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1 **The potential of floating macrophytes as feed and phytoremediation resources to improve**  
2 **the environmental performance of giant gourami production in Indonesia: A Life Cycle**  
3 **Assessment**

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15 **Abstract**

16 Over the past few decades, Indonesian freshwater aquaculture has intensified, raising many  
17 issues on the associated environmental impacts. In this study, a life cycle assessment (LCA)  
18 approach was used to assess the environmental performance of Indonesian giant gourami  
19 (*Osphronemus goramy*) aquaculture. We compared two contrasting fish production systems: a  
20 traditional monoculture (MoC) or a co-culture with the macrophyte *Azolla filiculoides* used as  
21 feed alternative to compounded pellets (CoC). Seven impact categories were considered: climate  
22 change, eutrophication, cumulative energy demand, land occupation, acidification, net primary  
23 production use (NPPU) and water dependence. The potential impacts were calculated in terms of  
24 1 kg of fish produced by the systems and were also expressed in terms of surface area (m<sup>2</sup> of the  
25 farm). Monte Carlo analysis was carried out to evaluate uncertainties in the assessed impacts  
26 based on data uncertainties for the systems. Results highlighted notable differences between the  
27 two production systems. CoC led to less impact relative to climate change, eutrophication and  
28 NPPU per kg of fish produced. These differences were even greater when reporting the impacts  
29 per surface area. Nonetheless, the main contributors of the seven impact categories remained  
30 constant between the two production systems (CoC and MoC). Overall, our findings suggested  
31 that using floating macrophytes as alternative feed resources, even in relatively low proportions,  
32 can improve the environmental performance of giant gourami production.

33 **Keywords:** Agroecology, Asia, Aquaculture, Freshwater, LCA

## 34 **1. Introduction**

35 With a production of 4.3 million tons of farmed fish in 2020, Indonesia is the third-largest fish  
36 aquaculture producer country in the world (FAO, 2022a). Since the government made  
37 aquaculture development a national priority in 2009 (Phillips et al., 2015; Rimmer et al., 2013),  
38 fish farming has shown the most rapid expansion of all Indonesian agronomic production  
39 sectors, with an average annual growth of 9% over the last decade (FAO, 2022b). This sector  
40 indeed plays a significant role in the national economy, ensuring food availability and security  
41 and improving living standards for rural communities (Rimmer et al., 2013). Excluding seaweed,  
42 freshwater fish farming represents 66% of aquaculture production in Indonesia (FAO, 2022b)  
43 and relies mainly on small-scale farms, which represent 90% of all fish farms (Maskur et al.,  
44 2013). Small-scale fish farmers usually practice aquaculture using low-input and low-level  
45 technology (Pouil et al., 2019). Nevertheless, the increasing demand for freshwater aquaculture  
46 products has driven a shift from low-intensive traditional culture methods to semi-intensive  
47 monoculture production systems. This intensification was accompanied by an increase in the use  
48 of commercial fish pellets (Edwards, 2015; Rimmer et al., 2013), involving a decrease in the  
49 proportion of non-fed Asian aquaculture by a factor of two since the 2000s (excluding China;  
50 FAO, 2022b). Such changes raise many issues on the actual effectiveness of current production  
51 systems regarding the use of resources and the associated negative environmental impacts. These  
52 negative impacts are further reinforced by the large amounts of solid effluents (faeces, feed  
53 wastes) and dissolved nutrients (waste products) discharged resulting from the use of  
54 commercial feeds, which contribute to eutrophication of wild aquatic ecosystems. Several  
55 freshwater reservoirs in Java, where fed fish farming has proliferated, experienced water quality  
56 degradations leading to the occurrence of disease outbreaks and fish death (Rimmer et al., 2013).

57 Indonesian aquaculture must become more sustainable to meet the national demand for  
58 aquaculture products and also limit their environmental footprint. Henriksson et al. (2019)  
59 evaluated possible interventions and innovations to mitigate environmental impacts related to the  
60 growth of Indonesian aquaculture. The authors found that improving feed efficiency for white  
61 leg shrimp, common carp and tilapia by 20% witnessed major reductions in environmental  
62 impacts. Such interventions imply the design of aquaculture systems that use fewer chemical  
63 products and commercial feeds, more renewable resources, and make the best use of ecosystem  
64 services without impairing their regeneration. Such systems can be called "ecologically  
65 intensive" (Griffon, 2010), having a high level of production per surface area but using  
66 ecological processes as much as possible to optimize natural resource use and develop ecosystem  
67 services (Bonny, 2011). In recent years, a growing effort has been made worldwide to implement  
68 ecological intensification in aquaculture (Aubin et al., 2019), particularly by increasing the  
69 complexity of rearing systems using different complementary species as in integrated multi-  
70 trophic aquaculture (Jaeger and Aubin, 2018). However, the proof of concept must be enhanced,  
71 and comparative studies of different production systems for the same species remain scarce.

72 Among the fish species reared in Indonesia, giant gourami *Osphronemus goramy* is one of the  
73 primary freshwater commodities of economic importance due to its high price and local demand  
74 (FAO, 2019). With its propensity to consume plant-based feeds, giant gourami is a valuable  
75 candidate for low-input aquaculture (FAO, 2019) and for integrating fish production and plant  
76 culture. Giant gourami production in Indonesia is mainly located on Java Island (79% of the  
77 national giant gourami production; BPS, 2013) and is strongly segmented (FAO, 2019). Rearing  
78 takes place in ponds exclusively dedicated to giant gourami or shared with other species  
79 (Kristanto et al., 2020). Slembrouck et al. (2018) evaluated *Azolla* as the best candidate among

80 five floating macrophytes species for the ecological intensification of small-scale fish farming in  
81 tropical areas. Recently, a study evaluated the feasibility of combining the production of the  
82 floating macrophyte *Azolla filiculoides* in giant gourami ponds (Caruso et al., 2021), with the  
83 macrophytes being used as an alternative fish feed. Compared with those from a conventional  
84 giant gourami monoculture system exclusively based on exogenous commercial feeds (Caruso et  
85 al., 2021), the use of *Azolla* proved to be economically advantageous by reducing the amount of  
86 commercial pellets used while maintaining a similar growth rate of the fish and biomass gain at  
87 the end of the trial. However, this study was not dealing with the environmental impacts of using  
88 such alternative production system compared to the conventional monoculture production.

89 A holistic approach is needed when considering the environmental impacts of production  
90 practices, and life cycle assessment (LCA) is considered a suitable tool for analysing aquaculture  
91 production systems (for review, see Bohnes et al., 2019). LCA is an international standardized  
92 method (ISO, 2006) designed to evaluate the global impact of a product or a process on the  
93 environment. Such evaluation implies the assessment of all the phases required for or caused by  
94 the product's existence; they include raw material uses and energy production, manufacturing,  
95 transport and emissions released into the environment at each stage until the product's end of life.  
96 Over the last two decades, LCA has been regularly used to assess the environmental impacts of  
97 aquaculture production (e.g., Aubin et al., 2006; Medeiros et al., 2017; Mungkung et al., 2013;  
98 Yacout et al., 2016). Several environmental impact categories are regularly used to assess  
99 aquaculture systems (Aubin et al., 2006; Bohnes et al., 2019; Henriksson et al., 2012;  
100 Papatryphon et al., 2004). One advantage of LCA is that it allows different products or systems  
101 to be compared using the same functional unit. Despite the usefulness of LCA, there are various

102 limitations to its application. One of the critical challenges is the necessity to incorporate  
103 uncertainty in the calculations to produce more robust outcomes (Mendoza Beltran et al., 2018).

104 The present study aimed to assess the potential benefits of ecological intensification in  
105 Indonesian fish farming. Thus, our objectives were (1) to evaluate the environmental  
106 performances of two production systems of giant gourami aquaculture, with or without the  
107 integration of a co-cultured floating macrophyte (*A. filiculoides*) and (2) to identify the sources  
108 of uncertainties in the environmental impacts. We hypothesized that the use of *Azolla* as an  
109 alternative feed should reduce net primary production use, energy demand and climate change  
110 impacts by partially substituting commercial feeds while decreasing eutrophication by improving  
111 nutrient recycling (Caruso et al., 2021).

## 112 **2. Materials and Methods**

### 113 2.1. Description of the production system

#### 114 2.1.1. Giant gourami production system

115 Although the giant gourami production cycle is not well standardized in Indonesia (Kristanto et  
116 al., 2020), its propagation relies on the natural spawning of captive brooders in ponds and  
117 includes the seven following production segments:

- 118 1) The hatchery phase lasts 10 days from egg incubation to 8-9 days after hatching.  
119 Larvae are kept unfed during this period.
- 120 2) The nursery phase lasts until juveniles reach  $2 \pm 0.5$  cm of length (40 days; Prakoso et  
121 al., 2019). Tubifex (*Tubifex tubifex*) worms, usually purchased on local markets, are  
122 the first and primary food provided during larval rearing (i.e., nursery phase).

