters defining the compound’s improvement performance on the fish farm and the parameters defining the farm’s fish mortality and environmental impact at the time of application were considered “uncertain parameters”. Note that the compound content in the microalgal biomass was considered as uncertain while it was implicitly treated as a risk parameter in Article II. The choice of where to set the limit between risk and uncertain parameters is ultimately subjective and further discussed in Article IV and Section 6.2.1 and 7.2.1.

ENSURe was then applied to try to evaluate if the totality probability of success was superior to a high decision threshold of 85 % , assuming a situation in which decision-makers would only consider further exploring the concept if the success probability was shown very high. I here only summarize the results obtained for the Global Warming impact category.

The most interesting results were obtained after combining 24 uncertain model's parameters into 5 aggregated parameters, thus reflecting the same uncertainty but a lower granularity level, to explore the uncertain space based on fewer dimensions. For Global Warming, four boxes were found, and therefore four distinct groups of technological configurations one should predict to ensure a total probability of success above 85%. The limits of these four boxes are presented in Table 3. Note how each box constitutes a different set of constraints ensuring the same total probability of success by tightening the constraint on some parameters and releasing it on others. It is important to understand that when a parameter is not constrained in this box, it means that no prediction is required for this parameter to ensure a probability as high as the decision threshold, as long as the other conditions of the box are met. In simpler terms, when a parameter is unconstrained, it implies that the conditional probability of success surpasses the decision threshold regardless of the possible values taken by the unconstrained parameter. This holds true as long as the constrained parameters within the box fall within their designated ranges.

Table 3: Limits of the boxes returned by ENSURe for the global warming impact with a decision threshold =0.85. The boxes constitute sets of conditions on the uncertain parameters. If it can be stated that the probability of the technological configuration being in one of these boxes is one, then the probability of success is superior to 0.85. A red name for a parameter indicates that higher values are more optimistic regarding the compound's performance and the potential for improvement in the fish farm. A green name indicates the opposite.

Aggregated parameter (aggregated factor in Article IV)	**Box 1**	**Box 2**	**Box 3**	**Box 4**
Compound content in the biomass ($g.g^{-1}$)	>0.2	>0.3	>0.2	>0.3
Overall Economic FCR reduction compared to projected alternative (%)	>22	>22	>17	>22
Overall Biological FCR reduction compared to projected alternative (%)	>15	Any [0,25]	Any [0,25]	>7
Dose of microalgal compound supplied to fish during their whole life cycle ($g.kg^{-1}$)	<2.5	<3.5	<2.5	<5
Impact per kg of currently used pharmaceutical (kg CO_2-eq. kg^{-1})	Any [10-1000]	Any [10-1000]	Any [10-1000]	Any [10-1000]
Level of losses experienced by the farm before application of the compound = % projected mortality / % current mortality	Any [1-6]	Any [1-6]	>3	>4.2

Overall, it could be observed that all boxes, including the ones discovered for other impact categories, featured very demanding set of constraints to ensure 85 % success probability for the technological concept. All boxes required full confidence that the compound content in the biomass is superior to 18 % (20% for the boxes on GW in Table 3) combined with high health management performance (% reduction of economic and biological FCR). Based on the technological configurations observed in the literature and the discussion with experts, it seems that meeting all conditions simultaneously is unlikely and the probability of the final technological configuration belonging to one of this box is substantially less than 1. It can therefore not be concluded that the total probability of success is superior or equal to 85%, which constituted a very high and therefore cautious decision threshold (cf. Discussion 6.3.1). As further detailed in Article IV and its appendix, it cannot be strictly concluded that the total probability of success is below 85 %, instead uncertainty remains and a conservative approach to decision-making could dismiss the concept. However, the evaluation of the total probability of success could be refined by changing the settings of the procedure and further exploring the uncertain space while using the law of total probabilities.

It is important to understand the far-reaching implications of this result. It is not surprising that ensuring 85% of success probability requires being sure that the compound's performance and/or the improvement potential in the farm will be very high. The results simply show that it would require being optimistic about the capacity of the currently prospected compound to fulfill its function (health management) to ensure a high probability of success given all the indeterminacies in the system. These indeterminacies exist from the background database parameter to the geographic production mix of the compound, encompassing the characteristics of the compound and host strain (diameter, density, strain-specific photosynthesis efficiency, thermal range, co-product properties, etc.) and the techno-operational setup of the PBRs. Decision-makers need to be even more optimistic about the performance of the compound considering that many of the previous indeterminacies still feature large distributions representing large scopes of possibilities, despite being treated as risk parameters.

6. DISCUSSION

This section discusses the modeling choices and their philosophical implications regarding the nature of technological development and the decision-making process. I also further explore the differences and connections between using ex-ante LCA for guidance within a specific R&D journey or using it to decide whether a technological concept should even be explored.

6.1. ON CHOOSING WHERE INCERTITUDE LIES

This work thoroughly explores incertitude in the ex-ante LCA of a very early-stage technology. More than a cold computation exercise, quantifying incertitude starts by deciding where incertitude lies, and therefore which aspects of the technological development are indeterminate. Indeterminacies do not exist as real-world objects on which analysts and stakeholders attempt to assess incertitude, instead, they are subjective realities entirely dependent on the questions that are asked, and the models that are used. In other words, indeterminacies and their associated incertitude are objects belonging to "model-land" (Thompson and Smith, 2019), a virtual construction built to study complex phenomena and inform decision-making. This section aims to "*escape model-land*" (Thompson and Smith, 2019) or at least exploring other model lands that could have been chosen in this PhD work and which would have changed the assessment of incertitude.

As previously exposed, predicting microalgae growth has kept some scientists busy their whole careers. The complexity of this exercise is magnified when attempting to predict productivity for commercial-scale reactors consistently functioning over long periods under changing weather and temperatures. A review of most available models for microalgae growth was performed at the beginning of this work to find the most suitable model to answer the research questions. Models with numerous parameters detailing biophysical phenomena were first considered. It became quickly apparent that choosing a more detailed kinetic model with parameters describing more phenomena than the thermodynamic approach would simply deport the incertitude to other parameters, often more numerous. For instance, the kinetic model from Krichen et al. (2020) uses a parameter *Khsx*, the half-saturation constant at the biomass concentration x, which is different for distinct species depending on their cell volumes but which also depends on the reactor's mixing to an indeterminate extinct. Using this model would thus require making a statement about how this parameter changes across the microalgal biodiversity and how it depends on the reactor. Little data exists to support this statement. Blanken et al. (2016) propose another kinetic model aiming at limiting the number of parameters that should be easily measurable for any microalgae. While still remaining complex, the model also needs to include an additional light correction factor to correct the Beer- Lambert law for light absorption. Without this correction factor, which needs to be calibrated for each reactor and strain, the mean absolute percent error (MAPE) when trying to fit the predictions of the growth rate with experimental data remains 36%. This illustrates how accurately modeling the light field is necessary for kinetic models, which is particularly difficult in outdoor large-scale PBRs under fluctuating conditions.

Another major issue with kinetic models in general is the need to compute the total productivity by integrating the differential equations of growth over time and over the whole reactor. This leads to drastic computing time when needing to simulate production in hundreds of thousands of scenarios in different locations. The analytical solution of kinetic differential equations to estimate maximal productivity proposed by Cornet and Dussap (2009) and Pruvost et al. (2012) and used in an LCA by Duran Quintero et al. (2021) could be used to limit computation time. This model is a light-limited growth model, which does not account for all other influences such as pH and nutrient gradients in the reactor.

Another crucial factor that influenced our modeling approach, as previously discussed in the state of the art and Article I, is the utilization of a thermodynamic model that assumes the attainment of 30% of the maximum strain-specific surface productivity within an indeterminate PBR techno-operational setup. This choice was made based on a review of the literature and available models, considering the various perspectives that exist regarding the uncertainty surrounding microalgal growth.

Typically, the literature focuses on addressing uncertainty related to the productivity of a selected strain within a specific PBR geometry and location. This uncertainty is what Wynne (1992) defines as "scientific uncertainty," which pertains to parameters associated with biphotonic interactions and biological processes in natural sciences. While this perspective is important, solely considering it may not fully capture the relevant aspects when attempting to anticipate the impacts of future technological development.

Indeed, as analysts of future environmental impacts stemming from the technology, our primary concern is not solely predicting how a strain will behave in a specific reactor, but predicting how the people in charge will upscale the technology and what will motivate them. Even if predicting productivity for all strains and locations and reactors was possible, this would not directly inform about the PBR design chosen by the industrial actors. It could be designed to target the maximum productivity, could be shaped to minimize the risk of culture collapse and target stability instead, or could be shaped to maximize economic gains which could be achieved by finding a compromise between productivity and energy consumption. This constitutes a “social uncertainty” in Wynne’s terminology. Overall, we merged the perspectives of scientific and social uncertainty by considering that the reactor would be designed in a certain way to maximize surface productivity, but that this specific design was indeterminate and would be found by bioengineers among the many potential configurations of vertical tubular PBR. 30% of this maximum productivity constitutes what is usually achieved in current large-scale productions (Hindersin et al., 2014; Williams and Laurens, 2010) and it constitutes an assumption on the capacity of bioengineers to find the good photo-biological formula for a strain and location (cf. State of the Art 2.2.2) (Mata et al., 2010).

In the models, this value of 30% was kept fixed but could have been considered as an uncertain parameter in Article IV to estimate which percentage of the maximum productivity should be predicted to ensure a certain probability of success.

In their LCA of *Nannochloropsis sp.* and *Scenedesmus sp.* productions, Posada et al. (2016) take another approach to the social and scientific uncertainty problem although they did not designate it as such because did not consider an ex-ante perspective. They start by estimating the design of a reactor to minimize energy consumption and then assume a certain productivity in this reactor. This is arguably a risky assumption to make when considering different strains and locations.

Overall, the modeling options for the same problem are numerous and all associated with different perspectives on where incertitude lies. Choosing a "good model" for decision-making thus becomes choosing a model which allows defining the incertitude unambiguously and making clear assumptions about the motivations of the actors in technological development. The model and the choices regarding where incertitude lies will therefore depend on the precise goal of the ex-ante LCA assessment.

6.2. IS TECHNOLOGICAL DEVELOPMENT CHAOTIC? TWO DIFFERENT PARADIGMS FOR THE USE OF EX-ANTE LCA

6.2.1. GUIDANCE AND PRECAUTIONARY PARADIGM

This work constitutes an archetypal example of using ex-ante LCA for decision-making before initiating the development of a technological concept with low *correctability.* The low correctability is mainly due to the first phase of the technology which is bioprospecting. What will be found will determine most of the upcoming R&D and integration by the market. In other words, initiating bioprospecting is taking the "risk" (in its common definition, i.e., related to hazard) of finding a compound of high value, whose production will be upscaled by industrials in potentially suboptimal locations and whose photobiological formula may require high energy consumption. This type of technological development pathways features possibilities of entrenchments and lock-in mechanisms early in the process (Brian, 1989; Hung and Tu, 2011).

By considering all potential strain-compound pairs and geographic production mixes while assigning them probability distributions, our approach takes on an externalist perspective. As analysts, we view technological development as a chaotic process that may evolve in unpredictable ways, potentially leading to unintended consequences. This viewpoint acknowledges the presence of ”*underlying values held by various actors, constellations of interests, and conflicting value frameworks*” (Liebert and Schmidt, 2010). While my approach can also provide direct guidance to the R&D stakeholders (cf. Article IV), my use of ex-ante LCA to evaluate what can be expected from a technological concept before initiating research constitutes a different paradigm related to the precautionary principle(Owen et al., 2013; Van Asselt and Vos, 2006).

The ”guidance paradigm” for the use of ex-ante LCA is summarized by the definition provided by (van der Giesen et al., 2020), which is “*performing an environmental life cycle assessment of a new technology before it is commercially implemented in order to guide R&D decisions to make this new technology environmentally competitive as compared to the incumbent technology mix*.” This use of ex-ante LCA constitutes the *responsive* dimension of RRI (Owen et al., 2013), in which technological development iteratively responds to ex-ante LCA guidance, and therefore to some extent to societal stewardship. According to this definition, the assessment assists decision-making along the R&D process so that every step of the development is optimized according to environmental criteria. In other words, the final and upscaled form of the technology is currently indeterminate but the ex-ante LCA will hopefully make R&D find the optimal development route. This does not mean that there is no incertitude in some parts of the modeling exercise, typically regarding the evolution of the alternatives and the general economic context which do not depend on the technology stakeholders‘ decisions. Nevertheless, the core issue revolves around an optimization exercise that aims at resolving the indeterminacies with the appropriate decisions.

In this paradigm, the analysts will typically study different scenarios regarding the indeterminacies at stake and inform R&D about the optimal choices. Assuming continuous guidance throughout the process, calculating probabilities of impact for the technology appears largely irrelevant, because indeterminacies encountered in the next steps of the R&D will also be subjected to optimization and consequent decision. The paradox lies in the fact that "probabilities" are commonly used in the context of natural or precisely defined mathematical systems and random experiments, which arguably evolve independently of analyst influence (an externalist standpoint), often exhibiting chaotic patterns. Assuming that technology is responsive to LCA guidance, presenting the future with probabilities becomes misleading since the guidance itself contributes to shaping that future. For probabilities to be propagated to impacts, stakeholders would need to specify the probabilities associated with their decision-making regarding the forthcoming indeterminacies encountered in the process. This represents a paradoxical externalist standpoint from the technology developers themselves.

In fact, the assessment of probabilities becomes more relevant when adopting an externalist standpoint that considers probabilities (if risk) or possibilities (if uncertainty) regarding the decisions that will be taken along the R&D process and market integration. This precautionary standpoint acknowledges that technological development and deployment do not necessarily follow guidance toward optimal environmental performances. Instead, this process is chaotic and its future could only be deterministically forecasted if we had perfect knowledge of all upcoming decisions and events from the beginning, which is equivalent to assuming a complete responsiveness of development and deployment to guidance. Typically, the parameters defining the geographic production mix for the microalgal compound, which are extremely influential on the environmental impacts, could be optimized in a guidance paradigm and the stakeholders could be told to develop the technology only in southern Europe (cf. Article I, II). However, it is more realistic, given the multiplicity of actors and interests in the current economic and regulatory system, that once an interesting compound is found, its production could develop anywhere.

6.2.2. USING *ENSURE* ONCE THE TECHNOLOGICAL CONCEPT STARTS BEING EXPLORED

By considering the location parameters as “risk” parameters when applying the ENSURe procedure, the discovered boxes show what we should be sure about to ensure 85% success probability if we assume that the production mix could develop equiprobably across Europe. Conceptually, this means that we find the conditions on parameters qualifying the discovered strain and developed compound that would ensure a certain probability of success while assuming a geographic deployment that remains beyond control. In a society where technological and production planning and stewardship are enforced to a further extent than nowadays, ENSURe could be instead used to find the conditions on the production mix that would ensure a certain probability of success. Decision-makers could then confine the deployment to the identified zone.

It is crucial to remember that total probabilities of success estimated with ENSURe are completely dependent on the probability distributions chosen for the risk parameters corresponding to the knowledge before initiating bioprospecting. As soon as one of the indeterminacy becomes determinate, i.e., as soon as the technological development and deployment are started with one strain, ENSURe must be applied again to estimate the total probability of success for this specific orientation of technological concept. For instance, setting a unique value for sensitive risk parameters such as the ones defining the strain’s thermal range may completely change the conditional probability of success associated with the boxes discovered when the strain was not found yet. ENSURe can thus also be used iteratively along the R&D process to continuously reevaluate the total probability of success.

6.2.3. A GALTON BOARD ANALOGY

Overall, this work and the questions that it raised allowed the differentiation of two main paradigms for the use of ex-ante LCA and the associated notion of probabilities: the guidance paradigm and the precautionary principle (cf. 6.2.1). To further illustrate these paradigms, an analogy can be drawn with a device designed to create chaotic patterns and reflect on probabilities: the Galton board (Chernov and Dolgopyat, 2008). On this device that Francis Galton imagined to illustrate the central limit theorem, small beads fall along a board covered with many pegs on which beads will bounce before being collected in different boxes. The complex bouncing patterns introduce chaos; it is impossible to deterministically predict the path of a specific bead. Yet, if the number of beads and ranges of pegs are large enough, the shape of the curve resulting from the stacked beads columns will tendentially remain the same. One can therefore probabilistically predict the position of a bead using the normal law for the traditional classic Galton bard (unbiased), or another law for any other organization of the board and pegs.

The analogy illustrated in Figure 12 starts by defining a bead and its trajectory as a technological concept or emerging technology and its development. A peg is an indeterminacy and a bead bouncing on it constitutes the resolution of this indeterminacy when the associated model parameter takes a single value because progress has been made in the R&D or new knowledge has been gained. The bead lands in a bin whose position represents the final impact of the technology.

In the guidance paradigm for ex-ante LCA, many pegs are considered “responsive” which means that the bouncing will always be on the left (tendentially lower impact) side because guidance will be provided to identify the associated choices. The bead does not cascade in a single fall but is stopped at every responsive peg and controlled. The ex-ante LCA exercise here mostly consists of controlling the chaotic development to make the bead tendentially fall to the left.

In the “precautionary” paradigm within which this work is mostly applied, the exercise mainly aims at deciding whether we should let the bead fall in the first place, and/or at studying what should be assumed regarding the bounces and the structure of the board to ensure an acceptable and “safe-enough” fall. All pegs must be considered unresponsive, and statements must be made regarding the possibilities and probabilities associated with the decisions that will be taken. The organization of the pegs and potential obstacles on the board represent the main assumptions regarding what drives the development such as the assumption that maximum areal productivity is sought by industrials. The objective is here to visualize and navigate the "environmental signal" of the indeterminate technology which is represented by the distribution of the beads in the bins.

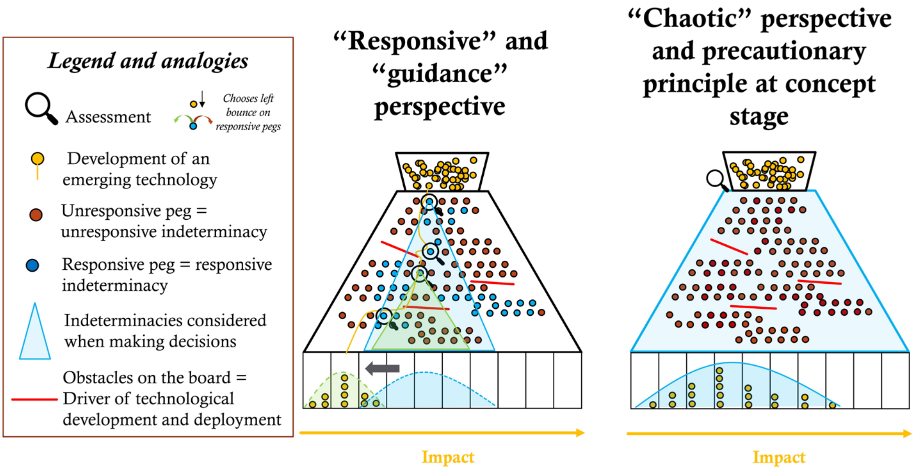

Figure 12: Galton board analogy for the guidance and precautionary paradigm in ex-ante LCA

These two paradigms for the use of ex-ante LCA are not necessarily exclusive and contradictory but represent two perspectives with different implications regarding what types of decisions are to be informed and at which level they are made. RRI and technological planning require an appropriate and unambiguous articulation of both perspectives as further discussed in the next section.

6.3. SHOULD TIME AND RESOURCES BE INVESTED INTO BIOPROSPECTING MICROALGAL COMPOUNDS FOR FISH HEALTH MANAGEMENT?

This work can provide support for the decision-making associated with this question, but some key aspects and limits must be discussed to make the best use of this support.

6.3.1. THE NEED TO DECIDE

The exercise of estimating what can be expected in terms of environmental impacts for a technological concept before initiating it implies such a high incertitude that "*concerns might be raised as to whether such an early stage approach is at all possible* " (Liebert and Schmidt, 2010). Yet, as Liebert and Schmidt (2010) state, we still know something most of the time, despite the ex-ante standpoint. This knowledge can be used to assess the environmental "signal" of a technology, based on which decisions can be made. The other option would be to suspend judgment, initiate further research into the concept, wait for more data, and guide technological development as much as possible, hoping for few lock-in mechanisms. However, this is not satisfying regarding the status of emergency for coordinated planning and action against the current ecological crisis (Derbyshire and Morgan, 2022; Ripple et al., 2021).

As shown in Article IV, the probability of success for the technological concept cannot be shown to be higher than 85% and additional iterations of the ENSURe procedure with different settings could refine this evaluation. I do not claim that 85% constitutes a necessary threshold before initiating technological research projects. This threshold is particularly conservative and cautious and would probability hinder technological progress in an inappropriate and disproportionate manner (Owen et al., 2013).

Using ex-ante LCA to decide whether a technological concept should be even explored, and not only to guide the R&D falls somehow within the "data before market" dimension of the precautionary principle (Owen et al., 2013). While the approach could be considered too radical and hindering "beneficial" technological advances, it must be acknowledged that current investment decisions are already made using models, probabilities, numerical indicators and thresholds to make decisions. Banks and investors choose to invest in projects based on their projections regarding the Return on Investments (ROI) (Magni, 2015). In medicine, and particularly in situations of emergency, patients are prioritized (Déry et al., 2020) and decisions have to be quickly made based on probabilities, either explicitly calculated or unconsciously estimated (Zehtabchi and Kline, 2010). Real option analysis is performed in companies to decide on orientations that will lead to uncertain consequences (Block, 2007; Bowman and Moskowitz, 2001; Lee, 2011). All these examples across fields involve to some extent a projection in an uncertain future and decisions which may eventually lead to "mistakes" but which aimed at being rational given limited knowledge and time. Note that even at higher political levels, the European Union imposes to the states an arbitrary 3% maximum deficit, with derogations granted based on projections regarding the state's future financial situation (Priewe, 2020). This is an example of rules and decisions based on arbitrary thresholds and projections based on criteria that are questioned (Priewe, 2020). Technological planning could be ruled by other criteria and thresholds involving environmental and social criteria, and this present work proposes the beginning of a path for this.

6.3.2. BEYOND THE ASSESSED SCOPE OF TECHNOLOGIES

The modeled scope of possibilities excludes some technological elements which should be kept in mind before making decisions.

First, the modeled possibilities do not include alternative microalgae production systems such as other types of PBRs (helical, horizontal, bubble columns) or indoor and greenhouse productions that could limit thermoregulation needs, but also limit productivity (Baliga and Powers, 2010) and induce high environmental impacts if the culture is artificially lit (Onorato and Rösch, 2020). Some novel disruptive systems resorting to biofilm cultivation seek to optimize the "active volume per area", i.e., the share of the volume which is photosynthetically active (Ferreira et al., 2020; Mantzorou and Ververidis, 2019; Morales et al., 2020). While most results are at the laboratory scale, biofilm culture looks promising and could greatly reduce water use, harvesting energy requirements and increase surface and volumetric productivity (Ferreira et al., 2020; Mantzorou and Ververidis, 2019; Morales et al., 2020). Such production systems however require strains with the ability to form consistent biofilms, and these biofilms are in general particularly sensitive to temperature changes (Mantzorou and Ververidis, 2019).

Heterotrophic and mixotrophic cultivations were not considered. They often show high productivities due to the additional organic carbon source and were shown environmentally performant compared to autotrophic cultures (Smetana et al., 2017). Heterotrophy and mixotrophy are however limited to some strains that can have requirements regarding the specific metabolizable carbon source (acetate, glycerol, glucose etc.) (Tuchman et al., 2006) for which supplies can be constrained.

Finally, as explained in 4.3, extraction and potential purification of the compounds were not modeled and could represent non-neglectable contributions to the environmental impacts (Pérez-López et al., 2014b).

6.3.3. BEYOND THE LCA SCOPE

Deciding on technological development and deployment implies considerations beyond the scope of LCA as partly addressed in 6.3.1. It is important to recognize that LCA, while a valuable tool, has its specific focus and limitations.

For instance, CLCA was here used to study the marginal changes due to the purchase of a small amount of microalgal compound by increasing the downstream demand for fish. The marginal change involves no sequestration of carbon via photosynthesis as the carbon stored in the microalgal biomass will be eventually released during anerobic digestion and the combustion of the biogas, or during animal respiration. The carbon balance was implicitly modeled as the characterization factor for all biogenic carbon flows in the models is 0 in the ReCiPe global warming impact category. Unlike for forestry and its long rotation time, the used CO_2, for which the marginal supply comes from monoethanolamine capture in industrial flue gases (Thonemann and Pizzol, 2019), is re-emitted not long after photosynthesis and the effect of postponing emissions can be neglected. However, this is true when considering the life cycle of only one product, but does not imply that a continuous production of large volumes of microalgal biomass over Europe could not constitute a substantial stock of carbon with fast rotation. These considerations are not accounted for in the LCA framework when studying marginal changes but could play a role in technological planning.

Regarding the effect of the compound on the fish farm, we did not assume nor model that new compound could replace the existing inputs of chemotherapeutants. These inputs were previously shown to have very little contribution to the total fish impact (Sanchez-Matos et al., 2022). However, these assessments were made despite a lack of data regarding the LCIs for these products and a high impact variability across pharmaceuticals (Emara et al., 2019; Jiménez-González et al., 2004; Parvatker et al., 2019). For this reason, the application of ENSURe in Article IV considered the impacts associated with the production of the existing pharmaceuticals used in the farm as uncertain parameters with very large ranges (from 10 to 1000 kg CO_2-eq per kg pharmaceutical (Parvatker et al., 2019)). The sensitivity of this parameter regarding the difference of impact between fish with or without the compound was found very low and no specific condition on this parameter was found across impact categories to ensure the threshold probability of success. This suggests that assuming potential substitutions of existing medicines with the new microalgal compounds would not substantially change the conclusions for the considered impact categories.

Nevertheless, no direct farm emissions of the pharmaceutical substances were considered and modeled for the existing pharmaceuticals and the new compounds. These direct emissions could cause substantial ecosytem and human toxicity impacts but little data exists regarding the biochemical transformations undergone by the substances in the farm before being released into the water bodies. The replacement rate and toxicity impacts of direct emissions for the current pharmaceuticals and new microalgal compounds could have been included as uncertain parameters in the ENSURe procedure to discover if a certain ratio of toxicity between the two alternatives was necessary to ensure the threshold probability of success.

An even more complex problem is the consideration of antibiotic resistance which is currently induced by the use of antibiotics. This resistance could potentially be reduced by replacing them with prophylactic or different antibiotic microalgal compounds. Recent progress in quantifying the antibiotic resistance enrichment has been made (Nyberg et al., 2021) and characterization factors are now proposed for different antibiotics and expressed in harmony with ecotoxicity impact category at midpoint level (Potentially Affected Fraction of species in the aquatic environment (PAF $m^3.day.kg^{-1}$)), and with human health at endpoint level (Disability Adjusted Life Years (DALY)). These characterization factors were recently used by Sanchez-Matos et al. (2022) in their fish farm LCA. They could also be included in the ENSURe procedure as uncertain parameters, as it is impossible to propose reasonable probability distributions for the antibiotic resistance enrichment associated with still unknown compounds.

Finally, our approach cannot encompass all the side benefits of technological research projects which may provide additional knowledge and know-how that will be useful for other environmentally beneficial projects. Serendipity, i.e., the fortunate discovery of important findings regarding questions or even domains that were not initially targeted, typically constitutes an unpredictable event along technological research that ex-ante LCA cannot consider. For example, bioprospecting for uses in aquaculture could eventually help discovering compounds for human health (Falaise et al., 2016).

7. CONCLUSION

This section answers the research questions, highlights the contributions of this work to the field and proposes directions for future research.

This PhD took up the challenge of tackling a main research question aiming at unraveling the expectations that could be held regarding the future environmental performance associated with the use of yet undiscovered microalgal compounds for fish farming. The succession of articles navigated indeterminacy at the different levels by answering three research questions.

The first research question was

What will be the environmental impact associated with the European production of a currently bioprospected microalgal compound?

This question was associated with the following **hypothesis**:

The impacts associated with the future production of a yet-undiscovered microalgal compound in Europe can be anticipated via probability distributions. These distributions can be obtained with a stochastic LCA approach based on process simulation which accounts for geographic, biological and techno-operational dimensions.

This hypothesis was validated using stochastic propagation of uncertainties in a LCA model based on microalgal cultivation simulation. Article I specifically studied the techno-operational uncertainty and how its propagation to the environmental impacts highly depends on the strain and location. Article II used the previous model and combined techno-operational uncertainty with bioprospecting and production mix uncertainties. It showed large but insightful distributions of impacts for the four assessed impact categories. If a single value was to be used for decision-making, median scores could be interpreted as the most reasonable expectation regarding the environmental impacts for the European production of undiscovered microalgal compounds. Sensitivity analysis highlighted that being able to know the compound content in the biomass and/or the geographic production mix would greatly reduce the uncertainty lying upon the environmental impacts.

The second question studied how the use of a bioactive microalgal compound could change the environmental impacts of fish production and was formulated as:

How does suboptimal biological performances in fish farms affect the life cycle environmental impacts of fish production?

Article III confirmed the following hypothesis:

Mortality and poor feed conversion ratios have distinct influences on the life cycle impacts regarding the timing of their occurrence during the fish production cycle.

A detailed LCI and parameterized modeling could quantify the current environmental opportunity cost of current suboptimal biological performances and show how the timing of the mortality within the fish growth cycle was highly influential on the opportunity cost. Mortality occurring at the end of the cycle was substantially more impactful than at the one occurring at the beginning. These results questioned the notion of environmental opportunity cost as calculated in (Just Economics, 2021).

Finally, the last research question was methodological and emerged from the observation of different levels of incertitude when answering the main research question.

How can ex-ante LCA be used for decision-making regarding deeply uncertain technological concepts?

The following hypothesis was explored in Article IV:

A distinction between risk and uncertainty is necessary to define meaningful probabilities in the output of ex-ante LCA models and provide assistance to decision-making.

This distinction was implemented in the ENSURe procedure which constitutes a methodological and theoretical advance for ex-ante LCA and its specific orientation to support decision-making. The procedure completed the ex-ante LCA models and uncertainty and global sensitivity analysis with theoretical considerations from post-normal science regarding different types of incertitude. Taking inspiration from scenario-discovery, ENSURe used the PRIM algorithm to navigate the uncertain space and inform on the conditions on the uncertain parameters that would ensure a certain probability of success. The probabilities approximated and evaluated by using ENSURe stem exclusively from risk and therefore reasonable levels of knowledge regarding the probabilities associated to the parameters' values.

Finally, the different results answer the main research question:

Which environmental performance and impacts can be expected regarding the use of yet undiscovered microalgal compounds for health-management in finfish farming?

This work validates the associated **main hypothesis:**

Handling of incertitude combined with stochastic ex-ante LCA approach can provide relevant estimates for the environmental impacts of the technological concept.

While Article I to III decomposed the uncertainty associated with the question, Article IV provided the theoretical framework to better handle incertitude and provided support to decision-making under conditions of deep uncertainty. The specific answer to the main question can be divided into three points.

First, the notion of "expected impacts", associated with "probability" is illuminated by the distinction between risk and uncertainty. As Stirling's uncertainty lies upon many indeterminacies in the future configuration of the technology, the quantification of this expectation is problematic, and probabilities can only be compared to thresholds. The use of ENSURe showed that the probability of a successful technological development, defined as a development which implies a lower impact for fish produced using the compound than without it, cannot be shown higher than 85%. Additional iterations of the procedure would be necessary to consolidate this conclusion (cf. Article IV and section 5.2.2). Ensuring a 85% success probability would overall require predicting a very high performance for the compound given the numerous other indeterminacies and their associated probability distributions.

