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RESEARCH ARTICLE

Comparative life cycle assessment of marine microalgae, *Nannochloropsis* sp. and fishmeal for sustainable protein ingredients in aquaculture feeds

Brandi McKuin[1,*], Anne R. Kapuscinski[1], Pallab K. Sarker[1], Nicolette Cheek[1], Jacqueline Lim[1], and Martin Sabarsky[2]

Fishmeal from small marine pelagic fishes reduces their availability for marine wildlife forage and artisanal fishing catches that support food security in lower income coastal nations. Fishmeal is primarily used in feeds for aquaculture, the world's fastest-growing food sector. Replacing fishmeal in aquafeeds with more environmentally responsible alternative ingredients can help feed aquaculture transition to more sustainable production methods. Protein from defatted marine microalga, *Nannochloropsis* sp., produced alongside polyunsaturated fatty acids (PUFAs) for the nutraceutical market lacks a comprehensive open-access analysis of environmental impacts of producing these products from biorefineries. This study compared life cycle impacts (global warming potential, water use, land use, marine eutrophication potential, freshwater eutrophication potential, and biotic resource use) of protein from fishmeal produced in a small pelagic fish biorefinery to protein from defatted *Nannochloropsis* meal. We conducted an attributional life cycle assessment using primary data provided by Cellana LLC to model biomass cultivation and harvesting at the Kona Demonstration Facility (Hawaii, USA) and literature data to model the downstream processing of biomass into a high-protein fishmeal replacement for the aquafeed market and concentrated PUFAs for the nutraceutical market. Material and energy inputs from a *Nannochloropsis* biorefinery included 2 harvesting scenarios (wet and dry biomass) and 2 scenarios for oil extraction and processing (i.e., oil fractionation and concentration of PUFAs): solvents or supercritical carbon dioxide. Results for aquafeed protein from defatted *Nannochloropsis* were that cultivation processes had the largest overall effect for all scenarios; urea and pure liquid carbon dioxide were environmental hot spots; and the processing scenario involving dry biomass followed by oil extraction and oil processing with solvent had significantly lower environmental impacts than protein from fishmeal from a small pelagic fish biorefinery for global warming potential, water use, marine eutrophication potential, freshwater eutrophication potential, and biotic resource use, but not for land use. These results suggest that aquafeed from marine microalgae can be an environmentally sustainable replacement for fishmeal if high-value metabolites are coproduced in a biorefinery.

Keywords: Biorefinery, Polyunsaturated fatty acids, EPA

1. Introduction

Global aquaculture production represents the fastest-growing sector among global food systems (Froehlich et al., 2018). While seafood production from wild capture fisheries has remained stagnant, global aquaculture production has increased by 500% since the late 1980s and provides an important source of protein and nutrition for a growing population (Food and Agriculture Organization [FAO], 2020). The rapidly expanding aquaculture sector involved a major transition from unfed to fed production using formulated aquafeeds, with fed aquaculture growing 158% from 2000 to 2018 when it comprised nearly 60 million MT (FAO, 2020). The production of aquaculture feeds is likewise expected to increase from 51.2 million tonnes in 2017 to 73.2 million tonnes by 2025 (Tacon, 2020). During this transition, aquafeeds relied on fishmeal and fish oil, derived from marine forage fisheries (e.g., anchovy, sardine, herring) for protein, lipid, and energy sources. Approximately 16 million of the 29 million tonnes of the forage fish annual global catch currently go into aquaculture feed (Cottrell et al., 2020). There are concerns about the sustainability of aquaculture's dependence on finite marine resources, given that it has been projected that at current rates of fishmeal and fish oil consumption, aquafeed demands could outstrip the supply of forage fish by 2037 (Duarte et al., 2009; Pikitch et

[1] University of California, Santa Cruz, CA, USA
[2] Cellana LLC, San Diego, CA, USA

* Corresponding author:
Email: bmckuin@ucsc.edu

al., 2014; Cashion et al., 2017; Froehlich et al., 2018; Shannon and Waller, 2021). Thus, there is a growing need for alternatives to fishmeal and fish oil ingredients in aquafeeds.

Marine microalgae show promise as potential replacements for fishmeal and fish oil in aquaculture feeds because of their elevated fatty acid profiles and high protein content (Kiron et al., 2016; Sarker et al., 2016a; Sarker et al., 2016b; Bélanger-Lamonde et al., 2018; Sarker et al., 2018; Sarker et al., 2020a; Sarker et al., 2020b; Bélanger et al., 2021). Recent studies have shown that whole cell biomass of *Schizochytrium* sp. is a highly digestible source of nutrients for rainbow trout and tilapia and is a potential substitute for fish oil in aquafeed (Sarker et al., 2016a; Bélanger-Lamonde et al., 2018; Sarker et al., 2020a; Bélanger et al., 2021). Docosahexaenoic acid (DHA)-rich oil from *Schizochytrium* sp. is being incorporated into salmon feeds by aquafeed companies (Tocher et al., 2019). *Nannochloropsis* sp. is another species that is commercially produced for aquaculture feeds and is rich in eicosapentaenoic acid (EPA) as well as crude protein, essential amino acids, lipids, and various minerals (Sarker et al., 2018; Sarker et al., 2020a; Sarker et al., 2020b).

Marine microalgae also show promise as an environmentally sustainable alternative to fishmeal and fish oil in aquafeed for several reasons reviewed by Nagappan et al. (2021). First, microalgae do not require arable land and have higher biomass yields than terrestrial plants or animals (Benedetti et al., 2018). A previous study reported the yield of *Nannochloropsis* (up to 6,171 kg ha^{-1}) is nearly an order of magnitude larger than soybean yields (up to 663 kg ha^{-1}) and 2 orders of magnitude larger than beef (80 kg ha^{-1}; Moomaw et al., 2017). Second, because marine microalgae can be cultivated using seawater or wastewater, they require less potable water than other agricultural products (Moomaw et al., 2017; Merlo et al., 2021). Moomaw et al. (2017) reported the freshwater consumption of *Nannochloropsis* (20 L kg^{-1}) is over 2 orders of magnitude smaller than soybeans (9,045 L kg^{-1}) and 4 orders of magnitude smaller than beef (148,077 L kg^{-1}). Third, microalgae have the potential to recycle carbon dioxide (CO_2) from flue gas and other industrial sources (Daneshvar et al., 2022). For example, Wilson et al. (2020) demonstrated that an algae-based system lowered the net carbon emissions of a slipstream of flue gas from a coal-fired power plant. Thus, marine microalgae cultivation does not compete with conventional food production for arable land and freshwater resources and could be more environmentally sustainable with respect to extensive cultivation of crops (Benedetti et al., 2018). Furthermore, by recycling flue gas from fossil-power power plants, microalgae have the potential to mitigate CO_2 emissions and enable the diversification and expansion of biologically derived products and commodities (Wilson et al., 2020).

Despite the potential advantages of microalgae as a substitute for fishmeal and fish oil compared with other alternatives, economic feasibility is constrained by small scale of production, company investments, regulation of novel food, and access to credit (Fasaei et al., 2018; Bussa et al., 2020; Mennella et al., 2020). It has been proposed that microalgae from biorefineries could allow high-value metabolites (e.g., pigments, omega-3 fatty acids, and specialty polysaccharides) produced in smaller quantities to be coproduced alongside high-volume but low-value products such as biofuels to improve the overall economic sustainability of these production systems (Rizwan et al., 2018). The microalgae biorefinery business sector initially focused only on biofuels is now evolving to consider other products including ingredients for aquafeeds (Khan et al., 2018). Although the economic feasibility of microalgae biorefineries has been well studied, the environmental impact of microalgae from biorefineries as an alternative source of fish feed has not (Parsons et al., 2020).

A useful tool to assess environmental impacts and ensure environmentally sustainable development of biorefinery-based production of microalgae is life cycle assessment (LCA; Bohnes and Laurent, 2019). Previous studies of the sustainability of microalgae biorefineries have focused on biofuels (Faried et al., 2017), while only a few studies have considered biorefineries that include microalgae as an aquafeed ingredient (Batan et al., 2010; Taelman et al., 2013; Barr and Landis, 2018; Beal et al., 2018; Ghamkar and Hicks, 2020). To avoid the unintended effects of transferring environmental burdens from the sea to land, a comprehensive set of relevant impact metrics considering natural resource depletion (e.g., biotic resource use, land use, and water use) and pollutant emissions (e.g., eutrophication potential and global warming potential) should be used to evaluate the consequences of replacing fishmeal with marine microalgae products before these substitutes are widely adopted.

Previous LCA studies have shown that different culture conditions, harvesting options, and downstream processing yield divergent results concerning microalgae's environmental performance (Bennion et al., 2015; Jez et al., 2017). Most LCA studies that have modeled microalgae oil extraction have considered conventional solvents (e.g., hexane or a combination of ethanol and hexane; Batan et al., 2010; Sills et al., 2013; Beal et al., 2015; Barr and Landis, 2018; Beal et al., 2018), while only a small number of studies have considered solvent free alternatives (Yuan et al., 2015; Posada et al., 2016; Tu et al., 2017). Supercritical CO_2 is a desirable alternative to conventional solvents because of its lower toxicity, higher selectivity (Lorenzen et al., 2017; Kwan et al., 2018), and higher yields compared to organic solvents like hexane. Supercritical CO_2 extraction has been found to yield extracts richer in neutral lipids and less rich in phospholipids (Elst et al., 2018). Solana et al. (2014) found little difference between the fatty acid composition between solvent and supercritical CO_2 extraction methods. Moreover, there is an increased interest in green extraction methods for extracting high-value functional ingredients such as polyunsaturated fatty acids (PUFA; Herrero et al., 2006).

Among the wide diversity of compounds from microalgae metabolites, omega-3 fatty acids (which are PUFAs) as a source of nutraceuticals or pharmaceuticals produced alongside other products have been identified as a way to make microalgae biofuels more sustainable (Trivedi et al., 2015). The demand for omega-3 fatty acids in the

nutraceutical and pharmaceutical industry has grown rapidly over recent years, due to the increasing scientific evidence supporting health issues, such as inflammation, heart disease, and mental development (Hamed et al., 2015). The health benefits of omega-3 fatty acids have been studied extensively and are attributed to the PUFAs EPA and DHA (Swanson et al., 2012), as well as their ratio to the omega-6 PUFA arachidonic acid (Simopoulos, 2010). Although microalgae biomass is rich in PUFA, the purity of product required for the nutraceutical (i.e., 60% or greater omega-3 fatty acids) or pharmaceutical market (i.e., 95% or greater omega-3 fatty acids) requires further concentration (e.g., chromatography, vacuum or molecular distillation, low-temperature crystallization, urea complexation, supercritical fluid fractionation, supercritical fluid chromatography, or enzymatic methods; Rubio-Rodriguez et al., 2010; Yves et al., 2017; Bonilla-Méndez et al., 2018; Catchpole et al., 2018). This concentration of omega-3 PUFA to increase purity is an essential step in the manufacturing process to ensure label claims for health benefits to consumers as well as the economic competitiveness of the nutraceutical and pharmaceutical markets for these products (van der Merwe et al., 2018).

