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Fate of commercial pellets and role of natural productivity in giant gourami ponds using stable isotope analyses

Simon Pouil, J M Mortillaro, Reza Samsudin, Domenico Caruso, Anang Hari Kristanto, Jacques Slembrouck

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17 **Abstract**

18 A better understanding of the fate of commercial pellets and natural resources in semi-
19 intensive aquaculture can help to reduce environmental impacts as well as the overall costs of
20 production in pond production systems. However, in these mixed systems, trophic structures
21 can be complex. Here, we investigated the trophic web structure in Indonesian giant gourami
22 (*Osphronemus goramy*) pond based on the analysis of the values of stable carbon and
23 nitrogen isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in 10 pond components of different trophic levels (i.e.
24 plankton of different sizes, macroinvertebrates, periphyton, fish, sediment and commercial
25 pellets). Isotope niche spaces (standard ellipse areas) were calculated for giant gourami at the
26 beginning (month 0), the middle (month 3) and the end (month 5) of the trial. Our results
27 showed that commercial pellets were the primary diet source for giant gourami throughout the
28 152-day trial in ponds. Nevertheless, differences in ^{15}N and trophic niche sizes at month 0,
29 month 3 and month 5 suggested slight changes in the giant gourami diet during the trial.
30 Altogether, our results highlight that, in a typical omnivorous species reared in semi-intensive
31 pond conditions, the endogenous resources of the pond are barely utilized and the diet is
32 mainly based on commercial pellets. Such findings highlight the need of optimizing the use of
33 both natural pond resources and commercial pellets.

34 Keywords: Formulated feed, Giant gourami, Intensification, Semi-intensive aquaculture,
35 Stable isotopes

36 **1. Introduction**

37 Pond aquaculture is one of the predominant production systems in aquaculture (Boyd, 2013).
38 This represents about 80% of finfish aquaculture. Most pond fish production is attributable to
39 cyprinid culture in China, India, and Southeast Asia, and tilapia culture and brackishwater
40 culture of milkfish (Tucker and Hargreaves, 2012). Traditionally, aquaculture production in
41 ponds is based on practices favouring primary productivity with limited inputs of feeds,
42 especially commercial pellets. Such aquaculture productions are generally reported to use
43 resources efficiently, especially when considering waste nutrients and ecosystem resources in
44 integrated agriculture-aquaculture systems (Nhan et al., 2007). Nevertheless, on a global
45 scale, the productivity of conventional earthen ponds where fish are fed with commercial
46 feeds and without supplemental aeration remains low (av. 2000 kg/ha/year), constrained by
47 the availability of suitable nutritional inputs (Bosma and Verdegem, 2011). The development
48 of tropical freshwater aquaculture in recent years has resulted in a shift from low-intensity
49 traditional culture methods to semi-intensive monoculture production systems, with
50 increasing intensification based on the use of commercial pellets (Edwards, 2009; Rimmer et
51 al., 2013; Tacon and Metian, 2015). Thus, worldwide, the percentage of non-fed species in the
52 total volume of production has declined from more than 50% in 1980 to 30% in 2018
53 consisting of 8 million tonnes of filter-feeding finfish (mainly silver carp *Hypophthalmichthys*
54 *molitrix* and bighead carp *H. nobilis*) and 17 million tonnes of aquatic invertebrates (FAO,
55 2020), mostly due to the intensification of Asian freshwater production.

56 Although commercial pellets are one of the main drivers behind the expansion of semi-
57 intensive, small-scale pond aquaculture (De Silva and Hasan, 2007; Edwards, 2015), there are
58 always growing concerns about their environmental impact. Nutrition and feeding will play
59 an essential role in the sustained development of this aquaculture. Thus, refine and optimized
60 feed formulations (e.g. Kabir et al., 2020) should be sought at the same time as improvement

61 of pond management and manipulation of pond productivity (e.g. Joffre, 2020) that maximize
62 both the quantity and the quality of production but limit negative externalities. In addition to
63 their environmental impact, formulated feeds are also the major expense in pond aquaculture,
64 and with the rising feed ingredient prices, feed costs may represent >80% of the total
65 production costs (Rana et al., 2009). Better understanding the fate of commercial pellets in
66 ponds can ultimately help to decrease environmental impact as well as overall production
67 costs, thus benefitting the small-scale farmers that dominate aquaculture production in
68 Southeast Asian countries. However, although commercial pellets are still one of the main
69 drivers behind increased productivity in a semi-intensive pond, a wide range of natural diet
70 sources are available (e.g. phytoplankton, zooplankton, macrobenthos, detritus, and insects).
71 This natural productivity may decrease the impact on the environment and reduce the
72 economic burden incurred by commercial pellets, thereby significantly contributing to the
73 ecological intensification of aquaculture (Aubin et al., 2019). The use of natural feeds can be
74 particularly advantageous for omnivorous species reared in a semi-intensive pond, such as the
75 giant gourami (*Osphronemus goramy* L.). This fish species is one of the main freshwater
76 commodities of economic importance and high heritage value in Indonesia. Its annual
77 production has grown exponentially over the past 15 years, reaching over 173,000 t in 2018
78 (FAO, 2021). Giant gourami is produced on small-scale farms (approx. 100,000 fish farmers;
79 BPS, 2013), usually in semi-intensive systems using organic fertilizers, to enhance the natural
80 productivity of ponds, and feed (i.e. terrestrial plants and commercial pellets) in highly
81 variable proportions (FAO, 2019). In these semi-intensive systems combining organic
82 fertilizers and feed, the real contribution of the different diet sources for fish growth is very
83 complex to determine.