Secondly, reasonable expectations of impacts were provided for the production of an undiscovered microalgal compound in Europe by considering all assigned probability distributions equivalent in terms of level of knowledge (without differentiating risk from uncertainty). The total impact probability distributions are displayed in Article II and section 5.1.2 and show median scores of 1.5 m^3 for water depletion; 96 kg CO_2-eq. for global warming; 0.017 kg P-eq. for freshwater eutrophication, and 0.007 kg 1.4-DC-eq. for terrestrial ecotoxicity (Figure 8).

Thirdly, the stepwise navigation of indeterminacy involving sensitivity analysis provided a thorough understanding of the main causes of risk and uncertainty and their interactions. For instance, it showed how the uncertainty lying upon the photobiological formula and the resulting PBR techno-operational setup propagated substantially more for strains with high thermal ranges in comparatively cold locations. It also demonstrated how anticipating a large geographic deployment of the technology decreases the uncertainty upon the impacts from a whole production mix.

7.1. CONTRIBUTION TO TECHNOLOGICAL DEVELOPMENT AND TO THE SCIENTIFIC FIELD

For the R&D stakeholders and the bioprospecting teams, this work provides a rough identikit of the ideal microalgal compound and host strain to maximize the probability of low environmental impact from the beginning of the project. Initial guidance can thus be provided to the technology developers and particularly to the biologists regarding the types of strains and compounds they should try to discover.

If possible, bioprospecting should focus on bioactive compounds that constitute a large share of the total biomass and strains that have large thermal tolerance ranges to minimize future thermoregulation needs. Within these candidates, the ideal compound should hopefully feature a substantial capacity to reduce the fish biological FCR, especially in the last stages of the production. If these conditions are met from the beginning, it will substantially increase the odds of an environmentally performant synergy with finfish farming even if the upscaling of the reactor and the geographic deployment occur without stewardship.

At a higher decision level, this work provided a detailed representation of the environmental “signal” of this technological concept, which is not only a set of impact values, but a set of probability distribution and probability thresholds associated with a deep understanding of the model’s sensitivities and hypothesis. This set of results constitutes valuable knowledge to decide on future orientations of the Blue Bioeconomy in Europe. It allows comparing this signal with the ones that could be obtained for other technological concepts with similar functions such as new vaccines, plant-based prophylactics, bacterial probiotics, humic substances, etc. (Lieke et al., 2020). This knowledge can also enlighten the enforcement of regulations and subsidies, for instance to restrict technological development and deployment to certain geographic areas or to promote the development of infrastructures to handle microalgal co-products according to their most beneficial fates.

This work also provides two new parameterized LCA models, ready to be used by the community. The model presented and developed in Articles I and II allows to simulate microalgal production and calculate its LCA in any location, season, vertical tubular PBP techno-operational setup, and for any strain and compound provided that some characteristics are known. The LCA model presented in Article III allows studying the influence of changes in mortality and/or biological FCR along the growth cycle. This enables projecting trout farming into the desired disease regimes or anticipating the environmental impacts associated with new health management strategies.

Article II constitutes, to the best of my current knowledge, the first study which consists of an LCA applied to a whole biological group (microalgae), over a whole geographic area (Europe). Among the various LCA studies on microalgae, the study is the only one to evaluate the environmental impact of a whole production mix producing the same strain and product. The production mix level constitutes the last step of technological deployment and is particularly relevant for microalgae productions. Our assessment thus takes into account that the supply of a microalga with a certain thermal range could feature high environmental impacts in a location, but a comparatively low environmental impact when assuming that the supply is shared between locations with different temperatures, irradiances, and energy mixes.

The fish farm LCA model and the study of its behavior questioned the notion of environmental opportunity cost which is a widely used notion in industrial ecology (Abu-Ghunmi et al., 2016; Eisen and Brown, 2022). The analysis demonstrated that simply multiplying the lost fish biomass by a single emission factor, typically associated with the production of 1 kg of commercially-sized fish, leads to a virtual impact that fails to capture the potential impact reduction achievable if the production had not experienced these losses.

Above all, this work proposes to deepen the ex-ante LCA approach to make it fit for assessing deeply uncertain technological concepts and all the directions that their developments and deployments could take. This deepening consists of several main aspects. First, I proposed a formalization of the use of ex-ante LCA not to guide R&D, but to decide whether a concept should be further explored under times of emergency and resource scarcity. I also included and assessed the geographical scope of deployment of the technology as part of the incertitude and "social uncertainty" regarding the future environmental impacts, which constitutes a step forward compared to considering locations as mere scenarios. Overall, the use of scenario discovery within ENSURe moves away from a discrete scenario development approach which overlooks a large share of the scope of possibilities and involves the biases and limits of expert elicitation. Finally, this work also contributes to building a bridge between the ex-ante LCA research community and the rest of the post-normal scientific realm, especially by distinguishing risk and uncertainty. This also participates in making ex-ante LCA comply to a better extent to the ethics of quantification (Saltelli et al., 2020) when attempting to provide decision support for technological planning under conditions of deep uncertainty.

The methodological and theoretical advances of ENSURe allow approximating probabilities even when uncertainty lies upon the decision-making exercise, which constitutes a substantial progress toward the use of numerical indicators and probabilities under conditions of deep uncertainty. While scenario discovery pre-exists this PhD work, its use combined with the distinction between risk and uncertainty and the law of conditional probabilities is, to our knowledge, a novelty that could be used beyond the LCA framework for model support to policy making.

7.2. FUTURE RESEARCH AND PERSPECTIVES

7.2.1. FOR THE ASSESSMENT OF FUTURE MICROALGAL COMPOUNDS AND FOR BIOPROSPECTING

More informed anticipation of the impacts of microalgal products and uses in the future would benefit from projections regarding weather and temperature in European locations in the following years. While average climate projections are available, geolocalized daily temperature profiles over the seasons require "morphing" current temperature data according to information on projected climates. This exercise has already been performed to estimate the future building energy demand in China (Zhu et al., 2013) and could be adapted for the microalgal sector. A warmer climate would likely decrease the heating thermoregulation needs but increase the cooling ones. Freshwater scarcity will favor marine and brackish water microalgae and further increase the attractivity of coastal locations for access to the resource.

Additionally, the prospective ex-ante LCA modeling could benefit from prospective background databases to project the technologies into the future economy. Indeed, the current marginal mixes considered in the current databases may be valid for short and midterm projections but will change in the future. Recent advances were made regarding the modification of the ecoinvent database according to the outputs from Integrated Assessment Models (IAM) run under conditions aligned with different Shared Socioeconomic Pathways scenarios (SSP) associated with Representative Concentration Pathways (RCPs) (Mendoza Beltran et al., 2020; Sacchi et al., 2022). SSPs describe possible broad socioeconomic orientations for human cities in the following decades and can be associated with RCPs which represent archetypes of greenhouse gas emissions and global warming trajectories. The methodology to derive prospective consequential databases from this approach is not established yet. Using these prospective databases in our assessment will add a new layer of complexity and uncertainty as anticipating the environmental impacts and obtaining probabilities of impact for decision-making would require exploring the indeterminacies in the IAM themselves. Above all, evaluating the probability of success for the technology will require stating about the probabilities of human societies heading toward each SSP and RCP scenario and some works are paving the way for this (Peters and Hausfather, 2020; Schwalm et al., 2020). Particular attention should be given to avoiding narrative mismatches regarding the LCA parameters and the assumptions underlying the SSP and RCP scenarios. Typically, modeling a large geographic deployment of microalgal production over Europe may not be compatible with all narratives regarding global socioeconomic orientations.

Another research direction is the application of the methodology proposed for the ENSURe procedure to make bioprospecting more target-driven by finding the strain-compound profile that would ensure a certain probability of success. In this work, we used ENSURe in a precautionary paradigm by differentiating parameters based on the level of knowledge supporting the assigned probabilities. The uncertain parameters were mainly chosen as the ones defining the compound's performance for health management in the fish farm, which are parameters that only become determinate long after the beginning of the technological exploration and can hardly be actively selected from the beginning. The compound's content in the biomass was the only uncertain parameter directly measurable after a strain is discovered while the other parameters defining the strain were considered as "risk" because data on microalgal biodiversity was available.

To discover which part of the biodiversity the bioprospecting biologists should try to focus on, all the parameters defining the strain should be treated as "uncertain" so that ENSURe could also find constraints on them to ensure conditional probabilities of success. Currently, the use we made of ENSURe considers that bioprospecting is an inherently random process that cannot be guided. This standpoint is valid to a large extent, especially when trying to isolate strains from the environment based on specific criteria. As documented by many, this selective isolation process is indeed a highly uncertain and complicated procedure (Andersen et al., 2013; Bhatnagar et al., 2011; El Hajji, 2010; Lacroux et al., 2022; Pandey et al., 2019; Pereira et al., 2011). Nevertheless, selective isolation from the environment is not impossible, and the many strains that are already known and available in the collections could be explored according to the found criteria. Considering the results obtained in my work, ENSURe would possibly find constraints on the sensitive parameters defining the strain's thermal range but not on the other strain-specific parameters. In an ideal scenario, ENSURe would find a box for which the strain's thermal range is constrained instead of some of the uncertain parameters regarding the compound's performance. This would mean that no predicting would be required regarding this aspect of the compound's performance to ensure a certain probability of success as long as bioprospecting is focused on strains with the thermal ranges identified in the procedure. The potential for such an approach is large and would empower biologists to limit the probabilities of their work being later adopted and deployed into commercial technologies with poor environmental performances.

Now that the AquaHealh project started gathering the first data on some candidate strains, the same models could be used for guidance. If this technological concept is indeed further explored, the wide distributions chosen for most of the parameters could be updated with new knowledge to narrow down the scope of possibilities. Note that this would only refine the estimates regarding the specific strain and compound that would have been discovered and showed interesting properties, but any new bioprospecting process for another compound would start with the same initial incertitude.

7.2.2. FOR EX-ANTE LCA AND TECHNOLOGICAL PLANNING

Updating knowledge and reasonable expectations according to new data and knowledge is at the core of Bayesian statistics, which could be better implemented in ex-ante LCA. The wide uniform distributions assigned to some parameters in our study could be considered as "uninformative" or "flat" priors in Bayesian theory, i.e., probability distribution which were informed by very little information (Van Dongen, 2006). The Bayes formula could be used to modify this prior according to new data. Progressively along the R&D process, the probability distributions for the parameters would hopefully narrow, and more targeted guidance could be provided. A direct implementation of Bayesian statistics in ex-ante LCA could provide a clear theoretical framework to update estimates along the technological development.[1]

In this perspective, the propagation of large uniform distributions for the uncertain parameters in ENSURe could simply be understood as the propagation of a flat prior, which already constitutes probabilistic information in Bayesian theory. Hence, in a Bayesian perspective, the difference between risk and uncertainty in Stirling's framework would become blurrier, as a problematic level of knowledge regarding the probabilities for a parameter would simply mean a very flat, uninformative prior. The output distributions resulting from the joint propagation of risk and uncertainty parameters could simply be interpreted as an output probability distribution stemming from priors which will have been differentially updated. However, this would not disqualify the approach proposed in ENSURe which allows refining the estimates and allows not to state about dependencies between parameters a priori. Overall, it would be interesting to see how Bayesian statistics and the conceptual distinction of risk and uncertainty can cohabitate and be implemented for better model-based support to decision-making and technological planning.

Better support to decision-making in ex-ante LCA under deep uncertainty, for instance with the help of procedures such as ENSURe, will also require further refining the definition of a "successful technological development". It is arguably not sufficient to base a decision on the probability of a concept eventually outperforming a baseline or an alternative, as it says nothing about the magnitude of the success or the failure. Ex-ante LCA and in particular its use for precautionary technological planning could therefore adopt the learning and methods from the field of Risk Analysis (Starr et al., 1976), which defines "risk" as the product of the probabilities of an event multiplied by the consequences of this event.

Due to the challenging incertitude it had to embrace, this work led to the proposal of a rather radical standpoint on the use of ex-ante LCA in a paradigm of technological planning under ecological and social emergency. Deciding to delve deeper into a technological concept would require politically agreeing on the objectives and values that technological planning and production should promote. To limit climate change to a certain temperature at the end of the century, or to ensure a safe-operating space

1. I refer the reader to the doctoral work of C.F. Blanco (2022) for the development of such Bayesian approach in ex-ante LCA.

   Blanco, C.F., 2022. Guiding safe and sustainable technological innovation under uncertainty : a case study of III-V/silicon photovoltaics [Doctoral dissertation, Faculty of Science, Leiden University). Leiden Repository. https://scholarlypublications.universiteitleiden.nl/ha. Leiden University.

within the planetary boundaries, decisions regarding investments of time and resources should not simply be made based on the probabilities of outperforming alternatives. Instead, they should be based on the probabilities of proposing solutions that contribute to achieving the predetermined objectives. In this sense, the research on absolute sustainability assessment should be continued and further integrated (Bjørn et al., 2016, 2015), together with the development of social LCA and subjective well-being measurements (Weidema, 2023, 2018).To some extent, the challenging articulation of these two paradigms constitutes the logical continuation of the direction paved by Rockström et al. (2023) who include justice considerations in the definition of safe and just planetary boundaries.

Adding this layer of modeling on top of midpoint impact categories will add scientific and social uncertainties, and involve ethical choices, making quantitative ex-ante assessment even more complex. Considering these advancements, it will become all the more essential to abide by the ethics of quantification (cf. 4.1)(Saltelli et al., 2020; Scoones, 2019), avoid black box models, and "*demystify the mathematics of uncertainty*" (Funtowicz and Ravetz, 1990). In the near future, the assessment of incertitude should hopefully become the norm and not a mere bonus step in LCA. Ex-ante LCA will not provide single impact values but quantitative environmental signals with the necessary information to navigate them and make the most reasonable decisions at different levels, from the societal technological planning to the R&D stage.

Finally, it will be necessary to further investigate how these different levels of decision can integrate these results and tools in practice. Pryshlakivsky and Searcy (2021) already highlight the existing gap between operational LCA tools and actual decision-making processes in organizations. The complexity and incertitude presented in the assessments often face organizational and cognitive barriers, leading to final decisions that deviate from well-informed rational choices. Building upon these observations, Pryshlakivsky and Searcy (2021) identify two overarching trajectories followed by the LCA community. One trajectory is seeking to simplify LCA, creating a streamlined version for internal company guidance at a micro level. The other trajectory, mainly undertaken by academia, heads toward an expansion of the scope of LCA for policy-making and technological planning, introducing new orders or incertitude. The authors argue that this higher level of LCA usage is still "*unproven and contentious*", mainly because it requires a more planned economy and because "*the move toward new economic paradigms is unlikely, particularly in the near-term*". The use of ex-ante LCA proposed in this work acknowledges both the necessity of this move, but also the current economic system which requires considering social uncertainties regarding the deployment of the technology depending on chaotic market mechanisms. Finding the institutional structures capable of effectively using this LCA approach will likely require political innovation requested by the contemporary environmental and social stakes.

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9. ARTICLES

Article I

Jouannais, P., Hindersin, S., Löhn, S., Pizzol, M., 2022. Stochastic LCA Model of Upscaling the Production of Microalgal Compounds. Environ. Sci. Technol. 56, 10454–10464. https://doi.org/10.1021/acs.est.2c00372

Environmental Science & Technology

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Stochastic LCA Model of Upscaling the Production of Microalgal Compounds

Pierre Jouannais, Stefan Hindersin, Sarah Löhn, and Massimo Pizzol*

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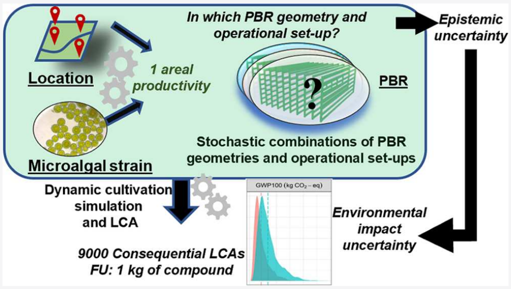

ABSTRACT: Microalgae are currently being investigated for their promising metabolites but assessing the environmental impact of producing these compounds remains a challenge. Microalgae cultivation performance results from the complex interaction of biological, technological, geographical, and physical factors, which bioengineers try to optimize during the upscaling process. The path from the discovery of a microalgal compound to its industrial production is therefore highly uncertain. Nonetheless, it is key to anticipate the potential environmental impacts associated with the future production of a microalgal target compound. This is achieved in this study by developing an ex-ante, parameterized, and consequential LCA model that performs dynamic simulations of microalgae cultivation. The model is applied to calculate the environmental impacts of 9000 stochastically generated combinations of photobioreactor geometries and operational setups. The demonstration of the model is done for a fictive microalgal strain, parameterized to resemble *Chlorella vulgaris*, and a fictive target compound assumed to be a carbohydrate. The simulations are performed in Aalborg, Denmark, and Granada, Spain to appreciate geographical variability, which highly affects the requirements for thermoregulation. Open-source documentation allows full reproducibility and further use of the model for the ex-ante assessment of microalgal products.

KEYWORDS: *life cycle assessment, uncertainty, upscaling, microalgae, photobioreactor*

1. INTRODUCTION

Microalgae are increasingly being produced for high-value compounds used as food supplements and nutraceuticals.[1,2] The detailed phylogenetic knowledge of the vast microalgae biodiversity[3,4] and recent discoveries in bioprospecting suggest that there is high potential for future large-scale production of microalgal compounds with interesting bioactive effects.[5−8] These compounds are very diverse and range from anti-inflammatory lipids to antitumoral peptides and immunomodulating or antimicrobial carbohydrates.[5,9]

In a context of ecological emergency, it is crucial to be able to estimate the environmental impacts associated with the future industrial production of a new microalgal compound as soon as the scientific community becomes aware of its existence. This estimate should therefore be provided ex-ante, for example, by means of life cycle assessment (LCA). Such a prospective LCA should also guide subsequent R&D toward improved environmental performance. However, upscaling of a microalgal compound production from a laboratory to an industrial scale for a new strain is subject to many uncertain factors that will eventually affect the assessment.

We identified four LCA studies of microalgae production that rely on primary data, all at a laboratory or pilot scale.[10−13] Other existing studies[14−27] make assumptions on biomass productivity under given conditions and can thus be defined asex-ante LCA because they attempt to predict performance at an industrial scale. These eighteen LCA studies focus on six real microalgal strains only, for which documented consensus on productivities in specific systems partly exists. Slegers et al.[28] criticize the use of these assumptions and recommend making assumptions on productivity that are coherent with the geometry of a photobioreactor (PBR) and the operational setup for a given strain because these two factors will eventually lead to different energy and material consumptions. Modeling tubular PBRs, Slegers et al.[29] showed how, for different locations and strains, the geometry of the PBR affects productivity. Discussing the potential of microalgae strains for different applications, Mata et al.[30] concluded that, for each strain, it is crucial to create an optimal "photobiological formula" in a given location. This formula can be defined as the combination of techno-operational parameters that will enable a stable and maximal

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production by providing, among other factors, a strain-specific optimal light exposition and use, nutrient accessibility, and minimal shear stress.[31]

Some of the complex biophysical phenomena playing a part in this formula have been mathematically modeled.[32−34] Nevertheless, most biophysical models of microalgal growth apply to a laboratory scale and cannot be reasonably applied to ex-ante LCA studies because of the high number of required parameters and computer simulation capacity. Williams and Laurens[35] argued that due to the complexity of the biophysical phenomena, the parameters maximizing the productivity of a strain that has never been cultivated at a large scale "*cannot be anticipated with any degree of certainty*".

In their parameterized LCA model, Schade et al.[15] assumed an overall photosynthetic efficiency PE = 3.4% for *Nannochloropsis sp.* and *Phaeodactylum tricornutum* in a PBR with a fixed geometry and operational setup. On the grounds of the previously exposed arguments, assuming such efficiency for any strain in the same PBR cannot be done with "*any degree of certainty*".

Certainty, however, is not required for an ex-ante LCA to be useful and necessary. Stochastic error propagation, scenario analysis, and sensitivity analysis based on probabilistic models are increasingly used in this context to explore and understand uncertainty.[36−38] Following this trend, the objective of this study is to introduce a parameterized and stochastic LCA model, which includes and assesses the uncertainty associated with the upscaling of any target microalgal compound production in photoautotrophy (inorganic carbon source and solar energy source) and in a vertical tubular PBR. The novelty of the approach lies in the combination of a dynamic cultivation simulation via established[15] and newly proposed deterministic calculation modules, with stochastic sampling for the parameters that could enable a certain energetic yield on solar light for a specific strain and location. The approach thus intends at adding robustness to decision-making when forecasting the impacts of microalgal compounds at a certain production volume. We tested the approach and analyzed uncertainty, sensitivity, and process contribution for a fictive strain and target compound (a carbohydrate) with realistic parameters cultivated in Granada, Spain, and in Aalborg, Denmark, to appreciate the effect of geographical variability.

2. METHODS

The deterministic and parameterized life cycle inventory (LCI) model simulates the cultivation of a strain and its specific compound (here defined as a "strain-compound" combination) in a specific location and vertical tubular PBR. Briefly, the cultivation consists in microalgae performing photosynthesis with solar light in a water suspension (culture) that contains the necessary nutrients, which is pumped through an outdoor circuit of tubes. The biomass concentration is maintained at a certain value in the reactor by harvesting the additional biomass every evening via centrifugation. The obtained microalgal slurry is eventually dried and the cells are disrupted to ease future extraction of the compound and use of the rest of the biomass. Since it is impossible to know, a priori, which combination of the PBR geometry and operational choices will allow the modeled strain to reach its estimated energetic yield[35] ($g \cdot kJ_{ground\ irradiance}^{-1}$), we generate a stochastic sample of the uncertain parameters associated with the PBR geometry and operation.

The LCI model is composed of modules based on biological, physical, and thermodynamic principles which simulate the main processes of the strain-compound production. The thermoregulation module, the wastewater module, and the algorithm for coproduct substitution were developed ex novo, and other modules were obtained by adapting or directly using equations from the literature. In particular, the productivity module is built on the work of Williams and Laurens,[35] and we adapted most of the parameterized modules for PBR operation previously developed by Shade et al.[15] We made these modules dynamic using the reactor's temperature evolution as an input to the modules' equations (Figure 1). The temperature at $t + 1$ depends on the temperature at t and on the interaction of some of the 20 techno-operational, 23 biological, 5 physical, and 5 geographical parameters used in the simulation. All of these parameters are defined as primary because of their assumed independence, which means that none of the primary parameters can be calculated based on other primary parameters and that the choice of a value for one does not affect the choice for another. For instance, the lipid content in the ash-free biomass is a primary biological parameter but not the carbohydrate and protein contents, which are secondary parameters estimated using lipid content and statistical observations within reported microalgal biomass compositions (cf. SI I.1). Once the LCI is obtained from the simulation of the cultivation, a static LCIA step is performed.

Figure 1B shows a map of the connections between primary parameters and modules. We refer the reader to SI I for details on the parameters, mathematical basis, behavior demonstration, and validation of each module.

2.1. Description of Modules.

2.1.1. Nutrients and CO_2. Following a previous LCA harmonization study,[39] we distinguished nutrient and CO_2 "demand", i.e., the amount needed for the strain growth, from "input" which is the actual supply to the culture given that only a fraction of the nutrients and CO_2 is assimilated. Input is obtained from demand using a utilization rate (cf. SI I.3). CO_2, N, and P demands are based on the strain biochemical profile (phospholipids, other lipids, carbohydrate, protein, ash) and on the average elemental compositions of each biochemical class.[40] Mg, K, and S demands are based on the ratios observed between N content and these elements in *Chlorella vulgaris*.[14] Other elements in the medium are neglected. The nutrients sources are indicated in SI I and SI II.1.

CO_2 that has not been assimilated is degassed and emitted to the atmosphere. Other nonassimilated nutrients are contained in the wastewater. No energy consumption is assumed for aeration as pressurized liquid CO_2 is used. O_2 degassing is also considered as a passive process resulting from culture mixing. Similarly, we assumed that the pH is maintained according to the strain requirements without involving substantial additional economic and environmental flows, as it can be controlled by piloting CO_2 and nutrient injection over the day.[41−43]

2.1.2. Water Use and Treatment. Water consumption depends on the PBR volume, the biomass concentration at harvest time, the water content in the dried biomass, and the productivity. After centrifugation, the supernatant is partly recycled (30%).

Wastewater management is mostly disregarded in other microalgae LCA studies. However, this management is of high importance to recover water but could also be associated with important impacts. In the model, the mass of nutrients that have not been assimilated is sent to wastewater treatment together

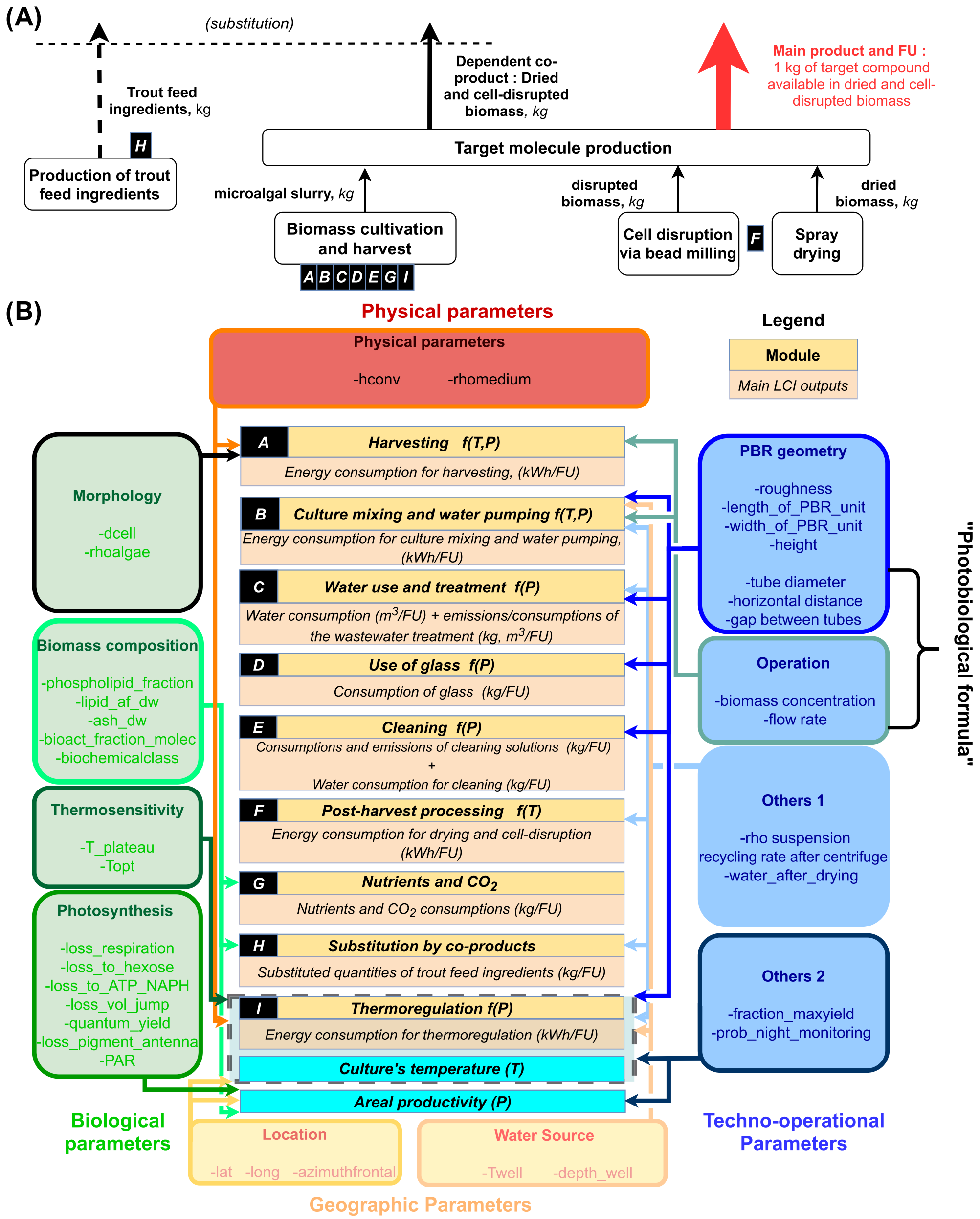

Figure 1. Simplified foreground product system (A) and mapping of the interactions between primary parameters and modules (B). (A) Letters (A–I) refer to the LCI modules described in B. The inputs and outputs of an activity labeled with "A" are calculated with the module labeled "A" in panel (B). cf. SI II.2 for an extended version of the foreground product system diagram. (B) Boxes with curved borders include independent primary model parameters classified into different types (physical, biological, geographical, techno-operational). Squared boxes represent model modules, where operations are performed using the primary parameters. Blue modules are submodules that provide secondary parameters for other modules (cf. SI I.3-4). The dotted line box containing "thermoregulation" and "culture's temperature" indicates that these two modules share the same equations and cannot be considered separately. $f(T)$ and $f(P)$ specify that the calculation of the LCI for this module is a function of the culture's temperature and productivity, respectively. Arrows between blocks of parameters and modules indicate that at least one of the parameters is used as input in the module's equations. For simplicity, some independent primary parameters are purposely omitted (cf. SI I.1).

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with the discharged water and we estimated an average composition for such wastewater. Additionally, the wastewater contains a small amount of microalgal biomass depending on the centrifugation efficiency. We modified a standard wastewater treatment activity from the ecoinvent consequential database (version 3.6, cf. SI I.4) so that the mass balances match the specific microalgal wastewater composition.

2.1.3. Culture Mixing and Water Pumping. Culture mixing is the result of maintaining a specific flow rate in the PBR with a centrifugal pump. The electricity consumption associated with pumping 1 m^3 depends on the height from which the water is taken (water source or centrifuge).[15] Daily energy for culture mixing depends on the dynamic viscosity,[15] which is approximated with a multiple linear regression on temperature and biomass concentration based on measurements from the literature.[44]

2.1.4. Harvesting. In the model, disk centrifugation is performed at the beginning of the night and uptakes the production of the day so that the initial biomass concentration in the morning remains at the targeted value. A harvesting flow rate of 5 $m^3 \cdot h^{-1}$ is assumed. We used an established empirical model[45] using strain-specific settling velocity and harvesting flow rate to estimate the energy consumption to centrifuge 1 m^3 of culture with a given separation rate. Following Stoke's law,[45] we modeled the strain-specific settling velocity as a function of the microalga's cell diameter, the densities of the cell and the medium, and the dynamic viscosity of the medium (which depends on the temperature).[44]

2.1.5. Thermoregulation and Culture's Temperature. Thermoregulation is crucial to maintain optimal and stable production by keeping a strain in its acceptable thermal range.[30,46,47] Our model allows estimating electricity requirements for thermoregulation in any location, season, and vertical tubular PBR geometry using data from the European Photovoltaic Geographical Information System (PVGIS).[48] Using time series from 2005 to 2016, PVGIS provides hourly values of direct and diffuse solar irradiance falling onto any tilted surface and hourly values of the outside temperature. We used this data to estimate convective thermal exchanges between the PBR and the surrounding air and to estimate the solar power captured by the PBR, for which we assume full conversion into heat.[49] Convective exchanges with the air are modeled following previous thermal modeling of PBRs,[10,50,51] and the convective exchange coefficient is included in the uncertainty and sensitivity analysis because of its unpredictable, wind-dependent value. We modeled thermal exchanges due to water replacement after centrifugation as well. The model is thus able to simulate temperature evolution over a day and allows calculating the thermal power needed to keep the culture within the strain-specific thermal range by cooling or heating. In the module, heating is provided by an electric heater, and cooling is performed via a perfect heat exchanger, whose cold source is the well or river from which the cultivation water is pumped. The flow rate of the heat exchanger is modulated to match the cooling requirement, and the pump electricity consumption is estimated. No water consumption is assumed for the cooling fluid as water is pumped back to the cold source with a neglectable temperature increase. It is also relevant to model a situation where no thermoregulation is needed at night, as the induced metabolic activity decrease may limit biomass loss through respiration for some strains.[51,52] The module was validated using primary data from a previous LCA study[12] and showed a good match of calculated and observed values for the same seasons and location (cf. SI I.4).

