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Research article

Upscaling and environmental impact assessment of an innovative integrated multi-trophic aquaponic system

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ABSTRACT

The increasing growth of the aquaculture sector has raised significant concerns regarding its environmental footprint, including nutrient discharge, substantial feed consumption, and high energy requirements. In response, innovative approaches such as aquaponics and integrated multi-trophic aquaculture (IMTA) are being developed as potentially more sustainable alternatives. This study aims to evaluate the environmental performance of an innovative Integrated Multi-Trophic Aquaponics system (IMTAcS) using the Life Cycle Assessment (LCA) approach. Given the experimental nature of the pilot plant, two distinct scaled-up scenarios were analysed: one utilizing an alternative feed (IMTAcS AF), and the other employing a commercial feed (IMTAcS CF). The functional unit was defined as 100 kcal and 1 kg of protein produced by the system, with a cradle-to-gate perspective defining system boundaries. Results revealed that IMTAcS AF has a higher global warming impact (0.234 kg CO₂ eq./100 kcal) compared to IMTAcS CF (0.207 kg CO₂ eq.). In both scenarios, electricity consumption was identified as the primary driver to environmental impact, exceeding 50%, in contrast to conventional systems where feed is the main hotspot. Moreover, while trends in impact categories such as net primary production use and eutrophication is opposite between the scenarios, the latter demonstrated substantial mitigation potential, attributable to the system's inherent nutrient recycling, in comparison with traditional aquaculture systems. While the findings are promising, certain limitations in the study (e.g. utilization of scaled-up data and inherent uncertainties analysed), with the scarcity of existing research, point to the opportunity for further exploration. This includes analysing real-scale implementations whenever feasible and conducting more detailed comparisons with traditional systems.

Acronyms

| Item | Acronym | Unit |
|---------------------------------------|---------|------------------------------------|
| Acidification | A | g SO ₂ eq |
| Alternative feed | AF | n/a |
| Climate change | CC | Kg CO ₂ eq |
| Commercial feed | CF | n/a |
| Eutrophication | E | g PO ₄ ³⁻ eq |
| Detritivorous filter-feeding organism | DFO | n/a |
| Feed conversion rate | FCR | n/a |
| Freshwater ecotoxicity | FEx | CTUe |
| Freshwater eutrophication | FE | kg P eq |
| Functional unit | FU | n/a |
| Geometric standard deviation | GSD | n/a |
| Global warming potential | GWP | Kg CO ₂ eq |

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| Item | Acronym | Unit |
|---|---------|-------------------|
| Human toxicity, cancer | HT-c | CTUh |
| Human toxicity, not cancer | HT-nc | CTUh |
| Integrated multi-trophic aquaculture | IMTA | n/a |
| Integrated multi-trophic aquaponic | IMTAcS | n/a |
| Land competition | LC | m ² y |
| Life cycle assessment | LCA | n/a |
| Marine eutrophication | ME | kg N eq |
| Net primary production use | NPPU | Kg C |
| Nitrogen | N | n/a |
| Overlap Area of Probability Distributions | PDF | n/a |
| Ozone depletion | OD | kg CFC11 eq |
| Particulate matter | PM | Disease increment |
| Phosphorus | P | n/a |

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(continued)

| Item | Acronym | Unit |
|----------------------------------|---------|-------------|
| Photochemical ozone formation | POF | kg NMVOC eq |
| Polyvinyl chloride | PVC | n/a |
| Recirculating aquaculture system | RAS | n/a |
| Resource use, fossil | RF | MJ |
| Resource use, mineral and metals | RMM | g Sb eq |
| Reversible heat pump | RHP | n/a |
| Technology-readiness level | TRL | n/a |
| Terrestrial acidification | TA | mol H+ eq |
| Terrestrial eutrophication | TE | mol N eq |
| Thermal-unit growth coefficient | TGC | n/a |
| Total cumulative energy demand | TCED | MJ eq |

1. Introduction

As the world's population continues to grow and the effects of climate change become more evident, the need for sustainable and environmentally friendly food production systems has become crucial (FAO, 2022). Traditional farming practices often degrade soil fertility, heavily rely on chemical inputs, and contribute to water pollution (Notarnicola et al., 2017). Similarly, the fishing industry faces challenges such as overfishing and habitat destruction. Aquaculture sector, which in addition to fish farming also involves crustaceans, shellfish and aquatic plants production, has emerged as a potential solution to address food security and overfishing issues. In particular, finfish farming is one of the fastest growing sectors in the world and its production through aquaculture has now exceeded wild-caught fish (FAO, 2022).

However, fish farming presents environmental weaknesses, and its expansion has raised several concerns (Gephart et al., 2021; Ceballos-Santos et al., 2024). On one hand, inland systems and Recirculating Aquaculture Systems (RAS) are characterized by a massive energy demand, both for water pumping and oxygenation using liquid oxygen. On the other hand, in offshore systems, the construction of aquaculture facilities, such as fish cages, can lead to the destruction of coastal habitats and seagrass meadows, which serve as critical areas for reproduction and nurseries for many marine species (Cao et al., 2013). Additionally, both farming systems consume a significant amount of feed, which, in most cases, is the main contributor to its environmental impact (Aubin, 2013). Finally, the release of excess nutrients, antibiotics, and chemicals into the surrounding waters can lead to water pollution and eutrophication (Nyberg et al. (2021)). To address these concerns, aquaponic systems, in which fish and plants are grown in a symbiotic closed-loop, and integrated multi-trophic aquaculture (IMTA) systems, consisting in cultivating multiple species from different trophic levels raised in closed proximity (Knowler et al., 2020), offer a potential solution for a more sustainable seafood production. In particular, in IMTA systems, the different incorporated trophic levels improve the efficiency and the ecological balance. This integrated approach allows waste from one species to become a valuable resource for others, helping remove excess nutrients from the water and reducing the risk of water pollution. However, this study focuses on a further innovative Integrated Multi-Trophic Aquaponic system (IMTAcs) that aims to integrate into a traditional aquaponic system (consisting of marine fish and halophytic plants), the rearing of detritivorous filter-feeding organisms (DFOs), such as mussels, clams and polychaetes, in fish wastewater, which contribute to water purification and become fish feed. This innovative approach maximises resource efficiency, reduces water consumption, recycles nutrients and minimises feed input, with a specific focus on the Mediterranean area. Even though the analysis of environmental performance in the aquaculture sector is gaining traction due to its rapid expansion and increasing focus on sustainability policies, further research needs to be conducted, particularly regarding aquaponic or IMTA systems. In this regard, the Life Cycle Assessment (LCA) is considered the most suitable approach to apply. LCA, a standardized

methodology defined by standards ISO 14040 and 14,044 (ISO, 2006a; ISO, 2006b), quantifies the potential environmental impacts on ecosystems, human health, and natural resources caused by products and systems throughout their entire life cycle. In aquaculture context, LCA has been applied to the production of seabass and seabream in the Mediterranean area (Zoli et al., 2023a; Jerbi et al., 2012; Abdou et al., 2017; Briones-Hidrovo et al., 2023); it has also been applied globally in the aquaculture sector (Song et al., 2019; Vázquez-Rowe et al., 2014), as well as to shrimp (Ziegler et al., 2011) and seafood production (Ruiz-Salmón et al., 2021) and capture (Cortés et al., 2022). However, although there are a few studies regarding aquaponic and IMTA systems (Bordignon et al., 2022; Jaeger et al., 2019; Forchino et al., 2017; Ghamkhar et al., 2020; Mendoza Beltran et al., 2017), there is still a lack of knowledge about the actual environmental benefits these systems can bring, especially on a commercial scale. In fact, as reported by Greenfield et al. (2022), very few studies (Boxman et al., 2017; Greenfield et al., 2021; Hollmann, 2017) have focused on commercial-scale aquaponic systems. Commercial aquaponics is still an emerging industry in its early developmental stages, and further studies are needed to guide its development, including from an environmental perspective. For this purpose, a prospective LCA (Cucurachi et al., 2022; Saavedra del Oso et al., 2023) is better suited than a standard LCA. A prospective LCA is defined as an LCA that scales up an emerging technology using likely scenarios (e.g., using expert help, extreme views, learning curves for similar technologies) of future performance at full operational scale (Cucurachi et al., 2018). In more details, a subset of LCA studies and methodologies is further referred to as “ex-ante LCA”, when it aims to assess the impacts of product and service systems that are in an early state of development, not yet commercialised and still emerging (Cucurachi et al., 2018).