PUFA concentration has largely been ignored in LCA studies, and therefore, little is known about the additional impacts this processing step may have in terms of energy use on the production process and whether it is a potential hot spot (Parsons et al., 2018). Perez-Lopez et al. (2014) conducted an LCA of EPA from the microalgae *Phaeodactylum tricornutum* but did not include the concentration step in their analysis. The LCA by Posada et al. (2016) modeled PUFA concentration with urea fractionation following the methods outlined in Medina et al. (1998). However, urea complexation does not fulfill the criteria of green extraction methods due to the use of hexane during the process (Marsol-Vall et al., 2020). The LCA by Barr and Landis (2018) compared the environmental impacts of omega-3 fatty acid, high protein feed, and biofuel production from algae to the impacts of the production of those products from a small pelagic biorefinery but did not include concentrating the PUFA to increase the purity (Barr and Landis, 2018). The LCA by Torgacheti and Padamati (2021) compared PUFA production from microalgae and farmed fish but did not include concentrating the PUFA to increase the purity.

Electricity consumption is one of the main drivers of environmental impacts throughout the production phases of microalgae production. Most microalgae biorefinery LCA studies have assumed that electricity would be provided from the grid (Batan et al., 2010; Sills et al., 2013; Beal et al., 2015; Barr and Landis, 2018; Beal et al., 2018). A small number of studies have coupled photovoltaic (PV) electricity and microalgae biomass production and found that shading microalgae ponds with PV panels can increase biomass productivity during hotter periods and produce local electricity for the process (Parlevliet and Moheimani, 2014; Morales et al., 2019). When PV panels are used as a source of electricity, it can increase the economic competitiveness of microalgae biofuels (Jez et al., 2017). PV electricity has also been found to have a lower environmental impact compared with grid electricity for nonfuel microalgae products (Smetana et al., 2017). Furthermore, it is a noteworthy option for operating microalgae facilities in remote areas that are far from the electric grid (Morales et al., 2019). Microgrids that combine multiple power solutions combined with energy storage could be a sustainable pathway for microalgae biorefineries; however, we are unaware of any LCAs that have modeled this approach in the literature.

The present study examined the consequences of substituting fishmeal for the aquafeed market and fish-oil derived PUFAs for the nutraceutical market with marine microalgae products and addressed 2 key knowledge gaps. First, we quantified the additional impacts that the isolation and concentration of PUFA processing step may have in terms of energy and material use. Second, we considered the environmental impact of a microgrid that combines multiple power solutions (i.e., PV panels and diesel-powered generator) combined with energy storage instead of grid electricity. We conducted an LCA that considered 6 impact categories: global warming potential, water consumption, land use, marine eutrophication potential, freshwater eutrophication potential, and biotic resource use. We used primary data provided by Cellana LLC to model biomass cultivation and harvesting at the Kona Demonstration Facility (KDF) and literature data to model the processing of the marine microalgae *Nannochloropsis* into a high-protein fishmeal replacement for the aquafeed market and concentrated PUFA for the nutraceutical market. Because the off-grid KDF utilized both diesel and PV energy sources, we modeled a standalone microgrid to generate power from a combination of diesel generators and PV and energy storage from lithium-ion batteries. We modeled the source of carbon for the cultivation of the microalgae as flue-gas carbon capture from diesel-powered generators and diesel-powered boilers. We modeled processing (**Figure 1**) to achieve 2 different biomass moisture levels (wet biomass and dry biomass at 23% and 95% cake solids dwt., respectively) and 2 different scenarios for oil extraction, isolation, and concentration of PUFAs (conventional solvent processing and supercritical CO_2 processing). The conventional solvent processing includes hexane for oil extraction, acetone for oil fractionation, and a combination of acetone and hexane solvents for the PUFA concentration. We sought to test whether processing wet biomass provides environmental benefits, compared to processing dry biomass in a biorefinery that recycles carbon from diesel-powered generator and diesel-powered boiler. We also sought to test whether solvent-free supercritical CO_2 processing methods (i.e., oil extraction, oil fractionation, and PUFA concentration) provide environmental benefits over conventional solvent processing methods. Finally, we compared environmental impacts of high-protein *Nannochloropsis* meal from a biorefinery to the benchmark, fishmeal from a small pelagic fish biorefinery (**Figure 2**).

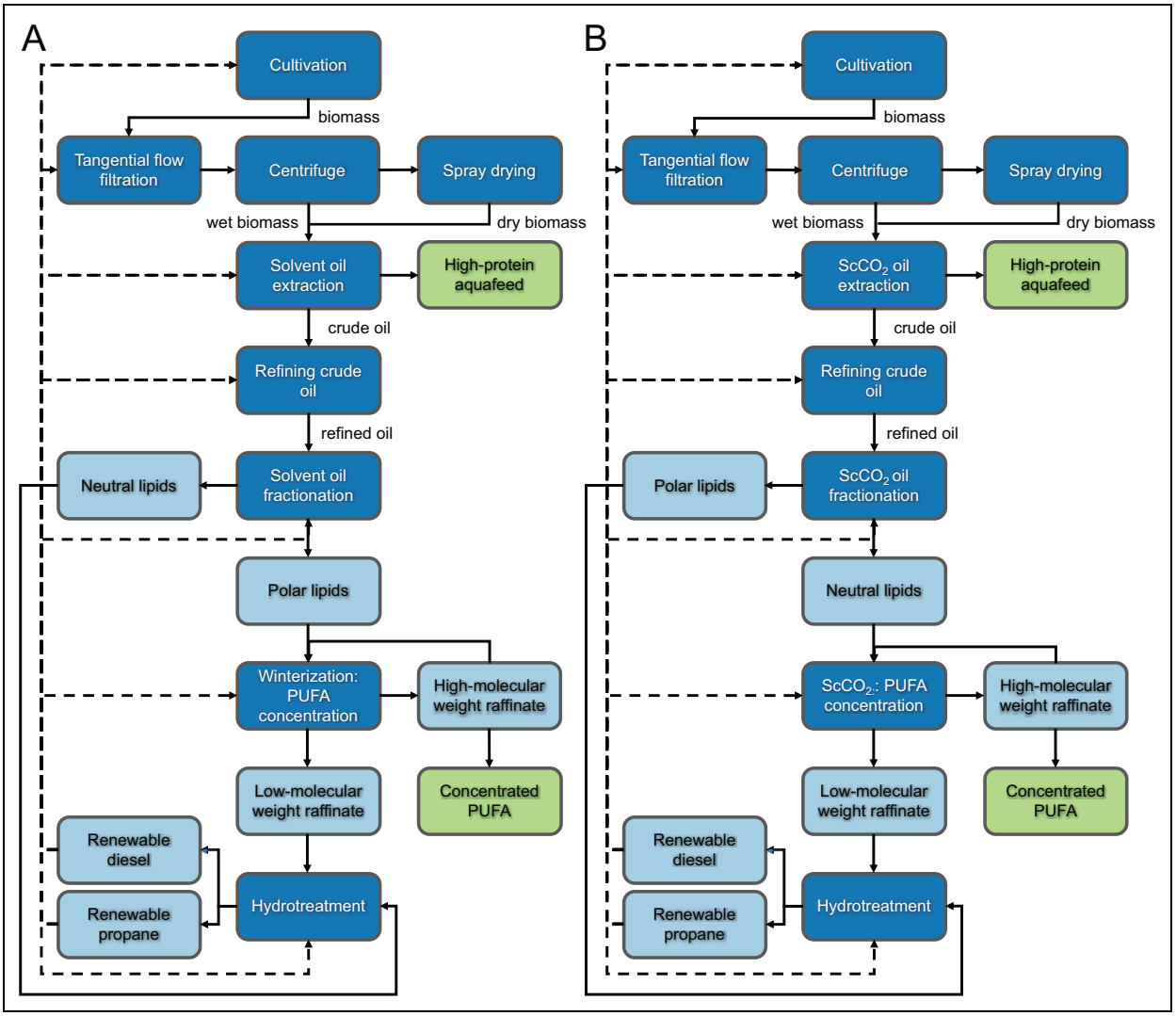

Figure 1. System boundaries and process-flow diagrams of *Nannochloropsis* biorefineries. Left panel (A): *Nannochloropsis* biorefinery following a conventional solvent scenario. Right panel (B): *Nannochloropsis* biorefinery following a supercritical (Sc) carbon dioxide (CO_2) scenario. The biorefineries include the production of defatted *Nannochloropsis* as high-protein aquafeed and concentrated polysaturated fatty acids (PUFA) as a nutraceutical with an omega-3 concentration of at least 60% (green boxes). The light blue boxes show key intermediary products. The dark blue boxes show the production processes including cultivation (e.g., growth in outdoor systems), wet biomass harvesting (e.g., tangential flow filtration and centrifuge dewatering until biomass is 23% cake solids dwt.), dry biomass harvesting (e.g., tangential flow filtration, centrifuge dewatering, and spray drying until biomass is 95% cake solids dwt.), oil extraction using either conventional solvent extraction methods (e.g., hexane) or supercritical CO_2 ($ScCO_2$) extraction methods, oil fractionation (e.g., separation of lipid classes into either neutral or polar lipids) using solvent or $ScCO_2$ methods, oil refining, polyunsaturated fatty acid (PUFA) concentration of omega-3 fatty acids (e.g., EPA and DHA) to at least 60% purity for the nutraceutical market using either solvent-based winterization methods or $ScCO_2$ methods, and hydrotreatment of the fraction of oil not included in nutraceutical oil product to produce renewable diesel and renewable propane. The dashed lines show flows of renewable diesel and renewable propane that offset fossil fuel to power production processes.

2. Methods

2.1. Data sources and general approach

We sourced *Nannochloropsis* cultivation and harvesting data from Cellana LLC. We sourced oil extraction, concentration of PUFA, and refining data from the literature. We conducted an attributional analysis of the environmental impact of the production stages of protein from defatted *Nannochloropsis* and a comparative analysis to the benchmark, protein from fishmeal produced from small pelagic fish.