- 123 3) The pre-growing I stage lasts 150 days until juveniles reach 30 g. Commercial feed  
124 (~33% of crude protein) is introduced at the beginning of this segment.
- 125 4) The pre-growing II stage, until juveniles reach 180 g (from the "jingo" to the  
126 "dampal" stage), is the first stage of the production cycle during which fish are able to  
127 eat plant materials (FAO, 2019). This production stage lasts 160 days.
- 128 5) The growing phase lasts 120 days. During this phase, fish are fed with commercial  
129 pellets and plant materials (mainly giant taro *Alocasia macrorrhizos* leaves). At the  
130 end of this production phase, fish of  $\leq 500$  g are sold, and the biggest fish are selected  
131 as future brooders.
- 132 6) Brooder selection lasts 210 days, where fish of  $\geq 2000$  g are sorted by sex and  
133 separated. Plant materials become the predominant feed at this stage, and commercial  
134 feeds are used as supplements.
- 135 7) The egg production period is continuous throughout the year. The average weight of  
136 brooders used for egg production is 3.6 kg, and they are usually kept for 10 years of  
137 production.

138 Note: all the values used in our analysis are average values gathered from surveys of  
139 approximately 40 giant gourami farms (Kristanto et al., 2020).

140 Except for hatchery and nursery phases, usually performed in plastic basins and wooden tanks  
141 covered by tarpaulin filled with stagnant water, all the production phases are performed in flow-  
142 through systems, mainly in earthen ponds of 300-1000 m<sup>2</sup> in size with gravity-flow water supply  
143 system from local reservoirs or upstream aquaculture farms. The production chain for giant  
144 gourami is shown in Figure 1.



### 145 2.1.2. Scenarios of production: experiment details and records

146 The system under study consisted of a typical small-scale Javanese polyculture fish farm where  
147 farmers can quickly shift from one species to another to better adapt to the market and the  
148 difficulties encountered. Kristanto et al. (2020) underlined that giant gourami is mainly produced  
149 by farmers producing other species in variable proportions. Thus, only about 50% of the surface  
150 is dedicated to giant gourami rearing. The production capacity is about 2 tons per year. All the  
151 production is sold on local markets (< 10 km of the production site). The farm included in this  
152 study is located in Bogor district, West Java, in the village of Babakan (-6°28'S; 106°42'E; alt:  
153 125 m). The farm size (i.e., 2100 m<sup>2</sup>) is in the range typically observed locally (Kristanto et al.,  
154 2020). While a non-negligible number of aquaculture farms are located in the peri-urban area in  
155 West Java (see Kristanto et al. (2020) for details), our study site is mainly bordered by rice  
156 fields.

157 The studied production cycle, which lasts 160 days (i.e., two production cycles per year),  
158 consisted in rearing giant gourami from 30 to 180 g of average fresh weight (i.e., from the  
159 "jingo" stage to the "dampal" stage). This phase corresponds to the pre-growing stage II  
160 described in section 2.1.1 (Figure 1). "Jingo" fish were purchased from the neighbourhood and  
161 were stocked at a density of 6 fish m<sup>-2</sup>.

162 The production system consisted of four earthen ponds, whose size varied between 353 and 482  
163 m<sup>2</sup> with depths ranging from 0.46 to 0.55 m. Each pond was equipped with PVC pipes for inlet  
164 and outlet water; anti-predator nets held by bamboo poles were placed 1 m from the banks.  
165 Ponds connected in series were filled by gravity with water from a reservoir located at <1 km  
166 from the farm. Before production, ponds were dried; the bottom was levelled and the banks were

167 consolidated with sediment accumulated during the previous production cycle. Then, unwanted  
168 species were removed and the water, transported by gravity from a reservoir located at <1 km  
169 from the farm, was limed and fertilized using organic fertilizer (typical composition: poultry  
170 manure mixed with rice bran at a 0.5:1 ratio on a dry weight basis; Purnomo et al., 2017).  
171 Production takes place in open-flow water (average water renewal 4-8% d<sup>-1</sup>).

172 Traditionally, giant gourami are reared in a monoculture system (MoC). To evaluate the  
173 possibilities of using floating macrophytes for ecological intensification of tropical freshwater  
174 fish aquaculture, as suggested by Slembrouck et al. (2018), an experiment was carried out  
175 throughout one 160-day production cycle in 2018 to test a new giant gourami rearing system  
176 based on co-culture with *Azolla* (CoC). Here, the two production systems (CoC and MoC) were  
177 operated as described below.

178 The MoC system fed fish exclusively on commercial extruded pellets of known composition  
179 (Table 2). According to fish growth, the daily ration was 3 to 5% of the estimated wet biomass  
180 which is slightly higher than what is usually done by farmers (FAO, 2019). The final feed  
181 conversion ratio (FCR) reached 2.8. Fertilizer was regularly added to the ponds (equiv. 6 kg m<sup>-2</sup>  
182 per year) to stimulate primary productivity. The annual fish production volumes reached 2 kg m<sup>-2</sup>  
183 in this production system, and mortality was 8% per production cycle.

184 In the CoC system, *Azolla* was cultivated in the space available between the banks and the anti-  
185 predator net (80 m<sup>2</sup> of production surface) (Figure 2) in earthen ponds managed as described  
186 above with slightly higher use of fertilizer than in the MoC system (i.e., 7.5 kg m<sup>-2</sup> an<sup>-1</sup>). Fish  
187 were fed for two days with extruded pellets as described above, then one day with *Azolla* at a  
188 food ration of 8% of the estimated fish biomass. Such rationing corresponds to the amount of

189 *Azolla* the fish can ingest within 24h. *Azolla*, coming from an initial stock maintained in two 33  
190 m<sup>2</sup> tarpaulin tanks, was stocked at an initial concentration of 400 g m<sup>-2</sup>, and *Azolla* growth was  
191 checked every three days (before distribution to the fish). *Azolla* was regularly sampled for  
192 chemical analyses (Table 2). During our experiment, stable production of *Azolla* was not met as  
193 initially planned, and its production regularly collapsed in the ponds. During the 160-day fish  
194 production cycle, only two cycles of *Azolla* production occurred for a total of 40 days of  
195 production. A complementary experiment was performed to understand better the limiting  
196 factors in the mass production of *Azolla* in ponds (Pouil et al., 2020). That experiment suggested  
197 that phosphorus (P) was likely the main limiting factor in *Azolla* production, with an average of  
198 0.4 μmol L<sup>-1</sup> measured in the ponds, with the optimal growth occurring at a concentration of 2.0  
199 μmol L<sup>-1</sup>. In addition, the occurrence of grazing snails (3-4 adult individuals m<sup>-2</sup>) may reduce  
200 *Azolla* production by 35% (Pouil et al., 2020). Thus, we built a scenario based on the  
201 extrapolation of the data (i.e., zootechnical and economic performance, chemical analyses)  
202 collected when co-culture was effective (i.e., 40 days) for the entire 160-day production period,  
203 and uncertainty related to the production of *Azolla* was included in the uncertainty analysis (see  
204 Section 2.6). The mortality and the annual fish production volume were comparable to the MoC  
205 system (i.e., 2 kg m<sup>-2</sup>), and the FCR reached 2.1 given the average reduction of ~25% of the  
206 extruded pellets used.

## 207 2.2. Assessment methodology

### 208 2.2.1. Goal and scope

209 This LCA study followed ISO standards (ISO, 2006) and aimed at evaluating the environmental  
210 relevance of ecological intensification of giant gourami culture by integrating a co-culture of

211 *Azolla*, which may provide the ecological services of water depuration and provision of  
212 complementary feed for fish. Environmental performances of earthen pond-based production of  
213 giant gourami aquaculture were evaluated through: (1) a traditional semi-intensified monoculture  
214 system as used in West Java province (MoC), (2) a co-culture production system including  
215 *Azolla* (CoC). This study is based on experimental results designed to compare the efficiency of  
216 the two rearing systems. The results were expressed per kg of fresh fish and m<sup>2</sup> of surface area.  
217 As an additional information, results expressed per kg of protein are also available in the  
218 Supplementary Material (Table S1).

219 The system boundaries used in this study were from "cradle to farm gate" (Figure 2). They  
220 included the production of capital goods (infrastructure and equipment), the production of  
221 organic fertilizers and lime, the production of fish feed (including the production of ingredients  
222 from agriculture or fishery sectors), the production of fish juveniles, farm operations (including  
223 the local emission of nutrients and pollutants), the use of energy sources, and the transportation  
224 of critical inputs (juveniles, fish feed, equipment), and the transport of fish from the farm gate to  
225 the local market by motorcycle or small trucks.

#### 226 2.2.2. Life cycle inventory

227 Inventory data for giant gourami fingerling production and brooder management were compiled  
228 mainly by collecting the data from fish farms and production centres producing giant gourami  
229 fry located in the Bogor and Tasikmalaya regencies (n=37, Kristanto et al., 2020). We also used  
230 experimental data from the experiments we performed on giant gourami egg production in the  
231 BPPSIGN Center, Tasikmalaya (Arifin et al. 2020; Slembrouck et al., 2019, 2020) and larval  
232 rearing in the RIFAFE, Cijeruk (Arifin et al., 2019; Prakoso et al., 2021).