The aim of this study is to investigate the environmental performance of the proposed innovative IMTAcs systems through the application of LCA methodology with an ex-ante approach. To this end, two different scenarios were modelled by upscaling experimental results, and their potential environmental impact was defined. The results of this study will be useful to guide the development of this new technology, identify its environmental strengths and weaknesses, and compare the results with existing systems, with particular regard to the Mediterranean technological-environmental context.

2. Materials and methods

2.1. Ex-ante Life Cycle Assessment methodology

The general procedure of conducting a Life Cycle Assessment (LCA) is widely recognized and standardized (ISO-14040, 2006a; ISO-14044, 2006b), and there are numerous handbooks and published guidelines to support it. However, in this study, an “ex-ante LCA” was performed. In this regard, the ex-ante LCA is applied on modelled upscaled future scenarios of emerging technology. The ex ante LCA follows the four phases of a typical LCA: (I) Goal and scope definition, (II) Inventory analysis, (III) Impact assessment, and (IV) Results interpretation. However, it involves additional considerations such as technology readiness level, system scale, and scenario types during goal and scope definition. Inventory analysis requires modelling future scenarios, often relying on predictive methods and diverse data sources. Impact assessment may include scenario techniques, while results interpretation emphasizes uncertainty analysis.

2.2. Description of IMTAcs system and experimental pilot plant

A prototype of the IMTAcs System was realised at the experimental facilities of University of Pisa in Tuscany (Italy). The prototype, outlined in Fig. 1, consisted of five sections.

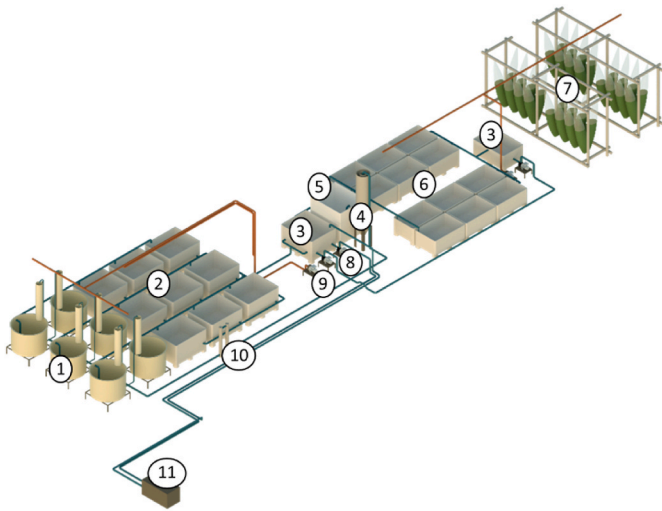


Fig. 1. Schematic diagram of the IMTAcS pilot plant at the University of Pisa (Italy). The different components of the system are listed: (1) fish rearing tank, (2) Polychaetes tanks, (3) sump, (4) sedimenter, (5) biofilter, (6) hydroponics section, (7) microalgae section, (8) pumps, (9) blower, (10) UV steriliser, (11) heat pump.

1. Aquaculture section: this section consists of six 500-L tanks in which Sea Bass (*Dicentrarchus labrax*) and Gilthead Sea Bream (*Sparus aurata*) were reared from a weight of 7 g–350 g, with a maximum density of 31 kg/m³. In the experimental trials, the final output of the system was about 77.5 kg of fish (221 fish). Rossi et al. (2021) have

identified these two species as highly adaptable for this type of system;

2. Storage/filter section: this section consists of nine 500-L tanks in which filter-feeding organisms (polychaetes) were reared, and it collects waste water from the aquaculture section;
3. Hydroponic section: this section included 12 tanks with a total capacity of 300 L in which halophytic plants (*Salicornia europaea* and *Beta vulgaris* subsp. *Maritima*) were cultivated. It also collects waste water from the aquaculture section. Puccinelli et al. (2022) identified these two plant species as the most suitable for saline aquaponics;
4. Microalgae section: this section, separate from the rest of the system, cultivates microalgae *Chlorella* (*Chlorella*) in polyethylene bags, providing an additional source of food for detritivorous-filter organisms. Microalgae cultivation was carried out using run-off from a separate aquaponics cultivation as growing media.
5. Filter/biofilter and recirculation system: this section consists of two tanks (sumps) for water storage and circulation and a nitrifying biofilter (1000 L) filled with 500 L of plastic carriers for bacteria growth.

The entire system was housed in a climate-controlled greenhouse with a surface area of 200 m². The system was equipped with a reversible heat pump to control water temperatures. A daily water replenishment of 0.12 m³ was carried out to compensate for evaporation losses and maintain a constant salinity level (set at 25 ppm).

From March to December of the year 2021, a complete production cycle of gilthead seabream (*Sparus aurata*) was conducted in the IMTAcS experimental plant.