84 Stable isotope ratios are a powerful analytical tool that can be used to directly assess the fish
85 diet in a pond and the fate of commercial pellets and other trophic resources. Carbon (C) and

86 nitrogen (N) stable isotope ratios are increasingly explored in pond aquaculture systems that
87 can be considered as simplified ecological systems (Feng et al., 2014; Gu et al., 1996; Nahon
88 et al., 2020; Yokoyama et al., 2002). Isotopes are typically measured as a ratio of heavy-to-
89 light isotopes, in this case, and reported as delta (δ) values. Isotopic fractionation of carbon
90 ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) takes place during nutrient assimilation by animals. $\delta^{13}\text{C}$
91 values of consumer tissues are usually similar to those of their diets, thereby helping to
92 identify feed sources (DeNiro and Epstein, 1978), and $\delta^{15}\text{N}$ values become enriched from
93 prey to consumer and can be used to estimate the trophic position of the consumer (Peterson
94 and Fry, 1987).

95 Our study aimed to use carbon and nitrogen stable isotope analysis to characterize the trophic
96 behaviour of giant gourami reared in a semi-intensive system in ponds. Here, intensified
97 aquaculture production was considered using organic fertilization and commercial pellets as
98 the unique feed resources for fish. To investigate the consequences of intensification as
99 currently practised by small-scale fish farmers in Indonesia, the ponds were fertilized and
100 managed according to the local fish farmers practices while commercial pellets were used as
101 an external feed resource

102

103 **2. Materials and Methods**

104 2.1. Pond systems and management

105 The experiment was performed on a small-scale fish farm located in the village of Babakan (-
106 $6^{\circ}28'S$; $106^{\circ}42'E$), Bogor district, West Java, Indonesia. Giant gourami juveniles were reared
107 for 5 months in a semi-intensive system consisting of four 0.5 m-deep earthen ponds whose
108 size varied from 353 to 482 m^2 . Fish (29 ± 6 g wet weight) were stocked at a density of 6
109 individuals m^{-2} in each pond and protected against predators using nets held by bamboo poles

110 implanted in the bottom of the pond. The structure of the ponds and management practices are
111 described in detail in Pouil et al. (2019). Briefly, empty ponds were limed and fertilized using
112 organic fertilizer (typical composition: poultry manure mixed with rice bran at a 0.5:1 ratio on
113 a dry weight basis). The quantity of organic fertilizer was based on the empirical knowledge
114 of local fish farmers. Fertilizer was then added four times during the first three months of the
115 experiment (equiv. 2.1-2.7 kg m⁻²).

116 Each pond was connected to the water inlet and outlet channels using polyvinyl chloride
117 (PVC) pipes and no aeration was provided. Each pond was connected to water inlet and outlet
118 channels using polyvinyl chloride (PVC) pipes. Water renewal was 6% per day in average
119 throughout the experiment the opening time was about 80% over the trial. Water quality data
120 were recorded as described by Pouil et al. (2019) and Caruso et al. (2021).

121 Throughout the experiment, fish were fed seven days a week with the same commercial
122 extruded pellets (gross energy: 15.38 ± 0.05 MJ kg⁻¹; see Table 1 for details). The daily ration
123 feeding rate was 3-5% (average of 4%) of the estimated fish biomass as proposed by Aryani
124 et al. (2017) and it was adjusted according to fish growth assessed as described in Section
125 2.2.1. Fish were fed three times a day by the same experienced fish farmer throughout the
126 experiment and feeding stopped and postponed in case no fish feeding activity was observed
127 to avoid waste.

128

129 2.2. Sampling procedures

130 2.2.1. Fish

131 Fish were sampled monthly from the beginning of the experiment. Every month, 50 fish from
132 each pond were caught in nets and individually weighed and measured to determine growth
133 and adjust the feed ration. At harvest, the remaining fish were counted to assess survival and

134 the total biomass of produced fish was determined for each pond. At the beginning of the
135 experiment, and then after 3 and 5 months, 5 fish per pond were sampled, euthanized and
136 pooled for the stable isotope analysis.

137 2.2.2. Macroinvertebrate species

138 Benthic invertebrate species were listed and their average biomass (fresh weight) was
139 estimated from a previous investigation (Pouil et al., 2019). Freshwater snails (*Pomacea*
140 *canaliculata* and *Filopaludina javanica*; 70 g m⁻²), bivalves (*Pilsbryoconcha exilis*; 110 g m⁻
141 ²) and aquatic insects (*Notonecta* sp.; <1 g m⁻²) are the dominant macroinvertebrate species in
142 the water column and no significant macroinvertebrate community was found in the first 10-
143 cm layer of the sediment. Sampling was done at the end of the experiment for stable isotope
144 analysis. Aquatic insects were collected directly from the water column using a 500 µm mesh
145 size landing net (40 x 20 cm). Samples were taken at three different locations in the pond
146 providing a composite sample. Benthic species were manually sampled using a PVC quadrat
147 (90 x 90 cm) when ponds were emptied. The quadrat was placed on the sediment and all the
148 individuals inside the quadrat were collected. This sampling procedure was repeated at three
149 sampling locations from the inlet to the outlet of each pond. All the samples were weighed,
150 shells of bivalves and snails were removed and tissues were rinsed in reverse-osmosis water
151 then transferred to labelled plastic bags and kept in a cooler for <1 h during transport to the
152 laboratory.