233 The inventory of the experimental phase was recorded during daily on-site monitoring of one  
234 entire production cycle from November 2017 to April 2018. The descriptive data concerning the  
235 studied sub-system are summarized in Table 1. The inventory covered farm operations, as well  
236 as the following processes: production of farm inputs such as energy carriers, fertilizers,  
237 juvenile, exogenous feeds (including ingredient production and processing), and farm equipment  
238 and infrastructures (i.e., equipment; construction of buildings and breeding infrastructures) and  
239 transportation at all stages. Water flow at the outlet of each pond was measured daily to estimate  
240 the water balance. The fate of nitrogen (N) and phosphorus (P) was based on a nutrient-balance  
241 experiment performed on-site involving chemical analysis of fish, fertilizers, feeds, inlet and  
242 outlet water, water and sediment (Pouil et al., 2019). Data for pond gaseous emissions were  
243 adapted from the literature. Gaseous fluxes are influenced significantly by factors such as  
244 drainage, primary productivity, and the availability of organic matter in aquaculture ponds (Yang  
245 et al., 2018). It is important to note that fish production plays a pivotal role in shaping these  
246 variables, especially in the context of our pond production systems, where the absence of  
247 drainage, the organic fertilization practice, and the provision of commercial feed are key  
248 determinants. Denitrification and ammonia volatilization were assumed to be equivalent to  
249 17.4% and 12.5% of the N inputs, respectively as found in channel catfish ponds (Gross et al.,  
250 2000). Values for methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) emissions were extracted from Yang  
251 et al. (2018) who measured fluxes in semi-intensive shrimp ponds: 3.09 mg m<sup>-2</sup> h<sup>-1</sup> and 0.5 μg m<sup>-2</sup>  
252 h<sup>-1</sup> for CH<sub>4</sub> and N<sub>2</sub>O emissions, and carbon dioxide (CO<sub>2</sub>) fixation in pond sediment was  
253 evaluated at 21.56 mg m<sup>-2</sup> h<sup>-1</sup> (Yang et al., 2018).

254 Background data (i.e., construction materials, fuel, rearing equipment) were based on the  
255 ecoinvent v3.8 database (Wernet et al., 2016) and adapted to the local context. The inventory

256 data for Indonesian electricity production were obtained from EIA (2021). Data on feed  
257 production were collected based on detailed reports supplied by the feed manufacturer in which  
258 all feed ingredients were quantified while proximate analysis were performed to determine  
259 nutritional composition (Table 2). The feed formulations were then validated regarding  
260 nutritional constraints according to Guillaume et al. (1999). Data on feed processing were  
261 supplemented with detailed information from a local feed producer. The inventory data for local  
262 fishmeal production (discarded fish) was collected from a local fishery processing plant  
263 dedicated to frozen fish (for export), surimi and fishmeal (Palembang, Sumatra Island). Heat  
264 energy from wood wastes was used for fishmeal processing (Mungkung et al., 2013). Inventories  
265 for crop-based feedstuffs were adapted from the ECOALIM dataset (Wilfart et al., 2016) to  
266 consider transportation to Indonesia. Distances for road and boat transport were estimated using  
267 Google Maps®. For organic fertilizer, we used the inventory for poultry manure on the global  
268 market available in the ecoinvent v3.8 database while inventory of rice produced in China, from  
269 the same database, was used for rice bran considering a mass allocation (Ayer et al., 2007).

### 270 2.2.3. Life cycle impact assessment

271 Midpoint impact categories were selected based on previous studies and recommendations for  
272 aquaculture LCA (Aubin et al., 2009; Bohnes et al., 2019; Papatryphon et al., 2004; Wilfart et  
273 al., 2013):

- 274 • acidification (mol H<sup>+</sup> eq), which aggregates substances that release hydrogen ions into  
275 the ecosystem;
- 276 • cumulative energy demand (CED; MJ), which aggregates the energy used from  
277 renewable and non-renewable sources;

- 278 • climate change (kg CO<sub>2</sub> eq), which aggregates greenhouse gas emissions;
- 279 • eutrophication (kg PO<sub>4</sub><sup>3-</sup> eq), aggregating nutrient emissions that lead to oxygen  
280 depletion, impacting aquatic and terrestrial environments and adding the theoretical  
281 oxygen demand (ThOD) calculation for solid wastes from fish farms (Aubin et al., 2009);
- 282 • land occupation (m<sup>2</sup> yr) which represents the terrestrial ground area used;
- 283 • net primary production use (NPPU, kg C) refers to the use of a net primary producer as a  
284 biotic resource in the sense of being unavailable for other purposes, as defined by  
285 Papatryphon et al. (2004);
- 286 • water dependence (m<sup>3</sup>) included the volume of water used in ponds as proposed for  
287 aquaculture production systems by Aubin et al. (2009) and the amount of water used in  
288 the other production phases.

289 The impact assessment was conducted according to (1) the International Reference Life Cycle  
290 Data System (ILCD) method for acidification and climate change, (2) the CML IA method,  
291 version 3.06 for eutrophication potential, and land occupation. Total energy use was calculated  
292 using the Cumulative Energy Demand method, version 1.10. Calculations were made using  
293 SimaPro software 8.5.4.0 (PréConsultants, Amersfoort, The Netherlands). As mentioned earlier,  
294 the ecoinvent version 3.8 database was used for background data, and the Agribalyse® database  
295 (Koch and Salou, 2014) was used for agricultural machinery, agricultural products and feed  
296 ingredients (ECOALIM dataset; Wilfart et al., 2016). For comparison purpose, we also  
297 performed the impact assessment using ReCiPe 2016 Midpoint (H) version 1.08 (Table S2 in the  
298 Supplementary Material).

## 299 2.6. Uncertainty analysis

300 The process for determining the uncertainty in the production systems was adapted for primary  
301 and secondary data. Among the primary data, the most important source of uncertainties in the  
302 CoC system was the production of *Azolla*. Considering the risk of predation and the variation in  
303 *Azolla* productivity due to the environmental conditions (light, nutrients) observed in ponds  
304 (Pouil et al., 2020), an uncertainty rate of 50% was applied in *Azolla* used as alternative feed in  
305 this production system. For other primary data (i.e., other inputs used and fish harvested), the  
306 standard deviation of the replicates was used in a normal distribution, as suggested by Medeiros  
307 et al. (2017). For secondary data, the pedigree matrix from Ciroth et al. (2016) was used to  
308 quantify uncertainty in the values selected from the literature (i.e. ThOD, N volatilization and  
309 denitrification and fluxes of CH<sub>4</sub>, CO<sub>2</sub> and N<sub>2</sub>O). We applied a lognormal distribution to the  
310 results generated from the matrix. Monte Carlo (MC) analysis was performed in SimaPro  
311 software 8.5.4.0.

### 312 2.7. Data analysis

313 To compare the CoC and MoC systems while considering uncertainty, we ran 1000 interactions  
314 in pairs in an MC analysis. This method indicated the number of times one system had higher  
315 impacts than the other. We considered that the two systems differed if one had higher impacts in  
316 at least 90% of the runs (Medeiros et al., 2017).

## 317 3. Results

318 Figure 3 presents the LCA impacts by category calculated per kg of fish produced (Figure 3A) or  
319 by m<sup>2</sup> of the farm (Figure 3B) for the CoC and MoC giant gourami production systems. Overall,  
320 we found similar trends regardless of the functional units chosen (i.e., product-based or surface-



321 based). Thus, values presented in the following sections are first provided by kg of fresh fish;  
322 values expressed by  $\text{m}^2$  are also given in Table 3.

### 323 3.1. Impact assessment

324 Among the impact categories selected, the MoC system had the highest impacts for all of them  
325 except land occupation (Figure 3 and Table 3), with  $4.7 \pm 0.2 \text{ m}^2 \text{ yr}^{-1}$  being required to produce 1  
326 kg of fish in MoC and  $4.8 \pm 0.3 \text{ m}^2 \text{ yr}^{-1}$  in CoC. The highest differences between CoC and MoC  
327 were for NPPU (23-27% less in CoC): the production of 1 kg of fish required  $13.8 \pm 1.3 \text{ kg C}$  in  
328 MoC, but the NPPU value of the CoC system was noticeably lower with  $9.1 \pm 0.9 \text{ kg C}$  (i.e.  
329 ~25% less NPPU required in CoC). The second largest differences in environmental impacts  
330 between the two production systems were for water dependence (12-17% less in CoC) with  
331 values of  $10.1 \pm 3.5 \text{ m}^3$  and  $8.7 \pm 1.7 \text{ m}^3$  in MoC and CoC, respectively. Similarly, implementing  
332 the CoC system reduced eutrophication (i.e., 11-16% less in CoC). Climate change showed a  
333 reduction of 10-15% in the CoC with, per kg of fish produced,  $6.4 \pm 0.3 \text{ kg of CO}_2 \text{ eq}$  compared  
334 with MoC where  $7.1 \pm 0.1 \text{ kg CO}_2 \text{ eq}$  were estimated (Figure 3). Differences between CoC and  
335 MoC were less pronounced for the other impact categories. The energy requirements for  
336 producing 1 kg of fish from the ponds were slightly reduced in the CoC, with  $130 \pm 7 \text{ MJ}$  vs  $139$   
337  $\pm 4 \text{ MJ}$  for MoC (i.e. 7-11% less in CoC; Figure 3). The gain provided by CoC in acidification  
338 was comparable, with values of  $0.14 \pm 0.01 \text{ mol H}^+ \text{ eq}$  for CoC and  $0.15 \pm 0.01 \text{ mol H}^+ \text{ eq}$  for  
339 MoC, equivalent to an impact reduction of 6-11% (Figure 3).