2.1.6. Postharvest Processing. To extend product lifetime and ease storage, the biomass is dried via spray drying and the corresponding energy consumption is calculated.[15] We excluded the compound extraction stage from the model as the extraction technology and related LCI were heavily dependent on the target compound, and in this study, we focused on the cultivation stage. However, cell disruption can be considered as a necessary step to release any compound from the microalgal biomass and ease its extraction. Including cell disruption in the product system therefore gives insight into the minimum impact of the postharvest processing. We considered 1 kWh of electricity to disrupt 1 kg of dried biomass via bead milling.[53]

2.1.7. Use of Glass. From the infrastructure, only the borosilicate glass of the tubes is considered. We assumed a life expectancy of 50 years[15,54,55] and a glass thickness of 2 mm[15,55] and calculated the total input of the glass material based on the PBR geometry.

2.1.8. Cleaning. To ensure a contamination-free culture, the PBR is cleaned with hypochlorite and hydrogen peroxide solutions (cf. concentrations in SI I.1) before and after the cultivation period.[10,15,21] This water is discarded in the environment with its hypochlorite and hydrogen peroxide content.

2.1.9. Substitution by Coproducts. The residual biomass generated when producing the target compound is considered a byproduct to be used as trout feed, thus substituting the production of such feed elsewhere. Microalgae have long been pointed to as potential new feed ingredients in fish farming.[6,56] However, a requirement for valid modeling in consequential LCA is that the substituting product fulfill the obligatory properties of the substituted one.[57] In our case, microalgae-based fish feed needs to provide the same fish growth performance as reference fish feed.

Nutritional studies suggest that microalgae biomass could be integrated into fish feed with an incorporation rate inferior to 10% without affecting fish growth performance if the biochemical profile (lipid, protein, carbohydrate, water, and ash) is kept constant.[58,59] In a conservative approach, we thus assume that the microalga can be incorporated at 10% in the new feed recipe provided that the biochemical profile of the feed remains the same. The constant biochemical profile is therefore considered as the obligatory property for the trout feed market. No further assumption on the digestibility of the microalgal biomass is made for the substitution.

As a result, the fractions of each ingredient in the new recipe are modified according to the biochemical profile of the incorporated residual biomass. The residual biomass composition depends on the biochemical class (lipid, protein, carbohydrate) and the concentration of the target compound in the biomass. The target compound is modeled as a generic compound of its class in terms of elementary and nutritional content. The new feed recipe and the resulting product substitutions are computed via nonlinear optimization under constraint to minimize the difference between the reference biochemical profile and the one from the new recipe (cf. SI I.4).

The reference trout feed recipe was defined after consulting with trout farmers in Denmark (cf. SI I.6). Each ingredient is eventually linked to the marginal market for feed energy and feed protein.[60]

2.2. Areal Productivity Module and Associated Uncertainty for the PBR Geometry and Operational

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Setup. A theoretical keystone of the model is the strain areal productivity ($g_{dw}\cdot m^{-2}\cdot d^{-1}$) module from Williams and Laurens,[35] which calculates a theoretical maximum energetic yield ($g_{ash\text{-}free\ dw}\cdot kJ_{ground\ irradiance}{}^{-1}$). The maximum energetic yield depends on the strain-specific biochemical profile (lipid, carbohydrate, protein), the oxidation level of the nitrogen source, and the strain-specific losses occurring during photosynthesis. Based on measurements from different PBRs and strains, Williams and Laurens[35] showed that only 30% of this theoretical energetic yield is usually obtained. Unlike the use of an overall "photosynthetic efficiency,"[15,61] which mixes inevitable biological losses and cultivation performance, this percentage can be argued to depend exclusively on the optimization level of the cultivation. In other words, it depends on how well the PBR geometry and operation parameters match the strain-specific "photobiological formula".[30] Optimizing a reactor geometry and operation for a given strain depends on many aspects and trade-offs. It is first necessary to set the culture to a light-limited regime (all other factors are optimal for the strain) and full absorption of the incident light without any dark zone in the tubes (*luminostat* mode).[49] Maintaining *luminostat* in fluctuating outdoor conditions is a challenge and depends on the location and season, PBR geometry, biomass concentration, and strain-specific radiative and absorbing properties. Furthermore, even if *luminostat* should remain an objective, maintaining a high concentration often allows a more stable production while limiting potential contamination and is thus a common operational choice.[55] In addition, distinct strains feature different tolerances to light intensity and regimes to avoid damage to their photosystems.[62,63] Finally, strains are differentially affected by shear stress resulting from pumping and turbulences, which are, however, necessary to generate quick dark–light cycles for the cells and avoid sedimentation.[31,64−67] All of these biophysical factors are affected by the choice of five PBR geometries and operation parameters: horizontal distance between stacks, vertical gap between tubes, tube diameter, biomass concentration, and flow rate[29,66] (cf. SI I.3 for visualization of the PBR geometry). The values of these five parameters that will enable a specific strain to reach 30% of its theoretical energetical yield are considered epistemically uncertain due to the complexity of the biophysical trade-offs previously exposed.

2.3. LCA Framework. The functional unit is the production of 1 kg of target compound contained in the dried biomass in which cells' membranes have been disrupted (first stage of extraction). A simplified version of the product system is available in Figure 1 and an extended version can be found in SI I.2. The model is designed to simulate the production of a target compound, which can be a protein, a lipid, or a carbohydrate, at any percentage. For the sake of model demonstration and to illustrate a plausible situation with low compound content, the model was parameterized to simulate a carbohydrate target compound that constitutes 10% of the total carbohydrate content in the microalgal biomass. Given the total carbohydrate content calculated based on the lipid fraction and the ash fraction (primary parameters, cf. SI I.1,3), the concentration of the compound in the dried microalgal biomass resulted in $0.0256\ g_{compound}\cdot g_{dw}{}^{-1}$.

The consequential LCA methodology is applied using marginal suppliers from ecoinvent 3.6 consequential as a background database (cf. SI II) and performing substitution with the coproduced biomass. We used the ReCiPe Midpoint (H) V1.13 method in the impact assessment. We also used the average European marginal electricity mix for both locations to isolate the influence of the climate and the PBR geometry on the environmental impacts from the variation due to different mixes.

2.4. Simulation Procedure, Uncertainty Propagation, and Sensitivity Analysis. The model is coded on Python 3.8 combined with the LCA package Brightway2[69] and is accessible on Github.[70] For a fictive new strain (whose parameters' values were chosen close to *C. vulgaris*, cf. SI I.1) and a fraction of maximum energetic yield achieved (0.3, cf. SI I.1), 9000 combinations of the six uncertain parameters (five related to the photobiological formula (cf. 2.2 and Figure 1) plus the convective exchange coefficient (cf. 2.1.5)) are stochastically generated with the extended Fourier amplitude sensitivity test (FAST) module from the SALib library.[71,72] Nine thousand LCAs are thus calculated and result in density curves for the considered environmental impact categories (Figure 2) and for the contribution analysis (Figure 3). Based on the same sample, FAST is performed to determine the shares of the output variability explained by each uncertain parameter.

We defined the intervals for the uncertain PBR geometry and operation parameters by collecting values observed for different strains and vertical tubular PBRs in the literature, including results from models. Values and intervals for all parameters are provided in SI I.1 together with sources. We assumed uniform distributions as it was not possible to justify a more refined uncertainty structure[68] with the information available.

The cultivation is simulated for a hypothetical large-scale plant in operation starting from April to the end of September in two different locations: a location near Aalborg, Denmark, and one near Granada, Spain. The assessment is made on an average square meter of this farm for which we assume a division in 900 m^2 PBR units defined by the geometrical parameters (cf. SI I.1-3). For each month, an average day is simulated, and the results are multiplied by the number of days of operation in that month.

3. RESULTS AND DISCUSSION

3.1. Average Environmental Impact Scores and Scenarios Comparison. Substantially different impact magnitudes are observed in Figure 2 for the presented scenarios. While cultivation in Granada without thermoregulation at night resulted in an average global warming impact (GWP100) of 537 kg CO_2-eq per FU, the worst scenario, in Aalborg with thermoregulation at night, reached an average of 3436 kg CO_2-eq. Such a difference is explained by a lower productivity in Aalborg (14 $g_{dw}\cdot m^{-2}$ versus 21 $g_{dw}\cdot m^{-2}$) due to the identical energetic yield and lower ground irradiance in Aalborg, but, above all, to higher heating requirements for Aalborg. Indeed, the heating energy per FU was on average 10 times higher in Aalborg with night thermoregulation than that in Granada without: 14107 $kWh\cdot kg_{compound}{}^{-1}$ versus 1230 $kWh\cdot kg_{compound}{}^{-1}$ (cf. SI II.2). Thus, on average, thermoregulation accounted for 90% of freshwater eutrophication (FEP), 84% of GWP100, and 67% of water depletion (WDP) in the case of Aalborg with night thermoregulation (Figure 3 and cf. SI II.3). The contribution of thermoregulation in the case of Granada without night thermoregulation still amounted, on average, to 51% of FEP, 37% of GWP100, and 20% of WDP.

Heating was by far the main contributor within thermoregulation, and this remained true for Granada without night thermoregulation with 1230 kWh per FU on average for heating and 5 kWh for cooling (cf. SI II.2). This asymmetry between cooling and heating can first be explained by the significant thermal inertia entailed by a large volume to heat during cold

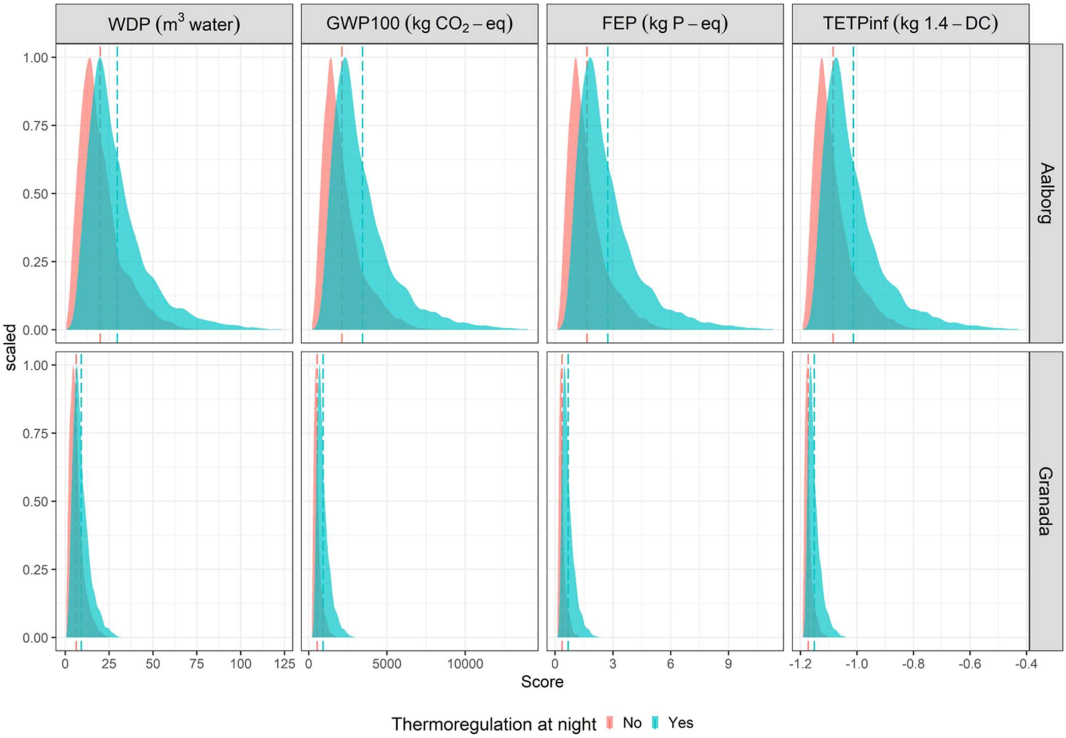

Figure 2. Impact score density curves for the cultivation of 1 kg compound in Aalborg and Granada, with or without thermoregulation at night. The dashed lines indicate the means. Each curve is the result of 9000 simulations and the densities are scaled to 1 for readability (cf. SI II.2 for statistical description of the model's outputs). WDP: water depletion, GWP100: 100-year time horizon global warming potential, FEP: freshwater eutrophication, TETPinf: terrestrial ecotoxicity.

hours and a vast exchange area to dissipate superfluous solar energy. Furthermore, the asymmetry may also be enhanced by the simulated cultivation period, which comprises relatively cold months (April, May, September), and using monthly averages, which trims daily peak temperatures. This could lead to a methodological underestimation of cooling as seen in the model validation (cf. SI I.4). Overall, validation showed good approximations of primary data obtained during PBR operation by Pérez-López et al.,[12] who also identified thermoregulation and heating as main hotspots.

Owing to the importance of heating, the absence of night thermoregulation resulted in a 40% impact reduction for GWP, FEP, and WDP for both locations (Figure 3 and cf. SI II.2).

Besides thermoregulation, cleaning, water mixing, and consumption of nutrients and CO_2 were found to contribute substantially to all impacts, in accordance with other LCA studies.[10,12,15]

Interestingly, and for all scenarios, terrestrial ecotoxicity (TETPinf) showed a negative score and therefore positive impact on the environment because the substitution of agricultural coproducts associated with high TETPinf impacts offsets the impacts of the cultivation.

3.2. Uncertainty Propagation Analysis and Sensitivity of the Model. For all scenarios and impact categories, the input uncertainty propagated into a similar density curve shape for the impact scores (Figure 2). For the two scenarios in Granada, the stochastic propagation resulted in standard deviations of the scores approximately equal to 50% of the average values for GWP100, FEP, and WDP, while this ratio amounted to 60% for Aalborg (Figure 2 and cf. SI II.2). For GWP100 and FEP, this standard deviation is associated with a ratio *minimum score/maximum score* of approximately 11 for Granada and twice as large for Aalborg. This is explained by the strong and positive correlation between the PBR volume and heating requirements (cf. SI I.9). Since these requirements are inherently higher in Aalborg and because the PBR volume depends on the uncertain geometric primary parameters, the input uncertainty propagates into a larger output dispersion.

In Figure 4, observation of FAST total-order sensitivity indexes showed that approximately 75% of the uncertainty on the environmental impacts is due to the input uncertainty on geometric parameters, which are, by order of contribution, horizontal distance between stacks, gap between rows, and tube diameter. It is notable that these parameters are ranked in the same order as the absolute values of their correlations with the PBR volume (cf. SI I.9), which confirms the sensitivity of the model to volume overall.

3.3. Comparison with Previous Studies. It must be noted that comparison with previous studies has strong limitations due to the use of different functional units (e.g., 1 kg of biomass or 1 MJ of biofuel), system boundaries, scenarios (e.g., season, cultivation system, strains), and modeling approaches (e.g.,

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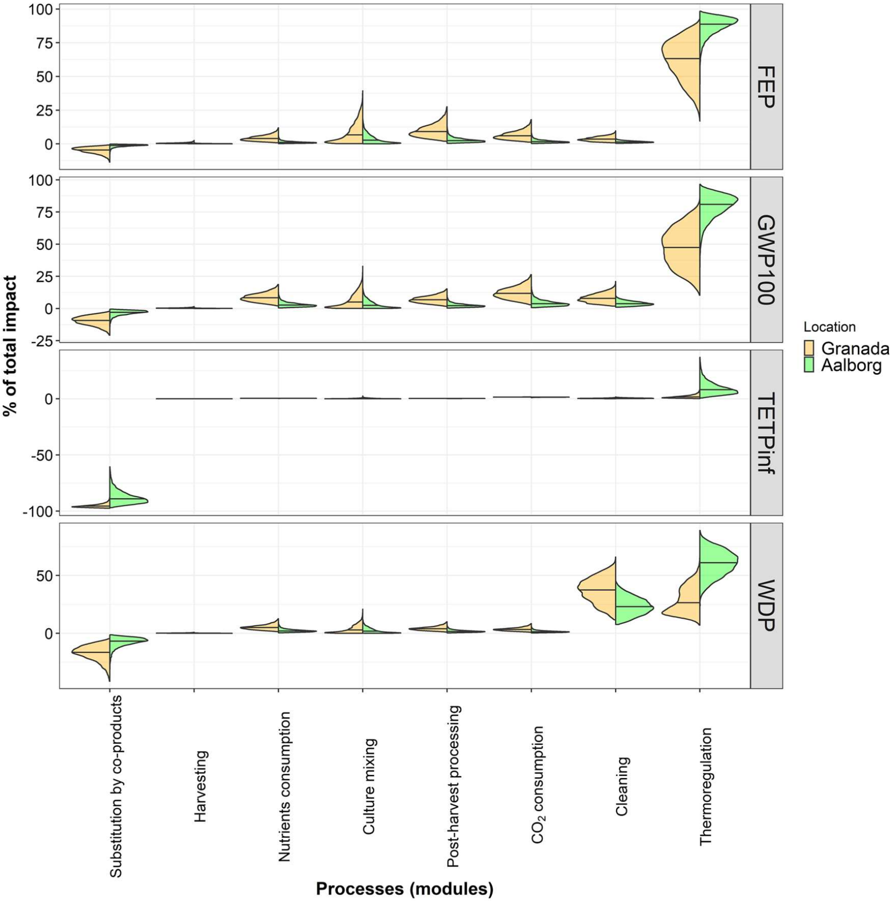

Figure 3. Density curves for the contribution of each process (module) to the total environmental impact. For Granada and Aalborg, each curve combines 9000 simulations with thermoregulation at night and 9000 simulations without thermoregulation at night. The horizontal lines within the areas indicate the medians. The "adjust" argument for the violin plot is set to 1. For a process x, the contribution is calculated in a simulation as $\frac{\text{impact}_x}{\sum_i^n |\text{impact}_i|}$, where n is the total number of foreground processes. Processes with negligible contribution are not shown: Water consumption and treatment, direct land occupation, water pumping and use of glass, water pumping. WDP: water depletion, GWP100: 100-year time horizon global warming, FEP: freshwater eutrophication, TETPinf: terrestrial ecotoxicity.

attributional instead of consequential, thus excluding the substitution of fish feed).

Pérez-López et al.[12] reported a carbon footprint of 574 and 214 kg CO_2-eq per kg of dry weight biomass in a thermoregulated vertical tubular PBR reactor for operation in Wageningen during fall and summer, respectively. A comparison of our results to these values can be proposed after dividing the latter by the compound concentration used in our study (2.6%) and accounting for 10% of impact substitution for the remaining biomass (Figure 3). This transformation returns 20 180 and 7523 kg CO_2-eq per kg of target compound. The authors, however, indicate that the electricity use of their pilot-scale PBR is inefficient compared to an industrial scale, for instance, with mixing pumps being approximately eight times less efficient than at an industrial scale as assumed in our model. Using this factor 8 returns 2522 and 940 kg CO_2-eq, which is closer to the average magnitude obtained in the present study (from 537 to 3436 kg CO_2-eq, cf. SI II.2 and Figure 2). For other studies including thermoregulation for their tubular PBRs, rescaling the reported results yields 7031[73] and 3379 kg CO_2-eq[23] per kg of target compound. Assuming perpetual CO_2 storage from flue gas in stable microalgal biochar as an end-product, the cultivation (without harvest) of 1 ton dry weight of *Tetraselmis chui* in a thermoregulated PBR in Australia was found to have a net negative impact of −222 kg CO_2-eq.[74] This transforms into −8.5 kg CO_2-eq per kg of target compound before harvest, which highlights the discrepancies between estimated impacts in different scenarios and assumptions.

For studies in similar systems but without thermoregulation, the transformation returns 2320 kg CO_2-eq per kg of target compound for a study[75] at a pilot scale, which may partly explain the relatively high value. Schade et al.[15] reported a carbon footprint of 1.7 kg CO_2-eq per kg of dried biomass that converts into around 59 kg CO_2-eq per kg of target compound after similar rescaling, which is lower than that in the current study. The difference is mainly due to the exclusion of thermoregulation and cell disruption and to the fact that Schade et al.[15] model CO_2 as directly taken from the atmosphere instead of liquid CO_2 produced with recovered CO_2 from industrial emissions. The complete LCI (cf. SI II.2) shows electricity and material consumptions close to the ones from Schade et al.[15] after rescaling.

Finally, it must be noted that the outdoor tubular PBR is not the only option to produce high-value microalgal compounds. A LCA study[13] reported 56.7 kg CO_2-eq per kg of dry biomass of *Tetraselmis suecica* grown in an indoor bubble column PBR at a pilot scale, which converts after transformation into 1993 kg CO_2-eq per kg of target compound. In another pilot-scale study,[21] the same technology for the diatom *Phaeodactylum tricornutum* gives 9035 kg CO_2-eq per kg of target compound after transformation, due to a comparatively low productivity, among other factors.

3.4. Minimizing Thermoregulation. This study indicates that thermal modeling of PBRs is crucial for meaningful ex-ante LCAs of microalgae cultivation and suggests that the assessment could benefit from even more detailed thermodynamic models.[50,51,76] Providing thermoregulation at an industrial scale is challenging. Cooling heat exchangers require a source of cold water near the plant, which limits the location possibilities, especially under warm and dry climates. Cooling could also be provided by spraying water on the tubes, but a previous LCA study[10] on *Haematococcus pluvialis* production in Lisbon shows that this water dissipation can be 10 times higher than the water consumption for cultivation. Greenhouses could be built to limit heat losses but the materials needed for a solid cover for *Arthrospira platensis* cultivation have been shown to contribute significantly to environmental impacts.[77] Besides technical solutions, cultivating strains able to bear or even benefit[51,78,79] from a temperature drop in the night would substantially improve the environmental performance as demonstrated in this study. Overall, our results suggest that the best option is using thermotolerant strains[80] or cultivating strains within climates and seasons that match the thermal ranges they strive in.[49] Finally, it must be noted that, if cooling energy consumption was underestimated by our model, the advantage of Granada over Aalborg for the modeled strain could decrease.

3.5. Minimizing Uncertainty on Environmental Impact Forecast. Because of the large influence of the PBR volume on the environmental impacts, the uncertainty on the ex-ante LCA is essentially due to the use of a model forecasting areal productivity rather than volumetric productivity. Thus, resorting to areal productivity models with fixed PBR geometries[15] to make forecasts on environmental impacts might be a misleading approach for new strains. Indeed, if the estimated areal productivity is only achievable in a slightly more voluminous PBR than expected, the environmental impacts could be much higher than forecasted.

Unfortunately, models estimating volumetric productivities at a large scale[29,34,49,81] require an extensive collection of parameters and consider only maximal productivities. The advantage of our approach is that it combines the results and knowledge gained from complex kinetic models with uncertainty estimates into a more easily applicable thermodynamic model estimating areal productivities.[35] Thus, we traded accuracy for comprehensiveness and applicability, and our model is able to simulate most strains and locations. As a result of this trade-off and because the results showed high sensitivity of the environmental impacts to the PBR geometry, the shapes of the estimated output uncertainties (Figures 1 and 2) are affected by different assumptions on input distributions for the PBR geometry parameters. One way to better approximate the output uncertainty would be to give a lower draw probability during the stochastic sampling to specific combinations of parameter values that are unlikely in the real world (for instance, high biomass concentration for high volume).

Another approach for the ex-ante LCA on microalgal compounds could be to consider uncertainty on the productivity reached by a new strain in a fixed PBR design.[29] However, assuming that this modeling is possible, an increase in demand and a high price for a new microalgal compound will likely incentivize bioengineers and companies to find the optimal PBR geometry and operational setup to maximize and stabilize productivity. Our stochastic model simulates most of the trade-offs that bioengineers may find and therefore provides the flexibility needed for ex-ante LCA.

4. OUTLOOK

We developed a model to account for biophysical epistemic uncertainty in microalgal compound production for strains that have never been cultivated at a large scale and propagated it to the results of an ex-ante consequential LCA. Despite high dispersion of the model's outputs, the four scenarios showed

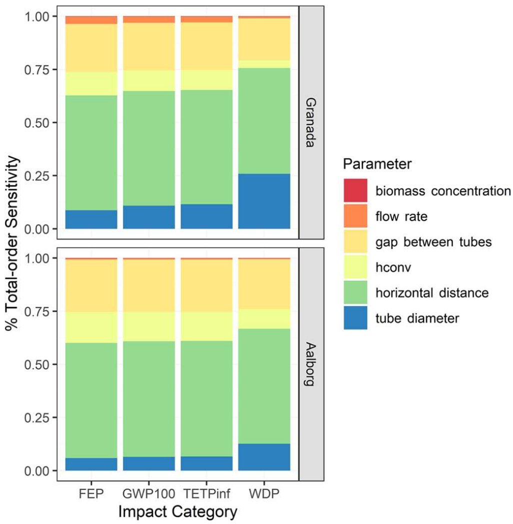

Figure 4. Contribution of each parameter to the total-order sensitivity for four impact categories. For a parameter x, the share is calculated as $\frac{\text{FAST total-order sensitivity index}_x}{\sum_i^n \text{FAST total-order sensitivity}_i}$ where n is the total number of parameters. Parameters are described in SI I. biomassconcentration: biomass concentration imposed at the beginning of a day ($g_{dw} \cdot L^{-1}$), flowrate: flow rate imposed in the PBR ($m \cdot s^{-1}$), gapbetweentubes: vertical distance between two rows of tubes (m), hconv: convective exchange coefficient ($W \cdot m^{-2} \cdot K^{-1}$), horizontaldistance: horizontal distance between PBR stacks (m), tubediameter: diameter of the PBR tubes (m).

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little overlapping between the probability density curves of environmental impact scores, and thermoregulation was identified as a key environmental hotspot. The identification of the uncertainty and its propagation highlighted that considering unique areal productivity for a unique PBR geometry and operational setup could lead to substantial errors when forecasting the impact of a new microalgal product. This error, however, would be reduced if the location and strain's thermal requirements entailed low thermoregulation needs. We focused here on the foreground uncertainty in the cultivation stage, but the model can be extended with information on background uncertainty, compound extraction LCIs, and different strains and scenarios. A slight modification of the model can also allow investigation of the whole microalgal biomass as a functional unit. As the location was confirmed as a highly influential factor for the environmental impacts, we recommend future users of the model to select appropriate regional marginal electricity mixes instead of an average European mix. In a rapidly evolving microalgae sector with promising prospects, the model was developed to investigate the consequences of an increase in demand for new microalgal compounds in Europe and to support decision-making and planning under substantial uncertainty.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c00372.

"**SI I**": Table of primary parameters; extended product system; modules' equations, behaviors demonstrations and validations; fish feed composition; elementary composition of microalgal compounds; and correlation heatmaps between model's outputs (PDF)

"**SI II: Sheet**" "**SI II.1**": Correspondence between foreground activities and background activities from ecoinvent 3.6 consequential; **Sheet** "**SI II.2**": Statistical description of the model's outputs in which Figure 2 of the article is based. The outputs include LCI figures and main simulation outputs such as areal productivities, geometric secondary parameters, centrifuged volume, etc.; **Sheet** "**SI II.3**": Statistical description of the contributions of each process (module) to the impact categories (Figure 3 of the article) (XLSX)

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Notes

The authors declare no competing financial interest.

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Article II

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Stochastic Ex-Ante LCA under Multidimensional Uncertainty: Anticipating the Production of Undiscovered Microalgal Compounds in Europe

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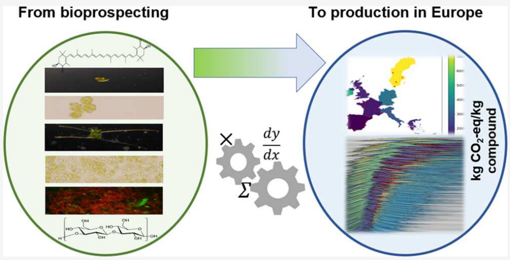
ABSTRACT: Due to their biodiversity, microalgae represent a promising source of high-value compounds that bioprospecting is aiming to reveal. Performing an ex-ante Life Cycle Assessment (LCA) to anticipate and potentially minimize the environmental burden associated with the European production of a bioprospected microalgal compound is subject to substantial and multifactorial uncertainty as the compound remains undiscovered. Given that any microalgal strain could potentially host the compound of interest, the ex-ante LCA should consider this bioprospecting uncertainty together with the uncertainty on the technology and the production mix. Using a parameterized cultivation simulation and consequential LCA model and an extensive stochastic pseudo Monte Carlo approach, we define and propagate techno-operational, bioprospecting, and production mix uncertainties for a microalgal compound being currently bioprospected in Europe. We perform global sensitivity analysis using different sampling strategies to identify the main contributors to the total output variance. Overall, the uncertainty propagation allowed us to define and analyze the probabilistic scope for the potential environmental impacts in the emerging production of high-value microalgal compounds in Europe based on current knowledge. These findings can support policy-making as well as actors in the microalgal sector toward technological paths with lower environmental impact.



KEYWORDS: *Life-Cycle Assessment, uncertainty, ex-ante, microalgae, industrial ecology*

1. INTRODUCTION

The biological diversity of microalgae makes them a promising biological group for biotechnological applications such as the production of organic products of high commercial value. Among these products, while microalgae-based third-generation biofuels have so far failed to compete economically with fossil fuels,[1] a few high-value microalgal compounds are already commercialized.[2,3] Recent discoveries from bioprospecting, that is, the search for compounds and properties within the biodiversity that could be valuable for human activities, range from antimicrobial to antitumoral lipids, proteins, and carbohydrates in microalgal strains.[4−10] This suggests that the European microalgae sector might develop substantially in the near future. Anticipating the environmental consequences of such a development is crucial as early-stage emerging systems are characterized by a high design freedom,[11] implying potential detrimental scenarios, and because sustainable development processes “are timely, anticipatory, integrative, flexible and action focused”.[12] Life Cycle Assessment (LCA) constitutes a robust holistic tool to quantitatively anticipate such impacts on a systemic level and offers flexibility and parameterization to project different scenarios.