153 2.2.3. Plankton

154 Plankton samples were taken according to Roucate and Quemeneur (2007) and Bayona
155 (2014) at the end of the experiment between 10:00 am and 12:00 pm. Briefly, PVC tubes (63
156 mm diameter, 1 m length) that slid along wood poles were capped at one end and placed at
157 three locations in each pond at the beginning of the experiment. This device made it possible

158 to rapidly sample the whole water column and prevent the escape of the swimming
159 organisms. At least three replicate samplings were performed in each pond for a final volume
160 of 10 L. The water collected was then filtered on 500 μm , 200 μm and 40 μm sieves. The >1
161 mm fraction, mainly composed of debris, was discarded and the other fractions were rinsed
162 with reverse-osmosis water. The plankton was thus divided into three different fractions based
163 on size: 0-40 μm , >40-200 μm and >200-1000 μm . Among the phytoplankton, Cyanophyceae
164 (mostly *Oscillatoria* sp. and *Microcystis* sp.) largely dominated the three fractions (from 86 to
165 98% of the counted cells). Zooplankton occurred in the two largest-size fractions. For the
166 >40-200 μm fraction, non-identified crustacean nauplii and *Trichocerca* sp. were the
167 dominant taxa (i.e. 28 and 25% of the individuals counted, respectively) while *Trichocerca*
168 sp., *Eurytemora* sp. and *Daphnia* sp. dominated in the >200-1000 μm fraction (i.e. 26, 23 and
169 21% of the individuals counted, respectively). The collected material was then transferred to
170 polypropylene vials (one per fraction and per pond) and kept in a cooler for transport to the
171 laboratory. Previous studies highlighted differences between lake plankton taxa and seasons
172 for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Syväranta and Rautio, 2010; Vuorio et al., 2006). Nevertheless,
173 because the photoperiod (12h00 \pm 30 min), the mean daily temperature (27-32°C) and the
174 water exchange only slightly changed over the 152-d trial, we assume that plankton had
175 isotopic signatures that varied little over the experiment. Thus, samplings were performed at
176 the end of the experiment following previous similar studies (Narimbi et al., 2018; Duffy et
177 al., 2011), as for all the other investigated trophic compartments, and were considered
178 sufficiently representative while avoiding minimizing disturbances for the fish.

179 2.2.4. Periphyton

180 Before the beginning of the experiment, two polycarbonate rectangles (20 x 50 cm) were set
181 at two locations in each pond. To sample periphyton at the end of the experiment, the
182 polycarbonate rectangles were removed from each pond and rinsed with osmosis water. Then,

183 the surface of each rectangle in contact with pond water (i.e. 1000 cm²) was carefully and
184 entirely scraped to collect the periphyton and transferred to polypropylene vials. The samples
185 were kept in a cooler during transport to the laboratory (see Section 2.3).

186

187 2.2.5. Sediments

188 Sediments accumulated during the experiment were sampled at harvesting in five different
189 locations in each pond using a 63 mm x 10 cm (w x h) PVC tube. For each pond, sediments
190 taken from the different locations were thoroughly mixed into a composite sample as
191 described by Pouil et al. (2019) and kept for stable isotope analysis.

192

193 2.2.6. Commercial pellets

194 Samples from each bag of commercial pellets were taken throughout the experiment (100 g
195 per bag). Samples were then pooled monthly for stable isotope analysis. Proximate analyses
196 were also performed on subsamples (n = 5) of commercial pellets as described in Section 2.4.

197

198 2.3. Sample preparation

199 All samples were transported in a cooler at 4-5°C to the laboratory of Nutrition and Feed
200 Technology of the Research Institute for Freshwater Aquaculture and Fisheries Extension
201 (RIFAFE). Fish were dissected and white muscle was sampled. All the commercial pellets,
202 fish flesh, macroinvertebrates and periphyton were dried at 40°C for 24 h to 48 h and then
203 manually ground into a fine homogeneous powder and transferred to Eppendorf® tubes.
204 Plankton samples were filtered through glass fibre filters (Whatman GF/F, porosity 0.7 µm,
205 47 mm diam.) using a filtration unit connected to a vacuum pump under low pressure, rinsed
206 with reverse-osmosis water and then dried at 40°C for 24 h. Before sample filtration, filters
207 were placed in a muffle furnace set at 450°C for 5 h to remove any trace of organic carbon or
208 nitrogen material. All the samples were kept under vacuum and sealed before analysis. To
209 remove ¹³C-depleted lipids (DeNiro and Epstein, 1977), fish tissues (lipids: 24% of dry

210 matter) were treated with cyclohexane, as described by Nahon et al. (2020) to avoid
211 influencing $\delta^{15}\text{N}$ values (Chouvelon et al., 2014), unlike other lipid extraction methods (e.g.
212 Post et al., 2007; Schlechtriem et al., 2003). Between 0.2 to 1 mg of each dried sample was
213 then weighed and packed into a tin capsule for simultaneous analysis of carbon and nitrogen
214 stable isotopes as described in Section 2.4.

215 2.4. Stable isotope analysis

216 The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of samples were analysed using elemental analyser/isotope ratio
217 mass spectrometry (EA/IRMS) in continuous-flow mode on a Delta V Plus isotope ratio mass
218 spectrometer with a ConFlo IV interface (Thermo Scientific, Bremen, Germany) interfaced
219 with a Flash 2000 elemental analyser (Thermo Scientific, Milan, Italy). Results are expressed
220 in the δ unit notation as deviations from standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and
221 atmospheric N_2 for $\delta^{15}\text{N}$, Peterson and Fry, 1987) using the following formula:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 10^3$$

222 where R is the ratio of heavy-to-light isotopes ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$). Analytical precisions were
223 $<0.10\text{‰}$ and $<0.15\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. Reference materials (IAEA-600,
224 USGS-24, USGS-61, USGS-62 for $\delta^{13}\text{C}$ and IAEA-N2, IAEA-NO-3, UAEA-600, USGS-61,
225 USGS-62 for $\delta^{15}\text{N}$) were used for multipoint calibration.

226 Proximate analyses of commercial pellets were performed following AOAC methods (1999).
227 Moisture was determined by weight loss upon drying at 105°C for 24 h. Crude protein was
228 determined using the standard Kjeldahl procedure (Foss Tecator Kjeltex 8400 and Kjeltex
229 Buchi); lipid content after acid hydrolysis was determined using the Weibull–Stoldt method;
230 crude ash by determining residue after heating at 550°C for 4-5 h in a muffle furnace and
231 crude fibre was determined as follows: commercial pellets were extracted with 1.25% H_2SO_4

232 and 1.25% NaOH and then dried; samples were weighed, incinerated and reweighed. Gross
233 energy content was calculated using energy value coefficients of 9 kcal for crude fat and 4
234 kcal for crude protein and carbohydrates.