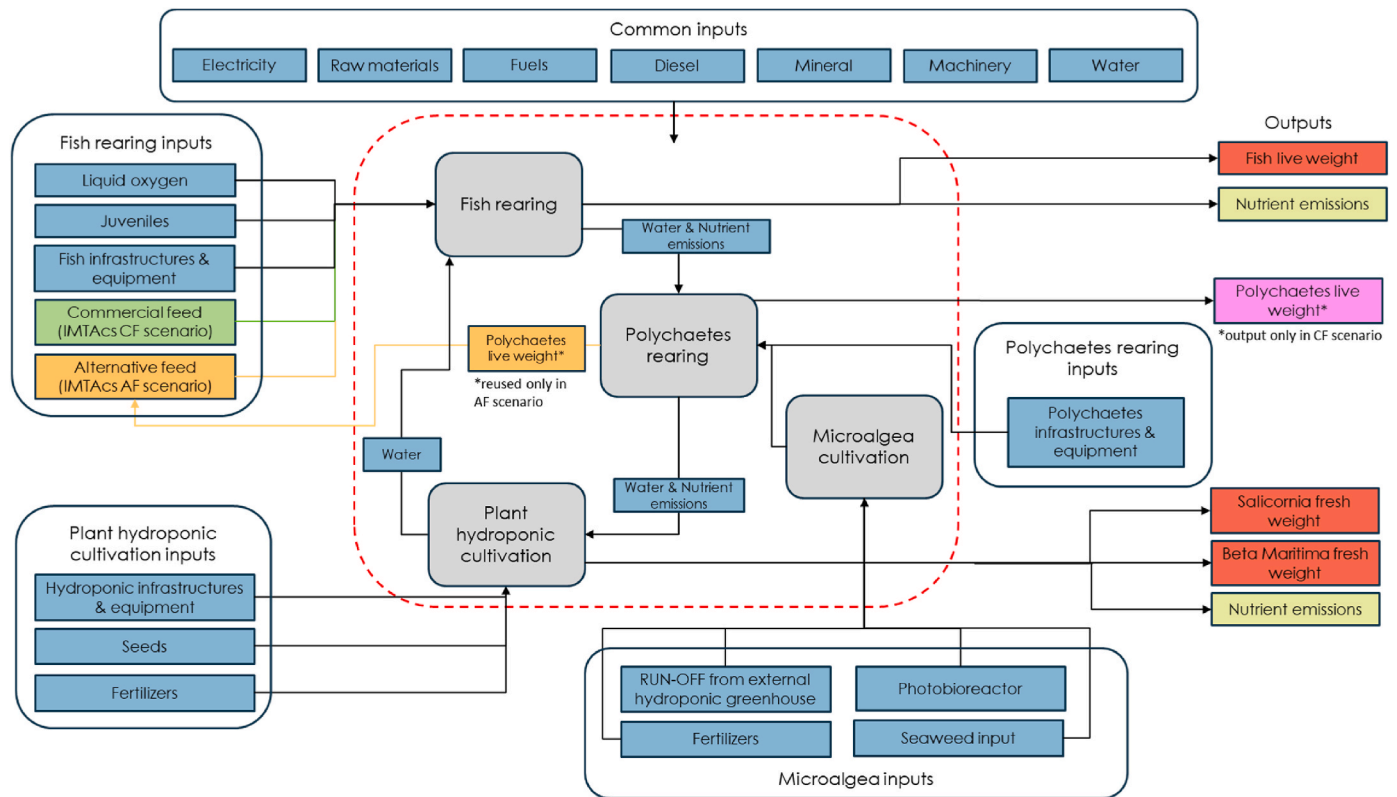


Fig. 2. System boundary schematisation of the study. The red dotted line represents the theoretical system boundaries. Different breeding sections that constitute the IMTAcS system are highlighted in grey. Common inputs for both evaluated scenarios are shown in blue. The commercial feed, considered only in IMTAcS CF scenario, is highlighted in green. The alternative feed, considered only in IMTAcS AF scenario, is highlighted in orange. Product outputs for both scenarios are highlighted in red. Nutrient emissions are highlighted in light green. The output related to polychaetes, included solely in the IMTAcS CF scenario, is highlighted in pink.

2.3. Goal and scope definition

The aim of this ex-ante LCA is to assess the environmental performance of an upscaled innovative IMTAcS system and identify its environmental strengths and areas of concern. To this purpose, two upscaled scenarios at a semi-commercial scale were constructed and analysed, based on the experimental results of a pilot plant. The two analysed different scenarios are distinguished based on the two fish feeding management tested during the experimental trials.

- IMTAcS Alternative Feed (AF): in this trial, the fish were fed with an alternative feed composed of mussels, clams, and self-produced polychaetes.
- IMTAcS Commercial Feed (CF): in this trial, the fish were fed with a commercial feed, and all the system's co-products (fish, DFO, and vegetables) were considered as outputs.

The definition of the inventory data for each scenario was carried out in two steps: first, primary data were obtained from the experimental trials conducted in the pilot plant. Then, based on these primary data and using secondary data (e.g. literature data, estimations and expert judgments), the final inventory of the upscaled scenarios was determined following the method described in section 2.4.3.

In defining the scale of the scenarios, a semi-commercial scale (TRL9, European Commission, 2014) was chosen because the upscaling began at TRL 6, which was the level of the pilot plant. Furthermore, this scale was selected because it falls between the pilot plant, from which some inventory data were obtained, and a fully commercial scale, which is considered less feasible in a limited timeframe. Indeed, for defining the temporal and geographical boundaries of the system, a time frame of no more than 10 years was considered, thus maintaining the current technological context of the Mediterranean region.

The functional unit (FU) chosen for this study is 100 kcal derived from the products of an aquaponic system with a production of 10 tonnes of fish/year. The functional unit serves as a reference unit to which all environmental impacts are linked and it must accurately represent the function of the analysed system. (ISO, 2006a). This selection better represents the food supply function of the IMTAcS system and aligns with the guidelines presented in the FAO document (McLaren et al., 2021). Furthermore, this choice allows for the consideration of all the different output products (fish, vegetables, and polychaetes) of the IMTAcS system and thus manage the multifunctionality of the analysed systems. For the calculation of the kcal produced by system, the following energy contents were considered.

- Fish: 2453 kcal/kg of whole body (Lupatsch et al., 2003);
- Salicornia: 303 kcal/kg (USDA);
- Sea beet: 220 kcal/kg (USDA);
- Polychaetes: 926 kcal/kg (primary data from commercial label).

Using a mass-based functional unit for these outputs would be impractical due to their significant chemical and physical heterogeneity. However, the analysis was also performed with 1 kg of protein produced as FU (see supplementary materials for further details). In this regard, the adoption of these FUs can facilitate comparison with other LCA studies. The system boundaries establish the conceptual limits of an LCA study and are crucial for determining which stages of the product's life cycle are included in the analysis. In this study, a "from cradle to farm gate" approach was adopted, which encompasses all activities from raw material extraction to the harvesting of fish, DFO organisms, and vegetables (Fig. 1). In this study system boundary includes the following processes: extraction, production and supply of raw materials and energy sources, such as minerals, fossil fuels, and lubricant oil; manufacturing, maintenance, and disposal of infrastructure components, including tanks, stands, pumps and filters; production and supply of juvenile fish; production of fish feed, considering agricultural

processes for plant-based ingredients and wild fisheries for marine-based protein, as well as the associated transportation; emissions resulting from the combustion of fuels; emissions related to fish metabolism, particularly nitrogen and phosphorus compounds. However, the production of juveniles for the polychaetes has been excluded from the analysis under the assumption that it is only necessary in the first year of system operation, as they can be self-produced thereafter.

2.4. Life Cycle Inventory

2.4.1. Data from experimental trials

The building of the inventory started from the data collected directly during the experimental tests. From these, the final analysed inventory, presented in the next sections, was then constructed. Primary data obtained from experimental trials mainly concerned the growth performance of organisms included in the IMTAcS system and the production ratio of the different trophic levels (summarized in Table 1).

It is important to underline that the tests between the two different diets did not lead to any differences in fish growth. The fish were raised from a size of 7g to a size of 350g with a maximum stocking density of approx. 30 kg/m³. The cycle was completed in 280 days with an average specific growth rate (Lugert et al., 2016) of 1.40 %/d. Considering the whole period the Feed Conversion Ratio (FCR) calculated on a dry matter basis was 1.4 (for both species). Primary data was also used for formulating the feeds used in the experiments (see Table 2).

From the hydroponic section, the production and nutrient uptake performances of the plants were obtained. Regarding salicornia and sea beet, several experiments have been conducted with a yield ranging from 1.50 to 9.70 kg/m² and 0.96–8.62 kg/m², respectively. The N uptake observed ranged from 0.03 to 0.50 g/m²•d for salicornia and from 0.05 to 0.63 g/m²•d for sea beet. While P uptake ranged from 0.01 to 0.05 g/m²•d for salicornia and from 0.01 to 0.03 g/m²•d for sea beet. As for the polychaetes, they were raised starting from a density of approx. 1400 individuals/m², producing 2.37 g/m²•d in 120 days.