2.1.1. Goal and scope of LCA

The goal of this LCA was to investigate a more sustainable alternative to fishmeal in aquafeeds. To achieve this goal, we evaluated the environmental impacts of using the marine microalga, *Nannochloropsis maritima* KA32, as

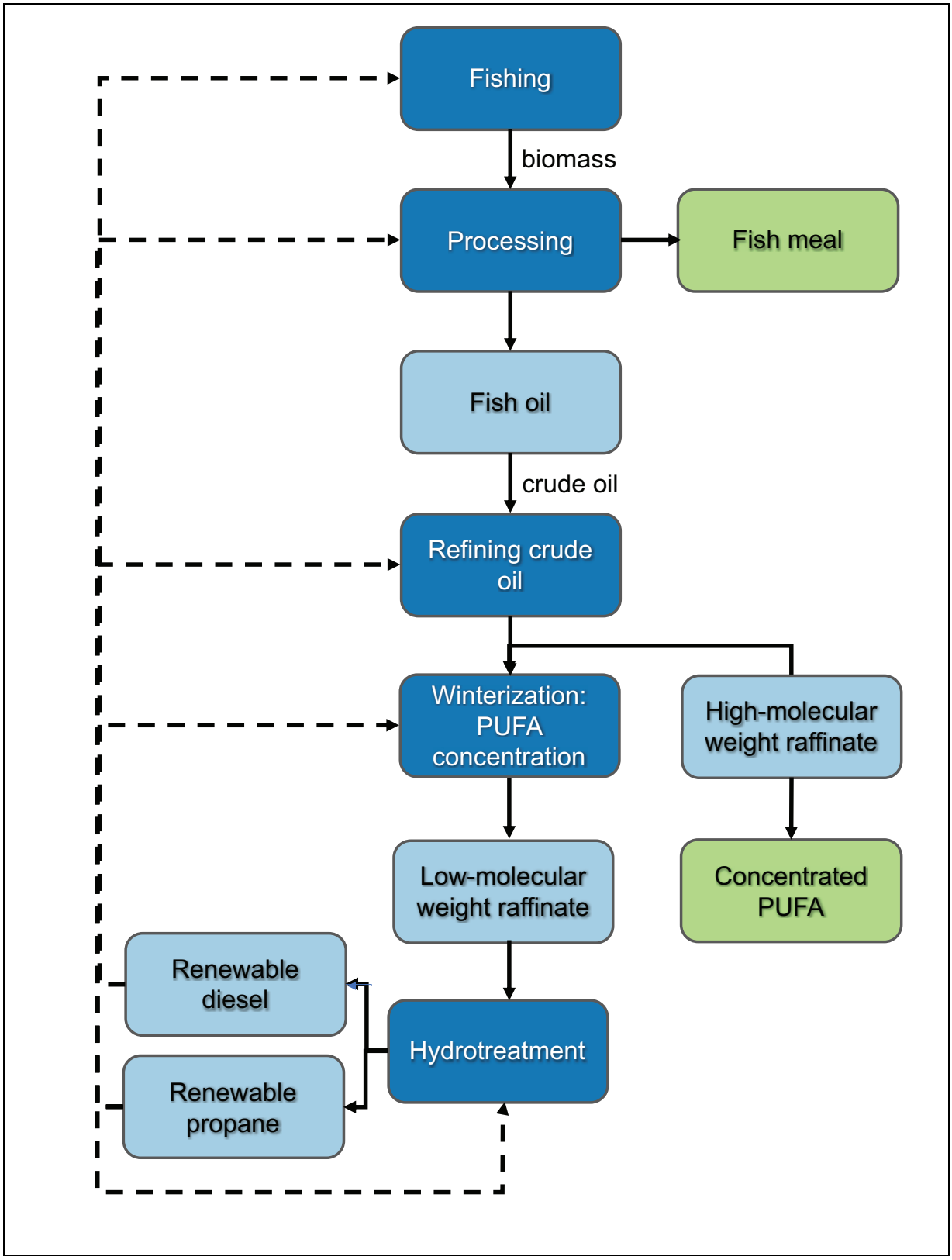

Figure 2. System boundaries and process-flow diagrams of small pelagic fish biorefinery. The small pelagic fish biorefinery we studied includes the production of fishmeal and concentrated polysaturated fatty acids (PUFA) as a nutraceutical with an omega-3 concentration of at least 60% (green boxes). The light blue boxes show key intermediary products. The dark blue boxes show the production processes including harvesting (e.g., fishing activities), processing of biomass into fishmeal and crude fish oil, oil refining, PUFA concentration using solvent-based winterization methods, and hydrotreatment of the fraction of oil not included in nutraceutical oil product to produce renewable diesel and renewable propane. The dashed lines show flows of renewable diesel that offset diesel fuel used in fishing activities, and renewable propane that offsets fossil fuel to power heat for production processes.

a substitute for the benchmark, fishmeal, and to provide guidance on how the aquafeed industry could further utilize ingredients resulting from marine microalgae biorefineries to decrease their environmental impact.

We conducted a contribution analysis of the input parameters to identify environmental impact hotspots. A hot spot is a life cycle stage, process, or elementary flow, which accounts for a significant proportion of the impact (Laurent et al., 2020). Additionally, we identified environmental impact reductions achievable by considering alternative production parameters in our sensitivity analysis.

2.1.2. Type of LCA

The LCA study consisted of 3 main parts: (1) inventory modeling analysis of *Nannochloropsis* production and processing with available data, (2) attributional life cycle impact modeling and hot spots identification compared to the benchmark product, and (3) sensitivity analysis and identification of more sustainable scenarios of *Nannochloropsis* production. The assessment followed the standard LCA approach (ISO 14040, 2006) and used professional SimaPro v.8.5.2.0 software (PRé Consultants B.V., Amsterfoort, The Netherlands) and adapted Ecoinvent 3.4 data sets for background data (e.g., electricity, water supply, heat generation, and crop ingredients; Wernet et al., 2016). We used the ReCiPe 2016 Midpoint (H) v.1.02 method (Huijbregts et al., 2017) to calculate the global warming potential, water consumption, land use, freshwater eutrophication potential, and marine eutrophication potential categories. Among LCA methods, the ReCiPe method is one of the most recent and advanced LCA methodologies with a broad set of midpoint impact categories and an impact calculation mechanism having a global scope (Goedkoop et al., 2013; PRé, 2023). It combines the strengths of both midpoint-based approach of CML-IA, and end-point/damage-oriented approach of Eco-indicator 99, which are globally recognized LCA methods (Goedkoop et al., 2013; Hauschild et al., 2013; PRé, 2023). For biotic resource use estimates, we made calculations from values provided in the literature (Lardon et al., 2009; Sarker et al., 2018; Zhang, 2018; Sarker et al., 2020b; see Supplementary Text S1.1.1 for detailed calculations).

Production of multiple products within the system boundaries of our analysis required a decision about which allocation method (e.g., economic, or biophysical) to use in the attribution of environmental impact to the coproducts. We chose economic allocation because it is the most commonly used allocation method in agricultural LCA studies, particularly for crop production and the livestock feed supply chain (Ardente and Cellura, 2012; Brankatschk and Finkbeiner, 2014; van der Werf and Nguyen, 2015; Mackenzie et al., 2017). In economic allocation, higher importance is placed on the more limiting coproducts generated and their relative demand, and, thus, acts as a proxy for the nutritional value of ingredients (Kok et al., 2020). Biophysical allocation, on the other hand, uses physical relationships between coproducts. Biophysical allocation systems, however, implicitly rely on economic value in that economic value informs whether to include or exclude whole sections of the mass balance in a model of an agricultural system (Mackenzie et al., 2017). For these reasons, we selected economic allocation for our analysis. The attributional LCA required the allocation of environmental impact between coproducts of cultivation inputs and oil extraction processes for *Nannochloropsis* and coproduct of small pelagic fish (e.g., anchovy, herring, and menhaden) to reduction for fish oil. We applied an economic allocation to the coproducts based on the coproduct yields and prices (see Supplementary Text S1.1.2 and Equation S1).

2.1.3. Functional unit

The functional unit is 1-kg crude protein. We estimated that 1.77-kg defatted *Nannochloropsis* sp. would replace 1 kg of protein from fishmeal based on a crude protein content of 49.7% and a dry matter basis of 56.3% (Sarker et al., 2020b). Further, we estimated that 1.47 kg of fishmeal would yield 1 kg of protein based on a crude protein content of 65.2% and a dry matter basis of 68.2% (Sarker et al., 2020b).

2.1.4. System boundaries, geographies, and scenarios

The life cycle boundaries encompassed all direct material and energy inputs related to the cultivation, harvesting, and processing systems from which the ingredients were derived—"cradle to factory-gate." We modeled the production of a high-protein fishmeal replacement for the aquafeed market and concentrated PUFAs for the nutraceutical market derived from a *Nannochloropsis* biorefinery. The cultivation and harvesting inputs of the *Nannochloropsis* biorefinery are based on previous experiments conducted by Cellana that took place at the KDF located in the State of Hawaii, USA. For downstream processing, we modeled 2 different biomass moisture levels (wet biomass and dry biomass at 23% and 95% cake solids dwt., respectively) and 2 different processing scenarios including conventional solvent methods (**Figure 1A**) and supercritical CO_2 methods (**Figure 1B**). As the benchmark for comparison, we modeled fishmeal produced from small pelagic fish on the global market (**Figure 2**).

2.1.5. Life cycle inventory of *Nannochloropsis* cultivation

Our inputs are based on Cellana's plans for a future commercial-scale facility, which are based on the performance of the KDF. Here, we describe the facility specifications, the nutrient inputs (e.g., nitrogen, phosphorous, and carbon sources), electricity demand, and capital goods.

The KDF has been described in other studies (Beal et al., 2015; Huntley et al., 2015; Barr and Landis, 2018). Although previous studies have included in-door cultivation inputs (e.g., inoculum for scale-up; Barr and Landis, 2018), our study includes only the outdoor cultivation inputs. The commercial-scale facility is expected to consist of a 4-stage outdoor system that operates in batch mode. The modular 4-stage system includes photobioreactors (PBRs) and 3 pond systems. The biomass yield was reported to be 0.289 ± 0.043 g l^{-1} (mean and standard deviation), the residence time for each outdoor growth

stage was 3 days, and the facility operated 330 days per year (Table S1; see Supplementary Text S1.2.1 for additional details of the facility specifications).

The biomass fractions of whole cell (dry weight) are based on the Algae Testbed Public Private Partnership 2015 state of technology seasonal macromolecular biomass fractions: 0.15 (± 0.03), 0.45 (± 0.03), 0.15 (± 0.04), 0.21 (± 0.03), and 0.05 (± 0.001), for lipids, proteins, carbohydrates, ash, and cell mass, respectively (mean and standard deviations of the spring, summer, fall, and winter values; Knoshaug et al., 2016). We calculated the ratio of dry weight to ash-free dry weight biomass as 1.26.

The nutrient inputs include urea as a source of nitrogen, triple super phosphate as a source of phosphorous, and CO_2 from flue gas as a source of carbon. From previous experiments at KDF, Cellana supplied 0.42-kg urea and 0.11-kg triple superphosphate (TSP) per kg biomass. Experiments conducted at the KDF demonstrated there was no significant difference in productivity between the control ponds using pure liquid CO_2 and the experimental ponds using diesel generator flue gas as the sole source of CO_2 (Anton, 2016). Thus, we assumed the CO_2 source is flue-gas carbon capture from diesel-powered generators and diesel-powered boiler facilities on-site. Because flue gas cannot be temporarily stored and algae do not perform photosynthesis and take up CO_2 at night (Chi et al., 2011), we assume that flue gas is only supplied during daylight hours (~ 12 h d^{-1}). While large-scale carbon capture from power plants assumes capture rates of 80 (% vol.; Davis et al., 2018), we assume that due to the smaller scale of ducting, there would be negligible losses. We assumed a demand of 1.83-kg CO_2 per kg biomass with an uptake rate of approximately 90%.