340 Contributions of the rearing system components (energy, exogenous feeds,  
341 equipment/infrastructures, farm operations, fertilizers, fish inputs and transport) varied according  
342 to the impact category and to the system (Figure 3 and Table 3). Overall, the ranking of the

343 different contributors among the seven impact categories remained constant between the two  
344 production systems (CoC and MoC). Results presented in Figure 3 show that for eutrophication  
345 and water dependence, farm operations contributed the most to the impacts (81-82% and 52-  
346 58%, respectively), and the second largest contributor for these impact categories was fish inputs  
347 (11-13% and 42-48%, respectively). For NPPU, climate change and CED, exogenous feeds were  
348 the main contributors (83-86%, 37-45% and 31-40%, respectively), followed by fish inputs (14-  
349 17%, 20-22% and 18-19%, respectively) and/or fertilizers (15-21% for climate change and 27-  
350 35% for CED; Figure 3). For acidification, the main contributors were ranked as follows: 32-  
351 34% for fish inputs, 30-32% for farm operations and 15-19% for exogenous feeds, accounting  
352 for ~80% of the total acidification. Interestingly, land occupation was mainly driven by  
353 fertilizers (49-60%) and exogenous feeds (28-38%; Figure 3).

### 354 3.2. Uncertainty analysis

355 Uncertainty analysis qualifies data according to their origin, considering the uncertainties linked  
356 to the production of macrophytes in the CoC and including the variabilities observed between  
357 ponds in the LCA. Considering a threshold beyond which the two systems differed if one had  
358 higher impacts in at least 90% of the MC runs, there were notable significant differences  
359 between the two production systems. The analysis showed that CoC leads to less impact relative  
360 to climate change, eutrophication and NPPU on a production basis (per kg of fish produced;  
361 Figure 4A). The differences are even more marked on a farm surface basis (per unit area (m<sup>2</sup>);  
362 Figure 4B), where, in this case, CED was also significantly lower in the CoC.

## 363 4. Discussion

### 364 4.1. Product-based vs area-based LCA approach

365 The functional unit is the basis of comparison in LCAs and influences allocation decisions at the  
366 farm gate (Henriksson et al., 2012). Here, we used a descriptive product-based functional unit  
367 (kg of fish) that is appropriate for comparisons between studies (Bohnes et al., 2019). Van Der  
368 Werf et al. (2020) highlighted the interest in combining product-based and area-based LCA  
369 when studying agricultural systems that provide a broad range of ecosystem services. For  
370 instance, although organic agriculture generally emits fewer pollutants per unit of land occupied  
371 than conventional agriculture (an area-based approach), it may have higher impacts per unit of  
372 product (e.g., land occupation, eutrophication and acidification). Thus, we also expressed the  
373 environmental impacts using an area-based functional unit (m<sup>2</sup> of farm), an original approach in  
374 LCA aquaculture studies (Bohnes et al., 2019; Henriksson et al., 2012). Our results show that the  
375 choice of the functional unit did not result in significant differences in environmental impacts for  
376 the LCA impact categories considered. Nevertheless, even if the conclusions remain similar  
377 comparing the two production systems (CoC and MoC), a greater difference between the  
378 systems was observed using the area-based functional unit for the following impact categories:  
379 acidification, CED and NPPU.

#### 380 4.2. Comparison of production systems

381 The responses in environmental impacts between the two studied production systems differed  
382 according to the impact category considered. The two systems remained similar considering land  
383 occupation and water dependence, although CoC showed slightly lower impacts for the latter  
384 category. Using CoC as an alternative to MoC was expected to reduce land utilization by  
385 decreasing the quantity of extruded pellets used. Nevertheless, the beneficial effects of reducing  
386 exogenous feed were attenuated by the higher quantity of organic fertilizer from the intensive  
387 poultry production system and rice culture (Purnomo et al., 2017) for *Azolla* production in CoC.

388 Such results highlight the need to better control the origin and the quantity of fertilizers used for  
389 pond fertilization.

390 Between the two production systems, the differences observed in acidification, CED, climate  
391 change eutrophication and NPPU were mainly explained by the lower use of exogenous feed in  
392 CoC (-25% on average per kg of fish produced). Our study confirms previous investigations  
393 highlighting that feed production is the major contributor to different impact indicators in semi-  
394 intensive aquaculture production systems (e.g., Wilfart et al., 2013; Yacout et al., 2016). The  
395 difference reflects the high impact of feed production, from the extraction of raw materials up to  
396 manufacturing processes, which require large amounts of energy and resources (Ghamkhar and  
397 Hicks, 2020). The processing of feed is by far the highest contributor to NPPU. Such results are  
398 in accordance with Mungkung et al. (2013), who pinpointed feed production as the unique  
399 contributor to NPPU (100%) in Indonesian tilapia-carp production systems. In addition to its  
400 predominant environmental impact, fish feed has a significant economic impact on the  
401 aquaculture farm because some ingredients are imported, which is frequently the case for small-  
402 scale fish farming practised in Indonesia (Slembrouck et al., 2018).

403 The importance of FCR, and hence of extruded feeds in general, has been highlighted by  
404 numerous LCA aquaculture studies. In literature reviews, Aubin (2013) and Bohnes et al. (2019)  
405 have concluded that feed production is the primary source of environmental impacts. In the  
406 present study, FCR for commercial pellets was relatively high in giant gourami compared with  
407 other omnivorous species like carp and tilapias (Fry et al., 2018).

408 In small-mouthed fish like giant gourami, uneaten feeds can represent 30% of the feed supplied  
409 (Bosma and Verdegem, 2011; Van Der Meer et al., 1997; Verdegem, 2013). In giant gourami,

410 Pouil et al. (2019) suggested that a significant proportion of the nutrients in pond sediments  
411 comes from uneaten commercial pellets. Nevertheless, FCR can be improved in the co-culture  
412 system (2.1 vs 2.8 for CoC and MoC, respectively) by using *Azolla* as a partial alternative feed  
413 resource. Here, we assumed that the improvement of FCR, calculated for commercial feeds, in  
414 CoC is related to the changes in feeding practices. The alternance between feeding with *Azolla*, a  
415 less nutritive resource, and commercial pellets may contribute in reducing the quantity of  
416 uneaten feed in particular. Indeed, Narimbi et al. (2018) also found very high FCR (6.4) in daily-  
417 fed GIFT tilapia while FCR was six times lower in weekly-fed fish suggesting wastage of feed  
418 when fish were daily fed. Lower FCR decreases the amount of feed delivered as well as the  
419 excess nutrients released in the water, influencing eutrophication.

420 Eutrophication is a primary concern among farmers and local populations due to the risks for  
421 water quality in reservoirs (Mungkung et al., 2013). A broad range of eutrophication potential  
422 has been reported in the literature for different aquaculture systems (i.e., 0.01 to 0.3 kg PO<sub>4</sub> eq  
423 per kg of fish; Ghamkhar et al., 2021; Medeiros et al., 2017; Mungkung et al., 2013; Pelletier and  
424 Tyedmers, 2010; Yacout et al., 2016). The differences in eutrophication between MoC and CoC  
425 can be identified through the nutrient concentrations measured in the water outlet with a notable  
426 decrease in P released from the CoC ponds (Table 1). Here, the differences in eutrophication is a  
427 consequence of the lower FCR in the CoC system that limits the impacts related to the farm  
428 operations (i.e. reducing on-farm nutrient emissions). Indeed, the nutrient emissions resulting of  
429 biological activity of fish, is the major contributor to eutrophication (see, e.g., Mungkung et al.,  
430 2013; Wilfart et al., 2013), and is driven by the quality and the amount of feeds used.

431 Interestingly, our results regarding the contribution of exogenous feeds in acidification and  
432 climate change differ from the literature. Indeed, in previous studies, exogenous feeds explain 64

433 to 95% of the acidification potential observed in different aquaculture pond systems, including  
434 Indonesian intensive tilapia monoculture (Pelletier and Tyedmers, 2010) or tilapia-carp  
435 polyculture (Mungkung et al., 2013) and Egyptian intensive and semi-intensive tilapia  
436 monoculture (Yacout et al., 2016). Similarly, most LCA aquaculture studies, carried out in  
437 production systems that are sometimes very different from fertilized ponds, report that feeds are  
438 drivers of climate change (Bohnes et al., 2019; Wilfart et al., 2023). In this study, we found a  
439 relatively low contribution of exogenous feeds to acidification (15-18%), with fertilizers and fish  
440 inputs contributing to more than 40% of the total acidification. A similar trend was observed for  
441 climate change, where fertilizers and fish inputs explain 35-43% of the impacts, with exogenous  
442 feeds also accounting for ~40%. Such findings can be explained by the origin of the organic  
443 fertilizer (i.e., poultry manure mixed with rice bran; Purnomo et al., 2017), a co-product from  
444 intensive agriculture used in large quantities in our production systems. Furthermore, the fish  
445 used in this experiment, as described in Section 2.1.1, are already six months old, meaning that  
446 their own production also required exogenous feeds and other inputs, which explains their  
447 significant contributions to the estimated global impacts on our production systems. Such  
448 findings differ from LCAs performed in aquaculture systems where the fish inputs are based on  
449 younger life stages (eggs or larvae for example).