Table 1

Primary data and parameters obtained from experimental tests and used as the basis for creating the final inventory.

| | | |
|--|-----------------------|-------|
| <!--Col Count:3-->Fish | | |
| Juveniles size | g | 7 |
| Harvest size | g | 350 |
| FCR | | 1.4 |
| Cycle duration | days | 280 |
| Specific grow rate | %/d | 1.40 |
| Max rearing density | kg/m ³ | 9.14 |
| Tank volume | m ³ | 12 |
| <i>Salicornia</i> | | |
| Cycle duration | Days | 56 |
| Density cultivation | Plants/m ² | 96 |
| Dry weight | % | 9.5 |
| N content | g/kg dw | 34.5 |
| P content | g/kg dw | 3.11 |
| <i>Sea beet</i> | | |
| Cycle duration | Days | 56 |
| Density cultivation | Plants/m ² | 96 |
| Dry weight | % | 8.34 |
| N content | g/kg dw | 47.45 |
| P content | g/kg dw | 3.68 |
| <i>Polychaetes</i> | | |
| Cycle duration | Days | 75 |
| Rearing density | #/m ² | 3700 |
| Final size | g | 0.5 |
| <i>Energy consumption</i> | | |
| Electricity for pumps and chiller/heater | kWh/m ³ d | 1.75 |

Specific growth rate (SGR) as %/d = 100 X (lnWf - lnWi)/d (Lugert et al., 2016), where Wf is the final body weight; Wi is the initial body weight; d is the observation period as days. Radiation use efficiency (RUE) as g/MJ.d = (Wg/∑GR)/d, where Wg is the weight gain; GR is the cumulative global radiation (MJ); d is the observation period as days.

Table 2

Nutrient characteristics and percentage inclusions of administered feeds (values in % as fed). In the CF scenario, different feeds were used depending on the size of the weights.

| | Commercial feeds | | | | Alternative feed |
|-------------------------------|------------------|--------|---------|--------|------------------|
| | Feed 1 | Feed 2 | Feed 3 | Feed 4 | |
| Fish sizes (g) | 7–35 | 35–125 | 125–250 | >250 | |
| Crude protein | 54.5 | 45.8 | 44.1 | 40.4 | 13.7 |
| Crude lipid | 15.6 | 16.2 | 18.1 | 21.1 | 1.5 |
| Crude fiber | 1.3 | 3.2 | 3.1 | 1.5 | – |
| Ashes | 12.2 | 10.4 | 10.2 | 9.3 | 1.9 |
| Carbohydrates | 10.5 | 19.6 | 19.5 | 20.9 | 4.6 |
| Phosphorus | 2.3 | 1.7 | 1.7 | 1.2 | 0.3 |
| Moisture | 7.5 | 8.0 | 8.3 | 8.4 | 77.8 |
| Ingredient inclusions | | | | | |
| Fish meal | 35 | 16 | 16 | 18 | |
| Fish oil | 8 | 4 | 4 | 5.5 | |
| Poultry by-product meal | | 25 | 20 | 29 | |
| Wheat Gluten meal | 22 | | | | |
| Wheat flour | | 9 | 10 | 21 | |
| Soy protein concentrate | 20 | | | | |
| Rapeseed meal | | 15 | 15 | | |
| Soybean meal | | 13 | 11 | | |
| Canola oil | | 6 | 8.5 | 8.5 | |
| Corn gluten meal | 7 | | | | |
| Pea seed | | | | 7.5 | |
| Blood meal | | 5 | 6 | 5 | |
| Soy lecithin | 5 | | | | |
| Yeast protein | 3 | | | | |
| Bacterial single cell protein | | 3 | 3.5 | | |
| Shrimp hydrolysate paste | | | 3 | 4 | |
| Mono ammonium phosphate | | 1 | 1.5 | 1 | |
| Whey protein isolate | | 0.5 | 1 | 0.5 | |
| Vitamin premix | | 0.5 | 0.5 | 1 | |
| Mussel | | | | 49 | |
| Clams | | | | 30 | |
| Polychaetes | | | | 20 | |

Primary data was also used to model the material composition of the used infrastructure. Fiberglass tanks were used for the fish, hydroponic, and polychaetes tanks. The materials used in the pumps primarily consist of cast iron and steel. PVC pipes were used for connections and the recirculation system. Data for these components were obtained directly from the supplier. Further details can be found in the “Life Cycle Inventory” excel file in Supplementary materials.

Regarding energy demands, in the Pisa system, sensors were installed to measure the energy consumption associated with the reversible heat pump (RHP) to control the temperature of the water (set point used for RHP were 25 °C during winter, heating mode, and 23 °C during summer, cooling mode) and the water pumps. This allowed calculating an energy consumption ranging from 1 to 2.5 kWh/m³•d depending on the season and external climatic conditions.

2.4.2. Scenario design: assumptions and data

Ensuring the availability and accuracy of data is crucial, particularly when considering the future potential and scale-up of the technology. To address data gaps, various strategies were employed, including the use of learning curves, economies of scale, secondary data, and realistic assumptions. Collaborations with technology developers and industry experts played a key role in identifying representative data or alternative data sources.

In order to create the scenario and the final data inventory (Table 3 and 4) the following approach was used, by solving questions.

Table 3

Main parameters and data of the scenarios developed (the entire inventory is given in the supplementary materials).

| | Unit | IMTAcs CF | IMTAcs AF |
|---|-------------------|-----------|---------------------|
| Fish section | | | |
| Annual productivity | tons/y | 10 | 10 |
| Initial fish body weight | g | 7 | 7 |
| Final fish body weight | g | 350 | 350 |
| N. juveniles | # | 28,572 | 28,572 |
| Max rearing density | kg/m ³ | 9.3 | 9.3 |
| N. batches | # | 13 | 13 |
| N. tanks | # | 30 | 30 |
| Tank volume | m ³ | 12 | 12 |
| Average water flow | m ³ /h | 325 | 325 |
| Average daily discharged water | m ³ | 19.2 | 19.2 |
| Liquid oxygen consumption | kg/y | 8866.67 | 8866.67 |
| FCR | | 1.4 | 1.4 |
| Commercial feed, feed 1 | kg/y | 1149.4 | |
| Commercial feed, feed 2 | kg/y | 2609.6 | |
| Commercial feed, feed 3 | kg/y | 4439.4 | |
| Commercial feed, feed 4 | kg/y | 5803 | |
| Alternative feed | kg/y | | 14,000 (dry matter) |
| Hydroponic section | | | |
| Salicornia annual productivity (fresh weight) | tons/y | 106.46 | 106.46 |
| Sea beet annual productivity (fresh weight) | tons/y | 31.46 | 31.46 |
| Surface hydroponic system | m ² | 10,000 | 10,000 |
| N fertilization | kg N/y | 322.39 | 168.11 |
| P fertilization | kg P/y | 0.23 | 7.71 |
| Average annual water volume in the section | m ³ | 2028.12 | 2029.14 |
| Polychaetes section | | | |
| Polychaetes live weight production | tons/y | 12.88 | 12.88 |
| Surface polychaetes rearing | m ² | 1000 | 1000 |
| Average annual water volume in the section | m ³ | 507.52 | 507.52 |
| Fertilization with microalgae | kg/y | 2655.61 | 2177.28 |
| Nutrient emissions flow | | | |
| Net N emissions from the system ^a | kg N/y | 310.37 | 465 |
| Net P emissions from the system ^a | kg P/y | 246.05 | 136.56 |

^a further details on the nitrogen and phosphorous emission flow can be found in the supplementary materials.