The cultivation energy requirements include energy for the paddlewheels, pumps, ultraviolet (UV) treatment unit, cleaning-in-place (CIP) generators, and air blowers (see Supplementary Text S.1.2.2 for details and summary in Table S2). Pumps are used to withdraw seawater and make water transfers. Paddle wheels are used to circulate the cultivation medium in the ponds. A UV system is used to disinfect seawater before it is used as cultivation medium. A CIP system generator is used to prevent fouling. Low-pressure air blower systems are to transport flue-gas CO_2 from the on-site diesel generators and to circulate the gas into the pond.

The capital goods we considered include the PBRs and the construction materials for the ponds. As reported in Monari et al. (2016), the PBR manufacturing contributes significantly to energy use and environmental impacts, and thus, we included these inputs (see Supplementary Text 1.2.3 for details). Due to the small contribution of construction materials associated with outdoor ponds (less than 1% of the total cumulative energy demand) found in Brentner et al. (2011), we omitted the construction of outdoor ponds in our analysis.

2.1.6. Life cycle inventory of *Nannochloropsis* harvesting

We considered 2 different biomass moisture levels: a "dry" scenario with a 95% cake solids (dry wt.) concentration and a "wet" scenario with a 23% cake solids (dry wt.) concentration. According to Cellana's harvesting process train, the contents of pond C are transferred to a tangential-flow filtration unit, followed by pumping to a decanter-bowl centrifuge, and finally, the sludge is sent to a spray-drying unit. Although previous KDF studies have used natural settling as a concentration step in the harvesting process train (Beal et al., 2015; Huntley et al., 2015), we do not make this assumption. Several factors including the small cell size of the genus *Nannochloropsis* (between 2 and 5 μm; Wang et al., 2014), the high lipid content, and the similarity of the density of the algal cells to seawater preclude natural settling as an effective concentrating step in the harvesting process train (Wiley et al., 2011; Milledge and Heaven, 2013). The preconcentrating step of tangential flow filtration produces a solids concentration of 3% (dwt.). The final solids concentration from the bowl-decanter centrifuge was reported to be 23% cake solids (dwt.) and the efficiency was reported to be 95%. For the dry biomass scenario, the biomass is dried with a ring dryer (spray-drying) unit (Table S3).

2.1.7. Life cycle inventory of *Nannochloropsis* oil extraction

We modeled cell disruption and oil extraction of biomass with 2 different biomass moisture levels (i.e., a "dry" scenario with a 95% cake solids dry wt. concentration and a "wet" scenario with a 23% cake solids dry wt. concentration) and 2 different lipid extraction methods (i.e., solvent extraction and supercritical CO_2).

Efficient cell disruption is an essential pretreatment step to maximize lipid recovery from microalgal biomass (Günerken et al., 2015). High-pressure homogenization is reported to be a scalable technology (Frank et al., 2011) and it has been shown to be an effective method for pretreatment and maximizing lipid yields from *Nannochloropsis* (Samarasinghe et al., 2012; Lee et al., 2017). Thus, we estimated cell disruption with high-pressure homogenization from a range of literature values (Frank et al., 2011; Davis et al., 2012; Tu et al., 2017; Table S4).

For the solvent extraction method, we assumed hexane was the solvent. We estimated the material and energy inputs for lipid extraction of wet and dry biomass with hexane solvents from the literature (Frank et al., 2011; Davis et al., 2012; Vasudevan et al., 2012; Passell et al., 2013; Sills et al., 2013; Azadi et al., 2014; Beal et al., 2015; Souza et al., 2015; Tu et al., 2017; Barr and Landis, 2018; Beal et al., 2018; Table S5).

For the supercritical CO_2 extraction, we calculated the energy consumption associated with the CO_2 pump, the CO_2 heater, and refrigeration of the CO_2 using the equations described by Attard et al. (2015; Equations S2–S4; Table S6; see Supplementary Text S1.3 for additional details). We estimated the CO_2 to oil ratio, CO_2 losses, reactor pressure, reactor temperatures, and process efficiencies from the literature (Garcia Alba, 2013; Yuan et al., 2014; Du et al., 2015; Monari et al., 2016; Posada et al., 2016; Tu et al., 2017).

2.1.8. Life cycle inventory of refining crude *Nannochloropsis* oil

The oils extracted from biomass undergo a series of refining steps, in which free fatty acids and other phospholipids are removed, a subsequent bleaching step to remove pigments, and a final further refining step to remove any volatile compounds associated with undesirable flavors or odors (Parsons et al., 2018).

We used literature values to model the process of refining crude *Nannochloropsis* oil (Table S7). The inputs for degumming (i.e., phosphoric acid), neutralization (i.e., sodium hydroxide), and bleaching (i.e., bleaching earth and activated carbon) were based on inputs for refining autotrophic microalgal oil (Togarcheti and Padamati, 2021). We calculated the electricity demand based on the loading volume (i.e., water, oil, and degumming and neutralization inputs) and the specific energy for centrifuge used to refine algal oil (Barr and Landis, 2018). Inputs for processing steam, water, and wastewater were adapted from the Agri-footprint and the Ecoinvent databases. Degummed oil yields, neutralized oil yield, and bleached oil yields were based on literature values for refined algal oil (Barr and Landis, 2018).

2.1.9. Life cycle inventory of oil fractionation

The isolation and concentration of PUFA from microalgal oil include the fractionation of the oils (into neutral, phospholipids, and glycolipids). We modeled 2 different methods of separation of lipid classes: solid phase extraction and supercritical CO_2 extraction.

For the separation of lipid classes (i.e., neutral and polar lipids) by solid phase extraction, we modeled acetone as a solvent to separate glycolipids and phospholipids from triacyl glycerides and other neutral lipids from wet (23% cake solids dwt.) and dry (95% cake solids dwt.) biomass using values from the literature (Table S8). We modeled a lipid to acetone ratio of 1:5 (w/w) to separate glycolipids and phospholipids from neutral lipids (Kokkiligadda and Srinivasa, 2017). We modeled electricity consumption and recovery efficiencies for the separation of neutral lipids from polar lipids with a disc-stack centrifuge (Fasei et al., 2018; Szepessy and Thorwid, 2018). We used first principles to estimate the heat required to evaporate acetone. We assumed solvent recovery efficiency of 99% (Stephenson et al., 2010). We estimated the relative fraction of lipid classes from the literature (Table S9; Kokkiligadda and Srinivasa, 2017).

For the separation of lipid classes by supercritical CO_2 extraction, our model was informed by patent and literature data (Hegel et al., 2017; Waibel et al., 2017; Table S8). We used the pressure (350 to 690 bar) and temperature ranges (60°C–90°C) reported by Waibel et al. (2017). We used a processing time of 60 min reported by Hegel et al. (2017). We calculated the energy consumption associated with the CO_2 pump, the CO_2 heater, and refrigeration of the CO_2 fluid using the equations described by Attard et al. (2015; Equations S2–S4; see Text S1.3 for additional details). We assumed the same CO_2 loss rate and process efficiencies as we did for supercritical CO_2 extraction of oil from dry algal biomass (Table S6). We used the CO_2 to oil ratio (i.e., sample loading volume of 364-μL algal oil; density of algal oil of 920 g L^{-1}; range of CO_2 flow rates: 5–15 mL CO_2 min^{-1}; density of CO_2 of 1.98 g L^{-1}) from a previous experiment (Montañés et al., 2013). We estimated roughly 50% neutral lipids from patent data (Waibel et al., 2017).

For lipids fractionated by both solvent and supercritical CO_2 methods, we estimated the fatty acid profile by lipid class from the literature (Table S10; Olmstead et al., 2013; Ryckebosch et al., 2014; Yao et al., 2015).

2.1.10. Life cycle inventory of concentration of PUFAs

We modeled 2 different methods of concentrating the PUFA: winterization and supercritical CO_2 processing.

For the winterization scenario, the refined oil is mixed with organic solvent and stored at low temperatures until the saturated fatty acids solidify, leaving the unsaturated fatty acids in liquid form. We estimated the solvent losses (acetone and hexane; Table S11), winterization temperature (Table S12), winterization time period (Table S13), and recovery yield (Table S14) from a range of experimental values (Mendes et al., 2007; Dueppen et al., 2010; Ruiz et al., 2016; Kokkiligadda and Srinivasa, 2017). We assumed the solidified fatty acids were separated by a decanter bowl centrifuge. We used first principles to estimate the heat to evaporate acetone. We assumed solvent recovery efficiency of 99% (Stephenson et al., 2010). We reviewed the literature to estimate the inputs for refrigerant losses associated with the winterization process (Bovea et al., 2007; Blowers and Lowenbury, 2010; Cascini et al., 2016; Table S15). The primary energy consumption for freezer storage during the winterization process was based on a linear relationship between the estimated energy consumption of a walk-in industrial refrigeration/freezer system and the temperature setting (Figure S1). We assumed a 14.5 m^3 walk-in industrial refrigeration/freezer system with an ambient temperature of 29.4°C, and we also assume the product enters the walk-in at ambient temperature (U.S. Cooler, 2019). The distribution of fatty acids in the recovered oil was based on values in the literature (Kokkiligadda and Srinivasa, 2017; Table S16).

For the supercritical CO_2 scenario, the free fatty acids are concentrated and isolated from shorter chain length fatty acids. Waibel et al. (2017) report that the PUFA in the neutral lipids can be concentrated with supercritical CO_2 by applying a pressure gradient under isothermal conditions. We modeled a pressure gradient (150–350) under isothermal conditions (50°C ± 10°C). To calculate the primary energy consumption, we assumed a stepwise increase in pressure over equal intervals for the duration of the extraction. We assumed the same reaction time (e.g., duration of 1 h) and the CO_2 to oil ratio that we used for the oil fractionation with supercritical CO_2. We calculated the energy consumption associated with the CO_2 pump, the CO_2 heater, and refrigeration of the CO_2 using the equations described by Attard et al. (2015; Equations S2–S4; see Text S1.3 for additional details). Waibel et al. (2017) report a total oil yield of 94.93%. The distribution of fatty acids in the recovered oil was based on patent data (Waibel et al., 2017; Table S17).

For both the winterization and supercritical CO_2 scenarios, we assumed the process would be repeated on the higher molecular weight raffinate until the omega-3 fatty acid purity was at 60%.

2.1.11. Life cycle inventory of hydrotreatment processes

We modeled upgrading of the lower molecular weight raffinate and the fractions of oil that were not used to concentrate PUFA (i.e., neutral lipids in the winterization scenario and polar lipids in the supercritical CO_2 scenario) to renewable diesel using the hydrotreatment process. We used the material and energy inputs for the hydrotreatment process from literature sources (Barr and Landis, 2018; Arguelles et al., 2021; Table S18).

