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Lacepède (1801) in Indonesian ponds**

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1 **Towards ecological intensification through the co-production of the floating macrophyte**
2 ***Azolla filiculoides* and the giant gourami *Osphronemus goramy* Lacepède (1801) in**
3 **Indonesian ponds.**

4

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18 **Abstract**

19 The feasibility of the co-culture production of giant gourami *Osphronemus goramy*, an
20 omnivorous with a herbivorous tendency fish, with high economic and patrimonial value in
21 Indonesia, and floating macrophyte *Azolla filiculoides* was tested. A three months trial was
22 carried out in an Indonesian small-scale fish farm from November 2017 to February 2018.
23 Four earthen ponds (from 353 to 482 m²) were used. Two ponds were managed as
24 conventional monoculture aquaculture; in the two others, 20% of the pond's surface was
25 dedicated to the production of *A. filiculoides*. The objective of this co-production was to
26 replace partially the commercial pellets with fresh, unprocessed *Azolla*. All ponds were
27 fertilized with chicken manure and stocked with juveniles of giant gourami (24-34 g) bought
28 in the local live fish market at a density of 6 individuals per m² under both management
29 schemes. Production of *Azolla* was discontinuous, covering only 40 days over 90 days of the
30 production cycle of Giant gourami. *Azolla* was harvested every three days producing 571 ±
31 359 g m⁻² (mean ± SD) of biomass. This discontinuity might be attributed to insufficient
32 phosphorous (P) fertilization and predation by other fish, aquatic snails, and insect larvae. In
33 the co-cultured ponds, a total of 15% of commercial pellets were spared by their
34 replacement with fresh *Azolla*. The specific growth rate (SGR) and the fish biomass gain were
35 similar for both systems. *Azolla* did not influence water quality. Both production systems
36 were negative economic net-benefice (-17% for conventional ponds and -4% for co-cultured
37 ponds), mainly because of high costs for seeds and sub-optimal feed performance. We
38 demonstrated that the co-production of *A. filiculoides* did not generate any significant extra
39 cost. We discussed the benefits of ecological intensification with macrophytes in semi-
40 intensive production systems in the tropics.

41 **Keywords:** alternative feed,

42 **Introduction**

43 Among agricultural food production systems, aquaculture has shown the highest growth
44 rate. The World Bank (2013) estimated that by 2030 aquaculture will supply over 60% of fish
45 intended for direct human consumption. Worldwide, most aquatic animal production
46 systems are located in Asian countries, where 9 out of 10 fish farms are located. This high
47 aquaculture activity mainly results from conventional intensification using commercial feed
48 and increased fish stocking density. Therefore, the share of unfed species in aquatic animal
49 production systems has decreased gradually from ca. 40 to 30% in 16 years (from 2000 to
50 2016; FAO 2018). Although this shift led to an increase in the worldwide production level, its
51 sustainability is broadly questioned, especially considering the environmental impact of fish
52 feed production (Tacon et al. 2009). Alternative production practices are thus required.

53 In contrast to conventional intensification, defined as the increase in the number of
54 production factors (labour and capital) per hectare, ecological intensification proposes to
55 increase crop production by maximizing the number of ecosystem processes or functions. It
56 emphasizes the importance of biodiversity and ecosystem services in the regulation of the
57 functioning of agroecosystems (Aubin et al. 2019). Initially developed in agronomy, the
58 concept of ecological intensification aims to design agricultural systems that use the
59 ecological processes and functions to increase productivity, strengthen ecosystem services
60 and decrease disservices (Aubin et al. 2019). Integrated multitrophic aquaculture (IMTA) is a
61 production system aiming to create a circular economy, minimising energy flows, losses and
62 environmental deterioration (Hughes and Black 2016). Two or more species of different
63 trophic levels are combined in the aquaculture system to increase yield and ecosystem
64 services. However, polyculture is an ancient practice in Asian aquaculture, often performed
65 in integrated agriculture-aquaculture (IAA), where polyculture fish ponds benefit from

66 alternative feed sources from surrounding agriculture (Nhan et al., 2008; Pucher et al.,
67 2016). Nevertheless, the modern conceptualisation of IMTA is mainly based on mariculture
68 in temperate countries (Troell et al. 2009), with few studies available on tropical freshwater
69 IMTA systems.

70 The evolution of aquaculture practices and their sustainability is a significant concern for
71 Indonesia. In Indonesia, more than three million fish farmers produced around 4.4 million
72 tonnes of finfish in 2018 (FAO, 2020). Freshwater farming represents 79% of finfish farming
73 in Indonesia. The giant gourami *Osphronemus goramy* (Lacepède)-an omnivorous fish with
74 high herbivorous tendency-, with a production of 173,000 t in 2018 (FAO 2020), is among the
75 most important freshwater fish produced, combining both very high economic and heritage
76 values. Traditionally, the giant gourami rearing is an integral part of the agroecosystem of
77 the Javanese countryside. Currently, this species is reared at low densities in shallow ponds
78 (Kristanto et al. 2020) using mainly commercial extruded pellets (usually 30-40% of crude
79 proteins) and raw plant resources (see FAO, 2019 for details), but nowadays, giant gourami
80 aquaculture becomes more and more intensified, with an increase in fish stocking density
81 and greater use of commercial feed which increases production costs and environmental
82 impact.