LCA studies on the environmental impacts of microalgae production address primarily bioenergy-oriented microalgae growth in Open Pond Raceways,[13−18] while high-value compounds' production would likely require stable and contamination-free photobioreactors (PBRs). When reviewing 18 existing LCA studies of microalgae,[19−27] we found that they consider only 7 well-studied strains. This is likely because the microalgal sector has historically focused on bioenergy applications and therefore primary data and assumptions on yields and operating conditions exist only for a restricted set of strains that are fit for bioenergy and have already been cultivated industrially (lipid-rich, robust, etc.). These data alone cannot support a comprehensive assessment of the future consequences of the microalgae sector's development, which could potentially take as many paths as there are microalgal

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strains and promising compounds to be discovered via bioprospecting. Furthermore, while previous bioengineering studies[28,29] model the potential for microalgal productivities in different PBRs and locations, previous LCA studies only assess productions localized in one or a few sites. However, it is reasonable to assume that an increase in demand for new microalgal compounds would, in the long run, result in the supply of microalgae from several different locations with distinct productivities, thermoregulation needs, and electricity mixes. In fact, 447 microalgae or cyanobacteria farms are already active in 23 European countries.[30]

Forecasting the environmental consequences of future developments in the European microalgae sector therefore requires extending the scope of the previous assessments to include many possible scenarios. This immense number of possibilities is inherent to bioprospecting, which implies that the desired property of a bioprospected compound is known (for instance, an anti-inflammatory compound) but not the organism which will produce it. Besides the case of bioprospecting, it is common[31] to consider a large number of scenarios in ex-ante LCA as it aims at anticipating the environmental impact of emerging technologies before these are actually implemented at an industrial scale.[32] A major challenge in ex-ante LCA is thus the need to generate Life Cycle Inventories (LCIs) for future production systems, which currently exist only at a low level of technological and market maturity,[33] such as pilot-scale applications. In a recent review of common practices in ex-ante LCA,[31] one-third of the 18 included studies resorted to process simulation to obtain LCIs at an industrial scale, mainly using technology-specific simulation software. In the case of microalgal compounds which are still being bioprospected, the upscaling anticipation can be done using parameterized physical and biological models to simulate photobioreactors and strains in different conditions. In fact, it is common practice[18,21,23−25,27,34] to simulate inventories for LCAs in the microalgal sector and recent studies show advanced models taking various biophysical phenomena into account.[35] Microalgae LCA models with extensive parameterization can be found associated with some forms of uncertainty and sensitivity analysis via stochastic sampling.[14,34,36]

These parameterized models, however, cannot cover the scope of possibilities associated with a microalgal compound that is not found yet and whose production upscaling and development in a European production market are indeterminate. However, the consequences of an increase in demand for high-value microalgal compounds must be assessed early on to inform both policy makers in the sustainability domain and the microalgal sector. The ex-ante assessment should put these stakeholders in a better position to evaluate the likelihood of microalgal high-value compounds' environmental superiority over alternatives as well as to identify the optimal scenarios and try to aim for them.

To do so, the present work aims at investigating the impacts of an increase in demand for a microalgal compound with a desired property that is currently being bioprospected in Europe (a bioprospected microalgal compound). The uncertainty space associated with this technological development is shaped by several forms of uncertainty that can either be epistemic, that is, due to a lack of knowledge, or aleatory because of stemming from inherently random processes.[37] We build on previous work[38] developing a parameterized model to simulate the microalgae cultivation technology from a life cycle perspective. The "techno operational uncertainty" addressed in this previous work is due to the impossibility of accurately predicting the productivity of a new specific strain in a specific photobioreactor and location. To tackle our research question, we here need to extend the parameterized model and the scope of previous LCAs in the microalgal sector to include other sources of uncertainty, namely, the lack of knowledge about the exact nature of the strain and the bioprospected compound, the associated PBR, and the geographic developments of the market. We anticipate that despite these large uncertainties, the stochastic propagations will provide insightful density-based representations of the environmental consequences of an increase in demand for yet undiscovered microalgal compounds. This work also contributes to the developments and discussions around uncertainty, global sensitivity analysis, and the associated terminologies in the field of ex-ante LCA.

2. METHODS

The approach consists in applying stochastically generated samples to our previously developed parameterized LCA model[38] on which minor changes were made (cf. Section 2.1) to assess the impacts for an increase in demand and production of 1 kg of bioprospected compound in Europe. The whole model is coded on Python 3.8 with the LCA package Brightway2[39] and is available on GitHub.[40] The background database is the consequential version of Ecoinvent 3.6. The impact assessment categories are global warming with a 100 year time horizon (GW100), freshwater eutrophication (FE), water depletion (WD), and terrestrial ecotoxicity (TETinf) from ReCiPe Midpoint (H) V1.13.

2.1. Deterministic Model and LCA Framework. The functional unit is 1 kg of bioprospected compound. Consequential LCA modeling was chosen as, by looking at the future effects of decisions and including only activities and technologies expected to be able to respond to future changes in demand, the consequential approach is prospective in nature and therefore well-suited to the assessment of emerging technologies. Moreover, the consequential approach reduces the number of normative assumptions needed, and since it is highly speculative to anticipate normative preferences in the future, this can be argued to be an advantage when performing ex-ante LCA. The foreground product system includes the cultivation of microalgae in an outdoor vertical tubular PBR with the associated energy consumptions for water pumping, mixing, thermoregulation by a heat pump, and centrifugation for biomass harvesting. The product system also comprises nutrients, CO_2, water, and glass consumptions. The extraction of the compound is not modeled, but cell disruption is accounted for as it likely constitutes the first step of the post-harvest processing, regardless of the biochemical class of the compound (protein, lipid, and carbohydrate).[41,42] Drying is modeled for the whole biomass so that the co-produced biomass (dependent co-product) is ready to substitute functionally equivalent products already on the market. The cultivation technology and the foreground product system are described in detail in Supporting Information I.2 and in our previous work and model,[38] for which a few modifications were made to the product system. First, thermoregulation of the PBRs was assumed to be provided by a reversible heat pump[43] with a coefficient of performance of 3, which we chose as an average value over locations and seasons instead of electric heating and a fluid thermal exchanger. Additionally, considering multiple potential strains with different characteristics made it necessary to model two additional possible substitution routes for the co-produced biomass (cf. Supporting Information I.3). This biomass can now enter the animal feed energy and feed protein markets[44] or be directly incorporated in fish feed after modification of the reference fish feed composition as previously modeled.[38] As a third possible substitution route, the co-produced biomass can be digested in an anaerobic digester for biogas production and substitution on the biogas marginal market based on a functional unit of 1 MJ of heating capacity. The biogas yields depend on the composition of the microalgal strains (cf. Supporting Information I.3.2.2).

The life cycle inventory results from a simulation of microalgal cultivation assumed from April to September within Europe. This simulation and the LCI result from the interaction of 27 parameters characterizing the strain and its compound, 22 techno-operational parameters characterizing the cultivation technology, the PBR geometry and setup, 3 geographic parameters defining the location, and 6 physical parameters. The cultivation simulation uses climatic data from the Photovoltaic Geographic Information System.[45] The simulation, its modules, the parameters, and all the equations are detailed in our previous work[38] and in Supporting Information I for the additions and modifications.

2.2. Types of Uncertainty and Assumptions. *2.2.1. Bioprospecting Uncertainty.* The "bioprospecting uncertainty" is caused by the lack of knowledge about the strain and the compound that will be found to feature the desired property (for instance, anti-inflammatory) and successfully upscaled after bioprospecting. The bioprospecting uncertainty is epistemic[37] in the first place as it stems from the lack of knowledge about which kinds of microalgal strains or compounds classes (lipid, carbohydrate, and protein) are more likely to possess the desired property. We have assumed that all strains and types of compounds have equal probabilities to possess the desired property as we currently lack arguments and data to hypothesize potential correlations between biological traits and desired properties. Therefore, the bioprospecting process was modeled as a random draw within microalgal biodiversity, whose result is subject to aleatory uncertainty. This is conceptually analogous to a draw in an opaque urn in which the balls would be the strains and their compounds whose proportions in the box depend on our knowledge on biodiversity (cf. Section 3.3).

To do so, we first use 27 parameters defining a "strain−compound pair", that is, a specific compound hosted in a specific strain. Some parameters define biological characteristics such as biomass composition, nitrogen source, or photosynthetic efficiency. Other parameters define if the strain−compound pair requires PBR thermoregulation at night or characterize the fate of the co-produced biomass (biogas, fish feed, or animal feed) that we consider strain-specific depending on cell-wall characteristics, digestibility, toxicity, and so forth.[46−48] Finally, compound-specific parameters define, respectively, whether the bioprospected compound is a lipid, protein, or carbohydrate, that is, the biochemical class of the compound; and the mass fraction of compound in such class, for example, measured as the mass of compound per total mass of proteins in the strain if the compound is a protein. The values of these compound-specific parameters are also characterized by uncertainty, which means that the same functional unit of 1 kg of bioprospected compound can be provided by different reference flows of cultivated microalgal biomass.

We modeled the random draw within the microalgal biodiversity by sampling random values for the parameters for which variation ranges are reported in the literature for the microalgal biodiversity (23 out of 27 parameters, cf. Supporting Information II.1). For 17 parameters out of 23, we modeled a uniform distribution within the range due to lack of further arguments to use other distributions.

2.2.2. Techno-Operational Uncertainty. This epistemic uncertainty was specifically addressed in our previous work[38] and is due to the unpredictable behavior and growth of a strain defined by its biological parameters in a specific vertical tubular PBR and location. More precisely, we used the model from Williams and Laurens (2010),[28] which estimates a maximum areal yield ($g_{dw} \cdot m^{-2} \cdot d^{-1}$) depending on the ground horizontal irradiance ($kJ \cdot m^{-2} \cdot d^{-1}$) and the strain-specific theoretical energetic yield ($g_{dw} \cdot kJ^{-1}$). Since Williams and Laurens (2010)[28] observe that real cultivations in PBR would often reach 30% of this maximum yield, in this study, the techno-operational uncertainty refers to the lack of knowledge about which PBR geometry and operational set-up will enable the modeled strain to reach this percentage. The techno-operational uncertainty was addressed by simulating random values of geometrical (e.g., tube diameter and distance between tubes) and operational (flow rate and biomass concentration) parameters defining the PBR for a strain and location. The values were sampled within ranges reported in the literature for different vertical tubular PBRs with different strains in various locations (cf. Supporting Information I.1). The sampled combinations were all assumed to have equal chances of enabling the strain to reach 30% of its maximum yield.

In addition to the geometrical and operational parameters, the uncertain vertical distance between the water source (river or well) and the PBR and the wind-dependent convective exchange coefficient ruling the thermal exchange between the PBR and the surrounding air were included in the techno-operational uncertainty.

In total, seven techno-operational parameters are therefore considered uncertain.

2.2.3. Geographic Locations and Uncertainty of Production Mix. Similarly to any agricultural crop for which an increase in demand in Europe will be answered by different producers in distinct locations (a mix), an increase in demand for the bioprospected compound will be met by distinct microalgae plants in Europe belonging to a "compound production mix". To anticipate the development of this compound production mix in Europe, we first assumed that the production would take place in the 10 countries which have the highest potential for microalgal biomass production as identified by Skarka:[49] Spain (ES), Sweden (SE), Italy (IT), Portugal (PT), United Kingdom (UK), France (FR), Greece (EL), Cyprus (CY), Ireland (IE), and Germany (DE). The author identified these countries as the best combinations of temperature, solar irradiance, and available land after exclusion of urban, mountainous, and protected areas. To account for indeterminacy regarding the plants' locations, a grid was first generated with random locations drawn every 2° of latitude in each country (28 locations in total). Mono-dimensional sampling was performed in each location, while multi-dimensional sampling only could add production mix uncertainty by sampling production mixes within the grid (cf. Section 2.3). To do so, random combinations of locations

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were selected within the grid in three different scenarios regarding the spread of the mix over Europe. Thus, in these scenarios, 5, 15, or 25 locations out of 28 were assumed to answer to the increase in demand.

In consequential LCA, the identification of the marginal suppliers and their shares in the mix are based on the study of market trends that are shaped by both political and economic factors.[50,51] In our case, due to the lack of data on market performance, we used areal productivity as a proxy and assumed that the plants in locations with higher areal productivities will have a higher chance to be part of the compound production mix. We then assumed equal production shares within this mix, once its locations have been determined: each plant produces 200 g of compound in a production mix for 1 kg if the mix contains 5 plants in different locations. A country-specific electricity mix was used when simulating each plant.

2.2.4. Independence Assumptions. The model was designed so that the sampled parameters are independent. For instance, our knowledge does not indicate if a specific strain has a higher chance to grow on nitrate instead of ammonium if it has a high optimal temperature and a short cell diameter. These three parameters were therefore sampled independently. Similarly, a cell diameter does not indicate if the strain has a higher chance to grow at the expected yield in a certain tube diameter, all other things being considered. Indeed, we did not model direct dependence between techno-operational parameters and productivity for a strain but instead treated this complexity as a part of the uncertainty (cf. Section 2.2.2) that could be reduced with more knowledge on the dependencies observed across species and locations. On the contrary, resorting to a parameter defining the content of the bioprospected compound in the biomass which would be independent of the biomass composition would lead to unrealistic scenarios. Thus, the biomass composition was first generated by randomly sampling a lipid content of the ash-free dry biomass and an ash content, from which the rest of the composition is calculated,[28] and the bioprospected compound constitutes a random fraction of a random biochemical class (lipid, protein, and carbohydrate).

2.3. Sampling Strategies for Uncertainty Propagation and Sensitivity Analysis. As shown in Figure 1, we implemented two random sampling strategies to propagate the uncertainty and perform sensitivity analysis.

The first strategy named "mono-dimensional sampling" mixes all types of uncertainties previously described (bioprospecting, techno-operational, cf. Section 2.2) by applying Sobol sampling to all parameters. With this sampling strategy, we obtained for each location of the grid 114,688 random combinations of the 23 biological and 7 uncertain techno-operational parameters. The global sensitivity analysis associated with mono-dimensional sampling allows ranking all parameters based on their influence on the dispersion of the impact scores in one location.

To be able to simulate the development of European production mixes producing the same strain−compound pairs in different locations, the second sampling strategy, namely, "multi-dimensional sampling", differentiates between uncertainty types. We first generated a Sobol sample to obtain a set of 5376 strain−compound pairs that could potentially display the desired property (bioprospecting uncertainty). The cultivation and production of each strain−compound pair was then simulated for each of the 28 locations of the grid, in 150 random PBR geometries and operational setups generated with Monte Carlo sampling on the 7 uncertain techno-operational parameters (techno-operational uncertainty). As the same strain−compound pairs were simulated in all locations of the grid, we could generate production mixes for all pairs by composing 400 random combinations of locations on the grid (production mix uncertainty). The probabilities for each location to be part of the marginal mix were weighted with the areal productivities (cf. Section 2.2.3). Unlike mono-dimensional sampling, multi-dimensional sampling allows assessing the uncertainty associated with the environmental impact caused by an unknown European production mix producing the same strain−compound pair after aggregating techno-operational uncertainty (cf. Figure 1). This sampling strategy also allows studying how sensitive is the impact of the entire European production mix (composed of the 28 locations of the grid) to the bioprospecting uncertainty, that is, to the parameters defining the strain−compound pair produced in this mix.

The global sensitivity analyses were performed with the Sobol methods from the python Salib package.[52]

3. RESULTS AND DISCUSSION

3.1. Uncertainty Propagation with Mono-Dimensional Sampling. The first notable observation is that the propagation of the uncertainty resulted in a very wide range of impacts scores, with, for instance, the global warming impact (GW100) per kg of bioprospected compound ranges from −100 to +89,000 kg CO_2-equiv/kg compound with mono-dimensional sampling (cf. Supporting Information I.3). This very large dispersion is mainly due to the uncertain content of the bioprospected compound in the biomass, which is part of the bioprospecting uncertainty. The bioprospected compound content is a secondary parameter as it results from the interaction of four uncertain primary parameters (cf. Section 2.2.4). The propagation of the uncertainty for these four parameters resulted in a target compound content ranging from 0.001 to 0.6 $g \cdot g_{driedbiomass}^{-1}$ (cf. Supporting Information II.2). The highest impact score in the range corresponds to extremely unfavorable conditions: a very low content of the bioprospected compound in the biomass (0.002 $g \cdot g_{driedbiomass}^{-1}$) coupled with a northern location (Sweden), a high strain-specific thermal range, and a large PBR volume involving low volumetric productivity and substantial PBR heating requirements. GW, freshwater eutrophication (FE), and water depletion (WD) impact scores all increase when the target compound content decreases because of a need to produce more biomass to provide the same functional unit, but a lower target compound content induces a higher terrestrial ecotoxicity (TET) impact on average (Figure 2). The observed overall trend in Figure 2, however, hides the specific trends for the three equiprobable substitution routes. Indeed, the overall trend is only due to the scenario in which the coproduced biomass substitutes fish feed. Despite constituting one-third of all the simulations, the strong positive slope observed for this scenario outweighs the slightly negative slope for the two other scenarios, which results in the trend observed in Figure 2 (cf. Figure S13 in Supporting Information I. 4.2.4.1). Fish feed substitution almost always implies negative TET scores (positive impact on the environment), which is partly the case for animal feed and never happened for biogas substitution. The environmental superiority of fish feed

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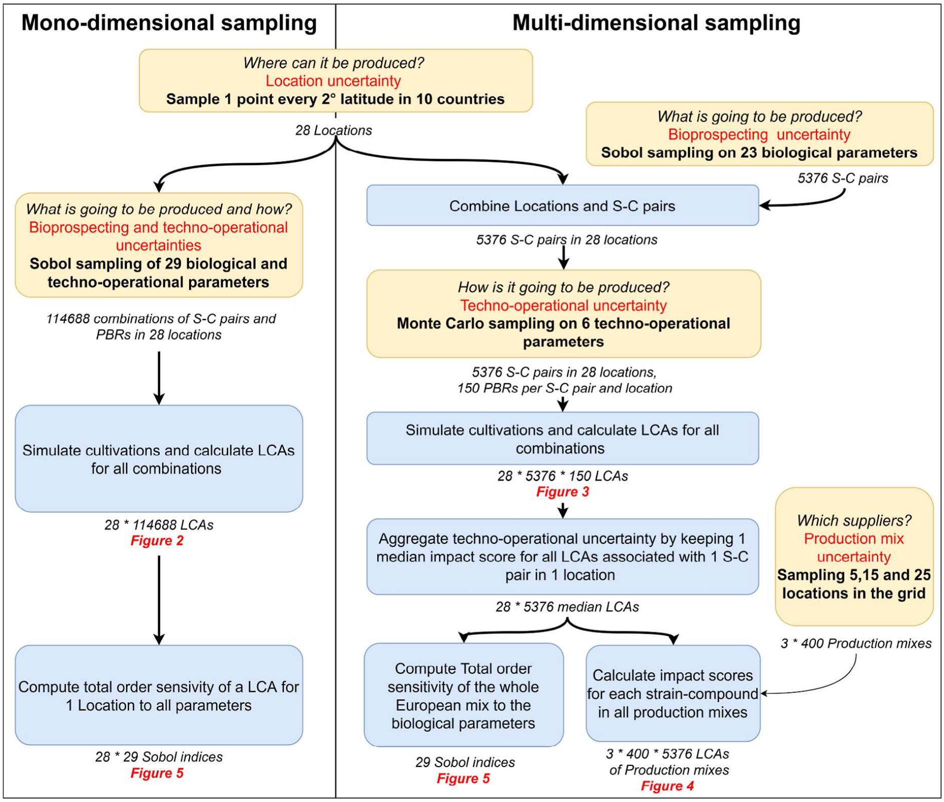

Figure 1. Visualization of the sampling strategies and the associated calculations. Acronyms: S−C = strain−compound. Blue and orange boxes, respectively, indicate a calculation step and a sampling step. We indicated the figures of the article under the results that they display.

substitution over biogas production had also been found in a previous study.[24]

3.2. Uncertainty Propagation with Multi-Dimensional Sampling.

3.2.1. Bioprospecting and Techno-Operational Uncertainties. In Figure 3, for each horizontal line representing a strain−compound pair, the density curves of the impact (horizontal axis in Figure 3) result from the propagation of the techno-operational uncertainty in the different countries. Despite a visible shift of the impact density curves across strain−compound pairs (vertical axis in Figure 3) and different countries (different colors in Figure 3), the significant overlap between strain−compound pairs indicates a large influence of techno-operational uncertainty.

It must be noted for GW, FE, and WD that the shifts of the curves along the horizontal axis are not mere linear transposition of the density curves but are associated with a higher standard deviation of the results for strain−compound pairs with higher average impact scores (cf. Supporting Information I.4.3). In other words, the dispersion of the impact scores due to techno-operational uncertainty varies with the modeled strain−compound pair. This heteroskedastic statistical behavior stems from two mechanisms particularly visible for GW. First, the same techno-operational input uncertainty is assumed for the cultivation simulations of all strain−compound pairs, which tends to result in a constant coefficient of variation (*standard deviation*/*mean*) and therefore a linear increase of the standard deviation across the mean impact scores of different strain−compound pairs (cf. Figure S16, Supporting Information I.4.3). Second, the coefficient of variation is tendentially higher for strains with higher optimum temperatures of culture (cf. Figures S15, S16, S20, Supporting Information I.4.3), which are also associated with high impact scores on average. This phenomenon was observed in our previous work[38] and stems from the fact that techno-operational uncertainty propagates more when thermoregulation can become a hotspot due to the combination of strain's thermal requirements and location.

3.2.2. Variability across Countries and Production Mix Uncertainty. Despite techno-operational and bioprospecting uncertainties, which cause substantial overlap between impact density curves (Figure 3a), the 10 countries can be ranked according to the average impact score for all simulations (Figure 3b and also observed in Figure 2 with mono-dimensional sampling). Latitude is an important determinant of the environmental impacts (cf. Figure S5, Supporting Information I.4.2.1.1) as it affects the horizontal irradiance and therefore influences biomass productivity. Latitude also determinates the outside temperature and therefore the energy required to thermoregulate the culture, in particular heating requirements, which was highlighted as an environmental

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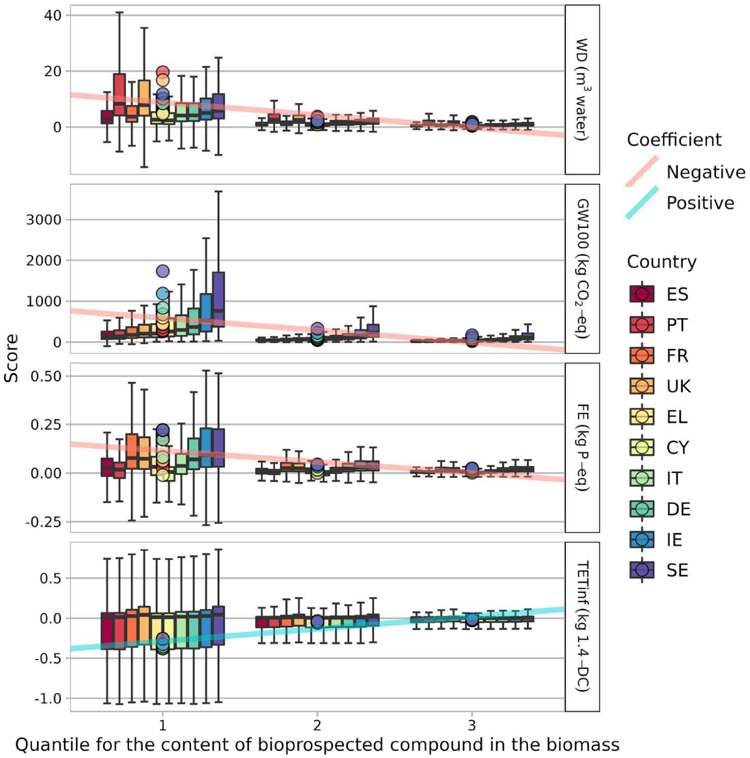

Figure 2. Boxplot of the environmental scores obtained in mono-dimensional sampling. The 3 211 264 LCA scores resulting from mono-dimensional sampling are divided into three quantiles applying to the dispersion of the uncertain bioprospected compound content in the biomass. Boundaries of the quantiles ($g_{\text{bioprospected compound}} \cdot g_{\text{driedbiomass}}^{-1}$): 0.001–0.090, 0.090–0.185, 0.185–0.60. The dots indicate the average scores per country. The regression lines are only displayed to highlight the bioprospected compound content influence on the scores. The equations of the lines are score = coeff × Q + intercept, with Q the quantile numbers 1, 2, and 3. More detailed plots can be found in Supporting Information I.4.2.4.1. WD = water depletion, GW100 = global warming with a 100 year time horizon, FE = freshwater eutrophication, TETinf = terrestrial ecotoxicity.

hotspot in other studies.[38,53,54] Thus, the impact scores for all impact categories and countries tendentially increase with the strain-specific optimal temperatures *Topt* (part of the bioprospecting uncertainty), and the regression slopes associated with $log(impact\ score) = f(Topt)$ are higher for northern countries (cf. Supporting Information I.4.2.1.2). Additionally, the impact scores per FU are strongly influenced by the impact profiles of the national electricity mixes (cf. Supporting Information I.4.2.2). To state an example, despite its southern location, production in Portugal is associated with the highest WD impact score among all countries due to the high WD impact of the Portuguese electricity mix (Figures 2 and 3). However, it is important to note that current marginal mixes used in consequential modeling only have a limited period of validity in the near-/medium-term future.

Differences between the impact scores of distinct countries do not necessarily imply additional uncertainty for the impact scores associated with the production of 1 kg of the bioprospected compound produced by a European production mix. Figure 4 shows how the dispersion of the impact scores for the strain–compound pairs varies across the generated production mixes. The differences observed between the distributions, which illustrate the production mix uncertainty, shrink when more locations are considered per mix. Indeed, the more locations there are in the randomly generated mixes, the closer the latter get to a full European mix composed of the 28 locations of the grid.

3.3. Sensitivity Analysis. Mono-dimensional and multi-dimensional samplings allow for a multifaceted understanding of a model's sensitivity to the different parameters. Regarding the impact scores associated with one strain–compound pair in one location, Figure 5 shows for mono-dimensional sampling that the uncertainty on the fraction of the bioprospected compound in the biochemical class (lipid, carbohydrate, and protein) dominates in the output uncertainty for all impact categories. This was expected as this parameter eventually affects the overall content of the bioprospected compound in the biomass and therefore substantially influences the reference flows in the product system. The uncertainty on the parameter assigning the substitution route also dominates the output uncertainty for TET and FE, which is to be put in relation to the significantly different LCA profiles of the three substitution routes (cf. Section 3.1 and Figure S13 in Supporting Information I.4.2.4.1,

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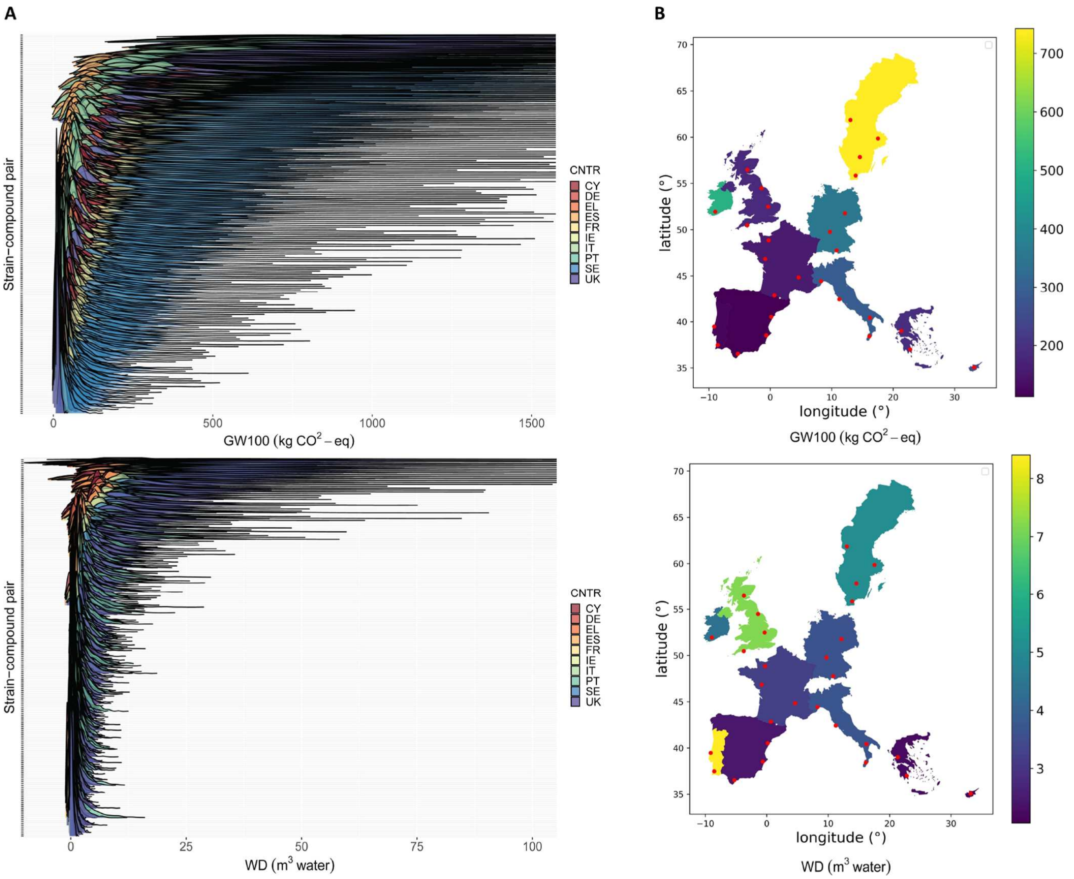

Figure 3. (A) Distinction of uncertainties with strain−compound-specific ridgelines and (B) mapping of average impacts scores per country. (A) Each horizontal line corresponds to one strain−compound pair for which the LCA scores in different locations and stochastically generated PBRs constitute the density curves. The plots only show 200 of the 5376 randomly generated strain−compound pairs in multi-dimensional sampling. The strain−compound pairs were divided into 200 quantiles regarding the dispersion of the GW impact scores and one pair was chosen per quantile and displayed in the figures. (B) The color gradients indicate the mean impact score per country, considering all simulations performed in each country. The red dots indicate the 28 locations of the randomly generated grid. The figures for TET and FEP are shown in Supporting Information I.4.1.

Supporting Information I.4.2.3). Eventually, the techno-operational uncertainty on the geometry of the PBR and the resulting culture volume (tube diameter, horizontal distance between stacks, and gap between tubes) accounts for 15−20% of the output variance for all impact categories. As also described in our previous work,[38] this is mainly due to the influence of the PBR volume on the thermoregulation requirements, together with the strain's thermal requirements (*Topt*, *Tplateau*) and location. The 22 other parameters explain around 60% of the output uncertainty, which makes it difficult to decide on which parameters could be fixed to a unique value without losing information on the output uncertainty.

While mono-dimensional sampling can spot uncertainty hotpots in the details of the model at the techno-operational level, only multi-dimensional-sampling can investigate the sensitivity of the model to a strain−compound pair at the European production level.

The latter as well shows that the fraction of the bioprospected compound in the biochemical class (*bioact_fraction_molec*) and the parameter assigning the substitution route (*random_market_subst*) dominate the output uncertainty (75%). The similar ranking of the common parameters between mono- and multi-dimensional sampling shows that the same strain−compound-specific parameters strongly influence the impact scores for a production both in a unique location and when the same strain−compound pair is produced all over Europe.