450 Recent studies reported that one of the leading production constraints in the Indonesian  
451 aquaculture sector, in addition to the dependence on imported feed ingredients (Henriksson et al.,  
452 2017), is energy sources (Henriksson et al., 2019, 2017). Previous studies have also pointed out  
453 that energy consumption during fish feed production is one of the significant contributors to  
454 CED (e.g., Mungkung et al., 2013; Pelletier and Tyedmers, 2010; Yacout et al., 2016). Overall,  
455 the estimated CED are comparable to other semi-intensive aquaculture production systems. For

456 instance, Yacout et al. (2016) found 238 MJ eq per kg of fish produced in an Egyptian Nile  
457 tilapia (*Oreochromis niloticus*) semi-intensive production system, compared with a tilapia  
458 intensive production system being almost fivefold more efficient in energy use, with 53 MJ eq  
459 per kg of fish production than a semi-intensive system. In this study, we found that ecological  
460 intensification performed in the CoC could also significantly improve energy efficiency in giant  
461 gourami pond production.

#### 462 4.3. Origins of the uncertainties

463 The uncertainty in this study was similar to other LCA aquaculture studies (Dekamin et al.,  
464 2015; Medeiros et al., 2017). Nevertheless, comparisons between studies remain challenging due  
465 to the heterogeneity in the studied systems and rearing practices and the origin of the data. Here,  
466 we compared similar production systems, except for including *Azolla* in the CoC system as a  
467 partial alternative to extruded pellets. However, the stable production of this floating macrophyte  
468 was more challenging than expected and inadequate in the present experiment, presumably due  
469 to the predation by snails and nutrient deficiency (Pouil et al., 2020). Nevertheless, the scenario  
470 built for CoC, designed based on experience gained in the production of *Azolla* on this farm  
471 (Caruso et al., 2021), made it possible to estimate uncertainties related to the exogenous feed  
472 gain in the implementation of such a production system. The uncertainties related to *Azolla*  
473 production mainly affect the impact categories in which exogenous feeds contribute strongly.  
474 Therefore, unsurprisingly, we observed higher variability in the CoC systems for NPPU, climate  
475 change, CED and, to a lesser extent, land occupation.

476 In addition to *Azolla* production, the other sources of uncertainties for all impact categories  
477 include variability among the two replicates of each system, the inherent variability already

478 accounted for in the ecoinvent database and the assumptions made for mass-balance modelling.  
479 Interestingly, most of the data we recorded from the replicates of the two systems were, overall,  
480 comparable. There was a relatively high uncertainty on water dependence, particularly for the  
481 MoC system. Indeed, the ponds used, connected in series and fed by the same river, experienced  
482 fluctuating flows during the production cycle. Although this only had a minor effect on fish  
483 production, the water inputs in the study were highly variable, especially for MoC.

484 Nutrients (N and P) fluxes can be an important source of uncertainties in LCA aquaculture  
485 studies. Here, most of the data used were extracted from chemical analysis of each compartment  
486 included in the production systems (i.e. water, feeds, fertilizer and fish), and the N and P  
487 quantities stored in sediments were taken from Pouil et al. (2019) that gives nutrient budgets in  
488 the same ponds with an MoC system of giant gourami. Assuming the occurrence of *Azolla* in the  
489 CoC was not drastically affected by particulate N and P in sediments (related to uneaten feed,  
490 faeces, plankton sedimentation, dead fish, etc.), the overall N and P values we used in the study  
491 are reliable. Digestibility determines the fate of nutrients and the amounts of nutrients in the  
492 water column (digestible wastes) and sediment (non-digestible wastes) (Medeiros et al., 2017).  
493 Here, we calculated ThOD, an interesting parameter to refine the impacts related to  
494 eutrophication as suggested by Aubin et al. (2009), using digestibility values for protein,  
495 carbohydrates, lipids, ash and fibre. Only protein digestibility values have been found for giant  
496 gourami in literature (Andriani et al., 2019). Thus, other digestibility information (i.e. fat,  
497 carbohydrates, ash, P) came from an experiment based on Nile tilapia, another omnivorous  
498 species (Schneider et al., 2004). This lack of data in the literature reduces the accuracy of the  
499 estimates. However, the release of nutrients (N and P) from the two production systems was



500 derived mainly from chemical analysis allowing a relatively accurate determination of the  
501 nutrient budgets.

502 Gaseous emissions to the atmosphere were the parameters with higher uncertainties in the  
503 pedigree matrix. The processes considered were denitrification and ammonia volatilization,  
504 which directly influence eutrophication. Denitrification and ammonia volatilization rates were  
505 based on Gross et al. (2000), a reference cited in some LCA aquaculture studies (e.g., Medeiros  
506 et al., 2017; Wilfart et al., 2013). This reference indicates a loss of roughly 40% of N inputs  
507 through these processes in channel catfish ponds. Nevertheless, in the literature, variability in N  
508 emissions in the air from aquaculture ponds is huge. For instance, NH<sub>3</sub> volatilization represents  
509 ~1% to total N input in feed for snakehead and bighead carp polyculture ponds (Hou et al.,  
510 2018), but NH<sub>3</sub> volatilization is equivalent to 10-66% of the N input in feed in shrimp ponds  
511 (Dien et al., 2018; Li et al., 2019; Lorenzen et al., 1997; Páez-Osuna et al., 1999). Such  
512 differences can be explained by the variety of the pond aquaculture systems studied, with diverse  
513 locations, species and rearing practices. Overall, these discrepancies highlight the need to  
514 consider such uncertainties of these processes in aquaculture LCA studies.

515 Other sources of uncertainties qualified through the pedigree matrix were the emissions of CH<sub>4</sub>  
516 and N<sub>2</sub>O and the CO<sub>2</sub> fixation in the ponds. Indeed, high variability of such emissions is  
517 expected in aquaculture ponds: reported pond emissions to air vary from 0.01 to 20 mg CH<sub>4</sub> m<sup>-2</sup>  
518 h<sup>-1</sup> and 1 to 252 µg N<sub>2</sub>O m<sup>-2</sup> h<sup>-1</sup> (Kosten et al., 2020; Yang et al., 2022; Yuan et al., 2019).  
519 Interestingly, high greenhouse gas (GHG) emissions from aquaculture ponds have been reported  
520 in some studies during the culture period (Chen et al., 2016, 2015; Datta et al., 2009), suggesting  
521 the importance of including them in LCA. In our analysis, we used values from Yang et al.  
522 (2018), which include measurements during the culture and non-culture period of Chinese

523 shrimp polyculture and earthen carp ponds based on a stocking density comparable to that in our  
524 study and a semi-intensive production strategy. Overall, we hypothesized that the presence of  
525 *Azolla* in the CoC system has no significant impact on CH<sub>4</sub> and N<sub>2</sub>O emissions from the ponds.  
526 Nevertheless, some sources suggest that *Azolla* plays a role in these flows. For example, previous  
527 studies have shown that *Azolla* application in flooding rice paddy can either increase (Chen et al.,  
528 1997; Ying et al., 2000) or have no influence or decrease (Ali et al., 2015; Kimani et al., 2018;  
529 Liu et al., 2017; Singh and Strong, 2016) CH<sub>4</sub> and N<sub>2</sub>O emissions from rice soils. Similarly,  
530 *Azolla* can also contribute to CO<sub>2</sub> sequestration in ponds (Hamdan and Hourri, 2022).  
531 Nevertheless, despite the discrepancy between these studies associated with the difficult  
532 comparison between rice paddies and aquaculture ponds, we considered the assumption  
533 reasonable. The overall conclusions of this study would not change if pond GHG emissions were  
534 excluded, but we include them in the LCA we performed given their importance to climate  
535 change, as previously suggested by Medeiros et al. (2017).

536

## 537 **5. Conclusion**

538 The development of Indonesian freshwater pond aquaculture in recent years has shifted from  
539 low-intensity traditional culture methods to monoculture production systems, with increasing  
540 intensification accompanied by the use of commercial feed pellets. Here, for most of the impact  
541 categories investigated, the co-culture system (CoC) with *Azolla* appeared to be more efficient  
542 than the semi-intensive monoculture (MoC) of giant gourami *O. goramy*. The observed  
543 improvements can be directly related to reducing the use of commercial pellets and a better  
544 control of the origin of the fertilizer used for enriching the ponds. Overall, we found that

545 environmental performances for giant gourami production can be enhanced by using floating  
546 macrophytes as an alternative feed resource, even in relatively low proportions. Nevertheless, the  
547 production of this macrophyte species has still to be optimized to enhance its efficiency for use  
548 as a feed supplement while the economic repercussions of a shift to a production system based  
549 on co-culture with *Azolla* have to be investigated.