83 Commercial fish feeds are widely acknowledged as the inputs with the highest economic and
84 ecological costs in aquaculture (Hasan 2010). Even though giant gourami is one of the most
85 expensive freshwater fish in Indonesia, its production costs have become increased eliciting
86 growing difficulties for small fish farmers. Using resources from the local environment may
87 be an appropriate alternative for reducing production costs and improving the sustainability
88 of small-scale giant gourami and other omnivorous freshwater fish in Indonesia. Ecosystem
89 services provided by macrophytes to aquaculture ponds may be useful for promoting the

90 sustainability of aquaculture systems. More than 50 species of macrophytes can be used as
91 feed in tropical and subtropical aquaculture (Mandal et al. 2010). Floating macrophytes can
92 also contribute to the remediation of aquaculture effluents (Sipaúba-Tavares et al. 2002;
93 Henry-Silva and Camargo 2006).

94 Several studies advocate the use of aquatic plants as an alternative resource for feeding fish
95 (Hassan and Edward 1992; Hasan and Chakrabarti, 2009). Using floating macrophytes as fish
96 feed has been studied for the Nile tilapia (Abou et al., 2007), the grass carp
97 *Ctenopharyngodon idella* (Valenciennes) (Domingues et al. 2017), and the silver barb *Puntius*
98 *gonionotus* (Bleeker) (Ahmed et al. 2015). Fish farmers traditionally include giant taro leaves
99 *Alocasia macrorrhizos* (Linné) or other plants in addition to commercial pellets in the diet of
100 giant gourami (FAO 2019). Gut weed *Enteromorpha* spp. was successfully tested as an
101 alternative feed for giant gourami (Anh et al. 2013); however, although floating macrophytes
102 are avidly eaten by this fish species, there are no field studies on the use of macrophytes in
103 giant gourami ponds.

104 This study aimed to evaluate the feasibility of combining *A. filiculoides* production and giant
105 gourami rearing in earthen ponds, where the produced floating macrophyte was used as an
106 alternative fish feed. Results were compared with those from a conventional giant gourami
107 monoculture system. This study was carried out under real production conditions, within a
108 typical Javanese fish farm, representative of the Indonesian freshwater production
109 dominated by small-scale farms (more than 90% of all fish farms; Maskur et al., 2013). The
110 giant gourami production cycle is highly segmented (FAO 2019). Here, the targeted rearing
111 phase is the first in the giant gourami production cycle performed in earthen ponds. This
112 phase generally lasts between 80 and 120 days (FAO, 2019). *Azolla filiculoides* (Lamarck) was

113 selected, among other species, as a good candidate for macrophyte-fish co-production
114 system in ponds from previous investigations (see Slembrouck et al. 2018).

115

116 **Materials and Methods**

117 *a. Pond description and management*

118 A pilot-scale trial was conducted for three months (90 days from November 2017 to
119 February 2018) in a small-scale fish farm located in the village of Babakan (-6°28'S; 106°42'E;
120 alt: 125 m), Bogor district, West Java, Indonesia. Our study used four earthen ponds with a
121 size between 353 and 482 m² with an average water depth of 0.5 m. Ponds were equipped
122 with PVC pipes for inlet and outlet water with an average daily water exchange of $6 \pm 5\% \text{ d}^{-1}$
123 (mean \pm SD). Furthermore, anti-predator nets held by bamboo poles were placed at a
124 distance of 1 m from the banks. Fish farmers install this fence to prevent the introduction of
125 unwanted species into the fish pond, such as marble goby *Oxyeleotris marmorata* (Bleeker),
126 snakehead *Channa* spp., and other predators such as the Asian water monitor *Varanus*
127 *salvator* (Laurenti) and the Asian small-clawed otter *Amblonyx cinereus* (Illinger).

128 Before starting the experiment, all ponds were dried, cleaned of unwanted species, limed
129 ($131 \pm 15 \text{ g m}^{-2}$, mean \pm SD), and fertilized with chicken manure mixed with 50% rice bran.
130 This organic fertilizer was given to all ponds before the start of the production cycle at $740 \pm$
131 88 g m^{-2} (mean \pm SD); further manuring was carried out five times during the production
132 cycle in all ponds with $314 \pm 40 \text{ g m}^{-2}$ (mean \pm SD) at each fertilization. These fertilization
133 practices and quantities were based on empirical fish-farmer experience for giant gourami
134 ponds. Finally, ponds were assigned to the experimental treatments in duplicates, two as
135 conventional ponds (B and D) and the other two as co-cultured ponds (A and C).

136

137 *b. Farmed organisms*

138 To stock fish in ponds, different batches of juveniles of giant gourami were bought in a live
139 fish market. Fish body weight ranged from 24 to 34 g fresh weight (FW), and the stocking
140 density was six individuals per m⁻². In the two co-cultured ponds (A and C), *Azolla* initially
141 seeded at a concentration of 400 g m⁻² wet weight, was grown in the space between the
142 banks and the anti-predator net (80 ± 5.6 m²; mean ± SD of production area; Fig. 1). *Azolla*
143 growth was monitored every three days using four floating 1 m² frames (1 m x 1 m)
144 randomly placed on the *Azolla* cover. *Azolla* collected inside the frames was gently wrung
145 outweighed and its biomass was then estimated by multiplying the estimated total surface
146 covered by *Azolla*. *Azolla* was harvested when density was higher than 400 g m² *Azolla* and
147 used to feed fish, in replacement to commercial pellets, at about 8% of the estimated fish
148 biomass.