Interestingly and in accordance with former observations (cf. Section 3.1), almost 100% of the output uncertainty for TETinf comes from the substitution mechanisms, determined by the biomass composition and substitution route. Thus, one could theoretically provide an educated estimate of the future TETinf impact of the production in Europe as soon as the biomass composition and the substitution market for a newly found strain−compound are known. Nevertheless, this

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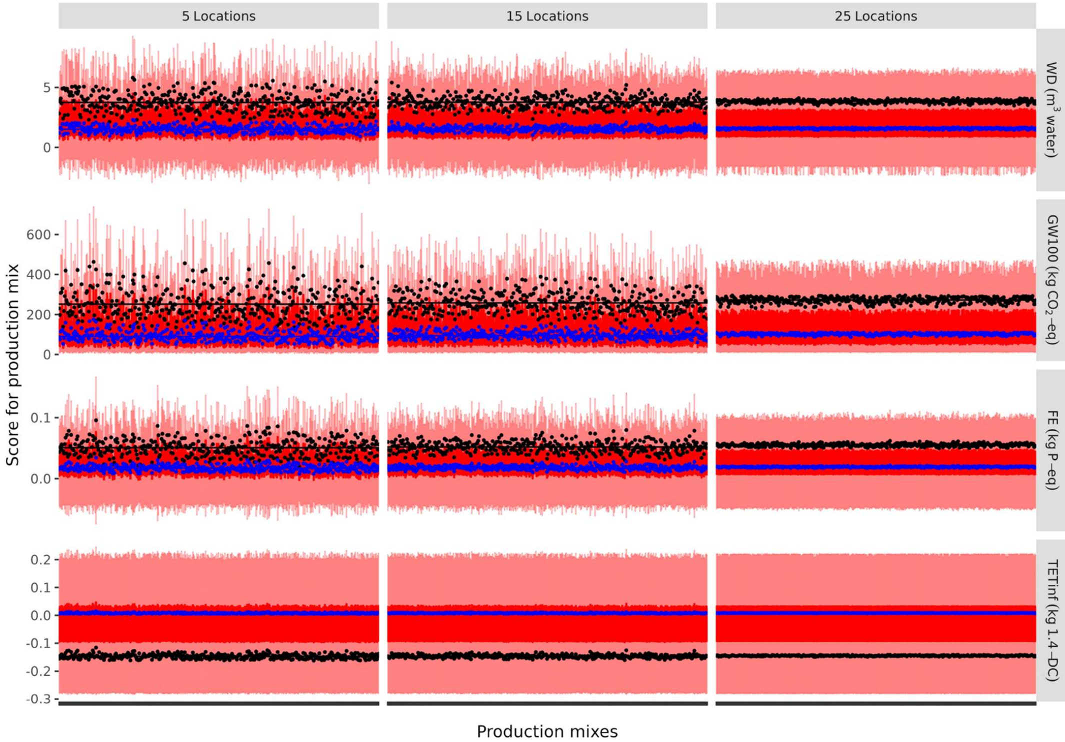

Figure 4. Production mix and bioprospecting uncertainties in aligned boxplots. The red area consists of a succession of narrow boxplots along the horizontal axis corresponding to 400 randomly generated production mixes. The range of each boxplot on the vertical axis corresponds to the dispersion of impact score per strain−compound pair produced in the production mix. The impact score for a strain−compound i in the production mix p is calculated as follows: $SMimp_{p,i} = \frac{\sum_{L=0}^{N_p} \text{median}([imp_{i,L,0}, ..., imp_{i,L,a}, imp_{i,L,150}])}{N_p}$, with N_p the number of locations in the production mix p, L the identifier of a location being part of p and $imp_{i,L,a}$ the impact score calculated for the production of strain−compound pair i, in location L and in PBR a. The black and blue dots, respectively, indicate the means and medians of each boxplot. The horizontal black and blues lines, respectively, represent the mean across boxplots and the means of the medians across boxplots.

estimate should be done keeping in mind the sensitivity of the model at the techno-operational level revealed by the mono-dimensional sampling.

3.4. Reflecting on Variability and Uncertainty. In this work, we have consistently used the word "uncertainty" to qualify the need to resort to a stochastically sampled set of values instead of using a static set of values for the parameters of our LCA model. The distinction between variability and uncertainty is key within the LCA community and more generally in modeling disciplines, which cannot settle for a mere deterministic assessment to support decision-making.[55−57] While variability is intrinsic to real-world phenomena and processes, uncertainty is often defined as being due to a lack of knowledge about the model and its parameters.[56−58] This semantic overlapping between variability and uncertainty depending on the formulation of the research question is well described by Frey.[55] Our case confirms and illustrates how uncertainty and variability merge in some cases, depending on the research question. The geographic variability becomes production mix uncertainty in our ex-ante LCA as it stems from an irreducible lack of knowledge a priori about the future development of the mix. Similarly, while there is a vast diversity of microalgal strains and compounds, this biological variability translates into uncertainty associated with our research question about the impacts of an increase in demand for ***a*** microalgal compound that is currently being bioprospected in Europe. The uncertainty is here similar to the one applying to the result of a random draw (aleatory uncertainty) within a diverse population expressing variability (the biodiversity). Finally, the techno-operational conditions should also be understood as part of the uncertainty rather than just variability as generating random PBR geometries and setups does not aim at representing alternative routes for a same compound production[57] but instead represent equiprobable scenarios for one strain to reach a specific productivity according to our limited knowledge.

To go further, the multi-dimensional strategy uses two independent loops as in the two-dimensional Monte Carlo simulation proposed by Michiels and Geeraerd[56] to distinguish variability and uncertainty. The difference is that we use this approach to distinguish bioprospecting uncertainty from techno-operational and production mix uncertainty. By doing so, we neglect biological variability by assuming that, once found and cultivated in a European mix, a strain does not

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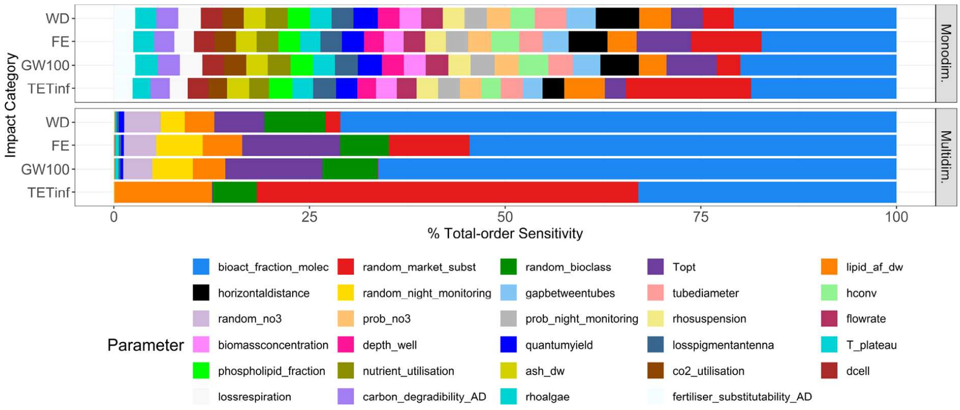

Figure 5. Shares of the Sobol total-order sensitivity for the uncertain parameters in multi-dimensional and mono-dimensional samplings. The parameters are detailed in Supporting Information II.1. In mono-dimensional sampling, the share of the total-order sensitivity for a parameter x is calculated as $\text{share}_x = \frac{\frac{\sum_{L=0}^{28}\text{indice}_{x,L}}{28}}{\sum_{i=0}^{p}\left(\frac{\sum_{L=0}^{28}\text{indice}_{i,L}}{28}\right)}$, with $\text{indice}_{x,\text{L}}$ the total-order sensitivity indice calculated in location L for parameter x and p the number of uncertain parameters in mono-dimensional sampling. In multi-dimensional sampling, $\text{share}_x = \frac{\text{index2}_x}{\sum_{i=0}^{p}\text{index2}_i}$ with indice2_x the total-order sensitivity index of the biological parameter x associated with the impact for a strain−compound in Europe (cf. Figure 1). The error bars and dispersion of the different indexes are shown in Supporting Information I.5.3.

express any phenotypical or genomic plasticity and features the same biological parameters' values in all locations. This is an oversimplification as the same strain producing the same compound in a European mix could, for instance, accumulate more or less lipids for different locations due to different light regimes.[59] In this sense, mono-dimensional sampling, while making it impossible to distinguish between the types of uncertainty, also tackles a less fixist[60] and therefore more realistic concept of "microalgal strain" by simulating a continuum of biological parameters in a continuum of different PBRs and locations.

3.5. Limits. Our forecast partly relies on our choices regarding the modeling of microalgal biodiversity. Adapting the generic model from Williams and Laurens[28] to represent a strain could be argued to be simplistic and not cover the immense diversity of microalgae. For instance, sinking rates can in reality vary from the Stokes' law estimates we used to estimate centrifugation energy consumption[61] depending on the microalgal taxa and cell shapes.[62] Adapting the centrifugation technology may be necessary for some strains.[63] Furthermore, using ranges and distributions for the biological parameters based on results obtained within the known biodiversity to simulate undiscovered strains could be a good example of survivor bias.[64] In fact, we believe this bias benefits the representation of uncertainty by taking into account the demonstrated difficulty to cultivate many strains which can be observed in their environment.[65,66] The discovered and upscaled strain will therefore likely be relatively similar to the ones we already know. Finally, and as previously mentioned (cf. Section 2.2.2), the model would benefit from further refinement of the interactions between techno-operational, biological, and geographic variables to limit the weight of unlikely combinations in the uncertainty representation.

3.6. Use of the Results for Decision-Making. The high dispersion of the results associated with a very *large LCA space*[67] and the complex overlapping of the uncertainties must lead us to question the usability of the estimates for decision-making. Ideally, the results should be used for planning and providing insightful indications on whether this technology will likely be beneficial and compete with alternatives. We can summarize the results by using the median impact score per kilogram of the bioprospected compound across production mixes and strain−compound pairs: 1.5 m^3 for WD; 96 kg CO_2-equiv for GW; 0.017 kg P-equiv for FE, and 0.007 kg 1.4-DC-equiv for TET (cf. Figure 4 and Table S3 in Supporting Information I.3 for complete statistical description). These values, however, are obtained by keeping one median score per strain−compound pair and location, thus aggregating techno-operational uncertainty (cf. Figure 1). A first comparison of magnitudes can be made with other bioactive compounds such as drugs from industrial chemistry whose impacts can range from 30 to 3000 kg CO_2-equiv per kilogram of drug.[68] Overall, if a solution based on a bioprospected microalgal compound was to be compared with an alternative technology for decision-making prior to technology development, the whole distribution of the results should be considered and different statistical measures could be used[69] (cf. Supporting Information I.3).

It must be highlighted that the results presented in this article can be understood as a *null model* by analogy with its use in ecology.[70] Thus, the patterns of the model's output densities are obtained for a set of standards' assumptions associated with our current level of knowledge. Additionally, the understanding of the uncertainty propagation combined with the sensitivity analysis allow anticipating the shape of the densities when other assumptions are made or more knowledge is gained. A key assumption supporting the *null model* is that bioengineers will find the combination of

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photobioreactor geometry and operational setup associated with 30% of the strain-specific energetic yields (cf. Section 2.2.1), as observed for cultivated strains by Williams and Laurens.[28] Running the model with more pessimistic or optimistic assumptions regarding the capacity of bioengineers to optimize photobioreactors for specific strains would shift the impact density curves. Another assumption is that there is no restriction on the possible biochemical class of the target compound (protein, lipid, and carbohydrate) and content in the biomass. Finally, we do not account for market mechanisms that could trim the density curves by making the worst cases economically non-viable, for instance due to very high energy consumption per functional unit. This assumption can be qualified as realistic as the context of high-value compounds does not exclude cases with high production costs provided that the market prices of the compounds follow.

4. OUTLOOK

Through a heavy stochastic simulation of microalgae cultivations across strains, technological settings, and locations, this work demonstrates the use of computational resources to investigate the uncertainty associated with the future environmental impacts of a technology at a very early stage. The stochastic approach, coupled with an explicit classification and separation of the uncertainties, allowed isolating the most important uncertain parameters but also to understand how techno-operational, bioprospecting, and production mix uncertainties interact with each other. It is key to note that, by propagating uncertainty regarding the LCA of one bioprospected microalgal compound, our approach eventually drew the LCA profile of a whole biological group (microalgae) and its sector (productions of high-value microalgal compounds) in a whole market (Europe). An even more accurate LCA portrait of the microalgal high-value compounds sector would benefit from including background uncertainty but also the extraction procedures which highly depend on the compound and strain. Overall, the approach can be generalized to technologies at a conceptual level of development for which the modelers know enough about the ruling biological and physical phenomena to determine the key model variables, draw dependencies, and eventually parameterize a model in which uncertainties are singled out. The value of the approach is enhanced when simultaneously applied to competing alternatives so that probability estimates resulting from the same method and same understanding of the uncertainties can be compared.

Finally, an additional step toward an educated decision-making process and planning would be to use the model to go beyond the presented *null scenario* and propagate uncertainties using prospective databases for market information and future marginal suppliers but also climate projections that could substantially influence the forecasts.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c04849.

Product system, description of additional calculations to the model, elementary composition of microalgal molecules biochemical classes, additional results and figures (PDF)

Details of parameters, statistical description of all models' outputs in mono-dimensional sampling, and correspondence between foreground activities and background activities (XLSX)

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Notes

The authors declare no competing financial interest.
The code of the model allowing reproduction of the figures is available at https://github.com/PJGilmw/Bioprospected_LCA.

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Article III

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LCA to evaluate the environmental opportunity cost of biological performances in finfish farming

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KEYWORDS : *LCA,* fish farming, mortality, industrial ecology, opportunity cost, trout, environment, aquaculture

ABSTRACT

Purpose

Mortality and suboptimal biological performance are a widespread problem in finfish farming. The associated losses constitute an environmental opportunity cost that needs to be thoroughly assessed to prioritize actions aiming at reducing the environmental impacts of finfish aquaculture. We here propose and demonstrate the use of a new parameterized and consequential LCA model of sea-trout production designed to assess the environmental opportunity costs of suboptimal biological performances, considering distinct mortalities and biological Feed Conversion Ratios (FCR) along the rearing process.

Methods

Primary data was collected in Danish and Italian farms to reconstitute the whole production process for sea-reared trouts. The level of detail allowed us to divide this production into seven different growth stages for which mortality and biological FCR can be assessed and modified. Excretion and valorization of fish sludge were modeled with a calibrated mass-balance model. Together with fish sludge, dead fish was modeled as valorized by anaerobic digestion. The foreground system was linked to the consequential version of ecoinvent 3.8 for which the embedded uncertainty was considered in MonteCarlo simulations. The model was used to assess the current environmental opportunity costs and evaluate the effect of losses happening at different timings along the rearing process.

Results and discussion

Results showed a low environmental opportunity cost for the current mortality rate of 5% as suppressing this mortality decreased impacts by 3.5% to 5% across impact categories. Decreasing the biological FCR decreased the environmental impacts proportionally. The timing of the losses was shown to greatly influence the environmental opportunity cost and the same mortality rate happening in the late stages had substantially more impact than in the first stages. The valorization of the dead fish showed a negligible contribution to the reduction of impacts in the current system but showed a substantial contribution in the case of higher mortalities, such as observed for other farms and foreseen in the future.

Conclusion

The model demonstrated that assessing an opportunity cost by multiplying the lost biomass by a unique impact factor, such a previously done, constitutes an oversimplification neglecting the losses timing and the fact that fish biomass is not a marketable product. Even though the current environmental opportunity cost for losses appeared neglectable, suboptimal biological FCR should be tackled. The model and approach can be used to project trout farming within future disease regimes and assess the trade-offs regarding fish-health issues and new treatments and practices.

TEXT

1. Introduction

Over the last decade, worldwide fish farming has caught up with fisheries and is now responsible for half of the global fish production and expected to play a predominant role in fulfilling the increasing population nutritional demand (FAO 2020). Farmed fish is indeed a valuable source of proteins and omega 3 that can be incorporated in diets with generally lower environmental impacts compared to other types of animal products (Tilman and Clark 2014; Poore and Nemecek 2018; Philis et al. 2019). Regardless of the species and production technologies, the key asset of fish farming over land animals production is a low Feed Conversion Ratio (FCR, calculated as feed input needed per fish output) as finfish are ectotherm animals that do not require as much energy to regulate their body temperature and do not require energy to fight gravity thanks to water support (Tlusty et al. 2018; Bohnes et al. 2019). Nevertheless, finfish farming is one of the fastest growing food sectors in the world (FAO 2020) and remain associated with environmental impacts. Three recent reviews (Bohnes and Laurent 2019; Bohnes et al. 2019; Philis et al. 2019) of the LCA literature cover more than 65 studies and offer a consistent overview of the efforts made to systematically assess the environmental impacts of the diversity of finfish farming systems. These critical reviews list better waste management, FCR improvement, and new farming practices as directions to decrease the environmental impacts. Overall, there is a consensus about the problematic lack of assessment of the fish health and biological performance-related aspects in LCA such as diseases, medicines, disinfectants, escaped and dead fish, and management of sludge among others. This gap is mainly due to difficulties to get detailed Life Cycle Inventories (LCI) at the fish farm level which imply aggregated inventories for “monolithic” and black-box fish farms models that are hardly parameterizable. The gap also stems from the scarcity of LCIs for the productions of interests such as chemicals and drugs, for which knowledge on emission and characterization factors also remain limited (Nyberg et al. 2021). Filling these gaps is even more relevant considering the general call for a *"new paradigm to help solve the global aquaculture disease crisis"*(Stentiford et al. 2017). Finfish aquaculture diseases are indeed plaguing the sector which is experiencing huge losses each year at a global scale. The estimates for the associated global economic losses amount to at least US$ 10 billion (Shinn et al. 2015; Just Economics 2021) per year and China is for instance loosing 15% of its production each year (Leung and Bates 2013) with similar figures for Norwegian salmon aquaculture (Bang-Jensen et al. 2019) or European finfish aquaculture in general (Shinn et al. 2015). The losses due to fish health issues can be associated either to routine mortality, disease outbreaks that can kill up to 100% of the fish, and FCR increase via stress and affections which weaken the fish without killing it (Murray and Peeler 2005; Monir et al. 2015; European Union 2018). Due to the raise of antimicrobial resistance, climate change and the intensification of production associated with a growing demand, the health issues are considered as a major threat to the development of the finfish farming sector (Bang-Jensen et al. 2019; Peck et al. 2020).

The environmental impact and societal cost that can be associated with health issues in finfish farming has rarely been looked at. These impacts and costs are due to the losses associated to a waste of resources, the additional treatment of biological waste, the cost of treatments and biosecurity measures, and the contamination of wild stocks (Skilbrei 2012; Monir et al. 2015; Abolofia et al. 2017; Just Economics 2021). Studies and reports have recently started to address the issue using a LCA approach. Philis et al.(2021) study and compare the environmental impacts of different biological delousing treatments for salmon production and show that such treatments constitute an insignificant contribution to the overall production's environmental impact for the considered categories (<1%). However, the study cannot assess the treatments efficiencies and their impact on salmon mortality, which hinders the comparison's equity between production with and without treatment. Cristiano et al.(2022) assess different options to valorize sludge and dead fish in a modern Norwegian smolt farm from a Life Cycle perspective. Besson et al. (2014, 2016) study the life cycle environmental performance gain associated with genetic improvement of the FCR and growth rate in an African catfish farm.

A recent report (Just Economics 2021) assesses the overall environmental, social and economic cost of salmon farms in the main producing countries. In particular, the cost of lice treatment and the environmental impact of the wild stock depletion due to lice spreading from fish farms are assessed. Furthermore, the economic opportunity cost of mortality is assessed by multiplying the lost biomass by the salmon price. Following this logic, the environmental opportunity cost could be defined and calculated as the lost biomass multiplied by a single emission factor for commercial-size fish. This approach would however be oversimplified because a fish dying at the beginning of its growth period will not be fed anymore while losing the fish just before slaughtering will constitute a complete waste of the investments and the associated impacts. These discrepancies could become particularly influent on the assessment for finfish species grown in systems where the FCR and inputs/outputs vary over the different growth stages, such as for anadromous finfishes (e.g. salmonids). Furthermore, multiplying the total lost biomass by the average impact to produce one kg of fish boils down to considering an indistinct "fish biomass" as a marketable product. Instead, a LCA must consider the demand for fish of a commercial size, and not for "fish biomass" in general which does not fulfill the obligatory properties on the market (Weidema 2003). This difference of perspective could considerably change the estimated opportunity cost. Modeling the distinct consequences of events happening at different growth stages and the responses of the production program has already been done for environmental or economic assessment (Bala and Satter 1989; Château and Chang 2010; Abolofia et al. 2017; Ferreira et al. 2021), but never adapted to LCA to our knowledge. Assessing the environmental opportunity cost of losses and being able to predict the environmental impacts of a farming system under different biological performances thus requires a new disaggregated and detailed LCA allowing modification of this performance at different stages in the production cycle. In particular, the model should be able to modulate separately the two main components of biological performance namely the mortality rate and the biological FCR, i.e., the ratio of feed input over the sum of dead and live fish productions, which differs from the economic FCR (feed/live fish).

To move further away from an opportunity cost estimate which does not consider market mechanisms and attribute an impact to losses in the past, consequential LCA constitutes a relevant approach to assess consequences of poor biological performances and take decisions accordingly. Only one consequential LCA of trout production has been published so far (Samuel-Fitwi et al. 2013) and the finfish aquaculture LCAs remain dominated by attributional approaches.

To address these needs, we present a parameterized and consequential LCA model for sea-trout production, with new primary data, which allows modeling of biological FCR and mortality rate changes at six different points along the rearing process. We use the model to assess the environmental opportunity cost of the current biological performance, i.e. of the mortality and of the suboptimal biological FCR. We also test the influence of different mortality timings on the environmental consequences of an increase in demand for Danish Sea-trout by assessing the sensitivity of the model to these timings. In addition to varying the biological performance in the foreground, we considered the uncertainty in the background and regarding our modeling of the valorization of losses and sludge via anaerobic digestion, which could influence the estimated costs and the sensitivity to the timings of the losses. Our study also presents the impacts and the contribution analysis of the system under its current biological performance.

In challenging times of change for the fish farming sector, this work aims at refining our understanding of the health-related issues in fish farming by increasing the detail and accuracy of LCA modeling and discuss the notion of environmental opportunity cost in LCA.

2. Methods

2.1) Fish farms under study and division of cultivation stages

The functional unit is defined as an increase in demand for 1 kg live weight of sea-reared rainbow trout *Oncorhynchus mykiss* at commercial weight which is 2.4 kg on average (noted as 2.4 lw. trout for simplicity). To get a satisfying level of division between the life stages, we reconstituted the whole production process by combining primary data from Italy from indoor hatchery to 0.08 lw. trouts (“hatchery”,” fry/fingerling”, “On-growing 1, 2 *3”* on Fig. 1) and from Denmark for life stages from 0.08 lw. trouts to the final sea-reared trout (“On-growing DK*”,* “Seafarm 1*”,* “Seafarm 2*”* on Fig. 1). Overall, this means that the very beginning of the fish growth is covered by Italian data and the produced young fish is an input to the first Danish stage for the rest of the fish growth. The Italian farm is in the north of Tuscany in west Apennine drainage and, with the exception of the hatchery, does not use electricity as the water flows and is oxygenated by gravity. The primary data provided for this farm represents average production figures over 2018, 2019 and 2020, for a total production of 100 tons per year.

The Danish on-growing stage ("On-growing DK" in Fig. 1) from 0.08 lw. to 1 lw. trouts is an outdoor semi-recirculating farm for which the business model imposes the overlap and commercialization of three sizes of trout during the same year. It produces 1 lw. trouts as main products that are sent to sea cages where they keep growing to the commercial weight (2.4 lw. trouts) that are then sold for consumption, and smaller 0.3 lw. trouts that are sold to other fish farms. It also co-produces larger trouts directly sold for consumption which are therefore functionally equivalent to the sea-reared 2.4 lw. trouts. The primary data for this cultivation stage covers two years of production (2019,2020) in a farm which produces 400 tons total live weight per year. Finally, the 1 lw. trouts are reared in Danish sea-cages for seven-eight months up to reaching their commercialization weight (FU). Roe constitutes 11% of the produced biomass but were included as part of the FU (1 kg of 2.4 lw. trouts with 11 % of roe mass). Data on sea cages were collected for seven Danish farms over 2019 and 2020 for a total production of 2500 tons per year. According to the production types clusters proposed by Philis et al. (2019), the reconstituted production process can classify as "land-based intensive flow-through" until the weight of 1 kg is reached and as "open sea-based rearing" until the commercialization weight is reached. The overall mortality rate calculated as the ratio of dead biomass over the total biomass bioproduction of sea-reared trout amounted to 5% (cf. Table 2), which is in the same order of magnitude as the average value reported for the whole Danish sector (6.4% for 2021 across all types of trout farming systems according to Danmarks Statistik (2021)). The sludge is filtered out and collected in all land-based stages and the dead fish in all stages.

To improve the granularity of the parametrization and model the current mortality as close as possible to reality, we further subdivided these "physical" cultivation stages in eight "virtual" fish growth stages based on direct information from the producers about mortality rates along the rearing process (cf. Fig. 1). These subdivisions are implemented such as the losses are happening at the very end of each virtual stage. The biological FCR of each physical cultivation stage was used to interpolate the feed inputs of the virtual growth stages. The other inputs and outputs to the virtual growth stages (electricity, oxygen, fuel, chemicals) were calculated proportionally to the feed inputs (cf. OR II.2). This implies that for each growth stage, we assumed that fish deaths occur after consuming all the feed and excreting all the residues. Thus, the mass balances between inputs and outputs are respected. Virtual growth stages are henceforth simply referred to as "growth stages".

2.2) LCA framework and product system

2.2.1) System boundaries, consequential modeling and stochastic LCA

We performed a consequential LCA using ecoinvent 3.8 consequential as background database. We therefore studied the consequences of an increase in demand for one kg of 2.4 lw. sea-reared trout and considered marginal mixes and technologies as responding to this increase in demand. The marginal Danish electricity mix was modeled at the fish farm level.

The LCA is stochastic and we performed MonteCarlo simulations considering the background uncertainty of ecoinvent and the uncertainty of the anaerobic digestion process in the foreground (cf. 2.5).

The scope of the system excludes slaughtering, considered neglectable in terms of environmental impacts, and starts with the eggs hatching without considering any burden for the egg production. The sludge and losses are valorized via anaerobic digestion and the substitution of natural gas on the Danish market is modeled.

We modeled the substitutions of corresponding growth stages for the co-produced 0.3 lw. and 2.4 lw. trouts in the growth stage “On-growing DK” (cf. Fig. 1). Thus, the model accounts for the business model of this farm and the mass balances are respected. Conceptually, the substituted 0.3 lw. trout production comes from another farm which shares the same first growth stage division as the main production line, but the division from 80 to 300g (“On-growing 2”) was modeled with Italian data only. For further reference, we name this substituted production “parallel production line” (cf. Fig. 1). The 2.4 lw. trouts substitute the functionally equivalent sea-reared trouts.

2.2.2) Life Cycle Inventory and product system

The complete life cycle inventories of the different growth stages and of the overall production are presented in Online Resource II.1. The collected data for the production’s economic inputs covers feed, oxygen, fuel for boats and on-site trucks and machineries, chemotherapeutants, electricity and treatments of dead fish sludge. No transport of the fish to consumer was modeled, neither between the land-based stages, because they were assumed close to each other. Table 1 shows the feed and fish inputs and outputs of the different growth stages and Table 2 displays the associated FCRs and mortality rates. The product system is shown in Fig. 1.

Even though chemicals and medicines inputs were provided, no inventories for the medicines exist in ecoinvent 3.8 and the few therapeutics inventories available in the literature (Jiménez-González et al. 2004; De Soete et al. 2017; Emara et al. 2019; Parvatker et al. 2019) do not cover the ones used in the trout production. This literature instead highlights that the use of other medicines as proxies can constitute a major source of error as chemically similar molecules can be associated with very different production routes (Parvatker et al. 2019). On the grounds of this, the inputs are not included in the inventory and impact calculations but the input amounts are presented in the life cycle inventory table in Online Resource II.1 for completeness.

The peracetic acid production was modeled based on the inventory provided by Echeverria et al.(2021). As no inventory was available for quaternary ammonium salt (biocide) production, we used benzyl chloride, one of its chemical precursors (Rossberg et al. 2006). Similarly, copper pyrithione was modeled as pyridine production and chloramine inventory was not considered. No direct emissions were modeled for the chemotherapeutants used in the farm either due to the absence of characterization factor for the substance and/or uncertainty regarding the fate of the substance through the farm and its biological processes such as biofilters, solid filters and lagoons (Emara et al. 2019).

The feed composition was provided by the producers and is given in Table 3. The feed contains 50% of plant-based ingredients, which is similar to the composition recently used by Sanchez-Matos et al. (2022) and significantly more than in the organic composition used by Samuel-Fitwi et al. (2013), (77% fish-based). The same heat and electricity inputs to the feed production as the ones used by Samuel-Fitwi et al. (2013) were considered. To model consequences of an increase in demand, we connected the poultry meal and hemoglobin meals to the marginal markets for feed and energy as modeled by Schmidt and De Rosa (2020) because these inputs are dependent co-products. Indeed, following the logic of consequential modeling, no increase in hemoglobin meal production will be induced by an increase in production for it, and the eventual consequence will be an additional demand for their equivalent in megajoules from the marginal producer of energy feed (maize and wheat) and in kilograms of protein on the protein feed market (soybean meal). The corresponding amounts of energy and protein are calculated in Table S1 in Online Resource I.6. Note that in the ecoinvent consequential database, fish oil and rapeseed oil which are part of the modeled fish feed are also eventually connected to these marginal feed markets in their product systems. The ecoinvent processes connected to the foreground system are shown in Online Resource II.3. Only direct land uses changes are considered in the ecoinvent processes.

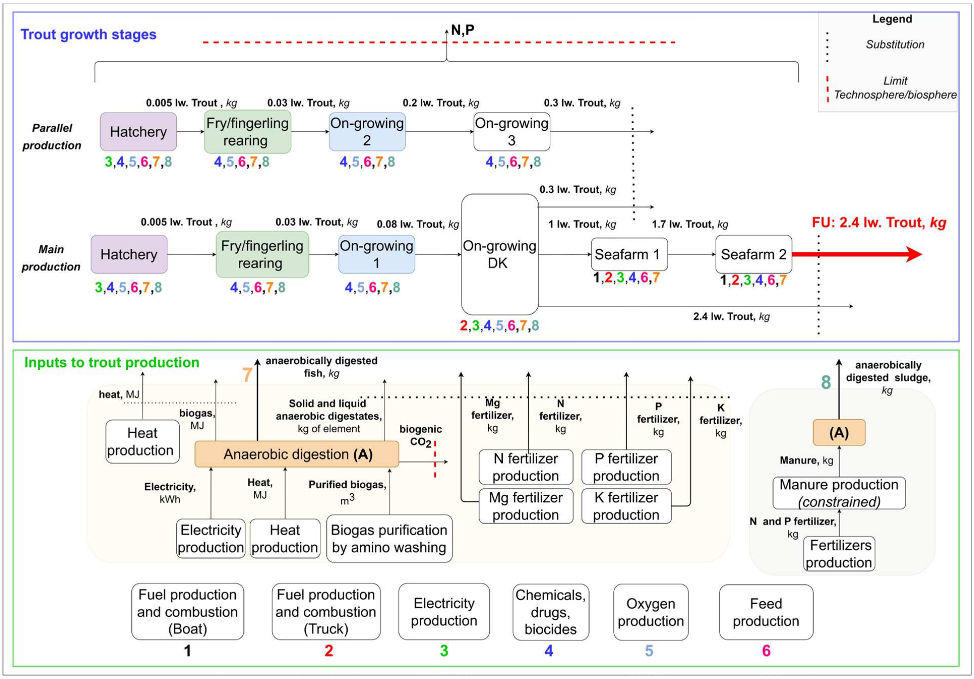

Fig. 1 Foreground product system. A colored number under a growth stage division (upper panel) indicates an input of the associated process in the lower panel. Growth stages with the same name and different numbers (e.g., On-growing 1,2,3) constitute virtual divisions of a same physical cultivation stage. l.w= live weight, DK = Denmark.