235 2.5. Data analysis

236 To assess potential changes in the giant gourami diet throughout the experiment, the standard
237 ellipse area (SEA) occupied by the fish in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ spaces were calculated for giant
238 gourami at the beginning (month 0), the middle (month 3) and the end (month 5) of the
239 experiment using the Stable Isotope Bayesian Ellipses (SIBER, version 2.1.4) package in R
240 (Jackson et al., 2011). Estimation of SEA is an approach amply described in the literature and
241 increasingly used in aquaculture-related studies (Nahon et al., 2020; Sardenne et al., 2020;
242 Weldrick et al., 2017). The SEA Bayesian metric is a bivariate equivalent of the standard
243 deviation containing the mean core population isotopic niche (approximately 40% of the
244 data). SEA metrics were chosen before convex hull metrics (TA: total area of the convex
245 hull encompassing the data points; Layman et al., 2007), which include outlier individuals.
246 SEA metrics are robust to variations in sample size; the corrected version (SEAc) was used to
247 circumvent the bias that arises when sample sizes are small. SEAc sizes were then compared
248 using a Bayesian approach based on Markov chain Monte Carlo methods (Jackson et al.,
249 2011).

250 The quantitative contribution of sources to consumer diets using mixing models was not
251 evaluated assuming that 1) exhaustive sampling of ingested sources was not possible and 2)
252 fish opportunism will provide underdetermined systems (i.e. with more sources than tracers),
253 thereby increasing model inaccuracy and bias despite being Bayesian (Brett, 2014; Stock and
254 Semmens, 2016). Thus, we focused on rough data.

255 One-way analysis of variance (ANOVA) was used to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among
256 the different kinds of sample collection as well as to test the difference in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
257 values in fish harvested at different times (i.e. month 0, month 3 and month 5). Tukey's post-
258 hoc tests were used to further examine pairwise differences. Assumptions of normality and
259 homoscedasticity of residuals were tested with Shapiro-Wilk and Bartlett tests, respectively.
260 Statistical analyses were performed using R software 3.6.3 and the level of significance was
261 set to $\alpha = 0.05$.

262

263 **3. Results and Discussion**

264 3.1. Zootechnical performance of giant gourami in ponds

265 In the present study, the aquaculture system can be classified as upper-level semi-intensive
266 (Pouil et al., 2019), with average daily rationing of 4% (3-5%) using commercial pellets
267 throughout the experiment, in line with current intensification trends in Indonesian freshwater
268 aquaculture (Udo and Steenstra, 2010). Zootechnical performance of giant gourami growth
269 was measured as partially described in Caruso et al. (2021) and Pouil et al. (2019). Overall,
270 giant gourami survival rates at the end of the experiment ranged from 83 to 100% and were
271 similar for three ponds (i.e. 100%), but for the fourth pond, a bacterial infection (presumably
272 caused by *Aeromonas* spp.) significantly decreased the survival rate compared with the other
273 ponds (83%, $P < 0.01$). Similarly, growth of giant gourami was consistent in the three ponds
274 with a final weight of 193 ± 45 g ($n = 50$ per pond) after 152 d of growth, but significantly
275 lower ($P < 0.01$) in the pond affected by the bacterial disease (155 ± 41 g). The specific
276 growth rate (SGR) was 1.22 ± 0.07 % d^{-1} over the experiment.

277 Azrita et al. (2020) reported that the weight of giant gourami juveniles (initial weight of 55 g)
278 increased by a factor of 2.75 over 90 days in semi-intensive production ponds (daily rationing

279 of 3% of commercial pellets) . In the present study, fish reached a comparable size after 60
280 days (i.e. ~47 g). During the following 90 days, fish weight then increased by a factor of 3.90,
281 i.e. a growth 1.4 times greater than that reported in Azrita et al. (2020). These findings
282 confirm that increased amounts of commercial pellets lead to a better expression of the
283 growth potential of this species (Aryani et al., 2017). Over the experiment, the FCR was 2.2
284 ± 0.1 while the protein efficiency ratio (PER) was 0.67 ± 0.02 , excluding the pond affected
285 by the disease.

286 Over the first 60 days of our trial, fish growth was very low with an average weight increase
287 of 64% while the feed conversion ratio (FCR) was very high (5.1 ± 0.8 , excluding the pond
288 affected by the disease) during this period. The reasons for this low growth remain uncertain,
289 but we assume that the size of the commercial pellets distributed at the beginning of the
290 experiment (i.e. 1-2 mm), commonly used by local fish farmers, was not suitable for giant
291 gourami of this size (i.e. 30 g, 12 cm in length). Small-mouthed fish like giant gourami can
292 leave uneaten feed, at amounts reaching 30% of the feed supplied (Bosma and Verdegem,
293 2011; Van Der Meer et al., 1997; Verdegem, 2013). Pouil et al. (2019) suggested that a
294 significant proportion of the nutrients in giant gourami pond sediments come from uneaten
295 commercial pellets. Interestingly, Narimbi et al. (2018) also found very high FCR (6.4) in
296 daily-fed GIFT tilapia while FCR was six times lower in weekly-fed fish suggesting wastage
297 of feed and indicating that feeding practices can strongly affect the feed efficiency of the fish.
298 The same study has shown that despite the high FCR, the growth of daily-fed GIFT tilapia
299 was significantly higher than that of weekly-fed tilapia. Here, we assumed that, even if the
300 fish were mainly fed with pellets from the beginning of the trial, the unconsumed commercial
301 feed heavily affects the FCR and consequently, the economic outcome. Nonetheless, the
302 growth recorded in the first two months of the experiment remains high with fish at the end
303 of the trial being 1.4x bigger than that reported in a comparable study (Azrita et al., 2020).