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813 **Figure captions**

814

815 **Figure 1.** Schematic view of the production chain of semi-intensive giant gourami aquaculture.

816 Weights and size provided are means are average values gathered from surveys of approximately

817 40 giant gourami farms (Kristanto et al., 2020).

818

819 **Figure 2.** System boundaries for giant gourami (A) monoculture (MoC) and (B) co-culture

820 (CoC) production systems.

821

822 **Figure 3.** Contribution of each input or production step in environmental impacts per (A) kg of

823 fish and (B) m<sup>2</sup> of the farm in traditional giant gourami monoculture (MoC) and co-culture with

824 the floating macrophyte *Azolla filiculoides* (CoC) systems. CED: cumulative energy demand;

825 NPPU: net primary production use.

826

827 **Figure 4.** Uncertainties in the environmental impacts per (A) kg of fish and (B) m<sup>2</sup> of the farm

828 in the traditional giant gourami monoculture system (MoC) and the co-culture system with the

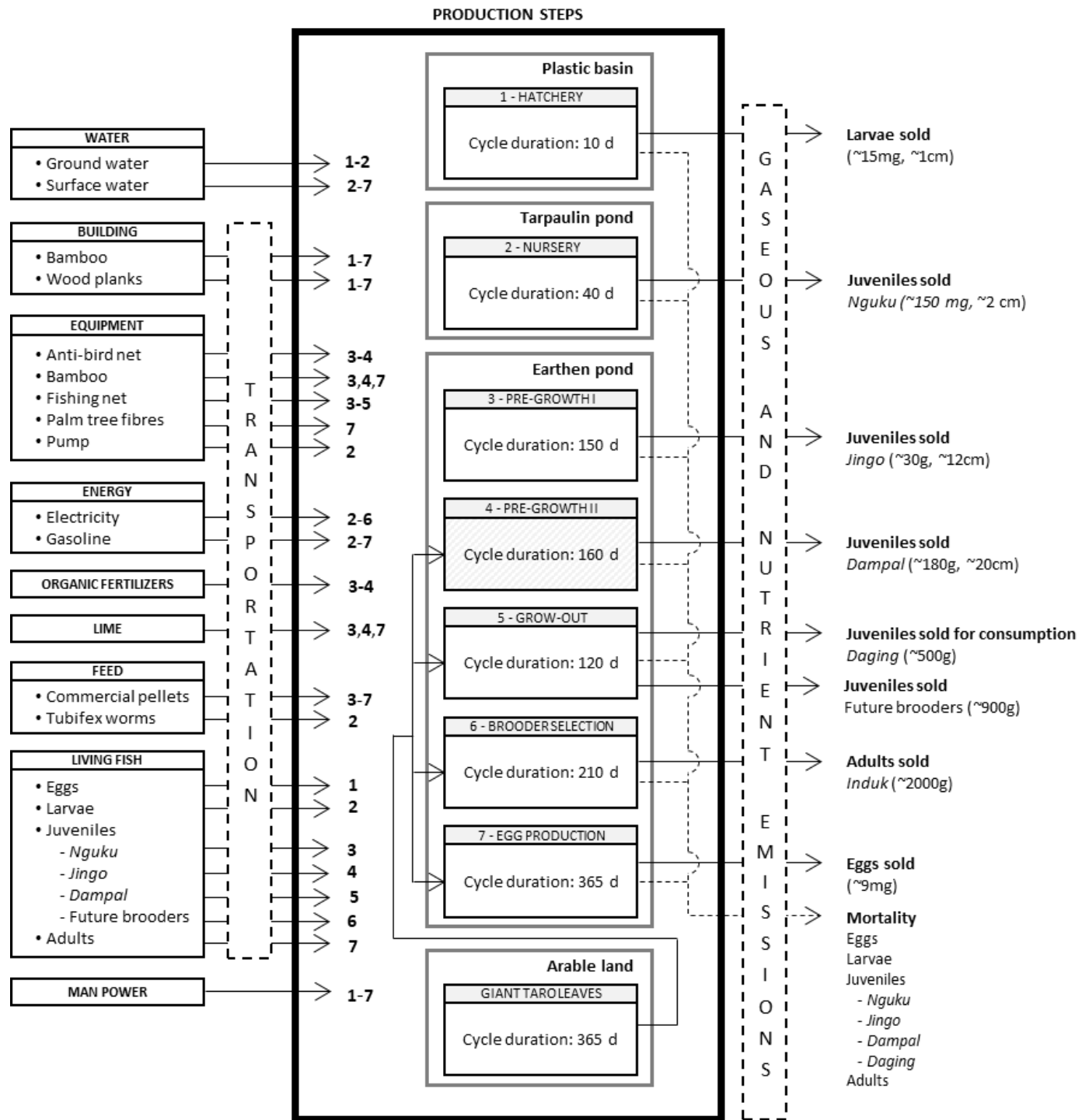
829 floating macrophyte *Azolla filiculoides* (CoC) for each impact category. Asterisks denote

830 significant differences between the two production systems. We considered that the two systems

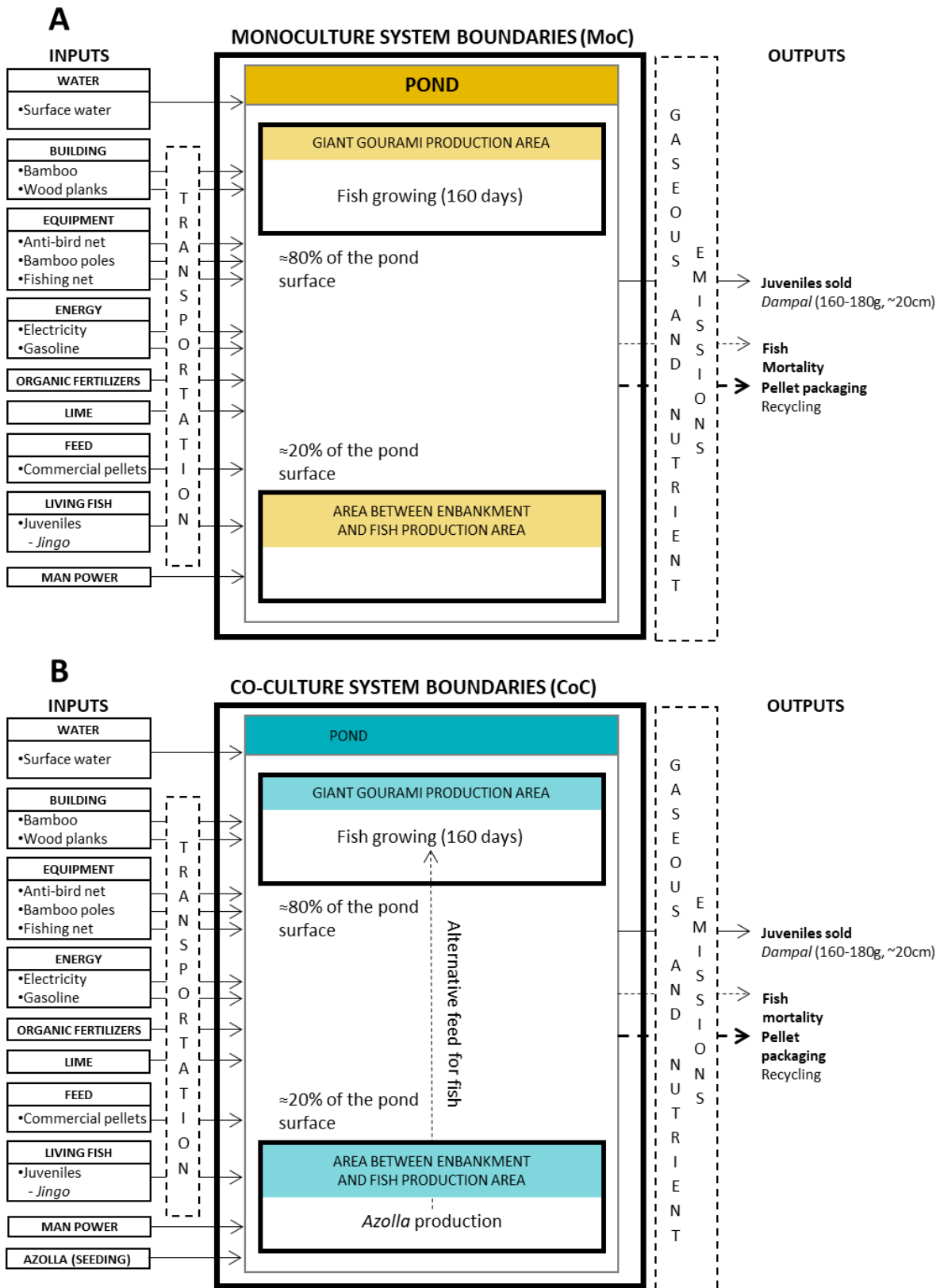
831 differed if one had higher impacts in at least 90% of the runs. CED: cumulative energy demand;