Table 1 *Fish and feed inputs and outputs across all growth stage divisions. The full life-cycle inventories are displayed in Online Resource II.1 under xlsx. format. The inventories are presented under the form of a technosphere matrix: A negative amount indicates an input. All amounts are scaled to a production of 1000 kg. For the total production of the FU (2.4 lw. trout, right column), the feed and dead fish amount, was calculated by considering the co-produced 0.3 lw. trout by "On-growing DK" as an output of 2.4 lw. trout for this stage*

	Hatchery	***Fry/ fingerling rearing***	***On-growing 2***	***On-growing 3***	***On-growing 1***	***On-growing DK***	***Seafarm 1***	***Seafarm 2***	***Total for FU***
0.005 lw. Trout (kg)	1000	-200	0	0	0	0	0	0	0
0.03 lw. Trout (kg)	0	1000	-187.5	0	-375	0	0	0	0
0.08 lw. Trout (kg)	0	0	0	0	1000	-216.33	0	0	0
0.2 lw. Trout (kg)	0	0	1000	-666.67	0	0	0	0	0
0.3 lw. Trout (kg)	0	0	0	1000	0	0	0	0	0
1 lw. Trout (kg)	0	0	0	0	0	1000	-548.36	0	0
1.7 lw. Trout (kg)	0	0	0	0	0	0	1000	-622.29	0
2.4 lw. Trout (kg)	0	0	0	0	0	686	0	1000	1000
Feed (kg)	-1125	-1033.33	-1487.5	-466.67	-875	-1513.79	-727.8	-521.04	-1288.3
Dead fish (kg)	166.67	0	250	0	0	41.4	75.94	0	50.49

Table 2 *Mortality rates and FCRs across all growth stage divisions calculated using inputs and outputs in Table 1. For the total production of the FU (2.4 lw. trout, right column), the rates and FCRs were calculated by considering the co-produced 0.3 lw. trout by "On-growing DK" as an output of 2.4 lw. trout for this stage*

	Hatchery	**Fry/ fingerling rearing**	**On-growing 2**	**On-growing 3**	**On-growing 1**	**On-growing DK**	**Seafarm 1**	**Seafarm 2**	**Total for FU**
Mortality rate: Dead/(Dead+ Live biomass)	0.14	0	0.2	0	0	0.02	0.14	0	0.05
Dead/Live biomass	0.17	0	0.25	0	0	0.02	0.17	0	0.05
Biological FCR (Feed input / (produced live + dead fish biomass))	1.13	1.29	1.4	1.4	1.4	1	1.38	1.38	1.23
Economic FCR (Feed input / produced live fish biomass)	1.13	1.29	1.83	1.4	1.4	1.03	1.61	1.38	1.29

__Table 3__ Feed composition. The N and P calculations are based on the contents of each ingredient and documented in Online Resource I.6

Ingredient	*kg·kg feed^{-1}*
Fishmeal	0.3
Rapeseed oil	0.15
Wheat	0.14
Fish oil	0.08
Soybean meal	0.13
Poultry meal	0.08
Hemoglobin meal	0.04
Wheat gluten	0.08
N	0.022
P	0.00415

2.2.3) Impact assessment

We assessed 10 impact categories with 9 impact assessment methods from ReCiPe (H) (Huijbregts et al. 2017) namely global warming with 100-year time horizon (GW100), terrestrial acidification (TA100), terrestrial, human, and freshwater ecotoxicity (TETinf, HTinf, FETinf), particulate matter emissions (PM), ozone depletion (ODinf.) and eutrophication, both for freshwater and marine ecosystems (FE, ME). For the latter category, which is particularly relevant for aquaculture systems, ReCiPe proposes a freshwater eutrophication method (FE), which only considers phosphorus emissions, and marine eutrophication (ME) method which only accounts for nitrogen emissions. This distinction is based on the hypothesis that only one element is limiting in each ecosystem type (Elser et al. 2007; Cosme and Hauschild 2016, 2017). As these distinctions are not commonly made in other LCAs of fish farms, we also used the generic eutrophication (Eutro.) potential from CML-IA to allow a comparison to other LCAs' results.

2.4) Joint modeling of mortality, biological feed conversion ratio and excretion

Parameterized mortality, biological FCR and excretion are modeled conjointly to ensure input and output mass balances. It is important to note that mortality and biological FCR can be modified independently as the biological FCR measures the overall conversion of feed into biomass, dead or live. The economic FCR instead, usually reported as "FCR" in other studies, is defined as the ratio of *feed/live fish* and is affected by different mortality rates. To ensure consistent modeling of the nutrient and mass balance while using the model from Papatryphon et al. (2005), we considered a fixed phosphorus (P) and nitrogen (N) content in the fish of 0.00415 kg P and 0.022 kg N per kg live weight fish (wet), as they represented average reported values in the literature (Shearer 1984; Skonberg et al. 1997; Bureau and Cho 1999; Bücker et al. 2020). Based on the same studies, magnesium (Mg) and potassium (K) fish composition were respectively fixed at 0.38 and 4.6 g · kg live weight $fish^{-1}$ and used to estimate the substitutions associated with the valorization of dead fish (cf. 2.5). The feed P and N content were calculated based on the content of each ingredient (cf. Table 3). Details about fish and feed composition are presented in Online Resource I.6.

Modulating the biological FCR necessarily implies a modification of the N and P excretion to close the mass balances, because the N and P contents in feed and fish and the feed digestibility are modeled as constant. The theoretical minimum biological FCR for each growth stage can therefore be calculated as the one which sets the liquid P excretion to zero, as we found P to be the limiting nutrient before N in the mass balances. The P excreted in the feces remain the same as its digestibility in the feed remains constant. Thus, for this minimal theoretical biological FCR, all digested P is assumed as perfectly metabolized and incorporated in the fish biomass. Note that this minimal biological FCR constitutes a necessary mathematical limit and theoretical performance optimum for the modeling purposes but is biologically unachievable. Finding this theoretical limit allows using the model within the theoretical boundaries. The analyst can thus modify the FCR by choosing the value of an improvement parameter which fixes the biological FCR to its minimum value when set to 1 and keeps the current FCR when set to 0 (cf. Online Resource I.1). Once the biological FCR is changed for a growth stage, the new fish biomass outputs are calculated accordingly and the new excretion is calculated by closing mass balances as previously explained.

The production of solid feces is calculated as the non-digestible part of proteins, carbohydrates and lipids in feed, shown in Online Resource I.6 and based on Aubin et al.(2011). Together with the non-ingested fraction of feed (0-0.05) a constant proportion of the solid feces is captured by solid filters and exported as sludge. In addition, a plant lagoon removes part of the N and P emissions before the water is returned to the river. The constant fraction of feces captured by the filter and the lagoon removal rate were calibrated by comparing the mass-balance model's output and the monitored outlet concentration in the "On-growing DK" stage (cf. Fig. 1).

Changes in mortality are modeled via two distinct parameters: loss level and loss reduction. Loss level (values from zero to one) is the fraction of the live production which dies in addition to the current mortality, while loss reduction is the fraction of losses that is avoided. In the model, any loss level combined with a loss reduction of one would lead to zero losses for the growth stage. The loss level parameter allows modeling a mortality due for example to a disease affecting the fish farm, while the loss reduction parameter allows simulating the effect of a new treatment or practice that increases the survival of the fish.

All losses were considered as dead fish that can be collected (cf. 2.5) and no escapees, cannibalism or bird predation were considered. For the growth stage division "On-growing DK" which produces three different sizes of trout, the losses and FCR modification are applied proportionally to the mass ratios of the three different flows.

As observed and modeled by Besson et al. (2016), the effect of FCR reduction on the life cycle inventory of a fish farm differs depending on what the current limiting factor is. If the limiting factor is the fish density in the tanks, improving the FCR allows only to reduce the amount of feed needed per kg of fish but does not reduce the needed amount of other production inputs. However, if the limiting factor is the nutrient discharge in the environment, a FCR improvement allows to obtain the same production while reducing all other inputs. Based on the Danish regulation limiting nutrient discharge for fish farms (Jokumsen and Svendsen 2010), the model assumes no current limit on the fish density and all inputs per kg of fish proportionally decrease when the FCR is improved.

The equations and parameters used to modify FCR, mortality and excretion in the different growth stages are presented in detail in Online Resource I.1 and I.2. The whole model and associated code is available on Github (Jouannais 2023).

2.5) Valorization of losses and sludge

Modeling the environmental effects of different fish farm biological performances required to consider the "valorization" of losses and sludge. The dead fish in the Danish farms considered in this study are currently collected and used as substrate for anaerobic digestion, thus generating biogas and recycling nutrients for field fertilization. This method can be expected to keep developing further as it constitutes an efficient valorization of nutrient and energy while neutralizing biohazard due to infected fish (Estevez et al. 2022). We modeled anaerobic digestion of fish sludge and dead fish by considering a fixed input of electricity and heat per kg of dry substrate calculated from the techno-economic assessment by Kratky and Zamazal (2020). This inventory covers the pretreatment of the substrate (hygienization and crushing), homogenization and fermentation at 28°C. Biotechnological research is still carried on to improve and stabilize the process and yields and operating conditions of fish waste anaerobic digestion vary substantially across studies (Chen et al. 2010; Ivanovs et al. 2018; Bücker et al. 2020). Based on seven yields reported in these studies and displayed in OR I.3.1, we modeled the uncertainty and variability of the methane yields obtained from this fermentation of dead fish with a triangular distribution ranging from 380 to 920, with a mode of 550 ml $CH_4 \cdot$ g dry dead fish $^{-1}$. While dead fish was modeled as a mono-substrate, the anaerobic digestion of fish sludge usually requires mixing it with another substrate to lower the N and P concentrations, avoid NH_3 accumulation during the process and suit the microbial communities. A volume ratio sludge/cow manure varying between 0.30 and 0.40 was modeled (Brod et al. 2017) and the associated CH_4 yield varying between 300 and 400 ml CH_4. g dry substrate $^{-1}$ was assumed (Estevez et al. 2022). CO_2 is also produced by the fermentation, and we assumed a fixed volume ratio CO_2/CH_4 of 0.465 (Bücker et al. 2020) for both fish and sludge. This CO_2, together with other gas present in minor fractions are degassed during the upgrade of this biogas to biomethane that we modeled with amino washing. The resulting biomethane substitutes heat based on its lower heating value.

The solid and liquid fractions of the fermentation's digestate contain the amounts of N, P, K and Mg calculated with the mass-balance model (cf. 2.4). Both fractions can be used for fertilization but the liquid, mineralized fraction presents the highest bioavailability for plants. Based on laboratory and field experiments on the mineralization rates and comparing agronomic efficiencies of different digestates, substrates and industrial fertilizer reported by Brod, E. et al. (2017) and Goddek, S. et al. (2018), we modeled uncertain substitution coefficients with industrial fertilizers with uniform distributions for N ranging 0.8 to 1, and from 0.2 to 0.8 for P, K, Mg. By definition an agronomic efficiency of 1 implies a substitution coefficient of 1. As fish sludge digestion requires an input of manure that would have been otherwise spread over fields without digestion, we modeled the overall fertilizer substitution resulting from the combination of manure with the fish sludge according to the differences in agronomic efficiencies between non-digested and digested manure reported by Brod, E et al. (2017). We refer the reader to Online Resource I.3 for the parameters and equation describing these substitutions.

2.6) Simulations and evaluation of the environmental opportunity costs

To demonstrate the use of the model and study the effects of different biological performances along the production cycle, we first estimated the current environmental opportunity cost of mortality and suboptimal FCR by calculating the impacts for the current system with the reported biological performance and when the system is pushed to its theoretical limits. We first estimate the environmental opportunity cost of mortality alone by simulating no mortality at all and calculating the difference of impact between this configuration and the current system. We then set the biological FCRs in all stages to their theoretical minimum values to estimate the environmental opportunity cost of the biological FCR. Finally, we assume no mortality and minimum theoretical FCRs to estimate the opportunity cost of the overall biological performance. All configurations share the same 1000 MonteCarlo iterations regarding the background and the anaerobic digestion parameters (paired sampling).

To study the distinct effect of losses occurring at different timings along the production cycle, we performed a series of six local sensitivity analysis by increasing the loss level by 15% in each of the growth stages separately. For example, the first sensitivity analysis considers 15 % loss level in the hatchery while the rest remains unchanged. We assess the change of the impacts for the functional unit in each of these six configurations.

For all configurations used to estimate the opportunity costs or in the sensitivity analysis, the modifications regard only the main production line while the substituted parallel line remains unchanged (cf. Fig. 1).

3. Results

3.1) Current environmental impacts

The boxplot on the left part of Fig. 2 shows the results of the stochastic LCA for the current system system's biological performance. The global warming impact (GW100) of the current system had a median score of 4 kg CO_2-eq. per kg live trout with a first and third quartile at 3.5 and 4.5 kg CO_2-eq. The median terrestrial acidification (TA) obtained a median score of 0.014 kg SO_2-eq.. As expected, the eutrophication impacts vary considerably depending on the choice of LCIA method because different methods consider fundamentally different impact pathways. The generic eutrophication freshwater and marine water eutrophication impacts had median scores of respectively 0.055 kg PO_4-eq., 0.008 kg P-eq. and 0.07 kg N-eq.. ODinf and PM respectively showed median scores of 1.5E-7 kg CFC-11-eq. and 0.006 kg PM10-eq. Regarding toxicity, HTinf, TETinf respectively scored at 0.9 and 0.025 kg 1.4-DC-eq. while FETinf showed a median score of 0.8 kg 1.4-DC-eq.with a substantially lower dispersion of the results than for the other toxicity categories. The contribution analysis is available in OR I.4 and shows that the feed production was responsible for most of the impact for GW100 (95%), PMF (85%), TA100 (75%), TETinf (99%), FD (88%), ODinf (80%), PMF (77%), while the direct emissions of N and P were the main contributors for FETinf and the three eutrophication categories. For HTinf, the electricity consumption was mainly responsible for the impact (120 %) while the substitutions occurring in the product system of the feed reduced the impact by 20%.

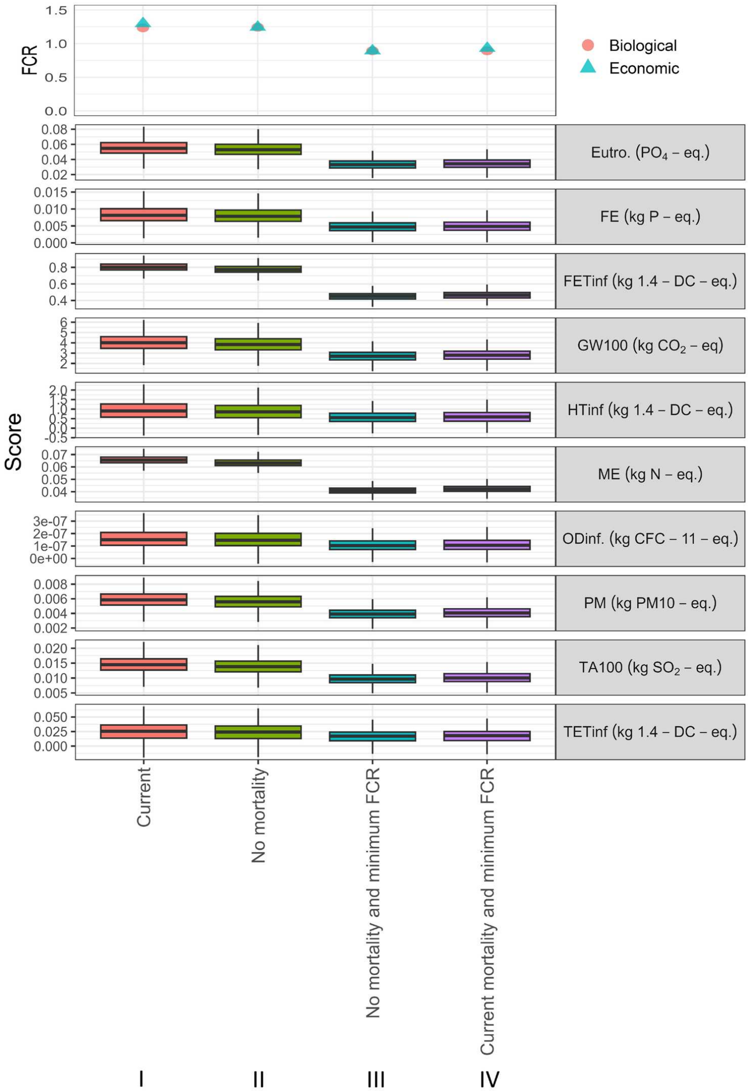

__Fig. 2__ Boxplots for the environmental impacts associated with an increase in demand for one kg of 2.4 lw. trout. The boxes limits represent the 1st and the 3rd quartile of the scores from the stochastic simulations. The edges of the vertical lines correspond to the minimums and maximums. Current: Unmodified primary data

3.2) Current environmental opportunity cost

The suppression of mortality (II in Fig. 2), currently distributed along the different growth stages (cf. Fig. 2), brings the economic FCR from 1.3 to 1.24. For the considered impact categories, the existing 5% mortality rate seems to be associated with a moderate environmental opportunity cost. When assuming no mortality (II in Fig. 2) the median impact scores are reduced for all impact categories, although to a different degree: from a minimum of 3.2% (ME, OD) to a maximum of 5.2% (HTinf) (cf. Fig. 2). This observation on the median scores remains valid for all paired stochastic iterations across scenarios as shown in Fig. S5 of Online Resource I.5. The losses have thus little weight on the environmental impact because they are generally low and because they are distributed along the growth stages and do not all concentrate at the very end of the life cycle (cf. 3.2). The valorization of the dead fish has a small contribution to the total impact (cf. Online Resource I.4), but additional simulations with higher mortality rates show that it can reduce impacts when assuming very low biological performances. For example, a reduction of 5% in GW impact was calculated when assuming a 95% loss level in fry/fingerling rearing to demonstrate the behavior of the model under extreme assumptions (cf. Online Resource I.4.1).

On the other hand, the opportunity cost of a suboptimal biological FCR in every growth stage is substantial (III, IV in Fig. 2). Simulating the same mortality rate with a theoretical minimum biological FCR for all growth stages (III in Fig. 2) reduced all median impact scores by at least 30% with a maximum 42 % reduction for FETinf (cf. Fig. 2 and Online Resource II.4). These reduction percentages show an absolute theoretical limit of what can be expected in terms of impact reduction for any attempt to improve the biological performance. When simulating the theoretical minimum FCR (III, IV in Fig. 2), the economic and biological FCRs decrease by 30% to reach the value of 0.9, which thus constitute the minimum theoretical FCRs for the whole production. Similar FCR values can be measured in very performant closed systems for salmonids (Samuel-Fitwi et al. 2013; Philis et al. 2019). As also reported by Paptryphon et al. (2004) and d'Orbcastel et al. (2009), all environmental impacts decrease in similar proportions together with the economic FCR. This observation holds for simulated changes of the FCR within the same production system and does not hold anymore across systems as Philis et al. (2019) report no correlation between FCR and impacts within the diversity of farming systems.

3.3) Sensitivity of losses in different growth stages

Fig. 3 shows for three impact categories that the effects of 15 % loss level (in addition to the current mortality rates) are hardly noticeable if losses happen before the trout's weight reaches 80 g (end of "On-growing 1"). The trends are similar for the other impact categories shown in OR I.5 and II.4. As expected, the later are deaths occurring in the production cycle, the higher is their negative influence on the environmental impact of the farm. Losses occurring in the last growth stage increase the environmental impact by 15-16% for all impact categories except for global warming, ozone depletion and human toxicity for which the impact increased by less than 13%. It's worth noting that a 15% loss level in the last stage constitutes a 85% production efficiency compared to the baseline for the activity delivering the functional unit. The expected impact increase would therefore theoretically be 17.6% (1/0.85). The fact that the impact increase is lower than this expected value is due to the valorization of losses which compensate for the efficiency loss (cf. Online Resource I.4.1). The magnitude of this compensation varies across impact categories. These results illustrate how the environmental impact due to the mortality rate depends on the growth stage that is affected by losses – and confirm that the model functions as intended.

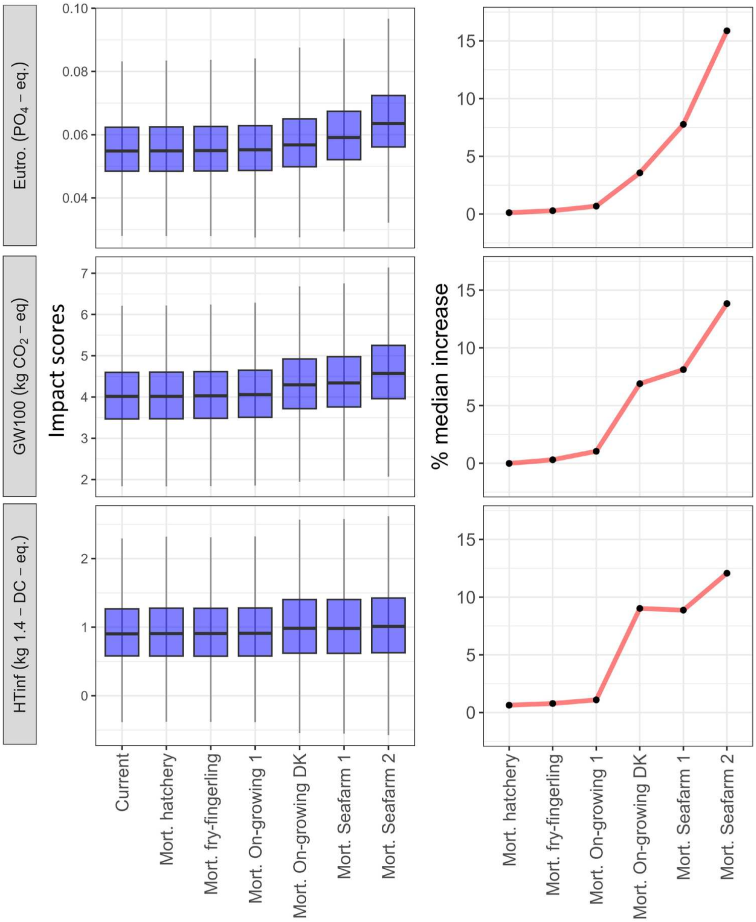

Fig. 3 *Boxplots for the environmental impacts associated with an increase in demand for one kg of 2.4 lw. trout for different timings of 15 % loss level. The left panel shows the impact score expressed in the corresponding impact category unit. The box limits represent the 1st and the 3rd quartile of the scores from the stochastic simulations. The right panel shows the percentage of median increase by setting the current median score as a baseline (0). The results for the other impact categories are presented in Online Resource I.5 and II.4. Legend of the horizontal axis: Current=Unmodified primary data. Mort. Growth stage X = Scenario with modified mortality in in the growth stage X*

4. Discussion

4.1) Comparison of the current system's impacts to the literature

Overall, the results show impacts similar to those reported in the review of 24 salmonid farms LCAs by Philis et al. (2019), even though a relevant comparison must consider the modeling choices, mainly attributional versus consequential modeling. In particular, the found median impact score of 4 kg CO_2-eq. kg trout^{-1} for global warming (GW) is higher than the average value of 3 kg CO_2-eq. kg trout^{-1} reported for open, sea-based system of salmonids in the review. The median terrestrial acidification (TA) score of 0.014 kg SO_2-eq. kg trout^{-1} is also the average value reported by the same authors for similar systems. Similarly, the median generic eutrophication impact (Eutro) of 0.055 kg PO_4-eq. kg trout^{-1} corresponds to the average value across the studies reviewed by Philis et al. (2019) for open, sea-based salmonid farms. The recent LCA of land-based rainbow trout production in Spain by Sanchez-Matos et al. (2022) reports a ReCiPe Freshwater (FE) impact of 0.007 kg P-eq. kg trout^{-1}, which is very close to the median value of 0.008 kg P-eq. kg trout^{-1} that we obtained for the same impact assessment method.

4.2) Feed contribution and influence of modeling choices

The feed major contribution to the impact is also documented in LCA studies of trout open sea-based systems, in opposition to fully recirculating aquaculture system RAS where electricity consumption usually dominates the contribution analysis (Samuel-Fitwi et al. 2013; Philis et al. 2019). It is worth noting that the fish feed modeling could benefit from more specific databases for agricultural products such as Agribalyse or Agrifoot-print, as used by Sanchez-Matos et al. (2022) for a similar trout production system, although consequential modeling should be kept consistent. However, our conclusions on the environmental opportunity costs and the importance of the timing of the losses would likely remain the same with these different modeling choices as Sanchez-Matos et al. (2022) also found a very important contribution of the feed to most impact categories (from 50% to 90% of the selected impact categories).

The high contribution of the feed to the impacts also suggests that the demonstrated model behavior can be generalized to a good extent to a model in which the production limiting factor would be fish density instead of nutrient discharge. As explained in 2.4, this would mean that an improved FCR would only reduce the feed input per fish output, while the inputs which are not proportional to the feed consumption would remain the same. This would therefore not change the results substantially for the impact categories where feed dominates. However, our assessment did not account for infrastructures that could constitute a non-neglectable (>5%) contribution to some impact categories, as found in the rare studies which consider capital goods (Bohnes et al. 2019). In this case, and assuming fish density as the limiting factor, the influence of an improved biological FCR would decrease while the influence of mortality, which increases all the necessary amounts of inputs per functional unit, would remain the same.

Overall, estimating direct N and P emissions using a mass balance model is very sensitive to the assumptions made regarding the N and P composition of the feed and the trout. Values ranging from 0.03 (Asmala and Saikku 2010) to 0.07 (Darzi 2021) kg P per kg fish produced are reported in the literature, for different fish species and conditions. As most LCAs rely on mass-balance models to estimate N and P emissions, a systematic reporting of the fish N and P contents considered the model would facilitate comparisons and potentially reduce the range of eutrophication results observed in the literature (Philis et al. 2019). Additionally, there is uncertainty and variability regarding the response of the filters when the biological FCR is modified. Instead of modeling a constant proportion of the excretion being filtered out, a fixed filtering capacity could be modeled which would possibly change the influence of FCR modification on the eutrophication impacts for the land-based parts.

4.3) Average opportunity cost versus opportunity cost in LCA

The results illustrate that changes in mortality rates are not directly proportional to changes in environmental impact. There are fundamental differences between assessing an environmental opportunity cost by multiplying losses by the impact associated to 1 kg of fish or by using a consequential LCA model. Indeed when neglecting the FCR variation across the growth stages divisions (cf. Table 2), that is both due to inherent fish metabolic differences (Wurts 2016) and to different rearing systems (Philis et al. 2019), and when assuming that all growth stages need the same inputs, then one kg of fish biomass virtually represents the same investment of feed regardless of the size and weight of the dead individuals. However, these assumptions constitute serious oversimplifications and describe fish production as a monolithic process instead of a succession of different stages with unique characteristics (e.g., land-based or sea-based, using or not a specific chemical, etc.).

Furthermore, calculating the opportunity cost with such a simplified model would return a *virtual cost* for the losses that happened, while estimating it with consequential LCA means estimating first the impact of an increase in demand for a farm subjected to a certain biological performance and the impact of the same production from a farm with better performance. The opportunity cost will then be the difference between the two impacts. The two approaches are fundamentally different in their framing as the simplified one does not aim at embracing consequences considering market mechanisms as it attributes a cost to an undifferentiated biomass, which is not a product in the LCA framework. In the present study, the market demand does not exist for fish biomass but only for 2.4 lw. sea-reared trout. This demand triggers chained demands for the different growth stages functioning as distinct processes with specific products, i.e., trouts of different sizes.

Due to these differences, the model should not be used to estimate the opportunity cost of a catastrophic viral outbreak (e.g. 80% of fish lost in the sea stage) as this would instead estimate the difference of impacts between a baseline and a farm that *consistently* produces trout with 80% mortality as a response to an increase in demand. The model is thus built to simulate the production under durable disease regimes as the ones associated with climate and ecosystem changes (Leung and Bates 2013; Reverter et al. 2020).

4.4) Towards full appraisal of the impacts of fish health issues

Despite the relatively low existing opportunity cost for the fish farms considered in this study, we believe that addressing mortality should not be considered a secondary effort and this cost could be higher in other fish production systems. Indeed, the reported value of 5% of losses used in this study are substantially lower than the value of 15% losses reported for Norwegian salmonid production (Bang-Jensen et al. 2019). Differences in the estimate methodology may be involved in the discrepancies but overall, 5% seems to correspond to a lower boundary for current losses in European finfish aquaculture (Just Economics 2021). 15% of losses are also reported for Brazilian fish farms (Tavares-Dias and Martins 2017). Parasitic diseases and the resulting higher biological FCR were found responsible for 80% of the economic loss associated to health issues in Bangladesh carp farms (Monir et al. 2015). The same modeling approach could be used to estimate the environmental opportunity costs for these other cases.

It could be legitimately argued that fish welfare and suffering of animals under human's responsibility should be reduced at all costs and regardless of the associated environmental impact

(Huntingford et al. 2006), which would make a thorough LCA on this topic pointless. This argument is not compelling, firstly because LCA does not necessarily aim at optimizing a process but can be used instead to help forecasting the environmental impacts of finfish aquaculture in the future and planning accordingly. Secondly, because fish welfare is also part of broader trade-offs. Reducing losses with new practices may lead to new impacts and the systemic approach of LCA aims at embracing this complexity, which is acknowledged by fish health experts themselves. The "Environmental impact of treatments in fish aquaculture" was ranked first among several topics regarding new treatments in a survey by Katharios et al. (2019) in which 124 fish health experts and stakeholders were asked to rank different potential research directions by order of importance.

The model here presented is designed to capture the opportunity cost of poor biological performances. It must be noted that this cost is only one part of the environmental trade-offs associated with health-management in fish farms, and improving biological performance will likely involve the environmental impacts of new treatments and practices. These impacts remain scarcely included in LCA studies but the recent use of ecotoxicity and antibioresistance characterization factors for different antibiotics (Nyberg et al. 2021) in a trout production LCA by Sanchez-Matos et al. (2022) is promising. They also use proxies for the production life cycle inventories of antibiotics despite the associated risk of error (cf. 2.3) which hinders a precise assessment of the trade-offs. Overall, the efforts made to better embrace the direct emissions and details of health-management at the fish farm level could be overshadowed by the uncertainty in the inventory. We therefore join Bohnes and Laurent (2019) and Philis et al. (2019) in advocating for a systematic assessment of uncertainties in the next LCAs for finfish aquaculture.

5 Conclusion

This study has shown that the current losses and their timing along the studied trout production constitute a minor environmental opportunity cost, while bringing the biological FCR closer to its minimal value would substantially decrease the environmental impact. The model is ready to be combined with inventories regarding the solutions undertaken to improve the biological performance.

Concluding, this work sheds light on one aspect of the trade-offs that exist in health-management in fish farming and can be used to estimate the environmental consequences of new treatments and farming practices, or project aquaculture into futures with different disease regimes, i.e., disease frequency and severity. These trade-offs are unavoidable when adopting a "One-Health" perspective (Stentiford et al. 2020), which embraces human, non-human and ecosystemic health as a whole and therefore requires a systemic environmental assessment for the management of biological performances in finfish aquaculture. Future research efforts to assess these trade-offs should focus on refining inventories and characterization factors for chemotherapeutants.

The availability of detailed primary data allowed us to estimate the environmental impacts associated with distinct timings for biological performance changes, but a complete understanding of the environmental consequences of disease regimes will require the combination of LCA with other modeling approaches such as epidemiology and agent-based modeling. Indeed, disease regimes do not tackle individual farms, thus reducing the biological performance of single production lines all other things being equal. A poor biological performance due to diseases in a farm is likely to propagate to other farms, eventually multiplying the environmental costs by spreading it to a whole sector (Jonkers et al. 2010; Peeler and Taylor 2011; Oidtmann et al. 2014; Ferreira et al. 2021), or even to humans (Ziarati et al. 2022). Sudden viral outbreaks such as infectious hematopoietic necrosis (IHN) on salmonids require the destruction of all the fish and temporary shutdowns of the farms (Bang-Jensen et al. 2019), which hinders the capacity for a production to answer to increases in demand. This is a problem for the economic performance of finfish farms and their capacity to ensure food supply first of all, and secondarily also for those working with modeling the impact of finfish farming, particularly when this requires the identification of marginal productions (Weidema et al. 1999) able to respond to such increases in demands. To fully appraise the consequences of poor biological performances in finfish farming, the scope of LCA studies should therefore be extended to consider the production of whole sectors, such as in our case, the entire European trout production, considering a network of distinct growth stages connected by epidemiology and market relationships (cf. Fig. 1 and "parallel production line"). While increasing the models' complexity, this would allow a better systemic understanding of the finfish-health issues' environmental consequences to facilitate the prioritization of efforts to tackle them and secure a sustainable food supply.