304 We did not find any significant change in water quality with DO concentrations
305 (measurement done between 08:00 and 10:00 AM) were from $5.0 \pm 2.4 \text{ mg L}^{-1}$ while TAN,
306 NO_2^- and NO_3^- values were 0.32 ± 0.25 , 0.04 ± 0.06 and $1.13 \pm 0.36 \text{ mg L}^{-1}$, respectively.
307 Water parameter values indicated that the pond water quality remained within appropriate
308 standards for tropical freshwater fish (Colt, 2006), especially for giant gourami, an air-
309 breathing species. Nutrient concentrations were 1.47 ± 0.45 and $0.06 \pm 0.04 \text{ mg L}^{-1}$ for N and
310 P (i.e. values in the range of those observed in semi-intensive pond aquaculture; Diana et al.,
311 2017). Overall, these results suggest that water quality 1) was not affected by the feeding
312 strategy used and 2) is not likely to explain the observed variations in growth and feed
313 efficiency during the experiment

314 3.2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of food web in ponds

315 At the base of the pond's food web, stable isotope values of small-sized plankton ($< 40\mu\text{m}$)
316 ranged between -28.28 and -27.32‰ for $\delta^{13}\text{C}$, and between 0.35 and 0.84‰ for $\delta^{15}\text{N}$ and
317 differed significantly from all other trophic components ($n = 4$, $P < 0.01$, Fig. 1), except for
318 similar $\delta^{13}\text{C}$ values shared with bivalves ($p = 0.99$). The $40\text{-}\mu\text{m}$ fraction mainly discriminated
319 primary producers in our ponds. It is therefore not surprising to find the lowest isotope
320 signature for this compartment in fish ponds as observed in Narambi et al. (2018).

321 The highest isotopic signature was observed for periphyton (i.e. between -24.38 and
322 -21.12‰ for $\delta^{13}\text{C}$, and between 9.83 and 11.46‰ for $\delta^{15}\text{N}$, $n = 4$). Periphyton is a micro-
323 ecosystem biofilm composed of autotrophic and heterotrophic microorganisms including
324 algae, cyanobacteria, heterotrophic bacteria and fungi (Wu, 2016). Thus, the isotope signature
325 of periphyton can be highly variable. Several studies have found that abiotic (i.e. water
326 velocity and light intensity) and biotic (i.e. species composition) factors can affect $\delta^{13}\text{C}$ and
327 $\delta^{15}\text{N}$ values in periphyton (Ishikawa et al., 2012; MacLeod and Barton, 1998; Trochine et al.,
328 2017; Trudeau and Rasmussen, 2003). Here, we observed high ^{15}N enrichment in periphyton.

329 None of the investigated compartments can explain this enrichment (Fig. 1). We assumed that
330 the high $\delta^{15}\text{N}$ observed in periphyton can be related to the direct use of chicken manure as a
331 nutrient resource.

332 In fed tilapia ponds, Narimbi et al. (2018) reported that chicken manure showed the most
333 enriched $\delta^{15}\text{N}$ value among the investigated pond compartments. Nevertheless, chicken
334 manure was not expected to contribute to the fish diet according to previous findings from the
335 same authors. Therefore, the isotope signature of chicken manure was not assessed in this
336 study. Altogether these results suggest that chicken manure may be not efficient for enhancing
337 the phytoplankton and zooplankton production in fish ponds, as already suggested by
338 previous investigations (Knud- Hansen et al., 1993; Narimbi et al., 2018; Pouil et al., 2019),
339 but it may contribute to the growth of other non-targeted species constituting other pond
340 compartments, such as periphyton communities. Further investigations are needed to confirm
341 this assumption.

342 Intermediate-sized plankton (i.e. 40-200 μm) showed an isotope signature similar to that
343 measured on the surface sediment (i.e. between -25.97 and -21.34‰ for $\delta^{13}\text{C}$, and between
344 3.29 and 5.90‰ for $\delta^{15}\text{N}$; $P = 0.99$, $n = 8$). Bivalves and insects showed typical isotope
345 signatures with relatively high $\delta^{15}\text{N}$ values (6.48 to 9.20‰ and 8.45 to 9.60‰ for bivalves
346 and insects, respectively; Fig. 1), suggesting that a part of the diet of these species was from
347 high $\delta^{15}\text{N}$ feed sources such as chicken manure. Such results are not surprising since
348 Unionidae bivalves preferentially feed on highly ^{15}N -enriched deposited matter (Raikow and
349 Hamilton, 2001), and the insect community is dominated by *Notonecta* sp. known to be top
350 predators in ephemeral water ponds (Dalu et al., 2016).

351 Some invertebrates including snails (mainly *P. canaliculata*) and large plankton ($> 200 \mu\text{m}$)
352 shared similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($P \geq 0.95$, Table 2 and Fig 1) and their isotope signatures
353 suggest that they feed on commercial pellets. Interestingly, in addition to fish, these two