Table 4

Environmental impacts in absolute terms of the two IMTAcs scenarios (var % calculated as (AF-CF)/CF).

| | Unit | IMTAcs AF | IMTAcs CF | Δ% |
|--------------------------------|------------------------------------|-----------|-----------|------|
| Global warming (GWP100) | kg CO ₂ eq | 0.234 | 0.207 | 13% |
| Acidification | g SO ₂ eq | 1.143 | 1.115 | 3% |
| Eutrophication | g PO ₄ ³⁻ eq | 1.275 | 1.735 | -27% |
| Land competition | m ² y | 0.114 | 0.088 | 30% |
| Total cumulative energy demand | MJ eq | 5.471 | 4.830 | 13% |
| Net primary production use | kg C | 0.038 | 0.147 | -74% |

1) **What is the size of the farm scenario?** The size of the scenarios has been set as a semi-commercial farm with a target production of 10 tons of fish per year. This assumption is derived from both the use of data from the pilot plant (which is less easily projected onto a fully commercial facility, e.g., producing hundreds of tons of fish) and the fact that the chosen time frame for the commercialization of the new system is rather short (10 years). A larger production of fish would complicate the management of fish metabolism emissions within the

system and require much more labour and difficulty in managing the production and the larger area dedicated to the hydroponic section;

- 2) *What is the balance between the different species and nutrients in the system?* The production ratio between fish and vegetables to optimize the valorisation of the dissolved nutrient from fish was extracted from experimental tests, and reached 1:10 (in line with some previous aquaponic study, Forchino et al. (2017) and Ghamkhar et al. (2020)).

Concerning nutrient flows, starting from the pre-established productivity target, the solid and dissolved emissions of nitrogen and phosphorus nutrients resulting from fish metabolism were estimated using a mass balance-based estimation model (Bureau and Hua, 2010). These emissions depend on the nitrogen and phosphorus content of the feeds administered, their digestibility, and the composition of fry and fish bodies.

From the estimated quantity of dissolved nitrogen, it was assumed that 25% of it would be lost through volatilization (Wongkiew et al., 2017), while the remaining 75% would be assumed to be fully available for plant uptake. Using the uptake rate recorded in experimental trials, the hydroponic system's surface area was estimated to maximize nitrogen absorption efficiency, resulting in 10,000 m². Then, using the yield per surface area from experimental trials, the annual production of salicornia and Sea beet was estimated (106.5 tons and 31.5 tons, respectively).

The growth of polychaetes has been modelled with the thermal-unit growth coefficient (TGC) using the equation proposed by Reid et al. (2020). The calculation of TGC coefficient is calculated according to literature data (Jerónimo et al., 2021) and it is set at 0.33. The uptake of N and P solid have been simulated using a mass balance approach using the coefficients calculated by Honda and Kikuchi (2002).

- 3) *What is the production cycle and schedule of the different species?* The scenarios envision a decoupled system with a production target at 10 tons of fish per year, with a maximum stocking density of 10 kg/m³. The scenarios also involve dividing the fish rearing into 13 batches with a production cycle of approx. 9 months (experimental result) and continuous production. Stocking density is a pivotal factor affecting fish welfare in aquaculture, especially in RASs where high densities are generally used to increase fish productivity (Espinal and Matulić, 2019). However, in general, the recommended fish stocking density is much lower in aquaponics than in RAS; in fact, in integrated systems a high number of batches, is desired because higher stability in the water composition determines greater efficiency in nutrient use by crops. Furthermore, in the case of a smaller number of batches, the risk is a non-overlapping of the two productions (fish and plants) with a consequent greater availability of nutrients from the RAS when the plants are not at their absorption peak. In Goddek and Keesman, 2020 the same assumption (i.e., a high number of batch) for tilapia production was made and the production was scheduled in 13 tanks in cohorts. For the hydroponic section, based on the experimental results, a year production was simulated using salicornia during spring/summer period and sea beet in autumn/winter. In both cases, the duration of the growing cycle is fixed at 56 days with a first harvest after 28 days then following harvests are considered each 14 days.

With regard to the polychaetes section, continuous production was considered, from a size of 0.03 g for 75 days.

- 4) *What is the design of infrastructures?* After establishing the production ratios and targets, the infrastructure design was developed. A maximum of 30 fish tanks of 12 m³ volume each were considered for the fish section. From this, the daily amount of water discharged from the system (approx. 20 m³) was estimated for filter system backwash and discharge to prevent accumulation of inorganic N in

the water. Based on the previously estimated hydroponic and polychaete system surfaces, the necessary infrastructure (tanks, growing media, substrate for polychaetes, pipes for the recirculation system, filters, degassing tower) was estimated (see "Life Cycle Inventory" excel file in supplementary materials);

- 5) *Estimation of consumable needs:* for the fish section, a total hourly recirculation flow of water was assumed (325 m³/h). Having 10 t of fish per year as a production target, this requires 200 kg of fry weighing 7g each reared up to 350 g. For fry modelling, literature data from García García et al. (2019) were used. The amount of feed to be distributed on a daily base was calculated with the equation proposed by Mozes et al. (2011) as a function of water temperature and fish weight using the coefficients proposed by Lupatsch and Kissil (1998). Thus the FCR calculated (1.4) was applied to calculate the consumption of each feed in the scenarios. The estimation of consumed liquid oxygen (8866.7 kg/y) was based on Mozes et al. (2011) and data from databases Ecoinvent V3.8 (Weidema et al., 2013.) were used for modelling.

For the hydroponic section, to meet the target production, a constant water volume of 2029 m³ is required, with an annual total water discharge of 12,516 m³. Additionally, a fertilization rate of 145.1 kg N/year and 57.7 kg P/year was considered in the scenario CF while 218.4 kg N/year and 27.8 kg P/year were considered in the scenario AF, considering both vegetable uptake and discharge of water on a daily basis. The integration of nutrient was calculated as a mass balance between the input from fish, and the output represented by plants uptake and water discharged. In particular, the different diets lead to a different nutrient balance and thus to different nutrient content of the water from the fish. However, with the aim of utilizing the entire plant size, phosphate and nitrogen fertilisation were added to ensure maximum yield in terms of plant production, which is therefore different in the two scenarios.

For the polychaete section, an average water volume of 506.21 m³ was considered, with an annual discharge of 2267.39 m³. As far as Polychaetes are concerned, no inputs were considered for their modelling (with the exception of the infrastructure required for their breeding) since they are self-produced and consumed directly in the system loop.

When considering the required water flow based on data from experimental trials, the energy requirements for water pumping and heating/cooling were estimated at a range of 1–2.5 kWh/m³ d. This energy consumption was applied exclusively to the fish section. In such systems, energy for handling, controlling (oxygenation and temperature) and pumping water for the fish section is the main source of energy consumption. Vegetables and DFFOs have no such requirements (artificial lighting for vegetables was not taken into account) and water is only piped into these sections and then discharged. as it is the section that requires more energy for water circulation, oxygenation, and temperature control.

Finally, about the type of data sources used, background data from databases were used for modelling the impact of feed ingredients, including modelling for bivalves in the alternative feed (AgribalyseV3.1), fertilizers used (Ecoinvent V3.8), hydroponic system seeds (Ecoinvent V3.8), and equipment and infrastructure materials (Ecoinvent V3.8). The Italian national electricity mix from Ecoinvent V3.8 was considered for energy consumption and no uptake of biogenic carbon dioxide was considered.