ASSOCIATED CONTENT AND DATA AVAILABILITY

All data for the reproducibility of the results can be found in the article and in the additional online resources and the github repository.

"Online Resource I" (docx): Main equations of the model, additional figures, feed and fish specificities.

“ **Online Resource II”** (xlsx):

- **Sheet "OR II.1":** Life Cycle Inventories, technosphere matrix.
- **Sheet "OR II.2":** Interpolation of the growth stages according to the timing of the losses.
- **Sheet "OR II.3":** Correspondence between foreground activities and background activities.
- **Sheet "OR II.4":** Numerical results associated with Figure 3 et 4.
- **Sheet "OR II.5":** Table of the model’s parameters.

The code of the model allowing reproduction of the results is available at https://github.com/PJGilmw/Fish_performance_LCA.(Jouannais 2023)

DECLARATIONS

The authors have no relevant financial or non-financial interests to disclose.

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Article IV

Jouannais, P., Blanco, CF., Pizzol, M., ENvironmental Success under Uncertainty and Risk (ENSURe): A procedure for probability evaluation in ex-ante LCA **(under review)**

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The Supplementary Information will be communicated to the committee and will be available online when the final version is published.

ENvironmental Success under Uncertainty and Risk (ENSURe): A procedure for probability evaluation in ex-ante LCA

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ABSTRACT

In a context of ecological emergency, ex-ante Life Cycle Assessment (LCA) can be used to prioritize investments into technological concepts that are considered likely to make human activities less damaging to ecosystems and people. Forecasts about the future environmental "success" of technological concepts once deployed on the market come with high incertitude and require careful appraisal of the distinct levels of knowledge associated with the technology's indeterminacies. This work presents a new algorithmic procedure named ENSURe (ENvironmental Success under Uncertainty and Risk) to apply ex-ante LCA when incertitude can be decomposed in risk, manageable with probability distributions, and uncertainty, for which the level of knowledge does not enable the definition of probability distributions. The procedure applies a scenario discovery algorithm to identify combinations of requirements on the most uncertain factors to ensure a minimum conditional probability of success which stems exclusively from risk. The analysis of these requirements allows stakeholders to state if the total probability of success of the technological concept is over a decision-threshold, thus allowing decision-making without forcing the definition of probabilities on uncertain factors. Our work thus aims at making ex-ante fit, theoretically and computationally, for decision-making under deep uncertainty. We demonstrate the use of ENSURe on a technological concept consisting in searching new microalgal compounds for health-management in fish farming.

KEYWORDS

Incertitude, deep uncertainty, prospective LCA, probability, microalgae, technology

TEXT

1. Introduction

In the wake of Collingridge's Social Control of Technology (Collingridge, 1980) and Beck's Risk Society (Beck, 1992) in the 1980-1990's, the field of Responsible Research and Innovation (RRI) (Owen et al., 2013) has problematized and advocated for a limitation of the undesired consequences of technological developments. Within this context, ex-ante LCA leaves aside the retrospective scope of conventional LCA and is being proposed as quantitative tool for such responsible innovation (Wender et al., 2014). Van der Giesen et al. (2020) describe the aim of ex-ante LCA as *"to guide R&D decisions to make a new technology environmentally competitive as compared to the incumbent technology mix"*. In this paradigm, ex-ante LCA does not forecast the future (Cucurachi et al., 2018) but explores scenarios to find the optimal technological choices to guide R&D accordingly through an iterative process. Using ex-ante LCA within such an iterative improvement paradigm constitutes the "responsive" dimension (Owen et al., 2013) of RRI in which technological development dynamically responds to societal needs and ex-ante LCA provides guidance. While ex-ante LCA here provides necessary guidance for existing innovation pathways (Genus and Stirling, 2018), it does not meet the need to make decisions about whether or not investing time and resources into initiating the exploration of broad technological concepts. Indeed, in an undisputed context of ecological emergency (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, 2019; Ipcc, 2022), a necessary policy-driven planning of technological development should reserve investments of limited time and resources to concepts that are likely to significantly improve the environmental performance of human activities.

While ex-ante LCA has been applied to specific, well-defined emerging technologies such as CO_2 reduction to formic acid production (Thonemann and Schulte, 2019), milk ultra-high pressure homogenization (Valsasina et al., 2017) or front-side metallization of photovoltaic cells (Vijver et al., 2020), we consider the use of ex-ante LCA in a context of urgent decisions about which technological concepts should be further explored. Initiating research regarding a technological concept can eventually lead to different technological outcomes because these concepts are generally formulated as broad and open questions such as *"What about using microalgae in fish farms?"* or *"Should we look for seaweed to improve cattle production?"*. The exact modalities, such as function, location, design and performance of the final technologies remain undefined. Ideally the decision makers of the innovation ecosystem (Carraresi and Bröring, 2022) in which such a technological concept emerges, from private entrepreneurs to politicians, should be informed early on about the probability of the concept to develop into a successful technology. Success here intended as improving environmental performance compared to a baseline situation. This exercise of projection of the uncertain consequences of a decision is already carried out by companies that forecast Returns on Investments (ROI) (Magni, 2015) considering incomplete information and perform Real option-analysis (Block, 2007; Lee, 2011) for strategic decision-making where the value of specific performance indicators is compared to defined thresholds. At the policy level also, the European states' budgets are for instance disciplined based on arbitrary threshold deficit values together with additional assumptions on future financial balance evolutions (Priewe, 2020). Ex-ante LCA could be used in this same logic across the innovation ecosystem and as soon as concepts emerge, replacing financial indicators by environmental and social ones. Decision makers would invest time and resources in the exploration of a technological concept based on a threshold that they would set as a minimum probability of success. Instead of trying to correct emerging innovation trajectories, ex-ante LCA here would assist in deciding whether a concept should even start being explored, acknowledging the chaotic nature of technological development (Brian, 1989; Hung and Tu, 2011), especially in the environmental domain (Pizzol and Andersen, 2022), and the difficulty to control it along a responsive process (Owen et al., 2013).

In this context, ex-ante LCA aims at projecting the limited knowledge about the possible outcome of a technological concept into the space of environmental impacts. The result of this projection, practically simulated via propagation (Mendoza Beltran et al., 2018) of the model's inputs distributions, should be presented under the form of a "probability of success" to decision makers. The term "probability" here needs to be understood as "reasonable expectation" as proposed by R.T Cox (Cox, 1946). This interpretation also overlaps with a Bayesian perspective as it reflects the degree of belief, ideally supported by knowledge regarding the realization of an indeterminate event (Blanco, 2022).

In a typical ex-ante LCA model, the result (output) is determined by the combination of several factors (inputs, also commonly referred to as parameters) that are indeterminate in the sense that they are *"not measured, counted or clearly known"*(Cambridge University Press, n.d.). In such ex-ante LCA model, some indeterminate factors come associated with reasonable levels of knowledge that allow to propose probability distributions regarding the future state of these factors. The propagation of these probability distributions therefore belong to the computation of "risk" in Wynne's and Stirling's classifications of incertitude (Stirling, 2010; Wynne, 1992). Yet, the level of knowledge about the probabilities for other indeterminate factors is so problematic (Stirling, 2010) that its projection into the impact space can deceive decision-making. Wynne (1992) and Stirling (2010) define the factors for which probability distributions can hardly be proposed, for instance because factors dependencies are supposed but cannot be modeled, as "subject to uncertainty" (instead of "risk"). The authors highlight the importance of clearly distinguishing "risk factors" from "uncertain factors" for sound decision support (Scoones, 2019; Scoones and Stirling, 2020; Stirling, 2010; Wynne, 1992).

As LCA is inherently a quantitative assessment, practitioners could be tempted to apply wide uniform distributions to uncertain factors (Bergerson et al., 2020), propagate these distributions together with the risk factors, and present the results under the form of "probabilities". While this is in principle a conservative approach to uncertainty quantification in traditional stochastic models, it still conveys a misleading overestimation of confidence and knowledge (Thonemann et al., 2020; van der Giesen et al., 2020). A conscientious way of dealing with uncertain factors is to include them within *what-if* scenarios (Pesonen et al., 2000) while acknowledging that no probabilities can be assigned to their realizations. Stochastic propagation for factors with non-problematic levels of knowledge can be performed within these scenarios to generate probabilities of success which are conditional to the realizations of the scenarios. In complex cases where uncertainty is deep and defining relevant and likely scenarios cannot be done a priori, scenario discovery algorithms such as PRIM (Patient Rule Induction Method) (Bryant and Lempert, 2010) can be used as a computational algorithm to detect scenarios of interest, which are sets of intervals for indeterminate factors associated with a high proportion of cases of interest for the output. The advantage of scenario discovery is that it does not require prior knowledge on the distributions associated with uncertain factors as it allows reflecting exclusively on the probability of occurrence of the scenarios of interest. Thus, the complex decision-making process can be summarized into simpler questions provided that the scenarios of interest are based on easily interpretable factors (Bryant and Lempert, 2010).

Summing up, the joint presence of risk and uncertainty when attempting to decide on broad technological concepts means that ex-ante LCA is performed under a condition of “deep uncertainty” (Kwakkel and Jaxa-Rozen, 2016; van der Giesen et al., 2020) which makes the calculation of probabilities of impacts rely on problematic levels of knowledge. However, even under deep uncertainty decisions still need to be made regarding the development of novel technological concepts. The question remains of how these decisions can be made without using probability distributions to represent uncertainty and still being able to compare a probability of technological success with a decision threshold.

In this work, we present ENSURe (ENvironmental Success under Uncertainty and Risk), an algorithmic procedure to unveil if the total probability of success of a conceptual technology, in relation to environmental performance, exceeds a stipulated threshold. By combining the forms of incertitude from Stirling and Wynne, the prospective modelling approach of ex-ante LCA, and the computational power of scenario discovery, ENSURe helps evaluating probabilities of success when both uncertainty and risk apply. We thus work towards more robust decision-making in the post-normal science age (Funtowicz and Ravetz, 1993), defined by high stakes and incertitude.

We demonstrate the ENSURe procedure with the case of the decision on whether time and resources should be spent on bioprospecting for new microalgal compounds (Jouannais and Pizzol, 2022) to enhance fish-health management. This case, which constitutes a deeply uncertain concept regarding the technological outcomes, is based on recent discoveries (Falaise et al., 2016; Krohn et al., 2022; Patel et al., 2021; Talero et al., 2015; Yaakob et al., 2014) showing interesting bioactivities of some microalgal compounds at laboratory scale and potential beneficial effects on fish health, resistance to diseases and growth performance (Lieke et al., 2020).

2. Methods

2.1) Definitions of uncertainty, risk and indeterminacy

The definition of "uncertainty" is subject of semantical debate. While Wynne (1992) defines different forms of "uncertainty" as risk, uncertainty, ignorance and indeterminacy, Stirling (2010) presents a typology of "incertitude" as risk, uncertainty, ambiguity and ignorance. The common categories cover the same concepts but Wynne's typology is ambiguous as it defines uncertainty as a type of uncertainty. In this work we pragmatically use "indeterminacy" as anything that prevents the modeler from using single deterministic values for factors in a model. We therefore interpret Stirling's typology of incertitude as "states of knowledges" regarding indeterminacy. Some factors in the LCA model are therefore "indeterminate" from an ex-ante perspective, which means that one type of incertitude applies on them. "Uncertain factors" are specifically subject to uncertainty intended in Stirling's terms: they are associated with problematic level of knowledge regarding their probability distributions. "Risk factors" are factors subjected to risk only.

2.2) Formalization of the decision-making problem

We name "a success" a technological concept eventually leading to a novel technology environmentally outperforming a baseline in specific impact categories. The baseline is case-specific and can be a fixed impact value or a comparison with an alternative projected in the future in absence of the concept. In the case under analysis the decision to incentivize or invest into this technological concept is taken only if it can be shown that the total probability of success is higher than a decision threshold. We name "total probability" the overall probability of success for the technological concept. By definition, this probability is inaccessible a priori as the decisions are taken under highly uncertain conditions but ENSURe will allow comparing it to the decision threshold of the decision-makers.

2.3) ENSURe procedure

The objective of ENSUre is to discover sets of value ranges for the uncertain factors that are associated with outcomes for which the probability of success is superior to the decision threshold. The space of technology configurations contained within such a set of value ranges is called a "box", following the original terminology by Friedman and Fisher (1999) who developed the algorithm to identify such boxes (cf. 2.3.3).

This procedure should allow to reflect only on the probability of the final technology having such a configuration instead of trying to define reasonable probability distributions for all uncertain factors a priori. Figure 1 illustrates the step-by-step algorithmic procedure of ENSURe which is further explained in the following paragraphs. A mathematical formulation of the procedure and underlying theory is available in SI A.1.

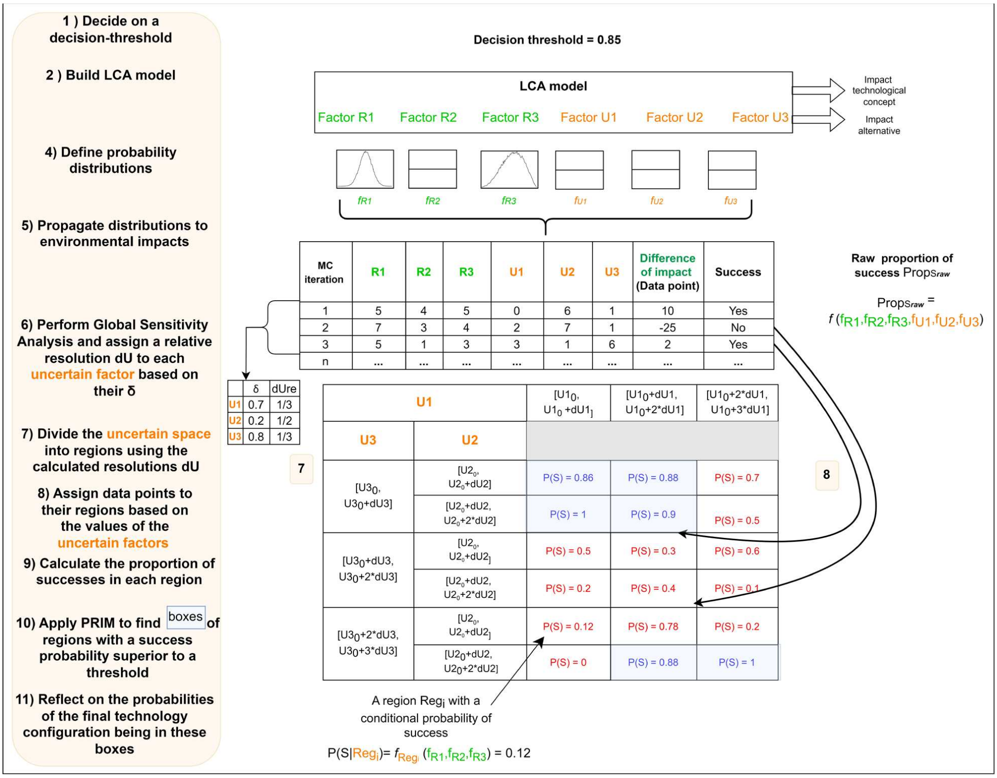


Figure 1: Step-by-step representation of the ENSURe procedure. P(S): probability of a success, here represented as equal to the proportion of successes. Ux_0 is the minimal value in the range for Ux. The presented mathematical notations are further developed in SI A.1.

2.3.1) Uncertainty and risk propagation

All indeterminate factors, whether they are subject to risk or uncertainty, are first propagated jointly via Monte Carlo simulation according to their distributions (cf. 4 and 5 in Figure 1). The uncertain factors are sampled only using uniform distributions defined within arbitrarily large boundaries - while abiding by physical and logical constraints (cf. example in 2.4.3). The life cycle impacts for the technological concept and for the alternative are calculated in pairs for each Monte Carlo iteration (cf. 5 in Figure 1). Each output of the model constitutes a data point, and success is considered when the developed technological concept has a lower impact than the alternative for the considered impact category.

2.3.2) Regionalization of the uncertain input space for conditional probabilities of success

The proportion of successes among the data points resulting from the joint propagation of all factors ($Prop_{S_{raw}}$,cf. 5 in Figure 1) cannot be interpreted as a total probability of success as this would boil down to treating uncertainty as risk. The only probabilities that should be assessed are those exclusively stemming from the propagation of risk. These probabilities are conditional to specific combinations of values for the uncertain factors.

We cannot assess all combinations of uncertain factors as this would lead to an infinite number of assessments. Therefore, the regionalization aims at assessing these conditional probabilities of success into small regions of the uncertain space, instead of for specific combinations of uncertain factors' values. To do so, each uncertain factor's range is divided into intervals of equal factor-specific length. For instance, a range from 0-100 m for a factor *Ua* is divided into 10 intervals of length 10m. This length is the resolution *dUa* for this uncertain factor. The resolution divided by the total range is called the relative resolution dUa_{re}, which is also the inverse of the number of intervals (e.g., factor *Ua* has a relative resolution of 1/10). This segmentation of all uncertain factor's ranges divides the entire uncertain space defined by the ranges of the uncertain factors into small y-dimensional regions *Reg* of dimensions {*dU1*, *dU2*, …, *dUy*} where y is the total number of uncertain factors (cf. 7 in Figure 1). Each region contains the data points for which the uncertain factors' values are between the limits of the region (cf. 8 in Figure 1). The smaller the regions are, the closer the proportions of successes within the region gets to conditional probabilities of success stemming exclusively from the propagation of risk factors. Indeed, the propagation of the chosen distribution for an uncertain factor *Ua* in such region is limited to the factor's variation over a small interval *dUa*.

To limit the number of regions to assess, we assign the number of intervals, i.e. 1/dUre, for each uncertain factor based on its sensitivity (cf. 6,7 in Figure 1). The latter is measured with the Borgonovo's delta index δ (Borgonovo and Iooss, 2016) regarding the difference of impact between the technological concept and the alternative. The analyst decides on a minimum and maximum relative resolution, i.e., a maximum and minimum number of intervals, to assign respectively to the most and least sensitive uncertain factors. The number of intervals for all the other uncertain factors is calculated between these two extremes as inversely proportional to their delta index δ via the equation presented in SI A.2. Thus, the more sensitive an uncertain factor is, the less it is permitted to vary in the regions in which the conditional probabilities of success are assessed. A value of 1 for the maximum relative resolution, implies that the probability distribution associated with the least sensitive factor is fully treated as risk (with a uniform distribution) and its influence on the probability assessment is considered neglectable. We can therefore approach true conditional probabilities of success, exclusively dependent on the propagation of risk factors, by decreasing the minimum and maximum relative resolutions.

2.3.3) Scenario discovery (PRIM) to reveal uncertain boxes of success

Once the regions *Reg* have been defined, the empty regions that happened not to contain any data point are discarded and the proportions of successes are assessed in the remaining ones (cf. 7,8 in Figure 1). Then, the PRIM (Patient Rule Induction Method) algorithm (Friedman and Fisher, 1999) is applied to discover boxes of regions, i.e., groups of regions, that are associated with a probability of success superior to the decision threshold (cf. 10 in Figure 1). The PRIM algorithm is designed to iteratively select "boxes" with a high predictive potential for an output of interest within a multidimensional input set. By applying a "hill climbing optimization procedure" (Friedman and Fisher, 1999), PRIM iteratively "peels-off" sub-boxes, i.e. discards sub-boxes of the input variables while maximizing an objective function to increase the predictive potential of the resulting box regarding the output of interest. The exact objective function and the settings ruling PRIM are chosen by the analyst depending on its goal (cf. 2.3.4). In this study we used the PRIM python implementation proposed by Kwakkel and Jaxa-Rozen (2016).

The total probability of success is evaluated by the analysis of the boxes identified by PRIM. While reasonable probability distributions cannot be proposed for the uncertain factors a priori, the focus can be placed on the identified boxes only, which correspond to spaces defined by some uncertain factors only, thus drastically reducing the complexity of the problem. If the decision-makers are now sure (Probability =1) that the final technology will feature a configuration within the identified boxes, this means that the total probability of success is superior or equal to their decision threshold. This is a direct conclusion from the law of total probability with conditional probabilities detailed in SI A.1. Otherwise, it cannot be strictly concluded that the probability of success is either superior or inferior to the threshold (cf. SI A.1), uncertainty remains and the ENSURe procedure can be repeated with different settings or decision threshold (cf. 2.3.4 and 4.2). However, if the procedure made the decision-making problem simpler so that it can now be stated that there is a probability P(β) of the technology eventually featuring a configuration in the boxes, the total probability of success is superior to P(β) multiplied by the decision threshold (cf. SI A.1).

2.3.4) Choices and trade-offs within the ENSURe procedure

The three main phases of ENSURe, namely the Monte Carlo sampling and model simulations, the regionalization of the uncertain space into regions of conditional probabilities, and the use of PRIM, can be configurated to optimize the procedure according to the objectives. The number of data points and regions quickly get constrained by available computational resources. Dividing the uncertain factors' ranges into more interval by decreasing the minimum and maximum relative resolutions allows a more precise distinction of risk and uncertainty (cf. SI A.1) but also creates a larger number of regions with few or no data points (cf. 4.1).

The objective function for the PRIM algorithm was chosen to be the "lenient" one proposed by Kwakkel and Jaxa-Rozen (2016), which is fit for different types of variables and considers the gain of density together with the loss of observations at each peeling step. Bryant and Lempert (2010) highlight how PRIM can be used as a "scenario discovery" tool to assist policy-making under deep uncertainty by selecting boxes with the desired trade-off between *density, mass, coverage, and interpretability*. The *density* is the proportion of observations (in our case the proportion of regions) of interest in the box, *mass* is the proportion of total observation contained in the box, and *coverage* is the proportion of total observations of interest. *Interpretability* refers to the fact that having too many factors constraining the boxes makes it difficult to interpret the results for stakeholders and policy makers (Bryant and Lempert, 2010). PRIM can be parameterized with the minimal density, mass and coverage that a box can feature.

2.4) Case study description

The technological concept under study is the bioprospecting of new microalgal compounds to enhance fish health in European fish farms. This technological concept is not a unique emerging technology, as initiating bioprospecting, i.e., searching compounds of interest within biodiversity, can eventually lead to substantially different technological configurations depending on what is found and how the market integrates it. The diversity of microalgal compounds together with the diversity of fish health issues leave the technological outcome of this technological concept uncertain. Initiating research on this concept could for example generate a German production of powerful microalgal antibiotics tackling a particular fish pathogen at very low doses, or the production in Southern Europe of nutraceuticals which are closer to feed supplements than medicines. The microalgal compound production part of the LCA model and the associated indeterminacies have been modeled and studied in previous works (Jouannais et al., 2022; Jouannais and Pizzol, 2022). The LCA model used in the present study is a combination of this model with a new parameterized model of a Danish trout farm (Jouannais et. al., 2023). We refer the reader to this work (Jouannais et al., 2022; Jouannais and Pizzol, 2022; Jouannais et al., 2023) for an exhaustive description. The alternative technology is the production of trout without the microalgal compound.

2.4.1) Parameterized product system

The functional unit of the consequential LCA is 1 kg live weight of sea-reared trout before slaughtering. The trout farm corresponds to a combination of primary data from Denmark and Italy reconstituting a detailed life cycle inventory with divisions between the different growth stages from inland hatchery to sea-reared trout of 2.4 kg live weight (Jouannais et al., 2023). The microalgal compound production, which can be an input to all trout growth stages, takes place in an indeterminate production mix stochastically sampled across 10 European countries previously identified for their high potential for microalgal biomass production (Jouannais and Pizzol, 2022; Skarka, 2012). The size of a mix (number of plants) and the producing locations are therefore indeterminate. Each location of a mix produces the microalgal strain and compound in a vertical tubular reactor whose techno-operational setup is indeterminate. The life cycle inventory for the microalgal compound is obtained via a parameterized dynamic and location-specific simulation of the microalgae cultivation.

The simplified product system is shown in Figure 2 together with three groups of indeterminate factors affecting different parts of the system. A first group covers the indeterminacy of the production and nature of the production of an indeterminate microalgal strain and compound in Europe. The second group reflects the indeterminacy of the trout farm's potential for improvement, which covers the level of losses (mortality) and the suboptimal feed conversion ratio experienced by the farm before using the compound, together with the unknown impact associated with the production of the chemotherapeutants used in the farms. This group also reflects the microalgal compound's improvement performance, i.e., the beneficial effect of the compound on the fish farm. The third group describes the indeterminacy in the background system.

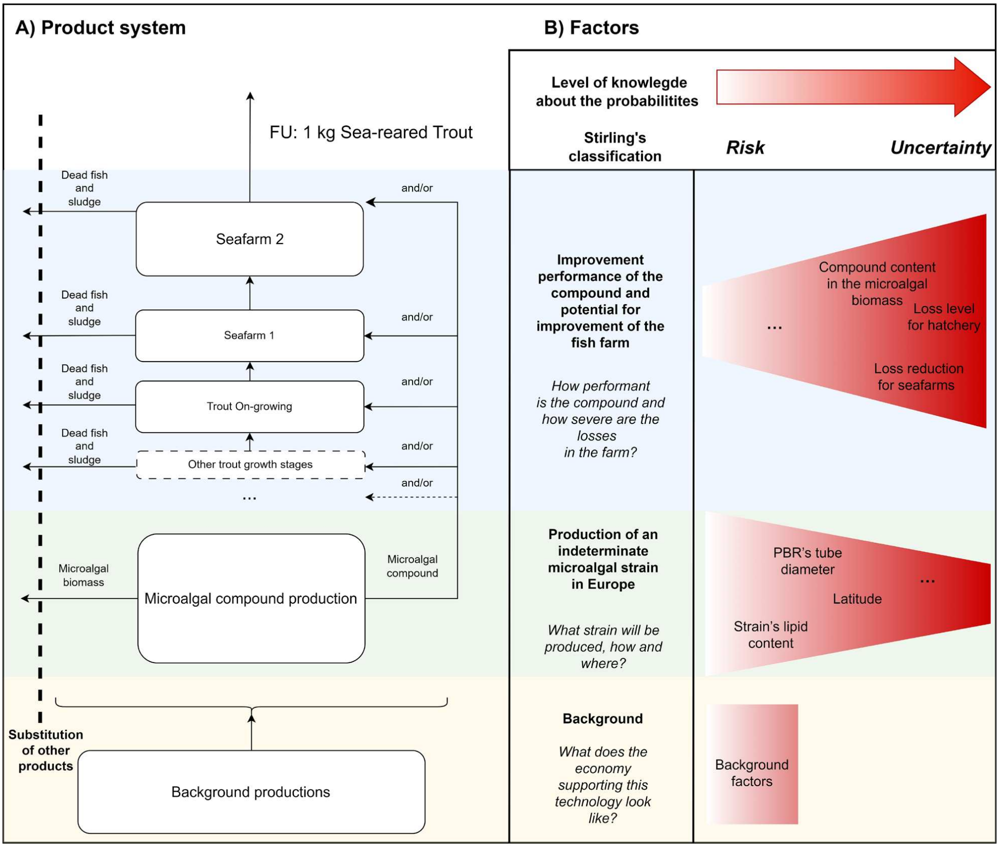


Figure 2: A) Simplified product system for case study. B) Groups of factors affecting the system and associated level of knowledge. *The groups of factors are located on the same level (same colored band) as the processes they affect in the product system. The trapeze's width illustrates the proportion of factors corresponding to the level of knowledge. No actual proportion is measured and only the trend is illustrated.*

2.4.2) Effects of factors on the compound's improvement performance and fish farm's potential for improvement

In the model, the use of microalgal compound can have a double effect to improve the biological performance in each fish growth stage. It can reduce the losses and can decrease the biological feed conversion ratio (FCR) based on two distinct factors. These effects on a growth stage are achieved with an indeterminate input of microalgal compound and reflect a vast range of bioactivities for the compound.

Furthermore, each growth stage division is parameterized so that it can be modelled as undergoing higher levels of losses than the current ones. This allows projecting the fish farms (with or without the compound) under different future regimes of health issues that the compound will tackle, thus modulating the farms' potential for improvement.

We refer the reader to Jouannais et al. (2023) for an exhaustive description of the fish farm model.

2.4.3) Risk and uncertain factors

While the distinction between risk and uncertainty can be obvious for some factors, the decision to categorize a factor in one category or the other is eventually subjective. This is illustrated by the purposely fuzzy delimitation chosen by Stirling who differentiates "problematic" and "unproblematic" levels of knowledge. As show in Figure 2, there happens to be an overlap between the three groups of factors in the product system and the associated levels of knowledge about probabilities. We used this case-specific overlap to draw a clearer distinction between risk and uncertainty factors.

Probability distributions can reasonably be proposed for the factors defining the production of an indeterminate microalgal strain in Europe which therefore classify as "risk factors". Indeed, data on microalgal biodiversity (lipid content, thermal requirements etc.) and techno-operational design of photobioreactors (tube diameter, flow rate etc.) can ground the definition of probability distributions as documented in previous works (Jouannais et al., 2022; Jouannais and Pizzol, 2022).

The background factors also classify as risk factors and the probability associated with these factors directly stem from the “uncertainties” (though not in Stirling’s terms) defined within ecoinvent. These probability functions are generated using a qualitative perdigree matrix in which the level of knowledge about the amounts reported is graded on a scale from 1 to 5 regarding the reliability, the completeness, the temporal, geographical and technological correlation and the sample size (Ciroth et al., 2016; Muller et al., 2016). The use of the pedigree matrix is a canonical example of treating incertitude as risk because a qualitative description of the level of knowledge is mathematically converted into a probability distribution.

The factors defining the improvement performance of the compound and the potential for improvement for the farm are considered uncertain. The knowledge associated with these paramters is scarce compared to other factors and no grounded guesses can be made about their distributions. The level of knowledge for these “uncertain factors” is so problematic that the propagation of arbitrarily large uniform distributions for the factors’ values allows a mere exploration of the input factor space. For instance, the uniform distributions chosen for the factors defining the loss reduction in the different growth stages range from 0 to 100 % and are therefore only constrained by logic, as losses cannot be reduced by more than 100%.

All the model’s factors are presented and classified in SI B.

2.4.4) Aggregated factors for compound’s improvement performance and farm’s potential for improvement

The LCA models used in this study are high-dimensional and allow to simulate many different configurations for the microalgae and fish farms. However, such a level of detail adds difficulty to the decision-making exercise. Indeed, it may require decision makers to form beliefs about many low-level factors that they may not be familiar with. For instance, assigning probabilities to certain regions for the FCR reduction in a specific fish growth stage (e.g., from 80g to 1 kg) is more difficult than reflecting on the overall FCR reduction for the whole production. Overall FCR values can indeed be compared to national statistics because they constitute widely-used performance indicators at the farm level.

Furthermore, applying regionalization (cf. 2.3.2) on many uncertain factors may lead to too many regions with very few data points depending on the computing resources and the total number of simulations that can be generated.

For these two reasons, namely interpretability and computability, we propose five aggregated factors that can be used to regionalize the uncertain space. These aggregated factors are calculated within the model and depend on the values of different uncertain factors to reduce the dimensionality of the problem and ease the decision-making exercise (cf. 3.3).