354 compartments are regularly harvested by fish farmers who use snails to feed poultry and other
355 fish (Pouil et al., 2020), and plankton is used to feed young gourami or other fish species
356 (Kristanto et al., 2020). Thus, although the main goal of using commercial pellets is to
357 improve fish growth, it can contribute to the entire food web through direct consumption of
358 food remains as suggested for snail and large plankton ($> 200 \mu\text{m}$) but also through nutrient
359 recycling which include several processes of respiration, oxidation, fermentation and
360 methanogenesis before fixation of the resulting CO_2 into organic carbon by primary producers
361 (Cam et al., 1991; Gamboa-Delgado, 2014; Nahon et al., 2020; Narimbi et al., 2018).
362 Nonetheless, the feeding strategy shall be improved and the alternate-day feeding could be
363 profitable for global performances of semi-intensive farms of giant gourami as in other
364 tropical species (Bolivar et al 2006; Amin and Hossain, 2008). Regular fasting inserted in a
365 feeding strategy may induce more efficient nutrient uptake from fish feed, and promote
366 foraging on natural unexploited resources from the pond by fish; Salger et al., 2020). Over the
367 past two decades, several studies showed that raising the carbon: nitrogen (C:N) ratio to 15-
368 20 can be efficient to enhance natural productivity of aquaculture ponds, and its subsequent
369 use by the farmed aquatic animals (Asaduzzaman et al., 2008; 2010; Hari et al., 2004; 2006).
370 Similarly, Kabir et al (2019) showed that the same effect can be achieved by altering the feed
371 composition and reducing the dietary protein to energy ratio (P:E) from the common
372 commercial levels of 19 g MJ^{-1} to 14 g MJ^{-1} . In the present study, we use a commercial pellet
373 with a P:E ratio of 21 g MJ^{-1} , leading to a C:N of 8.2 and fish were fed at satiation level.
374 Under these production conditions that mimic the current farmers' practices, the system is
375 overruled by a high quantity of high protein exogenous feed. Under such circumstances, the
376 system may turn into a hypertrophic heterotrophic ecosystem, dominated by bacterial nutrient
377 cycles. Chlorophyll-a measured in the ponds were $344 \pm 223 \mu\text{g L}^{-1}$ (i.e., on average, 18-fold
378 the levels measured in the inlet water), similarly to concentrations found by Wang et al.

379 (2016) in tilapia and milkfish ponds. In the present study, the average chlorophyll-a level is
380 negatively correlated with the water renewal (data not shown) indicating that water exchange
381 tends to dilute the planktonic and microbial population in the pond. We found that
382 phytoplankton communities were dominated by cyanobacteria that poorly contributes to the
383 natural food chain based on phytoplankton and zooplankton, usually beneficial for fish.
384 Nevertheless, a recent investigation suggested that giant gourami can exploit bacterial bio
385 floc as an additional feed, at least for young life-stages (Amriawati et al., 2021).

386 Giant gourami stable isotope values ranged between -24.22 and -23.51‰ for $\delta^{13}\text{C}$ and
387 between 6.30 and 7.10‰ for $\delta^{15}\text{N}$ (Table 2, Fig. 1) with significantly higher $\delta^{15}\text{N}$ signatures
388 ($P < 0.01$) in fish sampled in month 5 than those sampled in the other months (i.e. month 0
389 and month 3). No differences were observed for $\delta^{13}\text{C}$ ($P = 0.21$). Overall, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
390 signatures in giant gourami sampled at the beginning, middle and end of the trial
391 demonstrated that fish consumed formulated feed throughout the experiment (Table 2, Fig. 1).
392 Fish were, on average, gradually ^{15}N -enriched by 3.58 , 3.65 and 4.04‰ from month 0 to
393 month 5 relative to the commercial feed, as suggested for omnivorous fish ($\Delta\delta^{15}\text{N} = 3.41 \pm$
394 0.20‰ , Zanden and Rasmussen, 2001). The narrow trophic niche width also confirmed that
395 fish consumed formulated feed, as has been observed for the common carp (*Cyprinus carpio*
396 L.) in semi-intensive ponds (Nahon et al., 2020).

397 Although full trophic niche overlap was recorded for fish collected months 0 and 3, fish
398 collected in month 5 did not overlap with the samples from the other months (Fig. 2A). Also,
399 the SEAc value of fish at month 5 was lower than the SEAc value of fish at month 3 (Fig.
400 2B), suggesting slight differences in the trophic niche of giant gourami over time.
401 Nonetheless, the turnover time of tissues under different diets has been demonstrated to reach
402 a half-life of 84 d for muscle (95% turnover: 371 d) in a cyprinid (*Barbus barbus* L., Busst
403 and Britton, 2018). A similar half-life of 88 d was reported by Zanden et al. (2015). Based on

404 this latter finding, our results suggest that the isotopic signature of fish from month 3 had not
405 reached equilibrium with the commercial pellets used throughout the experiment. Overall, our
406 results confirm the preferential consumption and retention of high-quality dietary sources
407 (such as formulated feeds) over natural sources in giant gourami, as previously reported for
408 the common carp (Schultz et al., 2012).

409

410 **4. Conclusion**

411 The high cost of commercial fish pellets and inadequate feeding strategies impede the
412 development of pond aquaculture especially in countries dominated by small-scale fish
413 farming. Currently, different strategies are employed to optimize the use of commercial
414 pellets in fish pond aquaculture, with a debate on the relative importance and advantages of
415 promoting the endogenous primary productivity of ponds or providing an feed supply. This
416 optimization is particularly applicable to omnivorous species, to capitalize on the endogenous
417 resources of the pond, although this strategy has not been amply studied. Here, using stable
418 isotopes, we demonstrated that in giant gourami, a typical omnivorous species, the diet is
419 mainly based on commercial pellets and the endogenous resources of the pond are barely
420 utilized. Such findings highlight the importance of optimizing the use of both natural pond
421 resources and commercial pellets.