2.5. Life cycle impact assessment

The conversion of inventory data into different midpoint impact categories (different effects on the environment) was performed both with the CML Baseline method (Guinée, 2002) and Environmental Footprint 3.0 (EF 3.0, Fazio et al., 2018). The CML method is the most widely used method in previous aquaculture studies and allows for easier comparison, however, results are also reported using the EF 3.0

method which has more European up-to-date characterisation factors (results reported in supplementary material).

In more detail, the following impact categories were assessed using the CML Baseline method: global warming potential (GWP), acidification (A), eutrophication (E) and particulate matter formation (PM); land competition (LC) impact category was also added retrieved from CML not baseline. In addition, calculations were performed for the total cumulative energy demand (TCED) and net primary production use (NPPU). TCED represents the energy consumption, measured in MJ, encompassing various energy sources. The calculations were based on lower heating values available in SimaPro® (Frischknecht et al., 2007). NPPU quantifies the amount of carbon (C) utilized in fish production, depleting it as a biotic resource, and is expressed in kg of C (Papatryphon et al., 2004). The carbon content of ingredients originating from terrestrial sources was determined using the carbon content of crops (g C per kg of crop dry matter) as documented by Tyedmers (2001). For fishery-derived raw materials, the carbon content was obtained from the work of Pauly and Christensen (1995).

Using the EF 3.0 method, the following impact categories were analysed: climate change (CC), ozone depletion (OD), photochemical ozone formation (POF), particulate matter formation (PM), human toxicity, not-cancer (HT-nc), human toxicity, cancer (HT-c), acidification (A), freshwater eutrophication (FE), marine eutrophication (ME), terrestrial eutrophication (TE), freshwater ecotoxicity (FEx), land use (LU), water use (WU), resource use, fossils (RF), resource use, minerals and metals (RMM).

2.6. Uncertainty analysis

In an LCA study, the quality of results relies heavily on the quality of data. In this study, the inventory data was built as described in sections 2.4.2, considering both upscaling and ex-ante LCA approaches. For this reason, it is crucial to evaluate the uncertainty of the created inventory and the resulting impacts. Additionally, at the early stages of studying a new technology, the range of results may be wide. Therefore, to provide a clearer understanding of the potential range of environmental impacts for the proposed innovative system, a Monte Carlo analysis was conducted, and the results are presented using a violin plot.

For the uncertainty analysis, the pedigree matrix in Simapro V9.4.0.2 was employed. The pedigree matrix allows for the conversion of qualitative expert judgments into a numerical scale. Following the method suggested by Ciroth et al. (2016), the geometric standard deviation (GSD) was calculated for lognormal distributions (see supplementary materials). The uncertainty analysis was applied to all processes created by the research team, while the pre-defined values were used for background data.

3. Results

3.1. Environmental impacts

Table 1 presents the absolute results of impacts calculated using the CML Baseline method for the two analysed scenarios and their difference in percentage terms per FU. The GWP of the two scenarios is 0.234 kg CO₂ eq for IMTAcS AF and 0.207 for IMTAcS CF, which is 13% lower than the first one. The IMTAcS AF scenario also shows higher impacts in acidification (3%), total cumulative energy demand (13%), and land competition (30%). However, it has lower impacts in eutrophication (-27%) and net primary production use (-74%). All these differences are primarily due to the use of alternative feed.

Eutrophication is lower because the production of ingredients for alternative feed (mussels, clams, and polychaetes) results in a net nutrient absorption, which has a positive effect in this impact category. Similarly, in net primary production use, the impact is lower as these ingredients have a lower trophic level compared to common protein sources used in commercial feed (fish meal and fish oil). The higher

impact of land competition is also attributed to the intensive bivalve farming in this impact category (see Fig. 2).

3.2. Environmental hotspot

Fig. 3 reports the contribution analysis of IMTAcS AF. The graph shows the sub-processes responsible for each analysed impact category and their corresponding contribution percentage (see Fig. 4).

As expected, electricity consumption is the main hotspot. It accounts for nearly 60% of the impact on Global Warming, around 45% of Acidification, and 50% of the Total Cumulative Energy Demand. This underscores the critical aspect of electricity consumption (or more broadly, the substantial energy demand) of these kind of systems. However, it's important to note that since the IMTAcS system is a pilot system, the energy consumption data might be overestimated.

Alternative feed has a limited impact on global warming (approximately 12%), but it is the primary hotspot for land competition (50%) and net primary production use (90% of the impact). The cultivation of mussels and clams at sea significantly impacts land competition due to typical mollusc farming systems. In particular, this impact is due to the use of wooden poles to be anchored in the sea to carry out the traditional sea farming. Additionally, net primary production use is typically tied to feed, although, as seen in the previous paragraph, alternative feed has a lower absolute impact compared to commercial feed in this impact category. The remaining 10% of net primary production use is attributed to fry production (largely influenced by the feed used for fry). Another advantage of alternative feed is its negative impact on eutrophication: mussel and bivalve molluscs farming involves substantial absorption of nitrogen and phosphorus compounds, which has a positive effect in this impact category, mainly due to nitrogen and phosphorus compound emissions in discharged water (about 64% of the impact).

The production of microalgae (including the electricity required for photobioreactor operation and photobioreactor manufacturing) has a significant impact on land competition (41%), while its impact remains below 12% in all other impact categories. Wastewater treatment affects global warming by 7% and eutrophication by 21%, while its impact in other categories never exceeds 9%. The infrastructure and equipment necessary for plant construction and operation contribute 12% to global warming, 10% to acidification, and 11% to total cumulative energy demand. Finally, salicornia and beet seeds, as well as fertilizers (whose use is very limited), have a nearly negligible impact (consistently around 1%).

The results of the contribution analysis exhibit a similar trend in the IMTAcS CF scenario (result not shown), with the only difference being a net impact of commercial feed on the Eutrophication category.

3.3. Uncertainty analysis

Monte Carlo simulation is a computational technique used to understand the uncertainty and variability in a model's outcomes by performing a large number of random simulations. In this study, Monte Carlo simulations are being used to assess the potential variations and uncertainties in the impact assessment of different scenarios. During a Monte Carlo simulation, various input parameters are randomly sampled from their probability distributions (often derived from uncertainties in measurements, assumptions, or other factors) to generate a range of possible outcomes. By repeating this process numerous times, a distribution of possible outcomes is obtained, which can help quantify the uncertainty associated with the results (Fig. 4). Uncertainty analysis qualifies data based on their source, considering uncertainties related to inventory construction and the upscaling of experimental data.

The analysis shows that the differences between the two scenarios are not as distinct. In fact, the distributions of impact values often overlap significantly. Considering that the two systems differ in at least 90% of the Monte Carlo simulations, one has a greater impact than the other, those differ only in the Net Primary Production Use impact