Four aggregated factors are used to summarize the improvement performance of the microalgal compound. First, we quantify the total dose of microalgal compound per functional unit ($g.kg^{-1}$). Second, we calculate the overall economic FCR as the ratio of feed input over the live, ready-to-sell fish output and calculate the overall economic FCR reduction induced using the compound, in % of economic FCR in the alternative production. We also calculate the overall biological FCR (feed/(dead + live fish)) reduction, in % of biological FCR and the compound content in the microalgal biomass ($g.\ g_{dried\ biomass}^{-1}$) . The potential for improvement in the farm is summarized in one aggregated factor namely the increase in loss level, i.e., the ratio of the loss level in the projected alternative over the current loss level.

These aggregated factors depend only on uncertain factors and therefore reflect exclusively uncertainty and not risk. Their mathematical definitions are available in SI A.3.

2.5) Specific ENSURe settings for the case study

For this case study, 500000 technology configurations were stochastically generated via random sampling, where in each configuration each model factor is assigned a different value. Each simulation output, i.e. each data point, is associated with its specific technology configuration.

The conceptual technology is defined as successful if the impact of trout production using the microalgal compound is lower than the impact without using it. The decision is based on four impact categories from ReCipe Midpoint (H): Freshwater ecotoxicity (FETinf), Global warming (GW), Terrestrial Ecotoxicity (TETinf), Freshwater Eutrophication (FE). In addition, the eutrophication impact category from TRACI was used to encompass nitrogen, and phosphorus emissions both in seawater and freshwater. No normalization or weighting was considered.

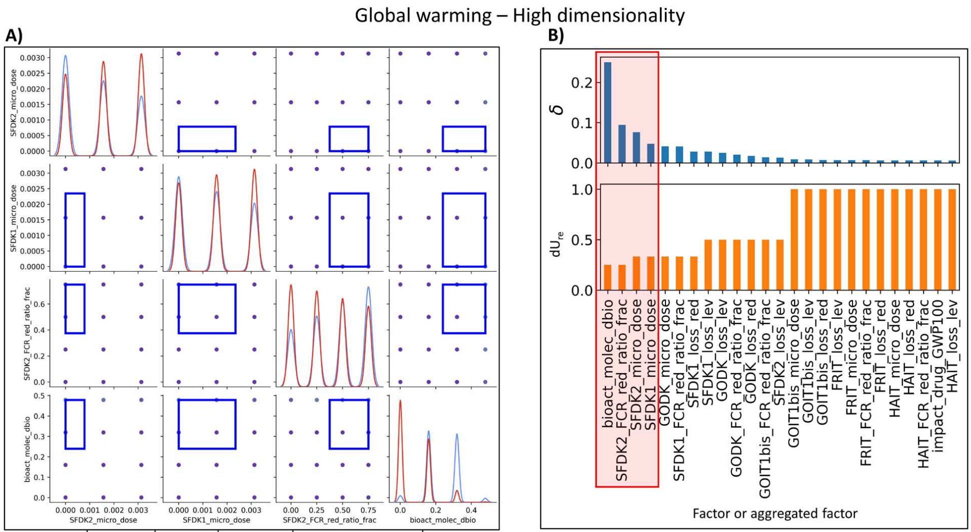
The regionalization step was first performed at what we defined as a "high dimensionality level", using 24 uncertain factors and one aggregated factor which constitute 25 dimensions. Using so many factors results in the definitions of a large number of regions, and the number of data points per region was expected to be too low in each region. Difficult interpretation of the boxes was also anticipated (cf. 2.4.4). Therefore, we also applied the procedure with a regionalization step at a "low dimensionality level" using only one factor and four aggregated factors. The influence of the 24 factors on the output of the model is still captured in the second case, because the aggregated factors are calculated based on these factors.

The decision threshold was set at 0.85. The minimal density, coverage and mass for a box to be discovered by PRIM were respectively fixed at 0.9, 0.01 and 0.01. The minimum and maximum relative resolutions were respectively set at ¼ and 1 to divide the uncertain space at the high dimensionality level, and 1/8 and 1 for the space at low dimensionality level .

3. Results

3.1) Discovering boxes at the high dimensionality level

When applying the regionalization at the high dimensionality level, the PRIM algorithm could identify at least one success box for each impact category.



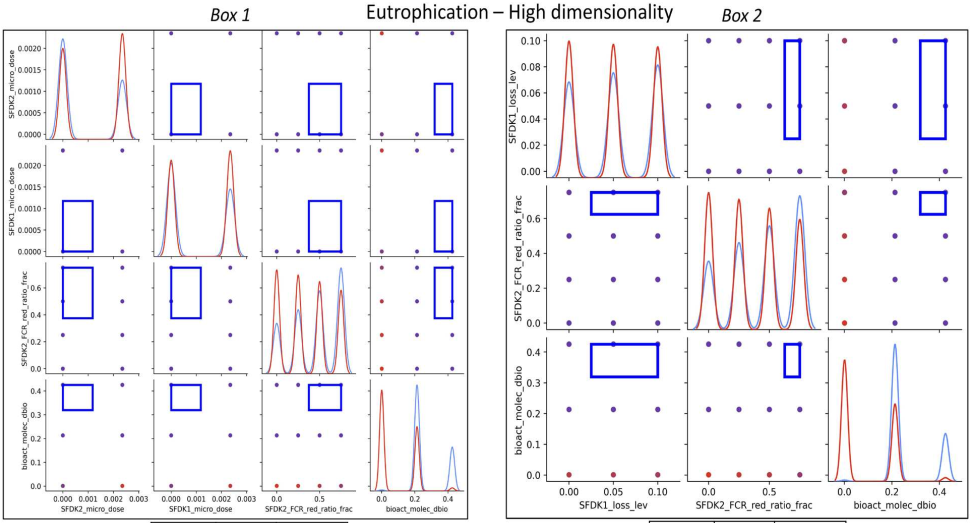
Density	Coverage	Mass	Number of regions (non-empty)
0.934	0.066	0.021	248832 (148230)

● Probability of success ≤ 0.85
● Probability of success ≥ 0.85

Figure 3: A) Box discovered by PRIM at the end of ENSURe, at the high dimensionality level, for the Global Warming impact*. The original graphic representation for PRIM proposed and programmed by Kwakkel and Jaxa-Roxen*(Kwakkel and Jaxa-Rozen, 2016) *was adapted to the present use. The dots were made transparent and then appear purple to see the stacking of the regions due to the one-dimensional projection. The blue rectangle corresponds to the limits of the box. A dot represents the*

lower boundary of a region which expands until the next dot (cf. 2.3.2). An assistance for box visual interpretation is presented in SI A.4 . ***B)*** **B*orgonovo's δ for each factor/aggregated factor describing the uncertain space and the corresponding assigned relative resolution*** dU_{re}*. The red rectangle contains the four factors/aggregated factors that were constrained by PRIM as visible in A).*

This means that we could identify one space of uncertain factors' values that would be robustly associated with a total probability of success superior to 85%. For the global warming impact (GW), only one box was found (cf. Figure 3 A). This success box is defined by three factors and one aggregated factor, out of the 25 uncertain factors used for the regionalization, and out of the 13 that were assigned a relative resolution lower than 1 (cf. Figure 3 B). The box's limits are a dose of microalgal compound lower than 3.1 $g.kg^{-1}$ output live fish in the first part of the last growing stage at sea (*SFDK1_micro_dose*), and lower than 1.5 $g.kg^{-1}$ in the second part of the sea stage (*SFDK2_micro_dose*). In addition to these limits, the biological FCR of this stage must be affected so that it becomes lower than the middle value between its current and minimum theoretical values (*SFDK2_FCR_red_ratio_frac>0.5*). Finally, the compound content in the microalgal biomass must be higher than 35 % (*bioact_molec_dbio>0.35*). As the discovered box displays only four limits, no other requirement applies on the other uncertain factors that can vary freely within their wide ranges while ensuring a probability of success superior to 85%. This means for instance that no predicting is required on the future increase in losses experienced by the farm, or on the compound dose delivered to any growth stage but the last one.



Number of regions (non-empty)
82944 (59791)

Box 1:

Density	Coverage	Mass
0.964	0.046	0.011

Box 2:

Density	Coverage	Mass
0.907	0.042	0.011

Figure 4: Boxes discovered by PRIM at the end of ENSURe, at the high dimensionality level, for the Eutrophication impact. *The Borgonovo's δ for each factor/aggregated factor and the assigned dU_{re} are presented in SI A.5. An assistance for box visual interpretation is presented in SI A.4 .*

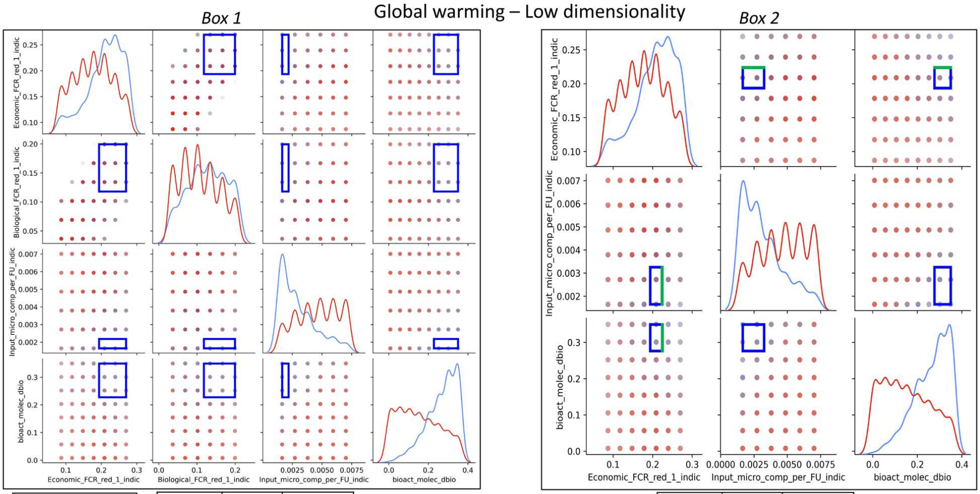
Figure 4 shows the two boxes that were found for Eutrophication, corresponding to two different ways of ensuring a 85% chance that the technology will outperform the alternative. The first box is obtained by limiting only four factors: the microalgal compound doses in the two divisions of the sea stage are both limited to 2.2 g . kg live fish $^{-1}$ (*SFDK1_micro_dose* and *SFDK2_micro_dose <0.022*), the biological FCR in the last part of the sea stage must be modified to at least 50% closer to its minimum value (*SFDK2_FCR_red_ratio_frac* > 0.50) and the compound content in the microalgal biomass must be at least 42 % (*bioact_molec_dbio* > 0.42). The second box informs us that it is also possible to reach a 85% probability of success by tightening the constraint on the biological FCR reduction in the last growth stage (*SFDK2_FCR_red_ratio_frac* > 0.75 instead of 0.5), removing the limits on the compound doses, and limiting the new level of losses in the first part of the sea stage to being at least five percentage points higher than it currently is (*SFDK1_loss_lev* > 0.05).

The boxes obtained for the other impact categories are presented in SI A.5.

While boxes can be identified at the high dimensionality level, their interpretation is difficult and their use for decision-making requires careful consideration. With 25 factors/aggregated factors and 500000 data points, the minimum relative resolution could only be set to 1/4 and the average number of points per region fell between 28 and 3 depending on the impact category. These numbers are too low to assess meaningful probabilities in each region (cf. 4.1).

3.2) Discovering boxes at the low dimensionality level

The low dimensionality level is only described by five aggregated factors and one factor, which allowed us to set the minimum the relative resolution to 1/8 with an average number of points per region varying from 100 (GW) to 290 (TETinf).



Number of regions (non-empty)
6048 (3901)

Box 1:

Density	Coverage	Mass
1	0.1	0.020

Box 2:

Density	Coverage	Mass
1	0.054	0.010

● Probability of success ≤ 0.85
● Probability of success ≥ 0.85

Figure 5: Boxes discovered by PRIM at the end of the ENSURe, at the low dimensionality level , for the Global Warming impact. *The Borgonovo's δ for each factor and aggregated factor and the assigned* dU_{re} *are presented in Figure 6. A green border on a box means that the PRIM outputs indicated a constraint which was shown substantially less significant than the others ,the actual box can therefore be interpreted as not constrained on this border (cf. SI A.7 for additional PRIM outputs). The "peeling trajectory" illustrating PRIM functioning is presented in SI A.6.*

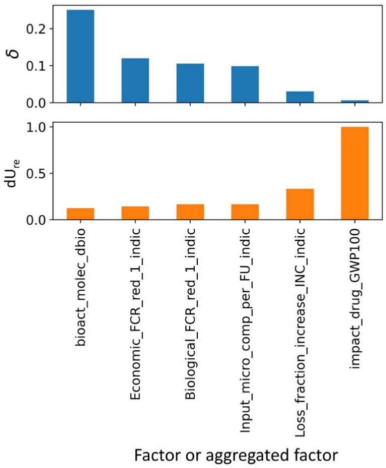


Figure 6: Borgonovo's δ for Global warming impact at the low dimensionality level and corresponding relative dUre

For GW, four boxes were found, and therefore four configurations under which the total probability of success would be superior to 85%. Two of the four boxes are shown in Figure 5 and the last two can be found in SI A.5. The first box covers 10% of the success regions with a density of 1 and is limited by a reduction of the economic FCR higher than 22% combined with a reduction of the biological FCR higher than 15%. This would need to be obtained with a dose of microalgal compound inferior to 2.5 g.kg live fish^{-1} during the whole life cycle and this compound should constitute at least 20% of the dried microalgal biomass. The second box features a limit on the compound dose which is less demanding than in the first one (< 3.5 g.kg $^{-1}$). Unlike the first box, it does not have any limit on the biological FCR but requires a higher compound content in the biomass (30%).

While ENSURe applied at the high dimensionality level on FE only allowed to find consistent boxes with a decision threshold at 65%, applying the procedure at the low dimensionality level generated 5 boxes at 85% (cf. SI A.5).

3.3) Example of conclusion for the technological concept

At the low dimensionality level and for the five impact categories, PRIM did not identify any box where the value of the factor "compound content in the microalgal biomass" is lower than 18%. This means that it is impossible to consider a probability of success superior or equal to 85% for the conceptual technology without predicting that the compound will at least reach this concentration in the biomass. The fact that this aggregated factor was limited for all boxes is due to its high sensitivity, as measured by Borgonovo's delta. The compound content in the microalgal biomass was the most sensitive aggregated factor for FETinf and GW. When considering these impact categories as outputs, the range for the compound content was divided into 8 intervals (relative resolution of 1/8 , cf. Figure 5,6), while the GSA processing only assigned it a relative resolution of ¼ when considering Eutrophication. This impact category was more sensitive to the economic and biological FCR reductions (cf. SI A.5).

Achieving a compound content of at least 18% can already be considered as a very demanding requirement but does not fully disqualify the conceptual technology from being explored, having set a total probability of success superior to 85% as threshold. Indeed, a compound content over 18% indicates that the compound should likely be a primary metabolite which is accumulated by the microalgae and associated with normal growth and development. While secondary metabolites are often bioactive because involved in meditating the interactions with other organisms, microalgal primary metabolites such as Beta 1-3 glucans and Poly-unsaturated fatty acids (PUFA) have also shown bioactivities and can accumulate up to more than 18 % of the biomass(Barsanti et al., 2001; Guedes et al., 2011)

In addition to stringent requirements about the compound content, the majority of boxes are defined by an ambitious reduction of the economic FCR by at least 20% (e.g., boxes 1,2, for GW in Figure 5 and 3,4 for GW, 2,3,4,5 for Eutrophication in SI A.5), regardless of the increase in the level of losses (e.g., box 1, 2 for GW in Figure 5) or by setting limits on them (e.g., box 2 for FE in SI A.5). The combination of these demanding requirements to ensure a total probability of success superior to 85% appears unlikely. It is impossible to state that there is a 100% probability for the conceptual technology to eventually meet them simultaneously by featuring a technological configuration in these boxes. Therefore, it cannot be concluded that the total probability of success for the conceptual technology is superior to the decision threshold of 85%. Note that it cannot be mathematically concluded that the total probability of success is strictly below 85%. Uncertainty therefore remains (cf. SI A.1). We further discuss this conclusion in 4.2.

4) Discussion

4.1) ENSURe's robustness and trade-offs

Applying the regionalization step on many factors at the high dimensionality level caused problems for the computability and therefore interpretability of the results. The model of the technological concept here used relies among other things on a dynamic simulation of microalgal cultivation in different European production mixes, which is computationally intensive. Simulating millions of data points requires large computing resources and time and we could only reasonably compute 500000 data points, corresponding to 325 000 000 LCAs and simulations of microalgal productions. The case study is thus particularly computationally demanding, but these computational limits can be generalized. Since ENSURe is limited by computing resources, trade-offs arise in terms of its performance. We propose three main criteria to evaluate the performance of the procedure The first criterion is *reliability* defined as the capacity of ENSURe to find boxes which do not contain false positive regions. In other words, reliability is high when the identified boxes only contain regions for which the conditional probability of success would asymptotically tend to being superior to the threshold with more data points. The second criterion is *purity* and represents the degree of distinction between risk and uncertainty achieved by the regionalization. Purity is minimal when the uncertain space is not regionalized, which is equivalent to setting all relative resolutions to 1, and increases with lower relative resolutions, thus approaching "pure" conditional probabilities in each region (cf. 2.3.2 and mathematical formulation in SI A.1) The third criterion *comprehensiveness* is maximized when all boxes, even the smallest ones, have been found. The trade-offs between criteria depend on the case study and its computing requirements and can be managed by modulating ENSURe's settings as illustrated below.

The number of regions within the uncertain space grows exponentially with the number of uncertain factors and intervals. Increasing the number of intervals decreases the average number of data points on which a probability of success is estimated in each region. At the high dimensionality level, dividing each of the 25 uncertain factor and aggregated factors' ranges into only four intervals would lead to 4^{25} regions with an average number of points per region being infinitesimal. Thanks to the use of Borgonovo's δ to assign distinct resolutions to factors, the average number of points per region was maintained at 28 for TETinf but due to different Borgonovo's δ, this number fell to three points per region for FETinf. This hinders *reliability* as it causes false negative and false positive regions assessed as "Success" based on very few data points, while more simulations could show a probability of success lower than 0.85 in these same regions. While increasing the minimum relative resolution from 1/4 to 1/3, thus dividing the range of the most sensitive parameter into 3 intervals instead of 4, would drastically increase the number of points per region, it would also allow a larger variation of the uncertain factors within these regions and reduce the *purity* of the procedure. If *reliability* is low, a lower minimal density can be set so that the algorithm can find boxes with a density inferior to 1, such as the boxes found for Eutrophication (cf. Figure 4). These settings can allow finding boxes that are meaningful despite the noise due to false positives and negatives.

The PRIM algorithm was here parameterized to find all boxes with a mass superior to 0.01, meaning that the minimum number of regions in a box is 0.01 multiplied by the number of not empty regions. As pointed out by Friedman and Fisher (Friedman and Fisher, 1999) who set the basis for PRIM, the lower the mass of a box is, the higher is the risk of a box delimitation being affected by noise ("over-fitting" problem). When PRIM is applied within ENSURe, noise is due to false positives and negatives among regions in the space. Thus, fixing the minimal mass value as small as possible would potentially lead to PRIM identifying all false positives as small boxes and hinder *reliability*. On the other hand, looking exclusively for success boxes above a large mass affects the *comprehensiveness* of ENSURe by potentially overlooking boxes. For example, when applying regionalization at the high dimensionality level, setting a minimum mass at 0.01 made PRIM leave 90 % of the success regions outside of the discovered boxes for all impact categories. This is probably because the success regions are sparsely distributed in the uncertain space due to the false negatives and positives. Therefore, PRIM cannot identify a unified and consistent box with a sufficient mass containing these regions.

In fact, for FE and at the high dimensionality level, no box at all could be found with a decision threshold at 0.85 but only at 0.65. The straightforward conclusion could be that the total probability of success for this impact category is not higher than 0.65, but the limitations addressed above prompt to remain cautious with this interpretation.

Figure 7 summarizes the trade-offs between *purity*, *reliability*, and *comprehensiveness* that are influenced by ENSURe's settings and the case study.

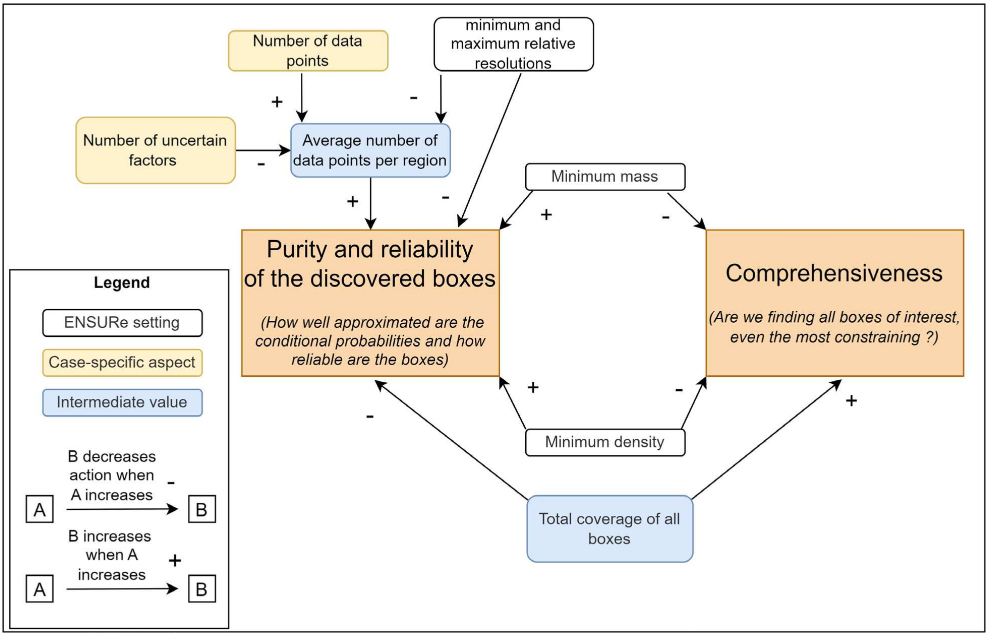


***Figure 7**: Effects of the case study and ENSURe's settings on the trade-offs between purity, reliability and comprehensiveness.* *An intermediate value stems from the combination of the case-specific aspects and the choice of the procedure settings. dU_{remin} and dU_{remax} respectively are the minimum and maximum relative resolutions.*

In addition to finding the right trade-offs regarding ENSURe's settings, insightful results can also be obtained by carefully reducing the ranges considered for the uncertain factors. This reduction can be done by first allowing PRIM to find large boxes and then using the boxes limits to define new ranges for the concerned factors in a new iteration of the procedure. This iterative approach allows discovering boxes with a lower resolution.

Decreasing the dimensionality of the problem by combining factors into aggregated factors is the solution we used to manage trade-offs in performance and provide results that have sufficient *reliability, comprehensiveness, and purity* for decision-making. It must be noted that reducing the dimensionality by using aggregated factors implies losing information at the most detailed level of the modeling. The same aggregated factor value can be obtained from different combinations of factors values. This means that the probabilities assessed with ENSURe are given considering that for one aggregated factor value, all potential combinations are equiprobable. Thus, the incertitude associated to these multiple combinations for one aggregated factor value is therefore treated as risk while the factors on which the aggregated factors are based were first identified as "uncertain". This particularity constitutes a limit to consider when using aggregated factors.

4.2) Refining the evaluation

For this case study in which the procedure could not prove that the total probability of success was over the threshold, decision-makers have multiple options. Firstly, they can settle for this result and dismiss the technological concept because ENSURe did not allow to state that the total probability of success was over the decision threshold. The modelers can also change the settings of the procedure to refine the evaluation. A first option is to decrease the decision threshold in the procedure until they can find a box associated with a probability of 1. This will inform about the minimum total probability of success and allow decision-makers to assess whether this probability is too far from the initial decision-threshold. Another option is to lower the minimum density chosen for PRIM to see if boxes with a slightly lower density can be associated with a probability of 1. This would mean that the total probability of success is not far from the decision-threshold.

The last option is to lower the minimum *mass* of discoverable boxes in PRIM which will allow the algorithm to find additional boxes on which decision-makers can reflect. However, these boxes will necessarily be associated to smaller shares of the uncertain space which implies more demanding limits on the ranges of the uncertain factors. These scenarios will probably be too specific to be likely. In addition, one must remain aware of the caveats that stem from low-mass boxes regarding *reliability* of the results (cf. 4.1).

Finally, the decision could be better informed if it was shown with certitude that the total probability of success is below the decision threshold. This can be done using the simple relationship *P(success)= 1- P(failure).* Thus, ENSURe can be applied to find boxes associated with a decision-threshold regarding the probability of a failure (e.g., 15%), which is equal to one minus the decision threshold for a success (1-15% = 85 %). This will allow showing whether the probability of failure is superior to a threshold (15%), and therefore proving whether the probability of success is inferior to one minus this threshold (85%).

4.3) Setting the context to talk about probabilities

While differentiating risk and uncertainty allows to better approach the notion of probability regarding the outcome of technological development, additional considerations are needed to approximate "*real-world probabilities*"(Thompson and Smith, 2019). As Wynne (1992) states, *"Science can define a risk, or uncertainties, only by artificially 'freezing' a surrounding context which may or may not be this way in real-life situations. The resultant knowledge is therefore conditional knowledge."* In this context a sound decision-making for policy-planning requires explicit hypotheses on the main drivers of technological development. Hence, the probabilities that we assessed via ENSURe can only be considered as such if we assume, among other aspects detailed in previous works (Jouannais et al., 2022; Jouannais and Pizzol, 2022), that microalgal producers will always seek to optimize the areal productivity of their plants and that bioengineers can find any strain-specific techno-operational setup to cultivate a microalgal strain at 30% of its maximum productivity (Jouannais et al., 2022). Once this context is established, the evolution of the conceptual technological can more accurately be pictured as a random process whose first step will be the discovery of a specific microalgal strain and compound.

4.4) Interpretation in a perspective of technological planning and guidance

The distinction between risk, which permits the definition of probability distributions, and uncertainty which does not, can also be made using a perspective of technological governance and planning. Let's examine our choice of considering the unknown locations of the compound's producing mix as belonging to risk, with markets developing equiprobably around random regions in Europe (cf. 2.4.1 and 2.4.3). It could be argued that nothing separates the nature of the incertitude applying to the unknown set of factors (size of the mix, latitudes and longitudes) describing the production mix (category 1) from the one applying to factors associated with the compound's effect on the farm (category 2). They could both be considered as equally uncertain, and the production mix incertitude could be included in the uncertain space within the procedure. Treating this incertitude as risk as we did means that the discovered boxes are associated with a total probability of success superior to 85 % if the geographical development can occur across Europe equiprobably. In other words, ENSURe here discovers boxes of uncertain factors that would allow making a safe-enough decision about the conceptual technology in a context where the geographical development is not constrained by regulations. This position constitutes a cautious stand, within which the evolution of the market associated with the technology is considered chaotic and uncontrollable (unresponsive) (Genus and Stirling, 2018) under the current economic system. We thus account for potential environmentally suboptimal configurations such as very valuable microalgal compounds being produced in countries requiring high fossil energy inputs per kg of compound because of low solar irradiance, low temperature and/or carbon intensive electricity mixes etc.

On the contrary, treating the geographical development of the production mix as uncertainty within ENSURe could allow finding boxes which are constrained on the production mix factors. This would for instance enable discovering minimal and maximal latitudes under which the technology should be confined to ensure a targeted probability of success.

In general, while we here presented a use of ENSURe to assist decision-making about initiating research on a technological concept in presence of uncertainty, with the previous example we argue that the procedure can also be employed for the guidance and planning of technological development. In this case, the factors being treated as risk constitute what decision-makers and regulators cannot, or do not intend to regulate. The factors treated as uncertainty represent political and technological freedom of maneuver for decision-makers and societal regulation of technological concepts.

4.5) Deciding whether to prioritize an unexplored conceptual technology

We have demonstrated the use of ENSURe on a technological concept on which a substantial incertitude applies. In this context, deciding to invest resources in the technological concept only if the total probability is shown higher than 85% constitutes a very conservative and cautious stand. Daily individual or political actions are taken with a lower level of subjective certitude and

ENSURe can be applied with lower decision thresholds. Furthermore, sound decision-making and technological planning could require more insightful decision criteria that do not consider only the probability of success but analyze the “risks” (in risk assessment terminology, i.e., “hazards”) of further developing an initial technological concept. Thus, ENSURe could be parameterized to not only consider the probability but also the magnitude of this success and the severity of a failure, i.e., how better and worse the final technology would be compared to the alternatives. This is particularly relevant when considering technological concepts associated with lock-in possibilities (Brian, 1989; Carraresi and Bröring, 2022; Hung and Tu, 2011) and possible suboptimal configurations regarding environmental impacts. A probabilistic view on innovation calling for cautious consideration of risks before exploring technological concepts is also provided in Nick Bostrom’s Vulnerable World Hypothesis (Bostrom, 2019), which depicts technological development as a random draw in an urn containing two types of balls. One type represents technological concepts that inevitably lead to the self-annihilation of humanity. The author exemplifies these concepts with theoretical “easy-nukes” but also global warming worsening directions. While microalgae-based solutions for fish farming could unlikely constitute a paramount threat to Humankind, it could be argued that all investment of time and resources in concepts with low chance of improving the status quo an constitute an irrational bet in times of ecological and social emergency. The use of ex-ante LCA within ENSURe enables an insight into Bostrom’s urn to cautiously preselect the balls we draw.

5) Conclusion

While Stirling’s risk propagation in LCA acts as projection of stakeholders’ belief and knowledge into the life cycle impacts space via the LCA model, ENSURe acknowledges that only partial projection is possible in presence of risk and uncertainty. ENSURe thus assists in evaluating the total probability of success, but not the full probability distributions for the impacts. We therefore keep uncertainty and risk differentiated without forcing a probabilistic approach to uncertainty and make ex-ante LCA comply with these key concepts in post-normal science.

By separating risk and uncertainty, ENSURe prevents ex-ante LCA modelling from falling further down into "model-land" (Thompson and Smith, 2019), in which assigning tentative probabilities to events, or mathematical relationships between factors sometimes constitutes an additional abstraction and a deceptive impression of quantifying all incertitude. Instead, the approach takes advantage of the fact that stakeholders often fail to simultaneously assign probability distributions to multiple factors that may be interdependent, but this does not prevent them from stating about the probability of reaching a certain scenario defined by several factors.

Our approach joins GSA to assist ex-ante LCA in providing insightful results for decision-making. While GSA supports LCA practitioners and decision makers in prioritizing their data collection efforts to reduce uncertainty in the output of LCA models, ENSURe informs them about which conditions should be met to ensure a certain probability of success and decide on further exploring a concept.

With ENSURe, ex-ante LCA can move forward into assessing broad technological concepts or any emerging technology for which incertitude is not limited to risk. While the other forms of incertitude, namely ambiguity or even ignorance (Stirling, 2010) still resist quantitative assessments, we tackle the need for early-stage decision making for a better planning of technological development under ecological emergency.

ASSOCIATED CONTENT

All data for the reproducibility of the results can be found in the article and in the Supporting Information and the github repository.

"Supporting Information A" (docx): Mathematical formulation of the procedure, definitions of aggregated factors, additional results and figures, assistance for the interpretation of the algorithm's outputs.

" Supporting Information B" (xlsx): Table of the LCA model factors classified by types and incertitude.

The code allowing reproduction of the results is available at https://github.com/PJGilmw/LCA_deep_uncertainty_fish_micro.(Jouannais, 2023)

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