2.1.12. Life cycle inventory of small pelagic fish biorefinery

We used data from a previous study to estimate the material and energy flows associated with fishing and processing of small pelagic fish into fishmeal and fish oil and refining the crude fish oil (McKuin et al., 2022). However, unlike McKuin et al. (2022), we assumed renewable diesel and renewable propane from the small pelagic fish biorefinery would partially offset the diesel fuel used in fishing and the thermal energy demands in processing the fish into fishmeal and fish oil. We modeled the concentration of PUFAs with the winterization process (Tables S11–S15) and upgrading the lower molecular weight raffinate to renewable diesel using the hydrotreatment process (Table S18). We estimated the fatty acid profile of the crude fish oil from the small pelagic fish biorefinery (Table S19; Homayooni et al., 2014). We estimated the fatty acid profile of winterized fish oil and the fraction that would remain in the higher molecular weight raffinate from literature values (Table S20; Homayooni et al., 2014). We assumed the winterization process would be repeated on the higher molecular weight raffinate until the omega-3 fatty acid purity was 60%.

2.2. Microgrid for Nannochloropsis *biorefinery*

We assumed a diesel generator, and solar-plus-storage technology microgrid would provide electricity for cultivation, harvesting, and processing operations. We assumed a diesel boiler would provide heat for drying and processing operations. We calculated the diesel generator load as a function of the daily CO_2 requirements for cultivating the algae and assumed the remainder of the load was met by solar-plus-storage technology. We simulated the solar-plus-storage load using HOMER Pro (v. 3.14.7880.21077; see Supplementary Text S1.4 for additional details).

2.3. Uncertainty analysis

We used established methods to calculate the uncertainty of several inventory parameters (McMurray et al., 2017). We fitted the distributions of selected inventory items with EasyFit Professional software (v. 5.6; Tables S21–S29). Using the best fit distribution, we ran Monte Carlo simulations of 10,000 samples. We ran a percentile bootstrap analysis of 1,000 replicates of the sample medians to estimate the 95% confidence intervals using the *boot* package in R (Canty and Ripley, 2020). We propagated the error for the selected inventory items using the derivative method (Bevington and Robinson, 2003).

2.4. Life cycle impact calculations

We used Equation S5 to calculate the life cycle impact results. The inputs to Equation S5 include the annual energy and materials used in the *Nannochloropsis* biorefinery (Tables S30–S55) and in the small pelagic fish biorefinery (Tables S56–S60), the life cycle impact characterization factors for the *Nannochloropsis* biorefinery (Table S61), and the small pelagic fish biorefinery (Table S62), the annual yield of protein from the defatted *Nannochloropsis* and fishmeal, and the economic allocation partitioning factors for protein from the *Nannochloropsis* biorefinery and protein from small pelagic fish biorefinery (see Supplementary Text S1.1.2). Additionally, we calculated offsets for renewable fuels (see Supplementary Text S1.5) and for flue gas from the combustion of fossil and renewable fuels that was captured and used as an inorganic source of carbon for cultivation of *Nannochloropsis* (Supplementary Text S1.6).

2.5. Hypothesis testing

First, we tested the hypothesis that protein from a *Nannochloropsis* biorefinery produced with wet biomass would have lower environmental impacts than protein from a *Nannochloropsis* biorefinery produced with dry biomass. Second, we tested the hypothesis that protein from a *Nannochloropsis* biorefinery produced with supercritical CO_2 processes (i.e., oil extraction, oil fractionation, and PUFA concentration) would have lower environmental impacts than protein from a *Nannochloropsis* biorefinery produced with solvent for the same processes. Third, we tested the hypothesis that protein for aquafeeds produced from a *Nannochloropsis* biorefinery would have lower environmental impacts than protein for aquafeeds produced from a conventional small pelagic fish biorefinery. We used an independent-sample, single-tailed, unequal variance student *t* test to test a one-sided hypothesis using Microsoft Excel. Significance was based on p values <0.05.

2.6. Sensitivity analysis

We conducted a sensitivity analysis of *the Nannochloropsis* biorefinery to identify the key parameters that have the largest impact on the results and to identify the parameters that contribute most to the output variability (see Supplementary Text S1.7.1 for additional details). We separately considered a sensitivity of alternative inputs including Productivity Enhanced Algae and Tool-Kits (PEAK) biomass growth performance, renewable fuel end use, alternative inorganic carbon source, electricity solely from PV panels and batteries, and alternative prices for defatted *Nannochloropsis* meal (see Supplementary Text S1.7.2 for additional details). We conducted our sensitivity analyses using a one-parameter-at-a-time approach (Laurent et al., 2020).

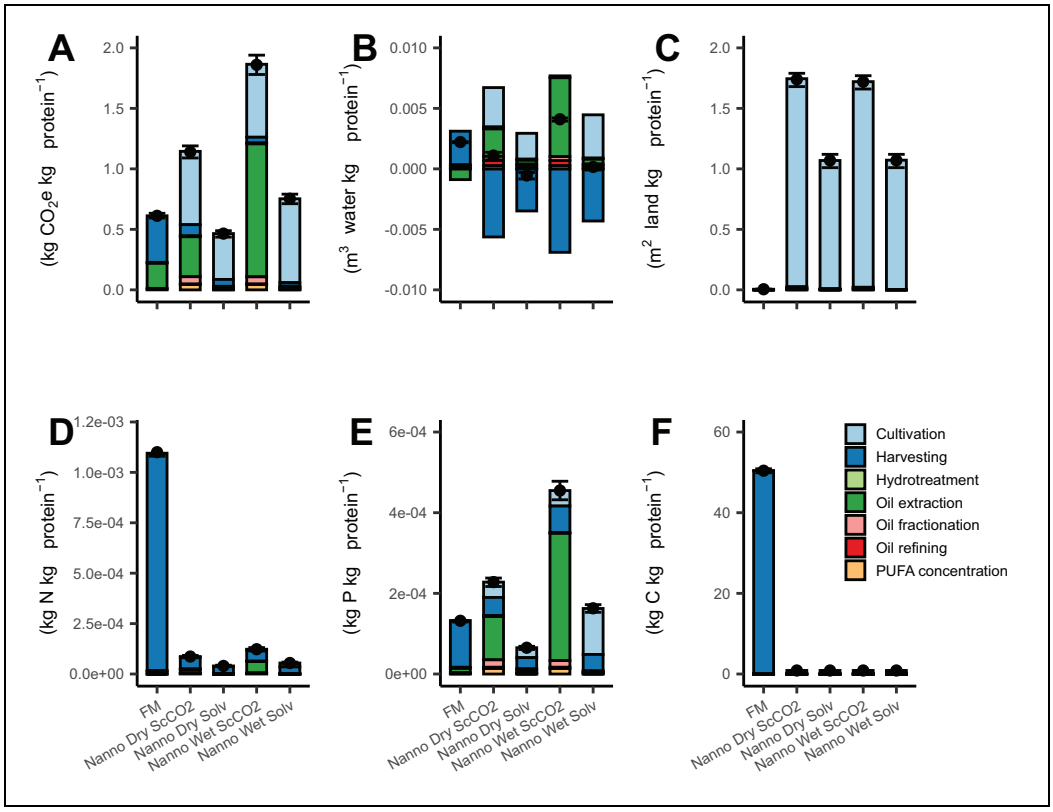

Figure 3. Life cycle impacts of protein from a defatted *Nannochloropsis* meal produced in a *Nannochloropsis* biorefinery compared with protein from fishmeal (FM) produced in a small pelagic fish biorefinery by production stage. Contribution analysis disaggregated by stages of the production cycle of FM protein from the small pelagic biorefinery and *Nannochloropsis* (Nanno) protein from the *Nannochloropsis* biorefinery processed with either dry or wet biomass. The *Nannochloropsis* biorefinery production stages include cultivation (e.g., growth in outdoor systems), harvesting (e.g., tangential flow filtration and centrifuge dewatering until biomass is at 23% cake solids dwt. in the case of wet biomass and tangential flow filtration), centrifuge dewatering, and spray drying until biomass is at 95% cake solids dwt. in the case of dry biomass, oil extraction using either conventional solvent (Solv) extraction methods (e.g., hexane) or supercritical carbon dioxide ($ScCO_2$) extraction methods, oil fractionation (e.g., separation of lipid classes into either neutral or polar lipids) using Solv or $ScCO_2$ methods, oil refining, polyunsaturated fatty acid (PUFA) concentration of omega-3 fatty acids (e.g., EPA and DHA) to at least 60% purity for the nutraceutical market using either Solvent-based winterization methods or $ScCO_2$ methods, and hydrotreatment of the fraction of oil not included in nutraceutical oil product to produce renewable diesel and renewable propane. The renewable diesel and renewable propane offset the fossil fuel demands to power the electricity and heat demands of the *Nannochloropsis* biorefinery. The small pelagic fish biorefinery production stages include harvesting (e.g., fishing activities), oil extraction (processing of biomass into FM and crude fish oil), oil refining, PUFA concentration using Solvent-based winterization, and hydrotreatment of the fraction of oil not included in nutraceutical oil product to produce renewable diesel and renewable propane. The renewable diesel offsets the demand for fishing fuel and renewable propane offsets the fossil fuel demands to power the heat demands of the small pelagic fish biorefinery. Top panel: (A) Global warming potential, (B) water use, and (C) land use. Bottom panel: (D) Marine eutrophication potential, (E) freshwater eutrophication potential, and (F) biotic resource use. The black circles represent the net value, and the error bars represent the 95% confidence interval.

3. Results

3.1. Environmental hot spots by production category

Here, we identify environmental hot spots across the 4 *Nannochloropsis* biorefinery scenarios (dry biomass and solvent processing, wet biomass and solvent processing, dry biomass and supercritical CO_2 processing, and wet biomass and supercritical CO_2 processing) and the small pelagic biorefinery by production category (**Figure 3**).

For the *Nannochloropsis* biorefinery scenarios, global warming potential and water use were dominated by cultivation processes across scenarios except for the wet biomass and supercritical CO_2 scenario, for which oil extraction was the dominant category (**Figure 3A** and **B**). There was a negative impact for water use due to water recycling in wastewater treatment in the harvesting process (**Figure 3B**). Across all scenarios, cultivation processes were dominant for land use and biotic resource use

(**Figure 3C** and **F**). For marine eutrophication potential, harvesting was dominant across scenarios except for the wet biomass and supercritical CO_2 processing, for which oil extraction was the dominant category (**Figure 3D**). For freshwater eutrophication potential, harvesting dominated the dry biomass solvent processing scenario, cultivation dominated the wet biomass and solvent processing scenario, and oil extraction dominated both the wet and dry biomass supercritical CO_2 scenarios (**Figure 3E**).

For the small pelagic biorefinery, global warming potential (**Figure 3A**), water use (**Figure 3B**), land use (**Figure 3C**), marine eutrophication potential (**Figure 3D**), freshwater eutrophication potential (**Figure 3E**), and biotic resource use (**Figure 3F**) were dominated by harvesting biomass (i.e., fishing activities).

3.2. Environmental hot spots by material and energy categories

Here, we identify environmental hot spots across the 4 *Nannochloropsis* biorefinery scenarios (dry biomass and solvent processing, wet biomass and solvent processing, dry biomass and supercritical CO_2 processing, and wet biomass and supercritical CO_2 processing) and the small pelagic biorefinery by material and energy categories (**Figure 4**). The classification of material and energy inputs at the individual level can help to identify hot spots that can inform targets for reduced consumption.