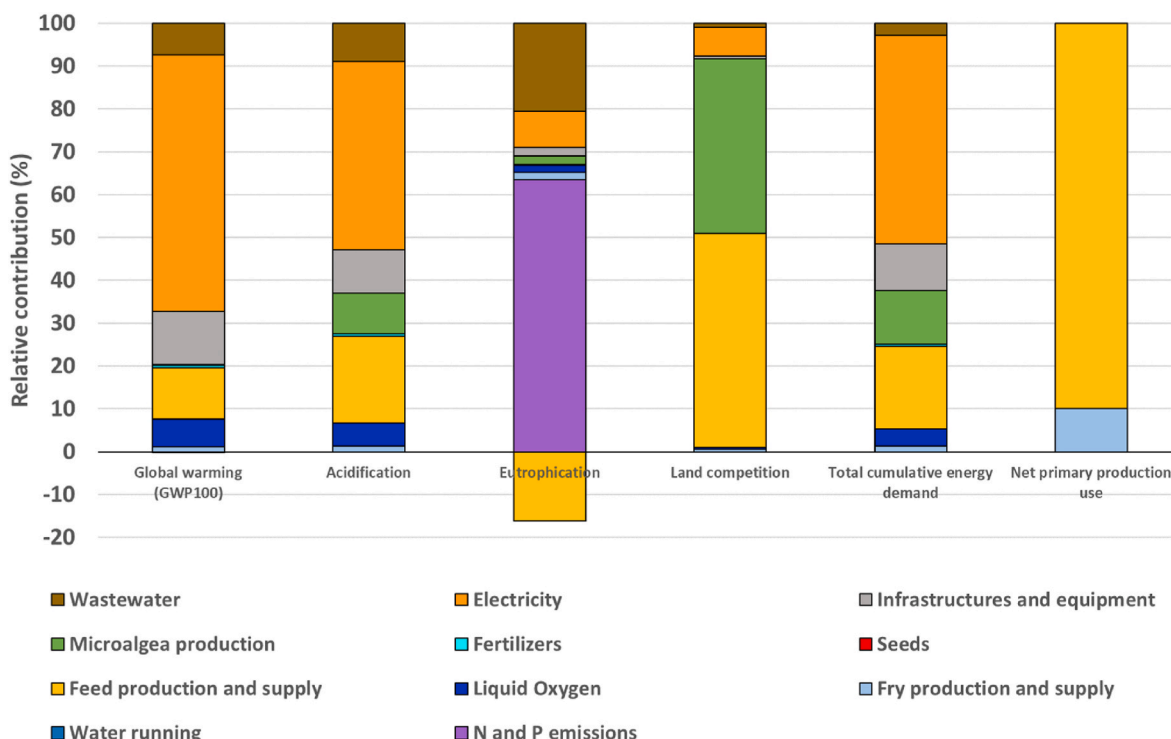


Fig. 3. Contribution analysis of IMTAcS AF scenario per 100 kcal FU. The graph shows the percentage impact share for each sub-process and impact category.

category. This is due to the particular sensitivity of this category to the types of feed used and the impact of the tested alternative feed is significantly lower in this category. In order to compare the results of the uncertainty analysis, a comparison was also made with the Overlap Area of Probability Distributions (PDF) method, described by Mendoza Beltran et al. (2018). The results of the comparison can be consulted in the supplementary materials.

4. Discussion

4.1. Methodological limits and interpretation

When conducting an ex-ante LCA, the lack of inventory data is a common challenge that is often addressed by utilizing secondary data, estimations, assumptions, and scenarios (van der Giesen et al., 2020). The necessity of making these assumptions and the high level of uncertainty associated with modelling choices implies that the outcomes of an ex-ante LCA study should not be treated as a final result but rather as a potential consequence that a technology may have under specific assumptions (Villares et al., 2017). For this reason, it is essential to evaluate not just the specific impact value but also the calculated range while considering data uncertainty.

Furthermore, there is no consensus among LCA practitioners on how to perform upscaling, and different procedures and adjustments may be required depending on the specific case study and the application of the analysed technologies (Tsoy et al., 2020). As suggested by Tsoy et al. (2020), in our study, various experts were involved in the upscaling process, including LCA expert practitioners, engineers, aquaculture technology experts, and animal nutrition experts.

Additionally, the results of upscaling, as well as the results of the environmental analysis, must take into account that at laboratory or experimental plant scales, experiments are typically conducted in batches and are less efficient, resulting in lower yields than typical continuous industrial-scale processes where efficiency gains have been integrated (Frischknecht et al., 2009). Furthermore, the experimental plant in question was constructed with overestimated capacities, hence

not operating at maximum efficiency. Therefore, the scenario results may potentially yield better environmental outcomes with an ex-post analysis of an actual commercial plant. This is especially true when considering that most impact categories are heavily influenced by electricity consumption. For materials, primary data from the experimental plant were considered, and calculations and projections were made based on the quantity required for the upscaling scenario. For these reasons, it is plausible to anticipate that in a future real and optimally optimized commercial plant, overall efficiency would be improved. In fact, as reported by Coulson et al. (1993) and echoed by Fusi et al. (2016) and Whiting and Azapagic (2014), the environmental impact of a larger-sized plant is consistently lower than that of a lab-scale or pilot plant due to the better efficiency between production factors and production yield.

In any case, ex-ante LCA does not predict the future, but it is certainly valuable for exploring the future by analysing a range of possible scenarios that define the space in which emerging technology could develop. Therefore, it should be considered as a tool to provide insights into the environmental performance of this new emerging technology and guide the design and development process toward improving future environmental outcomes.

4.2. Environmental results interpretation

Of the scenarios analysed in this study, neither was found to be environmentally better than the other in an absolute sense. In fact, depending on the impact categories analysed, the IMTAcS AF scenario may be better or worse than the IMTAcS CF scenario. Regarding Global Warming, IMTAcS AF was worse than IMTAcS CF. In this regard, it's crucial to highlight that the impact modelling of alternative feed was conducted based on a traditional bivalve farming system. Consequently, it's not difficult to envision how the farming and transportation of a substantial quantity of this low dry matter feed could influence environmental performance in Global Warming. Nevertheless, through experimental trials, growth performances were tested, and the viability of the alternative diet was examined. Since the results from this