832 NPPU: net primary production use.



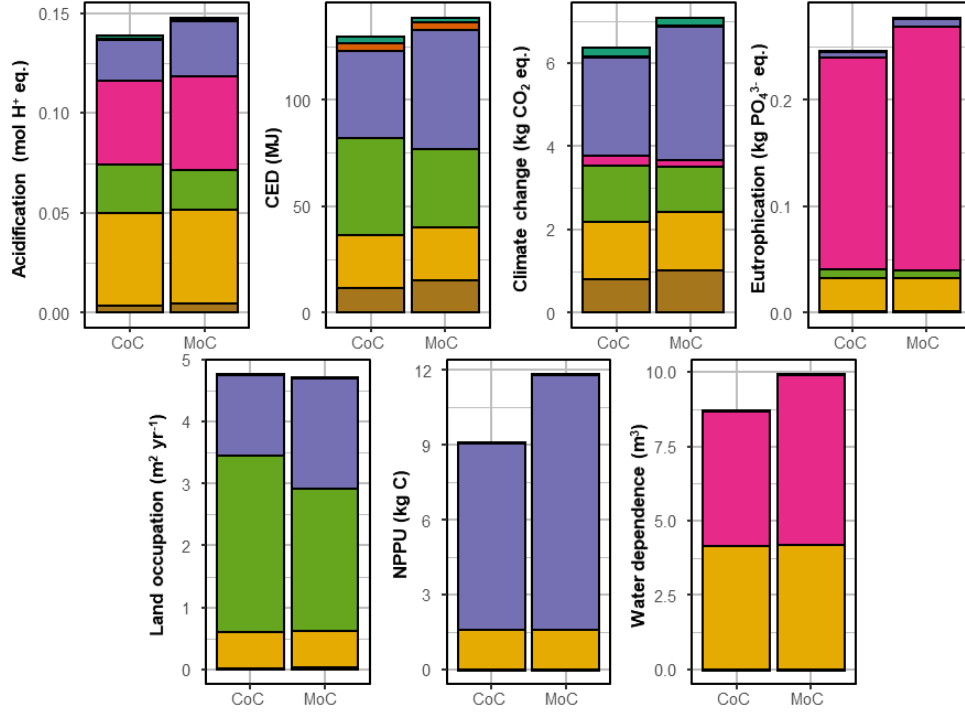


833 **Figure 1**

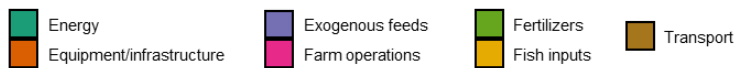
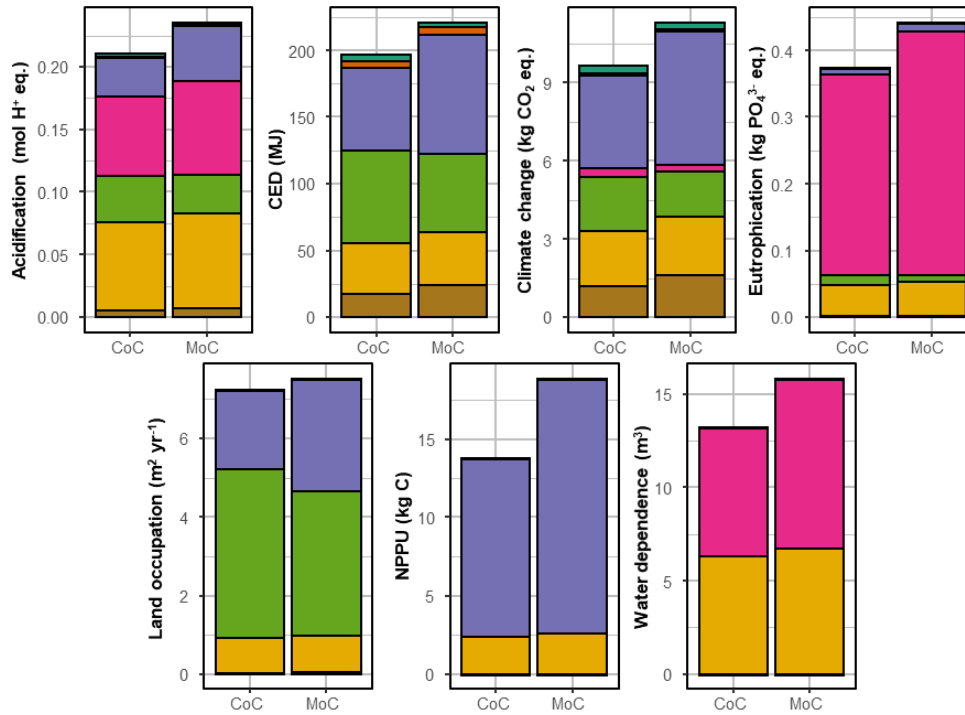


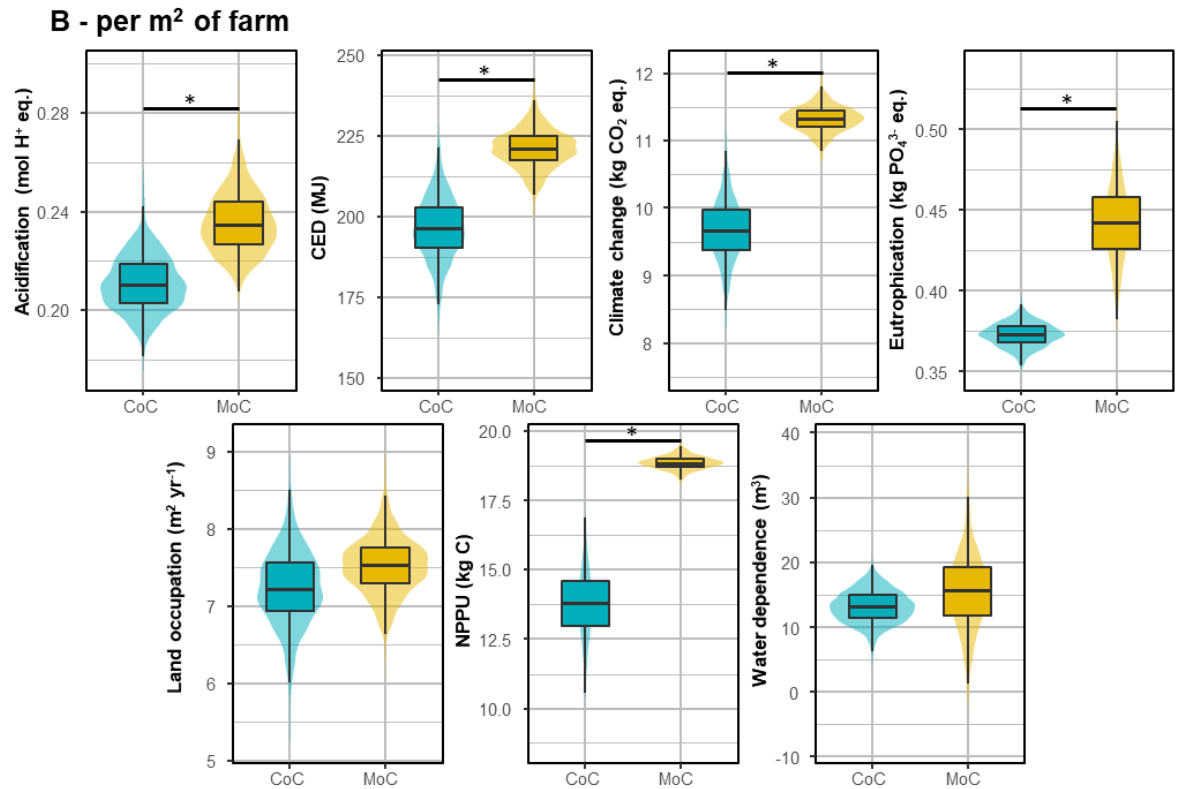
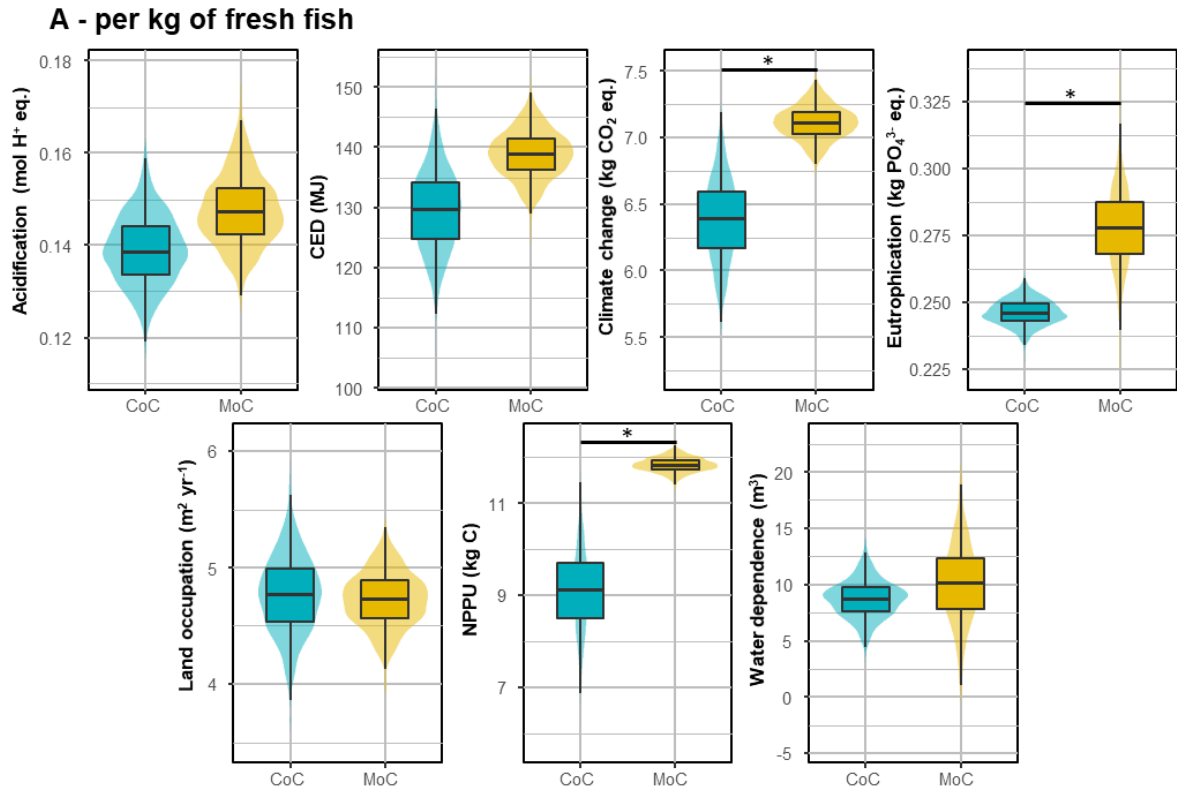
834 **Figure 2**