149 In conventional ponds (B and D), fish were fed three times per day and seven days per week
150 with commercial floating pellets (HI-PRO-VITE 781, proteins: 33%, lipids: 5%, humidity: 8%,
151 see Table 1 for details) at a daily feeding rate of 3-5% of the estimated fish biomass as
152 proposed by Aryani et al. (2017).

153

154 *c. Environmental conditions and analysis*

155 Air and water temperature and light intensity were recorded continuously throughout the
156 experiment using a data logger (Onset HOBO). A rain gauge placed on the production site
157 recorded the daily rainfall.

158 Water was sampled at the inlet channel and at the outlet of each pond every two weeks, as
159 described by Pouil et al. (2019). Dissolved oxygen (DO, mg L⁻¹), pH, temperature (Celsius),
160 and conductivity (μS cm⁻²) were recorded in situ between 07:00 and 08:00 AM with a multi-

161 parameter probe (HI9829 Hanna). Water samples were transported to the laboratory in a
162 cooler at 4-5°C to determine the concentrations (mg L⁻¹) of ammonium (NH₄⁺), nitrite (NO₂⁻),
163 nitrate (NO₃⁻) and orthophosphates (PO₄³⁺). These analyses were performed using a
164 spectrophotometer Hach DR 2010.

165 *Azolla* samplings were carried out every four days for proximate analysis. For each bag of
166 commercial fish feed, a sample of 500 g was taken and then mixed with other samples for
167 monthly proximate analysis. All the samples were analysed following AOAC methods (AOAC,
168 1999). Total P in *Azolla* and commercial feed was determined using an inductively coupled
169 plasma optical emission spectrometer (ICP-OES).

170

171 *d. Evaluation of fish production*

172 Fifty fish were individually sampled every 30 days, their wet body weight and total length
173 were recorded, and condition factor (K_{Fulton}) determined according to Froese (2006):

$$174 \quad K_{Fulton} = \frac{Weight (g)}{Lenght (cm)^3} \times 100$$

175 Survival rates (SR in %), the specific growth rate in body weight (SGR, % d⁻¹), and feed
176 conversion ratio (FCR) for commercial pellets distributed were calculated at the end of the
177 experiment.

178

179 *e. Statistical analysis and accounting data*

180 Due to technical constraints, only two ponds were used to evaluate each of the production
181 systems, the statistical analyses were done to assess the differences between each of the
182 four independent ponds. After checking for data normality using a Shapiro-Wilk test and the
183 equality of variances using the Brown-Forsythe test, parametric one-way ANOVAs were used
184 to assess differences of water parameters between the four ponds. A non-parametric

185 Kruskal-Wallis one-way ANOVA on ranks was used when assumptions for using parametric
186 tests were not met (K_{Fulton} , fish weight). Comparisons have been performed using Tukey's
187 test, or Dunn's test for non-parametric Kruskal-Wallis. The quantity of *Azolla* harvested were
188 compared between the two co-production ponds using a t-test. Correlations between water
189 quality, environmental conditions and *Azolla* harvested were assessed using Spearman's
190 rank correlation. Fish survival in ponds was tested using a chi-square (X^2) analysis. The
191 significance level for all analyses was set at $\alpha = 0.05$.

192 All costs were recorded (commercial feed, fish seed, labour, etc.) and production costs were
193 calculated for conventional and co-cultured ponds.

194

195 **Results**

196 *a. Environmental parameters and water quality*

197 Throughout the experiment, air temperature ranged between 20.4 and 43.5°C, and water
198 temperature varied between 25.7 and 38.6°C. The average daily diurnal (12D) light intensity
199 was 23,000 lux with maximum values reaching 176,000 lux. Rainfall ranged from 0 to 75 mm
200 day⁻¹. No significant difference in water parameters appeared between conventional and co-
201 cultured pond. Water pH varied from 6.0-7.3 and conductivity from 86 to 136 $\mu\text{S cm}^{-1}$.
202 Dissolved oxygen (DO) ranged from 0.8 to 6.4 mg L⁻¹. Nitrogen (N) chemical forms were
203 similar among the ponds (i.e. 0.3-3.3 mg NH₄⁺ L⁻¹, 0.0-0.1 mg NO₂⁻ L⁻¹ and 0.0-17 mg NO₃⁻ L⁻¹,
204 as well as orthophosphates (i.e. 0.9-13.2 mg PO₄³⁺ L⁻¹). Spearman correlations between
205 lightness, rainfall, air temperature, water quality and *Azolla* productivity were not significant
206 ($\alpha = 0.09$ to 0.46).

207

208 *b. Azolla production*

209 During the experiment, only two periods of *Azolla* production were completed (first period:
210 20 November to 12 December and second period: 24 January to 15 February). Throughout
211 the three-months experiment, 40 days of *Azolla* production was observed and the coverage of
212 *Azolla* inside the productive area varied from 5 to 100%. The yield of harvestable *Azolla* every
213 three days was significantly higher in pond A than pond C ($697 \pm 396 \text{ g m}^{-2}$ vs. $445 \pm 275 \text{ g m}^{-2}$;
214 $\text{mean} \pm \text{SD}$; $p = 0.039$). Proximate and P analysis in *Azolla* throughout the experiment are
215 shown in Table 1.