For the *Nannochloropsis* biorefinery scenarios, global warming potential was dominated by urea (as a source of nitrogen for cultivation) only for the dry biomass and solvent processing and all other production scenarios were dominated by pure liquid CO_2 (as a supplemental source of CO_2 for cultivation in the wet biomass and solvent processing; as solvent for supercritical oil extraction, oil fractionation, and PUFA concentration for the dry and wet biomass and supercritical CO_2 processing scenarios; **Figure 4A**). For water consumption across the scenarios considered, wastewater (for harvesting, oil refining, and hydrotreatment processes) was dominant and resulted in negative values due to water recycling at the wastewater treatment plant (**Figure 4B**). For land use across the scenarios considered, the classification "other" was dominant owing to direct land occupation for the cultivation of biomass (**Figure 4C**). For marine eutrophication potential, wastewater was the dominant contributor for the dry biomass and solvent processing, wet biomass and solvent processing, and the dry biomass and supercritical CO_2 processing, but CO_2 was the dominant contributor for the wet biomass and supercritical CO_2 processing scenarios (**Figure 4D**). For freshwater eutrophication potential, CO_2 was the dominant contributor across all scenarios except the dry biomass and solvent processing scenario for which electricity from PV was dominant (**Figure 4E**). For biotic resource use, the classification "other" was dominant owing to direct use of *Nannochloropsis* biomass (**Figure 4F**).

For the small pelagic biorefinery, global warming potential was dominated by diesel fuel used to power the main and auxiliary engines of fishing boats (**Figure 4A**). For water consumption, water supply (for oil refining and hydrotreatment processes) was dominant (**Figure 4B**). For land use and freshwater eutrophication potential, the classification "other" was dominant owing to antifouling paint used for the fishing boats (**Figure 4C** and **E**). For marine eutrophication potential, the classification "other" was dominant owing to copper wire used in construction of the fishing boats (**Figure 4D**). For biotic resource use, the classification "other" was dominant owing to direct use of fish biomass (**Figure 4F**).

3.3. Sensitivity analysis

3.3.1. Sensitivity analysis of standard deviations of input parameters

Here, we evaluate the parameters that contributed most to the variance in the standard deviations of the *Nannochloropsis* biorefinery scenarios, which vary by processing stage and environmental impact metric (Figures S2−S36). For cultivation, the input parameters that contributed most to the variance include urea, high-density polyethylene, biomass yield, energy for water pumps, and TSP (Figures S2−S6). For harvesting, biomass yield, tangential flow filtration, and spray dryer contributed most to the variance (Figures S7−S11). For oil extraction, electricity for solvent processing, carbon dioxide to oil ratio for supercritical processing, solvent oil extraction efficiency, and carbon dioxide losses for supercritical processing contributed most to the variance (Figures S12−S16). For oil refining, phosphoric acid and oil recovery yield contributed most to the variance (Figures S17−S21). For oil fractionation, acetone losses, carbon dioxide losses, and carbon dioxide flowrate (Figures S22−S26). For PUFA concentration, acetone losses, carbon dioxide losses, and carbon dioxide flowrate (Figures S27−S31). For hydrotreatment, hydrogen and electricity for hydrotreatment processing contributed most to the variance (Figures S32−S36). See Supplementary Text S2.1 for extended results of the sensitivity analysis of the input parameters by processing stage.

3.3.2. Sensitivity analysis of alternate parameters

Here, we present the results of the sensitivity analysis of the alternate parameters including PEAK biomass performance, renewable fuel end use, alternative inorganic carbon source, electricity from PV panels, and an alternative price for defatted *Nannochloropsis* meal (Figures S37−S41). The alternate parameters that contributed most to the overall environmental impact results by processing stage were: inorganic carbon source for the global warming potential across all scenarios (Figure S37); inorganic carbon source for water use across all scenarios (Figure S38); PEAK biomass performance for land use across all scenarios (Figure S39); inorganic carbon source for the marine eutrophication potential for the dry biomass and solvent processing, and dry and wet biomass and supercritical CO_2 processing scenarios (Figure S40a, c, and d), and PV electricity for the wet biomass and solvent processing scenario (Figure S40b); and inorganic carbon for freshwater eutrophication potential for the dry biomass and solvent and supercritical CO_2 processing scenarios (Figure S41a and c), and PV electricity for the wet

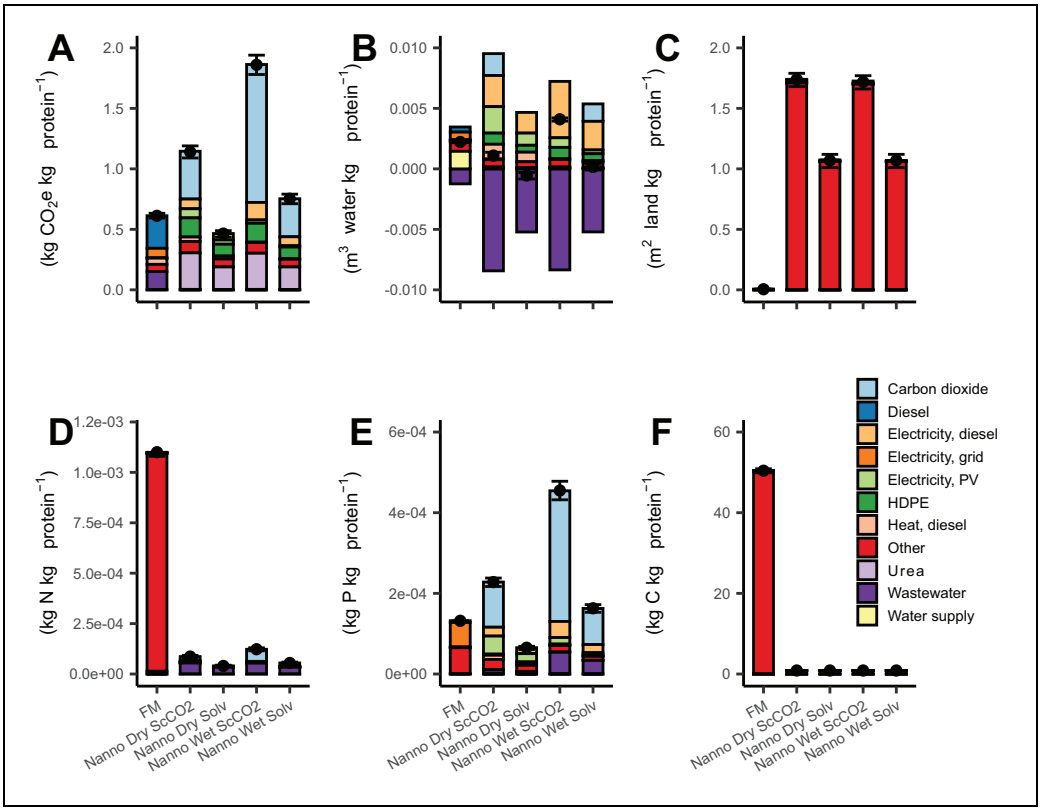

Figure 4. Life cycle impacts of protein from a defatted *Nannochloropsis* meal produced in a *Nannochloropsis* biorefinery compared with protein from fishmeal (FM) produced in a small pelagic fish biorefinery by material and energy source. Contribution analysis disaggregated by material and energy source of FM protein from the small pelagic biorefinery and *Nannochloropsis* (Nanno) protein from the *Nannochloropsis* biorefinery processed with either dry (biomass is at 95% cake solids dwt.) or wet biomass (biomass is at 23% cake solids dwt.) and either a solvent (Solv) or supercritical carbon dioxide ($ScCO_2$) scenario. The energy sources include electricity from a diesel generator, heat from diesel generator, electricity from the grid, electricity from photovoltaics (PV), and diesel fuel to power the main and auxiliary engines of fishing boats. The materials include carbon dioxide (source of supplemental inorganic carbon for cultivation and as a solvent for supercritical carbon dioxide processes including oil extraction, oil fractionation, and polyunsaturated fatty acid concentration), high-density polyethylene (HDPE) used to make PBRs and fishing nets, urea (source of nitrogen in the cultivation of *Nannochloropsis*), wastewater (from processing FM and fish oil, from harvesting *Nannochloropsis*, from refining the crude fish oil and crude *Nannochloropsis* oil, and from hydrotreatment of refined fish oil and refined *Nannochloropsis* oil to renewable fuels), water supply, and other materials. The other materials include triple superphosphate for the cultivation of *Nannochloropsis*, lithium-ion batteries as energy storage for the *Nannochloropsis* biorefinery microgrid, materials for the small pelagic fish biorefinery (e.g., engine oil, batteries, and materials for the hull and structure of the fishing boat, such as concrete, copper, and steel), materials for the fishing nets (e.g., lead, nylon), materials for refining the crude oil (e.g., phosphoric acid, sodium hydroxide, sulfuric acid, bleaching earth, nitrogen for cryogenic separation, and activated carbon), organic solvents (e.g., hexane and acetone for conventional solvent extraction, oil fractionation, and winterization), leaked refrigerant for the winterization process (e.g., R404a), and hydrogen for the hydrotreatment process. Top panel: (A) Global warming potential, (B) water use, and (C) land use. Bottom panel: (D) Marine eutrophication potential, (E) freshwater eutrophication potential, and (F) biotic resource use. The black circles represent the net value, and the error bars represent the 95% confidence interval.

biomass and solvent and supercritical CO_2 processing scenarios (Figure S41b and d). See Supplementary Text S2.2 for extended results of the sensitivity analysis of the alternate parameters.

4. Discussion

To our knowledge, this study is the first open-access LCA of a *Nannochloropsis* biorefinery that models the concentration of PUFA for the nutraceutical market using winterization and supercritical CO_2 methods. Furthermore, it is the first LCA of a *Nannochloropsis* biorefinery that models a stand-alone microgrid with battery storage for power generation. We have reported environmental impacts of a *Nannochloropsis* biorefinery, where we modeled: 2 different biomass moisture levels (wet biomass and dry biomass at 23% and 95% cake solids dwt., respectively); 2 different scenarios for oil extraction, isolation, and concentration of PUFA (conventional solvent

processing and supercritical CO_2 processing); and a standalone microgrid to generate power from a combination of diesel generators and PV, and energy storage from lithium-ion batteries. Comparing the 4 *Nannochloropsis* processing scenarios modeled, the dry biomass and solvent processing had the lowest overall environmental impacts across all categories (**Figures 3** and **4**). The environmental impacts of fishmeal protein from the small pelagic biorefinery were higher than the protein from the *Nannochloropsis* biorefinery in certain impact categories, depending on the scenario, except for land use (**Figures 3** and **4**). These results are in line with other studies that have shown that the replacement of fishmeal with alternatives alone will not prevent burden shifting but will also require strategies to provide alternatives with minimal energy requirements (Ghamkhar and Hicks, 2020; Maiolo et al., 2020). Under our model assumptions, we tested our hypotheses and identified several environmental tradeoffs of replacing fishmeal protein with defatted *Nannochloropsis* meal. In the following, we discuss our hypotheses tests and the limitations of our analysis.