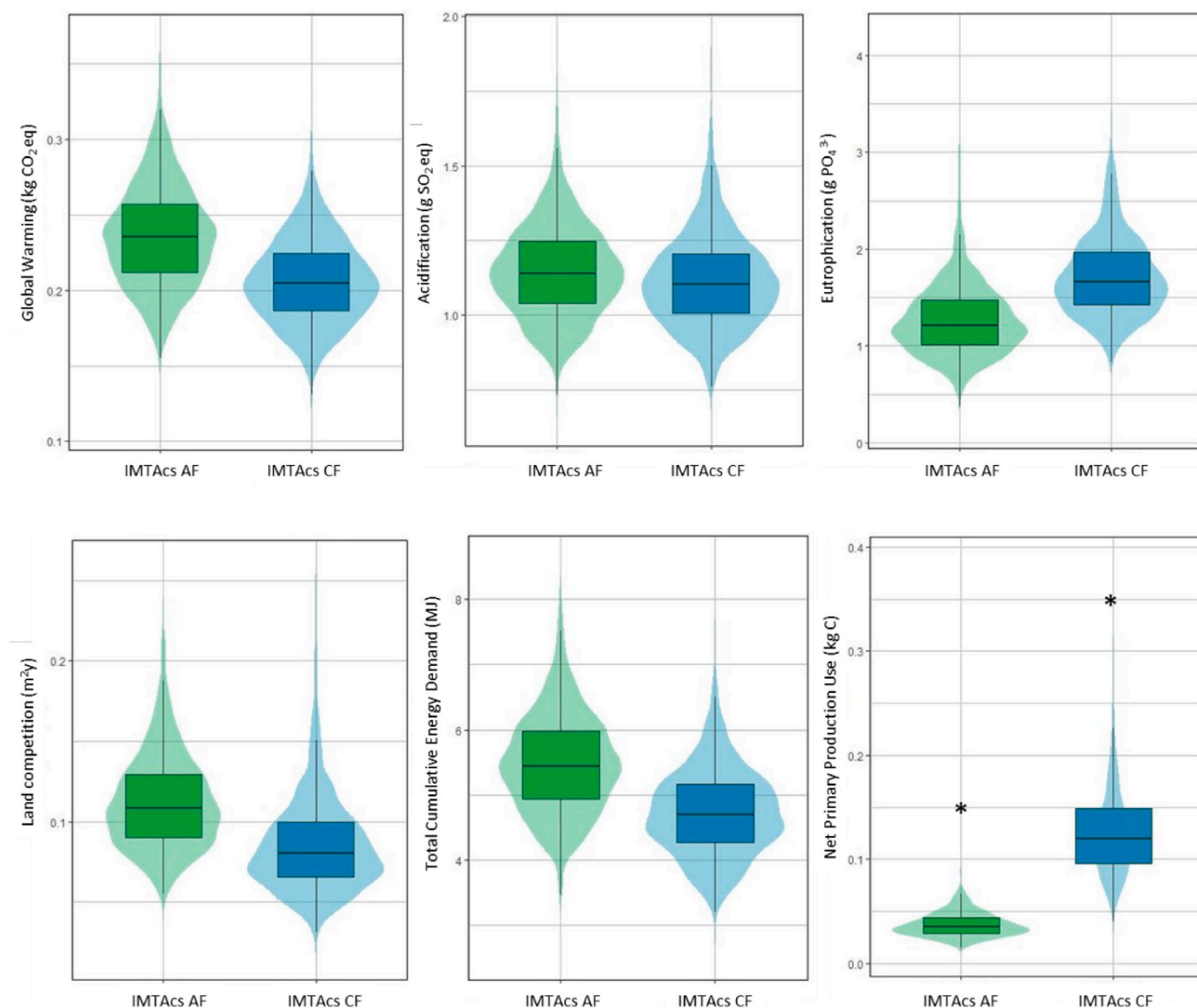


Fig. 4. Violin graphs showing the results of the Monte Carlo analysis simulations with the relative distribution of values. "*" indicates that the two scenarios differ if one scenario is more impactful in at least 90% of the Monte Carlo simulations.

perspective were promising, a potential next step in the IMTAcS system could involve integrating and introducing a new farming section within IMTAcS to self-produce also a portion of bivalves. Nonetheless, the results indicate that a combination of the two scenarios (e.g., farming bivalves and administering the self-produced bivalves and polychaetes, partially replacing commercial feed) could lead to further environmental improvements by leveraging the strengths of both scenarios analysed in this study.

Although to our knowledge, this is the first instance of an LCA study on an aquaponic system reporting results while considering data uncertainty, LCA has already been adopted to analyse certain aquaponic systems (Cohen et al., 2018; Forchino et al., 2017; Jaeger et al., 2019). However, most of these are on a small scale or involve primarily theoretical systems, with only one (Boxman et al., 2017) conducted on a commercial operating system. In the literature-reported studies, infrastructures and fish feed were identified as the primary hotspots within aquaponic systems contributing to their environmental impact. In regions with cold climates like Northern Europe and Europe (Forchino et al., 2017; Körner et al., 2021), the Midwest United States (Ghamkhar et al., 2020; Xie and Rosentrater, 2015), and Canada

(Valappil, 2021), the consumption of energy for heating systems has emerged as a noteworthy factor amplifying the environmental impact. This study aligns more closely with this latter aspect. In fact both energy for system operation and water pumping, as well as energy for maintaining a predetermined temperature range, were considered. This is why energy consumption (specifically electricity in this case) stands as a major hotspot. However, this suggests that if in the future, as is expected, the share of renewable electricity increases, it may also bring environmental benefits in such systems. Regarding infrastructures, even though infrastructure has a relatively minor impact, it is still notable and certainly more pronounced compared to a traditional aquaculture system, following the trend seen in the cited studies.

Another difference from traditional aquaculture systems is the impact of feed. As extensively demonstrated, feeds almost always represent the primary hotspots for impacts related to aquaculture systems across different species and contexts (Aubin, 2013; Bohnes et al., 2019). In this study, the feed contributes significantly less compared to traditional aquaculture systems in terms of Global warming, acidification, eutrophication, and total cumulative energy demand. This is attributed to the excellent Feed Conversion Ratio (FCR) observed in the

experimental trials. However, this result is in line with the few LCA studies in a context similar to IMTAcS. In fact, although with a different fish species (trout), Forchino et al. (2017) and Bordignon et al. (2022) reported that the feed impact share in the aforementioned categories consistently remains between 5% and 19%.

In absolute terms, comparison with other studies in the literature is difficult, mainly due to the different methodological choices made. First of all, the functional unit represents a major challenge to standardise LCA studies in the aquaculture/aquaponic sector. As reported in Zoli et al. (2023b), to facilitate comparisons of different LCA studies in aquacultures and between different species and, as reported in McLaren et al. (2021), to better capture the function of a food-producing system, an energy-based FU should be included in the study. However, so far most studies in the literature used a functional unit based on mass (or produced fish or vegetables, Bhakar et al., 2021; Ghamkhar et al., 2020; Körner et al., 2021), which hampers both the comparison between studies and different species and the understanding of the true function of an aquaponic system, i.e. the co-production of various food sources.

Regarding the comparison with traditional aquaculture systems, especially in Italy, Zoli et al. (2023a) reported a carbon footprint impact of 0.12 kg CO₂ eq per 100 kcal produced (with EF 3.0 method) by an offshore farm of sea bream and sea bass. Therefore, when comparing the results to a commercial and optimized company, the IMTAcS system impact can be more than 50% higher. However, when considering a different impact category, such as eutrophication, IMTAcS shows significantly lower levels of freshwater, marine, and terrestrial eutrophication compared to those reported in the same study, even achieving reductions of up to 100%. This highlights, on the one hand, that there is ample room for improvement and energy optimisation, and, on the other hand, the undeniable beneficial impact that such systems have on eutrophication categories (by recycling nutrients). To explore this further, next steps should involve comparing the results of this study with specific case studies about traditional farming systems for sea bass and sea bream, representative of the Italian region analysed. In this way, it will be possible to compare the studies by standardising the methodological choices so as to obtain a better overview of the ranking that these systems might have with respect to traditional ones, also analysing if and how the LCA results might change depending on the functional unit chosen.

5. Conclusions

This study offers a comprehensive assessment of the environmental performance of an innovative IMTAcS system, focusing on two different diet management approaches. However, in both cases, the results highlighted the importance of energy demand as a primary hotspot in such systems, emphasising the need for strategies to mitigate energy consumption or utilise renewable energy. It is evident that the environmental rank of the two scenarios depends on the specific impact categories, emphasising the need for a holistic approach to environmental optimisation. This suggests further refinement and optimisation of IMTAcS are essential to realise their full environmental potential. Furthermore, the study highlighted the potential of IMTAcS to reduce eutrophication impacts in an absolute sense by recycling water and nutrients compared to traditional aquaculture systems.

Conducting an ex-ante LCA thus led to the a priori identification of the main environmental constraints and this could guide construction and plant choices in order to develop a more environmentally sustainable system. However, future research directions could include exploring further scenarios or analysing a full-scale system inventory and conducting comparative analyses with traditional aquaculture systems to provide a more environmental overview. In addition, methodological considerations, such as evaluating the results using different functional units, should be carefully addressed to improve the robustness of the results.

Finally, holistic sustainability assessments including economic and

social dimensions could be further steps in the analysis, also with a view to guiding decision-making in aquaculture and food production in the future.

CRedit authorship contribution statement

Michele Zoli: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Conceptualization. **Lorenzo Rossi:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Jacopo Bacenetti:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Joël Aubin:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

On behalf of all the Authors.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2024.122327>.

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