216

217 *c. Fish production parameters and costs*

218 Fish survival rates at the end of the experiment ranged from 83 to 100% and were similar
219 between three ponds (ABC) (i.e. 100%), while for pond D a disease, presumably due to a
220 bacterial infection caused by *Aeromonas* spp., significantly decreased the survival compared
221 with the other ponds (83%, Chi-square= 1317.082 with 3 degrees of freedom. ($P = <0.001$)).
222 In co-cultured ponds, fresh *Azolla* was distributed for 13 feedings and the quantities fed
223 ranged from $10.2 \pm 3.7 \text{ kg}$ for pond A and $7.0 \pm 3.4 \text{ kg}$ for pond C ($\text{mean} \pm \text{SD}$) according to
224 *Azolla* availability and fish growth. *Azolla* was generally completely eaten by fish within 24 h.
225 Partial replacement of pellets with *Azolla* did not influence giant gourami growth. The
226 average final body weight was highest in fish produced in co-cultured ponds $p < 0.001$, (Fig.
227 2). However, the K_{Fulton} index was significantly higher in fish from conventional ponds (Fig. 3).
228 SGR was similar in all ponds but the FCR - based on commercial pellets- was high in all ponds
229 and reached up to 6.9 in pond D. Fish production data were summarized in Table 1. In co-
230 cultured ponds, *Azolla* production lowered commercial feed needs by 65 and 58 kg for ponds
231 A and C, respectively. These quantities represented 14.8% of the total amount of commercial
232 feed distributed in these ponds.

233 Both conventional and co-cultured ponds were showing a negative financial net-benefit.
234 Nevertheless, the loss was four times less for co-cultured ponds than under conventional
235 management (Table 3). For both types of ponds, the major production cost was the price of
236 juvenile fish, which accounted for more than 52% of total production costs. Commercial feed
237 was the second most important production cost (27-28%) but in co-cultured ponds, *Azolla*
238 production lowered commercial feed needs by 65 and 58 kg for ponds A and C, respectively.
239 These quantities represented 14.8% less of the total amount of commercial feed distributed
240 in these ponds. Assuming a price of 0.67 US\$ kg⁻¹ for pellets, the use of *Azolla* results in a
241 sparing of 104 US\$ per production cycle for the two co-cultured ponds. Fish were sold for
242 stock growth-out fish ponds. The selling price was higher according to the size of fish, and
243 the best price, determined through inspection of fish by the buyers, was reached by fish
244 from co-cultured pond A. Other aspects regarding the budget are shown in the accounting
245 table (Table 3).

246

247 **Discussion**

248 The use of floating macrophytes may represent an interesting alternative for the partial
249 replacement of commercial feed in small-scale fish farming. Reasonable replacement,
250 usually less than 30% of commercial feed, with *Azolla* and other macrophytes, may
251 significantly lower both economic costs and the environmental impact of omnivorous fish in
252 tropical aquaculture without or only minimal impact on fish growth (Abdel-Tawwab 2006;
253 Abou et al. 2007a). In another experiment (unpublished data), the substitution rate of
254 commercial pellets with about 30% *Azolla* induced a significant impairment in the growth of
255 giant gourami. However, in the present study, the commercial feed replacement rate
256 reached 15%; and similar SGR and fish growth indicated that inclusion of fresh *Azolla* at this

257 rate in the diet has no negative impact on growth and zootechnical performances in
258 juveniles of giant gourami. Overall, fish production parameters were similar in both systems.
259 The survival rates can be considered very high for this species, except for one conventional
260 pond affected by a disease.

261 The feeding rate used in this field trial ranged from 3 to 5% of fish biomass according to fish
262 size was higher than the feeding rate generally used by small-scale fish farmers (2-3% of fish
263 biomass, FAO, 2019). Nonetheless, despite the relatively high feeding rate, giant gourami
264 juveniles showed a low growth rate during the experiment in both production systems. Fish
265 farmers are well aware of this slow juvenile growth. They strongly restrict the distribution of
266 commercial feed to fish during this growth period because, in their view, fish do not benefit
267 from the commercial feed; however, the reduction of feeding rate is often due to cash flow
268 issues and to lower the production costs. Nevertheless, the very high FCR, observed
269 especially in smaller fish in our ponds, corroborate this empirical observation of fish farmers
270 and suggest that specific improvement in feeding strategies and feed formulations are
271 required for the early stages of giant gourami in rearing ponds.

272 Co-production of *Azolla* can reduce commercial feed distribution and promote ecological
273 intensification. Indeed, beyond economic returns, lowering the amount of commercial feed
274 is key to reduce the impact of aquaculture on the environment. In Indonesia, industrial feed
275 represents the input with the most substantial impact in aquaculture and changes in feed
276 composition, using less fishmeal and more local plant-based materials, may improve the
277 environmental footprint as shown in carp and tilapia production (Mungkung et al. 2013). The
278 same authors stated that the reduction of FCR from 2.1 to 1.7 may decrease eutrophication
279 by about 22% (Mungkung et al. 2013). In the giant gourami monoculture system, the use of
280 commercial feed is far to be efficient since only 15% and 3% of the N and P introduced in the

281 ponds respectively were recovered in harvested fish (Pouil et al. 2019). Thus, the use of
282 commercial pellets should be critically evaluated from the economic and ecological point of
283 view. However, in integrated systems, like our co-culture production system, FCR is not the
284 only relevant indicator to look at for reducing environmental impacts. Indeed, in such
285 systems, when larger amounts of poor feeds are used, the unfed parts of those feeds can
286 enhance the primary productivity of the pond, particularly when the management is
287 optimised by reducing water flow and efficiency of fertilizer (Pucher et al., 2015; 2017).
288 However, this field study showed that *Azolla* cultivation on a large production scale might be
289 challenging; optimized production practices may require specific conditions (see Brouwer et
290 al., 2018). These conditions may be complex to implement entirely in fish ponds.