4.1. Hypotheses tests

First, our results did not support the first hypothesis that processing wet biomass (23% cake solids dwt.) would have lower environmental impacts than dry biomass (95% cake solids dwt.) under our model assumptions. Instead, we found the opposite result. The wet biomass had significantly higher environmental impacts than did dry biomass across the 2 biorefinery production scenarios (solvent and supercritical CO_2 methods to extract and process oil) except land use (**Table 1**; **Figures 3** and **4**). The wet biomass scenarios avoided the energy consumption for drying the biomass in the harvesting phase of production, but the material inputs throughout the production phases were higher as a consequence. In the case of the solvent processing, the avoided drying energy resulted in less diesel-powered flue gas available as an inorganic carbon source and the need for supplemental pure liquid CO_2. Additionally, processing wet biomass instead of dry biomass in the oil extraction phase also required higher amounts of electricity from diesel power instead of from PV and batteries and higher amounts of heat. Furthermore, there were higher CO_2 losses for wet biomass than dry biomass. Previous studies have found that excess water in the biomass might act as a barrier in the diffusion transfer of the target product to supercritical fluid (Pourmortazavi and Hajimirsadeghi, 2007).

Second, our results did not support the second hypothesis that oil extraction processing using supercritical CO_2 would have lower environmental impacts than solvent extraction under our model assumptions. Similar to the results for our first hypothesis, we found the opposite result. The supercritical CO_2 processing had significantly higher environmental impacts than did solvent processing across the 2 biorefinery production scenarios (wet biomass at 23% cake solids dwt. and dry biomass at cake solids 95% dwt.; **Table 1**; **Figures 3** and **4**). Supercritical CO_2 methods had higher electricity and heat demands than oil extraction from solvent methods and larger impacts from losses of pure liquid CO_2 than from organic solvent losses. The increase in environmental impacts, however, should be weighed against other benefits of solvent-free methods. For example, liquid CO_2 is nonvolatile, nontoxic, and safer than organic solvents (Fizal et al., 2020).

Finally, our third hypothesis was supported by the results that aquafeed protein from a *Nannochloropsis* biorefinery would have a lower impact than fishmeal protein from a small pelagic biorefinery. Fishmeal protein from the small pelagic biorefinery had significantly higher environmental impacts than did the *Nannochloropsis* biorefinery in the dry biomass and solvent processing scenario for all environmental indicators except land use (**Table 1**; **Figures 3** and **4**). Notably, the biotic resource use of fishmeal protein from the small pelagic fish biorefinery was 2 orders of magnitude larger than the biotic resource use of protein from the *Nannochloropsis* biorefinery (all scenarios; **Figures 3** and **4**). These results highlight the potential of defatted microalgal biomass to replace fishmeal from wild fish catch and thus mitigate ocean resource depletion (Zhang and Kendall, 2019).

4.2. Comparison to previous studies

Although a handful of environmental impact studies have evaluated *Nannochloropsis* biorefineries that include either whole or defatted biomass as an aquafeed ingredient (Batan et al., 2010; Taelman et al., 2013; Barr and Landis, 2018; Beal et al., 2018), direct comparisons between this study and previous work are complicated by key differences: cultivation and processing methods, functional units, system boundaries, methods of accounting for the multiple products from the biorefinery (e.g., biophysical and economic allocation), different LCA databases (e.g., Agri-footprint, Ecoinvent), and different life-cycle impact assessment methods (e.g., ReCiPe, TRACI). For example, Batan et al. (2010) estimated the net energy ratio and greenhouse gas emissions of a *Nannochloropsis* biorefinery with cultivation in bioreactors only instead of in outdoor ponds, and the functional unit was 40 billion gallons of microalgal biodiesel with defatted *Nannochloropsis* for aquafeed as a coproduct. Barr and Landis (2018) conducted an LCA of a *Nannochloropsis* biorefinery with similar cultivation methods as this study, but the functional unit was one metric tonne of omega-3 fatty acids with biofuel and high-protein meal as coproducts.

Two environmental impact studies we reviewed, however, had *Nannochloropsis* biomass as the main product from the biorefinery (Taelman et al., 2013; Beal et al., 2018). Taelman et al. (2013) estimated the resource demands and carbon footprints of a *Nannochloropsis* biorefinery with cultivation in bioreactors, and the functional unit was 1-MJ exergy of dry matter biomass for the aquafeed market. In that study, the carbon footprint results were varied depending on the scenario (i.e., pilot plant 2012, pilot plant 2013, and first production scale 2015): 0.09–1.76 kg CO_2e per MJ exergy, dry matter biomass. We converted the results to the carbon footprint on a per unit dry matter basis using the conversion, 1-kg dry biomass is equivalent to 23.07-MJ exergy (Taelman et al., 2013), resulting in carbon footprints of 37.8-, 14.8-, and 2.08-kg

Table 1. Statistics for study hypotheses related to biomass moisture, processing scenario, and biorefinery type

Scenario	Biorefinery	Mean ($n = 3$)	Scenario	Biorefinery	Mean ($n = 3$)	p Value [a]
Global warming potential (kg CO_2e kg protein^{-1})						
Wet solvent[b]	*Nannochloropsis*	7.53E−01	Dry solvent[c]	*Nannochloropsis*	4.65E−01	2.30E−04
Wet $ScCO_2$[d]	*Nannochloropsis*	1.86E+00	Dry $ScCO_2$[e]	*Nannochloropsis*	1.14E+00	4.64E−04
Wet solvent[b]	*Nannochloropsis*	7.53E−01	Wet $ScCO_2$[d]	*Nannochloropsis*	1.86E+00	1.10E−04
Dry solvent[c]	*Nannochloropsis*	4.65E−01	Dry $ScCO_2$[e]	*Nannochloropsis*	1.14E+00	1.23E−04
Small pelagic fish	Small pelagic fish	6.12E−01	Dry solvent[c]	*Nannochloropsis*	4.65E−01	7.05E−04
Water use (m^3 water kg protein^{-1})						
Wet solvent[b]	*Nannochloropsis*	1.60E−04	Dry solvent[c]	*Nannochloropsis*	−5.43E−04	1.33E−02
Wet $ScCO_2$[d]	*Nannochloropsis*	4.10E−03	Dry $ScCO_2$[e]	*Nannochloropsis*	1.10E−03	2.89E−04
Wet solvent[b]	*Nannochloropsis*	1.60E−04	Wet $ScCO_2$[d]	*Nannochloropsis*	4.10E−03	7.13E−05
Dry solvent[c]	*Nannochloropsis*	−5.43E−04	Dry $ScCO_2$[e]	*Nannochloropsis*	1.10E−03	1.14E−03
Small pelagic fish	Small pelagic fish	2.22E−03	Dry solvent[c]	*Nannochloropsis*	−5.43E−04	1.59E−03
Land use (m^2 land kg protein^{-1})						
Wet solvent[b]	*Nannochloropsis*	1.07E+00	Dry solvent[c]	*Nannochloropsis*	1.07E+00	5.00E−01
Wet $ScCO_2$[d]	*Nannochloropsis*	1.72E+00	Dry $ScCO_2$[e]	*Nannochloropsis*	1.74E+00	3.39E−01
Wet solvent[b]	*Nannochloropsis*	1.07E+00	Wet $ScCO_2$[d]	*Nannochloropsis*	1.72E+00	6.66E−05
Dry solvent[c]	*Nannochloropsis*	1.07E+00	Dry $ScCO_2$[e]	*Nannochloropsis*	1.74E+00	5.52E−05
Small pelagic fish	Small pelagic fish	5.16E−03	Dry solvent[c]	*Nannochloropsis*	1.07E+00	4.45E−04
Marine eutrophication potential (kg N kg protein^{-1})						
Wet solvent[b]	*Nannochloropsis*	5.46E−05	Dry solvent[c]	*Nannochloropsis*	4.00E−05	5.83E−03
Wet $ScCO_2$ [d]	*Nannochloropsis*	1.23E−04	Dry $ScCO_2$[e]	*Nannochloropsis*	8.66E−05	1.84E−03
Wet solvent[b]	*Nannochloropsis*	5.46E−05	Wet $ScCO_2$[d]	*Nannochloropsis*	1.23E−04	5.79E−04
Dry solvent[c]	*Nannochloropsis*	4.00E−05	Dry $ScCO_2$[e]	*Nannochloropsis*	8.66E−05	6.77E−04
Small pelagic fish	Small pelagic fish	1.10E−03	Dry solvent[c]	*Nannochloropsis*	4.00E−05	2.23E−07
Freshwater eutrophication potential (kg P kg protein^{-1})						
Wet solvent[b]	*Nannochloropsis*	1.63E−04	Dry solvent[c]	*Nannochloropsis*	6.52E−05	2.56E−04
Wet $ScCO_2$[d]	*Nannochloropsis*	4.55E−04	Dry $ScCO_2$[e]	*Nannochloropsis*	2.28E−04	2.88E−04
Wet solvent[b]	*Nannochloropsis*	1.63E−04	Wet $ScCO_2$[d]	*Nannochloropsis*	4.55E−04	1.37E−04
Dry solvent[c]	*Nannochloropsis*	6.52E−05	Dry $ScCO_2$[e]	*Nannochloropsis*	2.28E−04	6.29E−05
Small pelagic fish	Small pelagic fish	1.32E−04	Dry solvent[c]	*Nannochloropsis*	6.52E−05	5.90E−05
Biotic resource use (kg C kg protein^{-1})						
Small pelagic fish	Small pelagic fish	50.4	Dry solvent[c]	*Nannochloropsis*	8.75E−01	1.67E−05

$ScCO_2$ = supercritical CO_2.

[a] p values estimated with the single-tail, unequal variance Student t test.

[b] The biomass moisture is wet (23% cake solids dwt.) and the processing scenario (e.g., oil extraction, oil fractionation, and polyunsaturated fatty acid concentration) is solvent-based.

[c] The biomass moisture is dry (95% cake solids dwt.) and the processing scenario (e.g., oil extraction, oil fractionation, and polyunsaturated fatty acid concentration) is solvent-based.

[d] The biomass moisture is wet (23% cake solids dwt.) and the processing scenario (e.g., oil extraction, oil fractionation, and polyunsaturated fatty acid concentration) is supercritical (Sc) carbon dioxide (CO_2)-based.

[e] The biomass moisture is dry (95% cake solids dwt.) and the processing scenario (e.g., oil extraction, oil fractionation, and polyunsaturated fatty acid concentration) is supercritical (Sc) carbon dioxide (CO_2)-based.

CO_2e kg dry matter biomass^{-1} for the pilot plant 2012, pilot plant 2013, and first production scale 2015, respectively. Beal et al. (2018) estimated the energy return on investment and the greenhouse gas impacts of a *Nannochloropsis* biorefinery with similar cultivation methods as this study. They reported a carbon footprint of 3.96-kg CO_2e per kg whole algae biomass.