**A - per kg of fresh fish**



**B - per m<sup>2</sup> of farm**





836 **Figure 4**

837 **Table 1.** Pond characteristics, rearing performances and electricity and water consumption used  
 838 in the LCA of the monoculture (MoC) and the co-culture with the floating macrophyte *Azolla*  
 839 *filiculoides* (CoC) of giant gourami (mean  $\pm$  1 standard deviation).

	CoC	MoC
<i>Pond characteristics and inputs</i>		
Surface (m <sup>2</sup> )	771	840
Depth (m)	0.5	0.5
Rearing structures	earthen ponds tarpaulin tanks	earthen ponds
Equipment	PVC pipes bamboo pools anti-predator nets fishing net happas	PVC pipes bamboo pools anti-predator nets fishing net
Fertilizers (kg)	5802 $\pm$ 580	5020 $\pm$ 502
Pellets (kg)	2874 $\pm$ 302	3811 $\pm$ 59
<i>Azolla</i> (kg)	2848 $\pm$ 1424	-
<i>Rearing performances</i>		
Initial body weight (g)	29 $\pm$ 6	28 $\pm$ 5
Fish stocking density (fish m <sup>-2</sup> )	6	6
Stocked fish biomass (kg yr <sup>-1</sup> )	274	297
FCR <sub>pellets</sub> (wet weight) <sup>1</sup>	2.1	2.8
Final body weight (g)	193 $\pm$ 52	183 $\pm$ 39
Fish productivity (tonne ha <sup>-1</sup> yr <sup>-1</sup> )	19.9	19.6
<i>Energy and water inputs</i>		
Electricity consumption (kWh yr <sup>-1</sup> )	422	403
Water (m <sup>3</sup> yr <sup>-1</sup> )	6985 $\pm$ 2544	9425 $\pm$ 5863
<i>Nutrient releases from water</i>		
N (mg L <sup>-1</sup> )	1.4 $\pm$ 0.5	1.5 $\pm$ 0.4
P (mg L <sup>-1</sup> )	0.167 $\pm$ 0.258	0.026 $\pm$ 0.031

840 <sup>1</sup>FCR<sub>pellets</sub> = feed conversion ratio, considering only the commercial feed (extruded pellets) and  
 841 calculated as the ratio between the amount of distributed pellets (kg) and the biomass gain (kg)  
 842 over the production cycle

843 **Table 2.** Ingredients and composition values of the feeds (n = 5 and n = 30 samples analysed for  
 844 pellets and *Azolla*, respectively) used in monoculture (MoC) and co-culture with the floating  
 845 macrophyte *Azolla filiculoides* (CoC). Except for moisture, composition values are expressed on  
 846 a dry matter basis.

<b>Composition (% , Mean ± SD)</b>	<b>Extruded pellets</b>	<b><i>Azolla filiculoides</i></b>
Moisture	7.95 ± 0.49	92.06 ± 1.17
Proteins	32.72 ± 0.23	20.29 ± 5.47
Lipids	5.29 ± 0.17	1.76 ± 0.61
Carbohydrates	47.29 ± 0.25	50.89 ± 4.72
Ash	10.01 ± 0.36	11.49 ± 1.75
Fibres	4.70 ± 0.20	15.58 ± 3.03
Phosphorus	1.47 ± 0.12	0.25 ± 0.13
<b>Ingredients (% [origin])</b>		
Fish meal	10 [Chili]	
Fish meal	11 [East Java]	
Soybean meal	15 [Argentina]	
Meat and bone meal	10 [Australia]	
Wheat bran	20.5 [Australia]	
Rice bran	25 [West Java]	
Fish oil	2 [East Java]	
Plant oil	2.5 [Banten/West Java]	
Choline chloride	0.5 [China]	
Premix vitamin	2 [China]	
Premix mineral	1.2 [China]	
Butylated hydroxytoluene (BHT)	0.3 [Central Java]	

857 **Table 3.** Results of the Monte Carlo analysis (n = 1000) applied to the two production systems: monoculture (MoC) and co-culture with the  
858 floating macrophyte *Azolla filiculoides* (CoC) for each impact category. We considered that the two systems differed if one had higher impacts  
859 in at least 90% of the 1000 runs (see values in bold in the last column).

Functional unit	Category	System	Median	Q1 (25%)	Q3 (75%)	Mean	SD	MoC > CoC (%)
<i>Fish weight (kg)</i>	<b>Acidification (mol H<sup>+</sup> eq.)</b>	CoC	0.139	0.134	0.144	0.139	0.007	
		MoC	0.147	0.142	0.152	0.148	0.008	78.8
	<b>Cumulative energy demand (MJ)</b>	CoC	129.787	124.828	134.099	129.565	6.653	
		MoC	138.986	136.27	141.493	138.897	3.889	88.6
	<b>Climate change (kg CO<sub>2</sub> eq.)</b>	CoC	6.394	6.165	6.595	6.383	0.306	
		MoC	7.108	7.027	7.191	7.109	0.125	<b>98.4</b>
	<b>Eutrophication (kg PO<sub>4</sub><sup>3-</sup> eq.)</b>	CoC	0.246	0.243	0.25	0.246	0.005	
		MoC	0.278	0.268	0.287	0.278	0.014	<b>98.3</b>
	<b>Land occupation (m<sup>2</sup> year)</b>	CoC	4.773	4.538	4.989	4.766	0.328	
		MoC	4.732	4.569	4.888	4.728	0.236	46.5
	<b>Net primary production use (kg C)</b>	CoC	9.123	8.489	9.714	9.116	0.87	
		MoC	11.833	11.731	11.949	11.837	0.157	<b>100</b>
	<b>Water dependence (m<sup>3</sup>)</b>	CoC	8.791	7.609	9.732	8.708	1.659	
		MoC	10.255	7.808	12.345	10.117	3.484	64.4
<i>Farm surface (m<sup>2</sup>)</i>	<b>Acidification (mol H<sup>+</sup> eq.)</b>	CoC	0.21	0.203	0.219	0.211	0.011	
		MoC	0.235	0.227	0.244	0.236	0.013	<b>93.8</b>
	<b>Cumulative energy demand (MJ)</b>	CoC	196.47	190.368	202.691	196.505	9.813	
		MoC	221.05	217.39	224.814	221.098	6.095	<b>97.9</b>
	<b>Climate change (kg CO<sub>2</sub> eq.)</b>	CoC	9.672	9.378	9.97	9.674	0.453	
		MoC	11.327	11.204	11.443	11.323	0.19	<b>100</b>
	<b>Eutrophication (kg PO<sub>4</sub><sup>3-</sup> eq.)</b>	CoC	0.373	0.368	0.378	0.373	0.007	
		MoC	0.442	0.426	0.458	0.442	0.024	<b>99.9</b>
	<b>Land occupation (m<sup>2</sup> year)</b>	CoC	7.227	6.937	7.562	7.237	0.48	
		MoC	7.529	7.292	7.751	7.52	0.374	67.9
	<b>Net primary production use (kg C)</b>	CoC	13.827	12.958	14.584	13.793	1.275	
		MoC	18.864	18.708	19.016	18.864	0.237	<b>99.9</b>
	<b>Water dependence (m<sup>3</sup>)</b>	CoC	13.168	11.37	14.957	13.165	2.463	
		MoC	15.663	11.911	19.249	15.519	5.814	64.0