291 Despite the monitoring of air and water temperature, rainfall, and light intensity, it was not
292 possible to show any correlation between these factors and the production of *Azolla* in
293 earthen ponds. However, an experiment carried out in the same area of the present study
294 showed that air temperature and sunlight positively impact *Azolla* production when the
295 fertilization level is high (Pouil et al., 2020). The growth of *Azolla* is also driven by levels of P
296 (Temmink et al., 2018), but not the level of N because, *Azolla* species can use atmospheric N
297 due to their symbiosis with the cyanobacteria *Nostoc* (ex. *Anabaena*) *azollae*. Thus, *Azolla*
298 growth may be vigorous even in natural N-limited conditions (Brouwer et al., 2017).

299 Organic fertilization with chicken manure is a widespread practice among giant gourami fish
300 farmers, who use organic manure mixed with rice bran to induce phytoplankton blooms and
301 zooplankton production. The amount of chicken manure used in this trial was determined
302 based on local practices and it was very high compared to the amount of chicken manure
303 generally used in Indonesia (i.e. 500 kg ha⁻¹; Supriyadi and Rukyani, 2000). Despite the high
304 organic fertilizer input, the water nutrient level was insufficient to guarantee the sufficient

305 growth of *Azolla*. In fact, in our conditions, we observed high P and N trapping in pond
306 sediments, likely due to the deep layer of mud on the pond bottom (Pouil et al., 2019).
307 Proximate analysis of *Azolla* showed the fluctuating level of P was generally below that
308 estimated as optimal for the growth of *Azolla* (Pouil et al., 2020), suggesting the P level was
309 not sufficient to ensure the continuous growth of this macrophyte species. The use of
310 poultry manure in aquaculture is therefore regulated by Indonesian rules (Supriyadi and
311 Rukyani, 2000). Bearing in mind the uncertain impact of chicken manure on *Azolla*
312 production in earthen ponds and its potential environmental and health impacts, we suggest
313 that the use of poultry manure should be thoroughly reconsidered for co-culture
314 gourami/*Azolla* ponds. However, fertilization can be improved in other ways. For example,
315 more appropriate pond cleaning can lower the nutrient trapping by sediment. The
316 availability of nutrients in water can be also enhanced by increasing the N and P fluxes from
317 the sediment into the water column. For instance, stocking the common carp *Cyprinus*
318 *carpio* (Linné) at a density of 0.5 fish m⁻² can have substantial effects on nutrient fluxes from
319 pond sediment due to the carp bioturbation activity, thereby increasing primary and
320 secondary production (Rahman, 2015).

321 Beyond nutrient deficiencies, other factors compromised *Azolla* production in ponds,
322 particularly unwanted grazing. Fish, especially small tilapia trapped between the
323 antipredator net and the pond bank, actively graze on *Azolla*, as also demonstrated by
324 several authors (El-Sayed, 1992; Abdel-Tawwab, 2006). Furthermore, *Azolla* underwent
325 predation due to the golden apple snail *Pomacea canaliculata* (Lamarck). This snail
326 proliferates in ponds with *Azolla* (0.07 kg m⁻² or approx. 3-4 adult individuals m⁻²) and can
327 ingest 50% of its weight per day of *Azolla* (Pouil et al., 2020). The small China-mark moth,
328 *Cataclysta lemnata* (Linné) (Order Lepidoptera, family Crambidae), is another source of

329 predation for *Azolla*. It has a heavy impact on the *Azolla* cover and can completely wipe it
330 out (Farahpour-Haghani et al., 2017). Eventually, all types of predation can seriously
331 compromise the productivity of *Azolla*, especially when its growth rate is low.

332 Several studies have shown that small floating macrophytes, such as *Azolla* spp. and *Lemna*
333 spp. may hold promise for bioremediation, particularly for dissolved N and P responsible for
334 eutrophication (Madsen and Cedergreen, 2002; Ferdoushi, 2008). Overall, water quality and
335 phosphates remained within the standards for tropical fish rearing throughout the entire
336 experiment (Fewtrell and Bartram, 2001; Kim et al., 2013). The absence of statistical
337 differences between conventional and co-cultured ponds suggest that the biomass of *Azolla*
338 and/or nutrient concentrations were too low to measure any effects. Water deterioration
339 may arise when the water surface is covered by extensive mats of floating macrophytes,
340 causing a reduction in water/air exchanges and light penetration for photosynthesis
341 (Slembrouck et al., 2018). That did not happen in our study, because the *Azolla* was
342 restrained between the pond's embankment and the antipredator net. The stability of
343 water parameters suggests that coverage of 20% of the pond with *Azolla*, does not affect the
344 water quality. Probably, the macrophyte surface could be increased by a reasonable amount,
345 as tested in tilapia ponds (Abdel-Tawwab 2006).