422

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431

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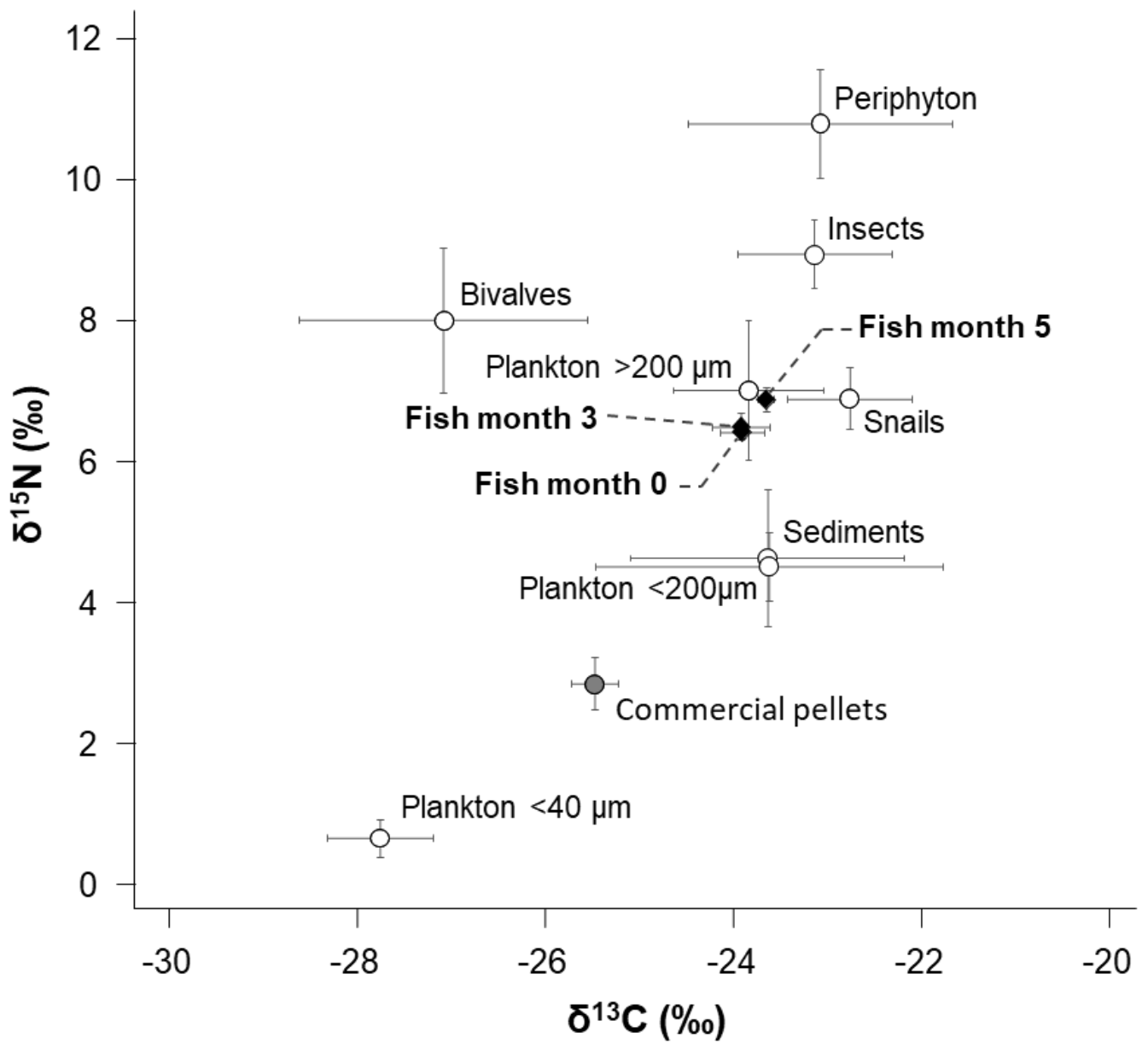
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655 **Captions to figures**

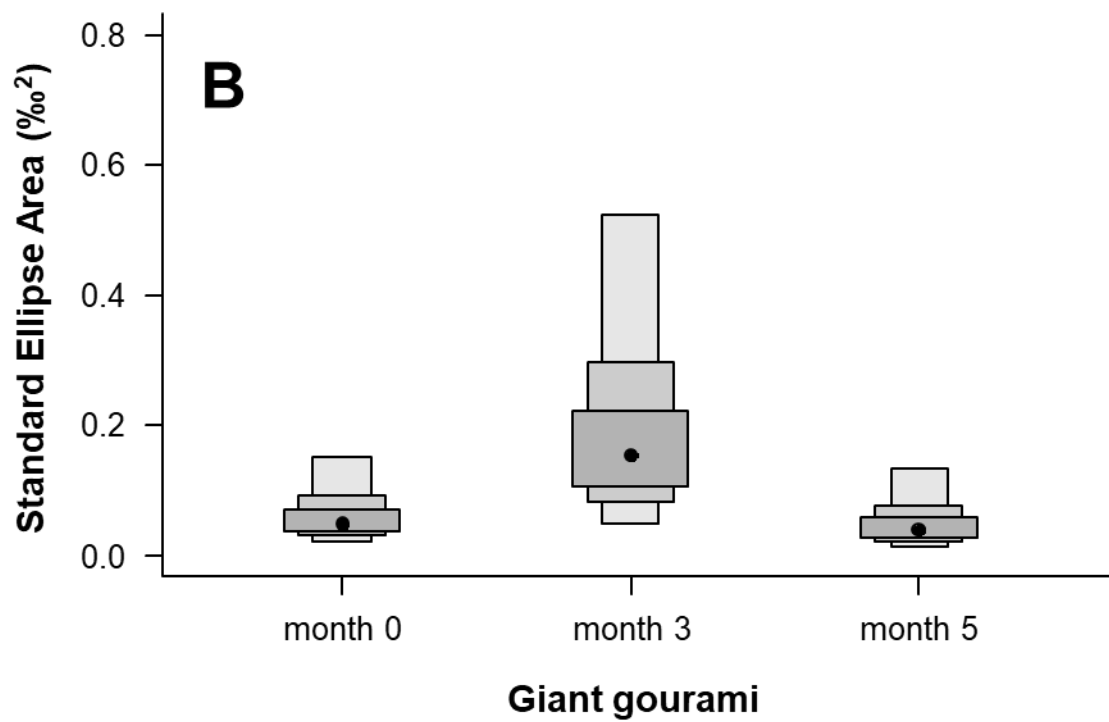
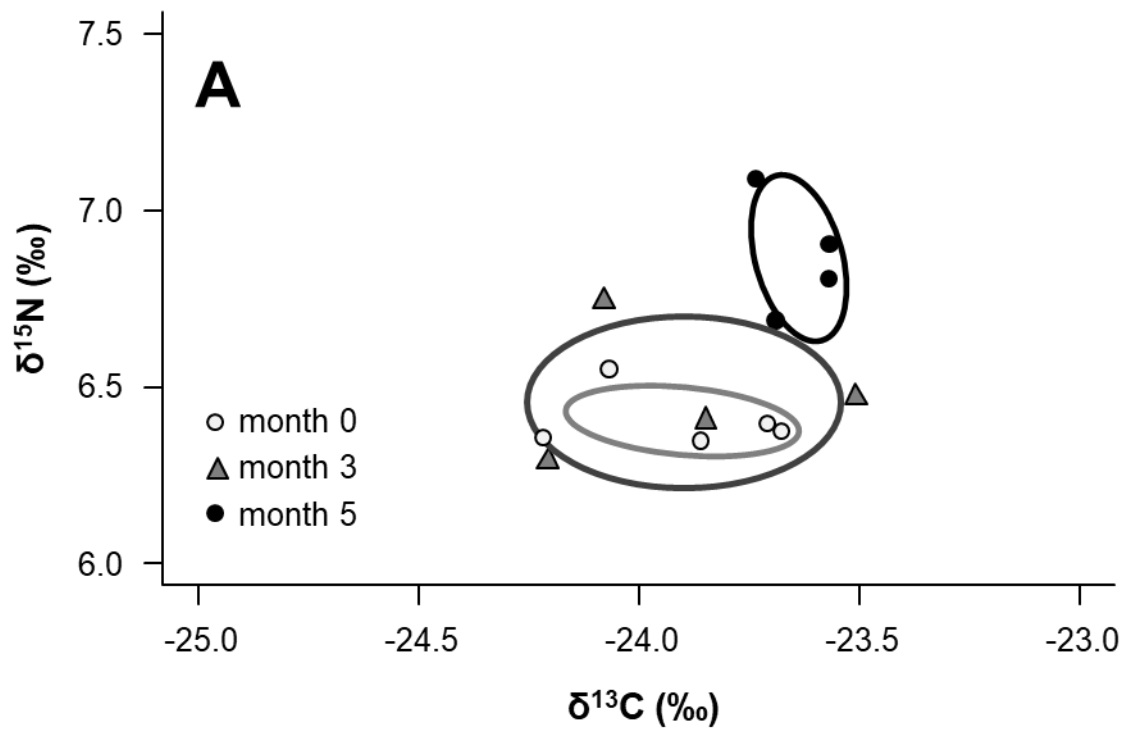
656 Figure 1. Dual stable isotope plot of $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) showing the isotope signature of
657 giant gourami (black) sampled at the beginning (month 0), the middle (month 3) and the end
658 (month 5) during the 152-day growing period in ponds, commercial pellets (dark grey) and
659 other pond compartments (white). Data are means \pm SD (n = 3-8).