We calculated the LCA results normalized by per unit defatted biomass instead of the functional unit (per unit protein), and the results were 0.38 ± 0.02, 0.62 ± 0.03, 0.85 ± 0.03, and 1.38 ± 0.05 kg CO_2e kg defatted *Nannochloropsis* biomass for the dry biomass and solvent processing scenario, wet biomass and solvent processing scenario, and dry biomass and supercritical CO_2 processing scenario, and wet biomass and supercritical CO_2 processing scenario, respectively (Figure S42). The best-case scenario of this study (i.e., dry biomass and solvent processing) were 1 to 2 orders of magnitude smaller than the reported results of Taelman et al. (2013), depending on the scenario, and one order of magnitude smaller than the reported results of Beal et al. (2018).

There are several reasons for the discrepancy between our results and these 2 other studies. First, differences are related to biorefinery approaches and multiproduct allocation methods. Taelman et al. (2013) did not consider a biorefinery approach with multiple product outputs. Unlike other studies that have multiple product outputs, Beal et al. (2018) did not make an allocation between the 2 coproducts (algal oil and algal meal) but instead attributed the greenhouse gas impacts to the whole algae biomass instead. In comparison, our study used an economic allocation that included high-value PUFA for the nutraceutical market. Second, pure CO_2 (100%) as food-grade compressed gas was modeled in Beal et al. (2018) and in the pilot scale scenarios in Taelman et al. (2013) instead of recycled CO_2 from flue gas that was considered in our study. Lastly, Taelman et al. (2013) and Beal et al. (2018) modeled grid electricity instead of the microgrid (diesel generator and solar PV) with energy storage that was considered in our study.

4.3. Study limitations

Our study revealed that flue-gas recovery from diesel-powered sources yielded lower emissions than the scenarios requiring supplemental liquid CO_2. Although we assumed renewable diesel would offset a portion of the diesel fuel required to operate the generators, using fossil fuels is not sustainable due to the finite nature of these resources (Bocken and Short, 2021). Recent studies have suggested recycling the carbon from flue gas to microalgae biomass would be more sustainable economically and environmentally if the power plant fuel was sourced from biomass instead of fossil sources (Beal et al., 2018; Cui et al., 2019). Other promising alternatives to sourcing CO_2 from flue-gas recovery include direct air capture of CO_2 combined with bicarbonate (Zhu et al., 2020). Methods using direct air capture of CO_2 coupled with PV power sources have the potential to significantly reduce the environmental impacts of inorganic carbon sources. Thus, future LCA studies should consider the environmental sustainability of these alternative sources of carbon.

We found that oil extraction and processing with solvents had lower impacts than supercritical CO_2 methods. Also, there are limitations to using supercritical CO_2 methods because the nonpolar CO_2 has limited dissolution power (Lee et al., 2021). Polar modifiers using solvents such as ethanol or hexane can be added to extract polar and membrane-associated neutral lipids such as phospholipids and glycolipids and could improve the viability of this technique (Lee et al., 2021; Morcelli et al., 2021). Additionally, we did not consider other alternatives to solvents including ionic liquids, deep eutectic solvents, switchable solvents, or integrated cell disruption and lipid extraction methods (e.g., ultrasound, microwave, bead-beating, surface, and pulsed electric field assisted methods; Lee et al., 2021). Moreover, in addition to oil extraction methods, there are alternative PUFA concentration methods, we did not consider including vacuum or molecular distillation, urea complexation, and enzymatic methods (Rubio-Rodriguez et al., 2010; Yves et al., 2017; Bonilla-Méndez et al., 2018; Catchpole et al., 2018). Future microalgae biorefinery LCA studies should consider these alternative methods.

The protein coproduct from defatted *Nannochloropsis* through an integrated biorefinery (i.e., dry biomass and solvent processing) was less environmentally impactful than protein from fishmeal from a small pelagic biorefinery in our study. Recent studies, that focused on algal biorefineries in the context of fuel production instead of aquafeeds, have pointed out that the possibility of maximizing biomass value through the extraction of very high added-value products is often limited by microalgae species, cultivation strategy, and other factors, as well as by their market size. The coproduction of high-value added products thus limits the application of this strategy in a multiple-commodity fuel-scale biorefinery (Levasseur et al., 2020; Wiatrowski et al., 2022). Although fuel production alongside niche products (e.g., pigments, omega-3 fatty acids, and specialty polysaccharides) may improve the viability of the algae industry in the near term, this production strategy presents a risk of saturating a small market and would not be a long-term sustainable concept to support commodity production volumes (Barkia et al., 2019; Wiatrowski et al., 2022). However, commodity chemicals and other compounds with otherwise substantial market sizes given a more elastic demand could change the viability of the algae industry in the long term (Wiatrowski et al., 2022). Thus, future LCA studies should consider alternatives to PUFA for nutraceuticals such as commodity chemicals (e.g., polyurethanes from unsaturated fatty acids) to improve the long-term sustainability of microalgae biorefineries for aquafeeds.

To handle the multiple product outputs of the microalgae biorefinery, we used an economic allocation method that has been used in other microalgae biorefinery studies (Beck et al., 2018). Several studies have reported that the choice of coproduct handling methods affects the LCA results of microalgal biorefineries (Beck et al., 2018; Cai et al., 2018; Sills et al., 2020). For microalgal biorefineries

where the primary economic driver for a system is a product that is a small fraction of the total mass produced by the system (e.g., nutraceutical oil being a higher-valued product than aquafeed protein or than biofuel), only a small impact will be allocated to the primary product (Sills et al., 2020). Although our study was a comparative study and we used the same allocation method for each biorefinery in our comparison, future LCA studies should consider alternative allocation methods for multiple product outputs (e.g., biophysical allocation).

This study focused on certain categories of environmental sustainability, but a more comprehensive sustainability assessment should also consider economic and social challenges (Govindan et al., 2021). Holistic sustainability assessments of alternative aquafeeds are beginning to emerge. For example, the comprehensive assessment by Ghamkhar and Hicks (2021) evaluates the sustainability of varying formulated aquafeeds based on their relevant economic, environmental, commercial, and technical aspects. However, the social challenges reported in the literature (e.g., violations of human rights, child labor, forced labor, discrimination, forced overtime, low wages, poor health and safety, and sexual harassment; Govindan et al., 2021) have not been applied as widely as environmental impact because social welfare is a relatively new LCA impact (Huertas-Valdivia et al., 2020). Notwithstanding, social welfare impact should be considered in future studies when making sustainability comparisons between protein from fish meal and marine microalgae. Lastly, although our study included several metrics that are linked to natural resource depletion (i.e., biotic resource use, land use, and water use) and pollutant emissions (global warming potential, and eutrophication potentials), future studies should include a more comprehensive set of pollutant emission metrics such as acidification potential and natural resource depletion metrics such as cumulative energy demand (Ghamkhar and Hicks, 2021).

In our study, protein from the *Nannochloropsis* biorefinery in the dry biomass and oil extraction with solvent scenario had lower environmental impacts than protein from fishmeal produced in the small pelagic biorefinery. Our analysis of environmental impacts of protein from fishmeal produced in the small pelagic biorefinery could be understated because our analysis did not consider the additional material and energy inputs associated with removing heavy metals, dioxins, polychlorinated biphenyls, persistent organic pollutants, and organochlorine pesticides that bioaccumulate in the fatty tissues of forage fishes (Oterhals et al., 2007; Sprague et al., 2010; Berntssen et al., 2016; Ng et al., 2018). Future comparative LCA studies should include the additional processing steps of removing toxins from fishmeal.

Ultimately, the goal of this LCA was to investigate a more sustainable alternative to fishmeal in aquafeeds. To accomplish this goal, it will be necessary to consolidate multiple criteria (e.g., the overall cost of aquafeed, its impact on final fish product, its impact on environment; Ghamkhar and Hicks, 2021). The results of this study can inform stakeholders in the aquafeed industry of the environmental dimensions and implications of substituting fishmeal protein from a small pelagic fish biorefinery with protein from a *Nannochloropsis* biorefinery. However, the other criteria such as the overall cost of the *Nannochloropsis* products and its technical potential (i.e., impact on the final fish product) are needed to fully evaluate the suitability of this ingredient as a substitute for protein from fishmeal. Future studies should be undertaken that also include the economic and technical potential of protein from *Nannochloropsis* meal as a substitute for protein from fishmeal using the framework of a decision-support tool.

5. Conclusion

We evaluated the environmental impacts of protein from defatted *Nannochloropsis*, a candidate ingredient for aquaculture feeds, and PUFA for the nutraceutical market produced from a biorefinery and compared these products to their analogs produced from a small pelagic fish biorefinery. Protein from defatted *Nannochloropsis* in the dry biomass and solvent processing scenario had significantly lower environmental impacts (i.e., global warming potential, water use, eutrophication potential, and biotic resource use) than protein from fishmeal produced by the small pelagic biorefinery except land use.

In addition to environmental benefits, replacing protein from fishmeal with defatted *Nannochloropsis* meal in aquafeeds would provide human health benefits. Unlike pelagic fish, marine microalgae do not accumulate heavy metal pollution and other contaminants of concern (Bélanger-Lamonde et al., 2018). Furthermore, there are human health benefits from elevated omega-3 fatty acid profiles of the flesh of farmed fish that were fed diets formulated with defatted *Nannochloropsis* compared with other high-protein alternatives to fishmeal (Naylor et al., 2009; Sarker et al., 2018).

There is an urgent need to understand the relative environmental performance of alternative feed inputs and identify trade-offs between different types and sources of inputs given the growing demand for feed inputs to aquaculture over the coming decades. Our comprehensive and open-access study of the environmental impacts can help accelerate the discovery of environmentally sustainable alternatives to fishmeal. The information presented here can be used to inform feed formulation decisions based on the environmental impacts of defatted *Nannochloropsis* and PUFA for the nutraceutical market as an alternative to these products produced in a small pelagic biorefinery. Furthermore, aquafeed companies can use this information to make targeted improvements in their production processes to achieve their environmental sustainability goals.

Data accessibility statement

The life cycle impact models (Appendix A), the microgrid simulations (Appendix B), the hypothesis tests (Appendix C), the uncertainty analysis (Appendix D), sensitivity analysis (Appendix E), and the files used to make the figures in the main manuscript are available in the DRYAD repository at the following link: https://doi.org/10.6071/

M3VM2S. The other datasets used in this article have been included in the supplemental material.

Supplemental files

The supplemental files for this article can be found as follows:

We have provided supplemental text (S1 and S2), supplemental equations (S1–S5), supplemental tables (S1–S62), and supplemental figures (S1–S42).

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Competing interests

The authors have no competing interests to declare. ARK is one of the Editors-in-Chief at *Elementa*. She was not involved in the review process of this manuscript.

Author contributions

Contributed to conception and design: BM, ARK, PKS, MS.

Contributed to acquisition of data: BM, MS, ARK, PKS, NC, JL.

Contributed to analysis and interpretation of data: BM, MS, ARK, PKS, NC, JL.

Drafted and/or revised the article: BM, MS, ARK, PKS, MS, NC, JL.

Approved the submitted version for publication: BM, ARK, PKS, MS, NC, JL.

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