346 Production costs were highly impacted by the cost of fish seed and then by the cost of
347 commercial feed, together representing 80% of overall production costs. The giant gourami
348 production chain is highly segmented, and fish can be sold several times before reaching
349 consumption size (FAO, 2019). The same study also highlighted that, in the pre-grow-out
350 phase, seed costs might be as high as 40% of the sales price. This prohibitive cost can be
351 explained by the low and highly variable fry availability for farmers (Arifin et al., 2013;
352 Kristanto et al., 2020). Availability of fry is recognized as one of the main impediments in

353 developing giant gourami aquaculture (Amornsakun et al., 2014; Slembrouck et al., 2019).
354 For this reason, small-scale fish farmers sometimes prefer to stop feeding the fish, whose
355 loss on fasting remains low and wait for higher selling prices. Considering the high cost of
356 giant gourami seed, the positive impact of reducing commercial feed was not noteworthy,
357 but it can be relevant in grow-out conditions, where the feeding cost represents between 50
358 % and 70 of the production costs, and the cost of juveniles per kg of fish produced is
359 proportionally less. During pre-grow-out, fish farmers greatly decrease or stop feeding fish
360 when they judge that feed costs are too high or when they run out of liquidities.

361 Although our conclusion is limited by the number of ponds available to perform the
362 experiment and the discontinuity in *Azolla* production, we demonstrated here that co-
363 production of *A. filiculoides* in the pond did not generate any extra costs. If *Azolla* can be
364 reliably produced, it is reasonable to expect a 20-25% reduction in feed costs. Thus, co-
365 culture *Azolla*/giant gourami may be more profitable for the grow-out period of fish, which
366 may require larger production surfaces and higher levels of dissolved nutrients. Enhancing
367 ponds' productivity and/or using ecosystem resources, such as macrophytes in co-
368 production, may be more affordable for small fish-farming. Further long-term investigations,
369 using a larger number of ponds, would be relevant to confirm our findings and improve the
370 co-culture production system's performances. We still recommend using co-produced *Azolla*
371 spp. or *Lemna* spp. in earthen ponds to giant gourami fish farmers, even considering the
372 difficulties encountered for its continuous production and some remaining uncertainties.

373 Indeed, macrophyte cultivation in a tropical earthen pond is not a risky initiative for fish
374 farmers as it does not generate extra costs and is not time-consuming. Furthermore, as
375 proposed here, it does not cause changes in production methods or competition for land or
376 water. Co-production of macrophytes may be an alternative source of feed for giant gourami

377 in small-scale fish farms and probably for other omnivorous fish. As IMTA is the production
378 of different organisms linked to the same trophic chain in the same environment (Rosa et al.,
379 2020), co-culture of macrophyte and fish in the same fish pond can be considered as a
380 simplified model of IMTA pond. The integration of macrophytes in IMTA pond systems may
381 be a promising way to sustain and promote production in these systems (Kibria et al., 2018).
382 This kind of ecological intensification in earthen pond aquaculture can lower production
383 costs and decrease the environmental impact from commercial feed, thus increasing the
384 sustainability of the small-scale aquaculture prevailing in Indonesia.