660

661 Figure 2. A) Standard ellipse area (SEAc) and B) density plot showing 95%, 75% and 50%
662 confidence intervals (from dark to light grey boxed areas) for giant gourami collected at the
663 beginning (month 0, n =5), the middle (month 3, n = 4) and the end (month 5, n = 4) of the
664 152 day growing period in ponds. Black dots correspond to the median SEAc.



665 **Figure 1**



666 **Figure 2**

667 **Table 1.** Chemical composition (% dry matter (DM) except for humidity, means \pm SD, n = 5)
 668 and components of the commercial extruded pellets (gross energy: 15.38 ± 0.05 MJ kg⁻¹,
 669 energy calculated using the energy value coefficients of 9 kcal for crude lipids and 4 kcal for
 670 crude proteins and carbohydrates) used to feed giant gourami in semi-intensive ponds.

Chemical composition	%
Humidity	7.9 \pm 0.5
Crude proteins	32.7 \pm 0.2
Crude lipids	5.3 \pm 0.2
Crude ash	10.0 \pm 0.4
Crude fibres	4.7 \pm 0.2
Carbohydrates	47.3 \pm 0.2
Components	%
Fish meal, Chili	10
Fish meal, East Java	11
Soybean meal, Argentina	15
Meat and bone meal, Australia	10
Wheat bran, Australia	20.5
Rice bran, West Java	25
Fish oil, East Java	2
Plant oil, Banten	2.5
Choline chloride, China	0.5
Premix vitamin, China	2
Premix mineral, China	1.2
BHT, Central Java	0.3

671

672 **Table 2.** $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm 1 standard deviation and corrected standard ellipse
673 area (SEAc, ‰²) of giant gourami collected in month 0, month 3 and month 5 and all the
674 potential diet sources. Values with different letters are significantly different ($p < 0.05$).

Sample	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	SEAc (‰ ²)
Giant gourami				
Fish month 0	5	-23.91 \pm 0.24 ^a	6.41 \pm 0.08 ^a	0.08
Fish month 3	4	-23.91 \pm 0.31 ^a	6.48 \pm 0.19 ^a	0.28
Fish month 5	4	-23.65 \pm 0.09 ^a	6.88 \pm 0.17 ^b	0.07
Other samples				
Bivalves	8	-27.08 \pm 1.53 ^a	8.00 \pm 1.03 ^{de}	-
Commercial pellets	4	-25.44 \pm 0.24 ^{ab}	2.84 \pm 0.37 ^b	-
Insects	4	-23.14 \pm 0.82 ^b	8.93 \pm 0.49 ^c	-
Periphyton	4	-23.07 \pm 1.40 ^b	10.78 \pm 0.77 ^f	-
Plankton < 40 μm	3	-27.65 \pm 0.55 ^a	0.66 \pm 0.27 ^a	-
Plankton < 200 μm	4	-23.62 \pm 1.85 ^b	4.51 \pm 0.49 ^{bc}	-
Plankton > 200 μm	4	-23.84 \pm 0.80 ^b	7.01 \pm 1.00 ^d	-
Sediment	8	-23.63 \pm 1.46 ^b	4.63 \pm 0.96 ^c	-
Snail	4	-22.76 \pm 0.67 ^b	6.89 \pm 0.44 ^d	-

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