385

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392

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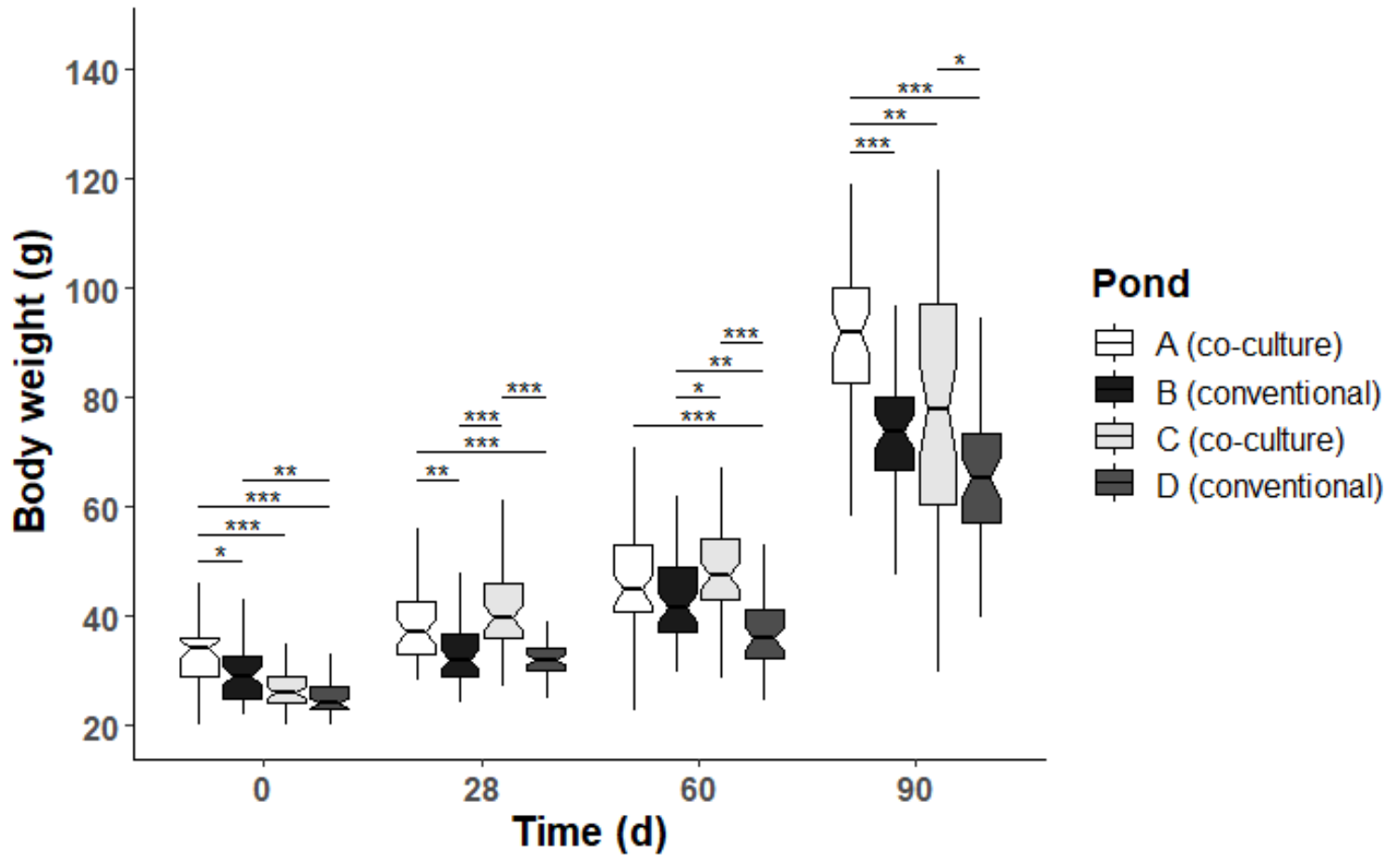
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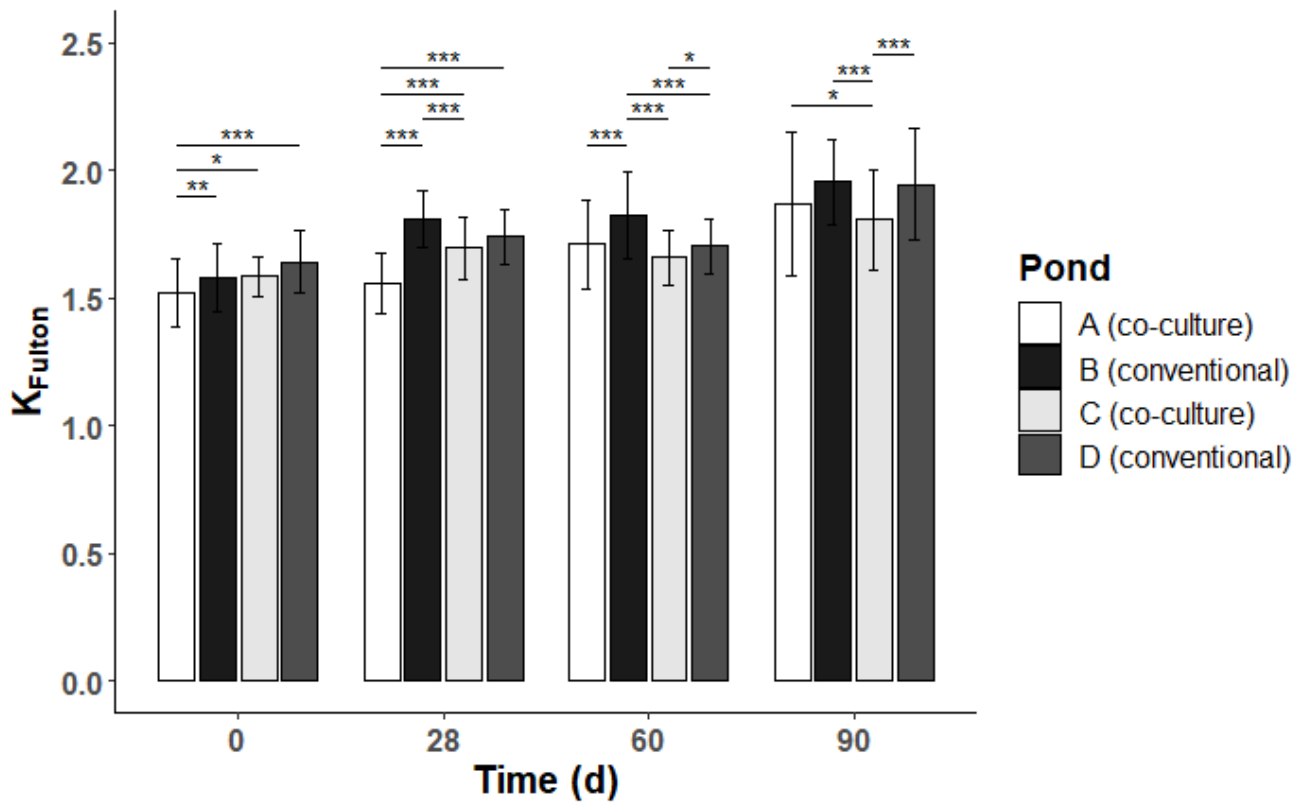
536



537 **Figure 1.** Space between the banks and the anti-predator net (1 m) in a conventional pond
538 (on the left) and the same space covered by *A. filiculoides* in a co-cultured pond (on the
539 right). Both types of ponds have fence nets to protect fish from predators (Photo IRD - J.
540 Slembrouck).



541 **Figure 2.** Body weight of giant gourami fed exclusively with commercial feed in conventional
 542 ponds (B and D) and fed with commercial feed and *Azolla* alternatively fed with *Azolla* in co-
 543 cultured ponds (A and C) (n = 50 fish per pond for each sampling time). A disease occurred in
 544 pond D during the experiment. Whiskers indicate the minimum and maximum, black lines
 545 indicate the medians, and boxes represent the 25th and 75th percentiles. Symbols denote
 546 significant difference (* p < 0.05, ** p < 0.01, *** p < 0.001).



547 **Figure 3.** Body condition factor K_{Fulton} of giant gourami fed exclusively with commercial feed
 548 in conventional ponds (B and D) and fed with commercial feed and *Azolla* alternatively fed
 549 with *Azolla* in co-cultured ponds (A and C) ($n = 50$ fish per pond for each sampling time).
 550 Data are means \pm SD. Symbols denote significant difference (* $p < 0.05$, ** $p < 0.01$, *** $p <$
 551 0.001).

552 **Table 1.** Composition of the different feeds used in the experiment (i.e. *Azolla* and
 553 commercial pellets). Proximate data are expressed in percentage of dry matter (DM) and
 554 energy is expressed as MJ kg⁻¹ fresh weight. Phosphorus (P) concentrations are expressed as
 555 g FW kg⁻¹. Data are given in means ± SD.

Parameter	Commercial pellets (n = 3)	<i>Azolla</i> (n = 22)
Dry matter	92.3 ± 0.3	7.6 ± 1.1
Crude proteins	32.6 ± 0.3	19.3 ± 6.0
Crude lipids	5.4 ± 0.1	1.8 ± 0.7
Ash	10.1 ± 0.4	11.6 ± 1.9
Fibre	4.6 ± 0.2	15.0 ± 3.3
Nitrogen-free extract (NFE)	47.2 ± 0.3	52.2 ± 4.7
P	13.9 ± 1.2	0.2 ± 0.1

556

557 **Table 2.** Production parameters for giant gourami juveniles (fish growth and feed efficiency)
 558 in two production systems: conventional ponds (B and D) and co-cultured *Azolla*/giant
 559 gourami ponds (A and C). Weights are expressed as fresh weight .

Parameter	Conventional ponds		Co cultured ponds	
	Pond B	Pond D*	Pond A	Pond C
Pond surface (m ²)	482	358	418	353
Initial number of fish	2970	2220	2590	2200
Biomass gain (kg m ⁻²)	0.35	0.13	0.33	0.20
Specific growth rate (% d ⁻¹)	1.04	1.11	1.06	1.25
Pellets distributed (kg)	461	312	368	328
<i>Azolla</i> distributed (kg)	0	0	118	82
FCR** pellets	2.7	6.9	2.6	4.8

567

568 * A disease occurred in pond D during the experiment.

569 ed** FCR: feed conversion ratio

570 **Table 3.** Accounting table for co-cultured (A and C) and conventional ponds (B and D). Data are
 571 expressed for 100 m² of pond's area and one production cycle (3 months) in \$US from Indonesian
 572 Rupiah (IDR). Conversion rate 1 US\$ = 13 388 IDR (February 2018).

573

	Conventional ponds (B + D*)		Co-cultured ponds (A+C)	
Fixed costs	Values (\$US) for 100 m ²	% of production costs	Values (\$US) for 100 m ²	% of production costs
Net: 0.06 \$US m ⁻² (depreciation over 4 years)	5.8	3	5.8	3
Bamboo poles: 0.03 \$US m ⁻² (depreciation over 3 years)	2.5	1	2.6	1
Seed: 0.19 \$US fish ^{-1**}	115.4	52	116.0	56
<i>Subtotal</i>	<i>123.7</i>	<i>55</i>	<i>124.4</i>	<i>60</i>
Variable costs	Values (\$US) for 100 m ²	% of production costs	Values (\$US) for 100 m ²	% of production costs
Lime: 0.23 \$US kg ⁻¹	2.6	1	2.6	1
Fertilizer: 0.07 \$US kg ⁻¹	16.7	7	16.7	8
Commercial feed: 0.67 \$US kg ⁻¹	61.9	28	55.7	27
<i>Subtotal</i>	<i>81.2</i>	<i>36</i>	<i>75.0</i>	<i>36</i>
Workforce	Values (\$US) for 100 m ²	% of production costs	Values (\$US) for 100 m ²	% of production costs
Pond preparation	4.6	2	4.3	2
Transport and seed counting	13.8	6	12.5	6
<i>Subtotal</i>	<i>18.5</i>	<i>8</i>	<i>16.8</i>	<i>8</i>
Total production costs	Values (\$US) for 100 m ²	% of production costs	Values (\$US) for 100 m ²	% of production costs
Sum of all costs	223.5	100	206.0	100.0
Production costs (\$US fish ⁻¹)	0.39		0.33	
Turnover				
Selling: 0.31-0.36 \$US fish ⁻¹ (according to fish size)**	186.2		196.4	
Net income \$US	-37.3		-9.5	
Deficit		-17%		-4%

574

575 * A disease occurred in pond D during the experiment.

576 **Prices for seeds and harvested fish can greatly vary throughout the year and are highly
577 dependent on fish quality evaluated by the buyers (FAO, 2019). Furthermore, according to the
578 demand/offer of juveniles of giant gourami, its price can highly fluctuate.

579