

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

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4	Simon Pouil <sup>1</sup> , Jean-Michel Mortillaro <sup>1,2,3</sup> , Reza Samsudin <sup>4</sup> , Domenico Caruso <sup>1</sup> , Anang Hari				
5	Kristanto <sup>4</sup> , Jacques Slembrouck <sup>1*</sup>				
6					
7	<sup>1</sup> ISEM, Université de Montpellier, Cirad, CNRS, IRD, EPHE, Montpellier, France				
8	<sup>2</sup> CIRAD, UMR ISEM, F-34398 Montpellier, France.				
9	<sup>3</sup> FOFIFA DRZVP, Antananarivo, Madagascar				
10	<sup>4</sup> RIFAFE, Bogor, Indonesia				
11					
12	* Corresponding author: Jacques Slembrouck				
13	ISEM, Institut de Recherche pour le Développement (IRD)				
14	Université de Montpellier, Place Eugène Bataillon				
15	Montpellier Cedex 05, France				
16	E-mail: jacques.slembrouck@ird.fr				

#### 17 Abstract

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

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

#### 36 **1. Introduction**

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

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

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

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

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

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

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#### 103 2. Materials and Methods

#### 104 2.1. Pond systems and management

The experiment was performed on a small-scale fish farm located in the village of Babakan (-6°28'S; 106°42'E), Bogor district, West Java, Indonesia. Giant gourami juveniles were reared for 5 months in a semi-intensive system consisting of four 0.5 m-deep earthen ponds whose size varied from 353 to 482 m<sup>2</sup>. Fish (29  $\pm$  6 g wet weight) were stocked at a density of 6 individuals m<sup>-2</sup> in each pond and protected against predators using nets held by bamboo poles implanted in the bottom of the pond. The structure of the ponds and management practices are
described in detail in Pouil et al. (2019). Briefly, empty ponds were limed and fertilized using
organic fertilizer (typical composition: poultry manure mixed with rice bran at a 0.5:1 ratio on
a dry weight basis). The quantity of organic fertilizer was based on the empirical knowledge
of local fish farmers. Fertilizer was then added four times during the first three months of the
experiment (equiv. 2.1-2.7 kg m<sup>-2</sup>).

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

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

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129 2.2. Sampling procedures

130 2.2.1. Fish

Fish were sampled monthly from the beginning of the experiment. Every month, 50 fish from each pond were caught in nets and individually weighed and measured to determine growth and adjust the feed ration. At harvest, the remaining fish were counted to assess survival and the total biomass of produced fish was determined for each pond. At the beginning of the experiment, and then after 3 and 5 months, 5 fish per pond were sampled, euthanized and pooled for the stable isotope analysis.

137 2.2.2. Macroinvertebrate species

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

153 2.2.3. Plankton

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

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

179 2.2.4. Periphyton

Before the beginning of the experiment, two polycarbonate rectangles (20 x 50 cm) were set at two locations in each pond. To sample periphyton at the end of the experiment, the polycarbonate rectangles were removed from each pond and rinsed with osmosis water. Then, the surface of each rectangle in contact with pond water (i.e. 1000 cm<sup>2</sup>) was carefully and
entirely scraped to collect the periphyton and transferred to polypropylene vials. The samples
were kept in a cooler during transport to the laboratory (see Section 2.3).

187 2.2.5. Sediments

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

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193 2.2.6. Commercial pellets

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

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#### 198 2.3. Sample preparation

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

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

215 2.4. Stable isotope analysis

The  $\delta^{13}$ C and  $\delta^{15}$ N values of samples were analysed using elemental analyser/isotope ratio mass spectrometry (EA/IRMS) in continuous-flow mode on a Delta V Plus isotope ratio mass spectrometer with a Conflo IV interface (Thermo Scientific, Bremen, Germany) interfaced with a Flash 2000 elemental analyser (Thermo Scientific, Milan, Italy). Results are expressed in the  $\delta$  unit notation as deviations from standards (Vienna Pee Dee Belemnite for  $\delta^{13}$ C and atmospheric N<sub>2</sub> for  $\delta^{15}$ N, Peterson and Fry, 1987) using the following formula:

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

where *R* is the ratio of heavy-to-light isotopes ( ${}^{13}C/{}^{12}C$  or  ${}^{15}N/{}^{14}N$ ). Analytical precisions were <br/>
<b

Proximate analyses of commercial pellets were performed following AOAC methods (1999).
Moisture was determined by weight loss upon drying at 105°C for 24 h. Crude protein was
determined using the standard Kjeldahl procedure (Foss Tecator Kjeltec 8400 and Kjeltec
Bucchi); lipid content after acid hydrolysis was determined using the Weibull–Stoldt method;
crude ash by determining residue after heating at 550°C for 4-5 h in a muffle furnace and
crude fibre was determined as follows: commercial pellets were extracted with 1.25% H<sub>2</sub>SO<sub>4</sub>

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

235 2.5. Data analysis

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

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

12

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

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#### 263 **3. Results and Discussion**

264 3.1. Zootechnical performance of giant gourami in ponds

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

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

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

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

We did not find any significant change in water quality with DO concentrations 304 (measurement done between 08:00 and 10:00 AM) were from 5.0  $\pm$  2.4 mg L<sup>-1</sup> while TAN, 305 NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> values were 0.32  $\pm$  0.25, 0.04  $\pm$  0.06 and 1.13  $\pm$  0.36 mg L<sup>-1</sup>, respectively. 306 Water parameter values indicated that the pond water quality remained within appropriate 307 standards for tropical freshwater fish (Colt, 2006), especially for giant gourami, an air-308 breathing species. Nutrient concentrations were  $1.47 \pm 0.45$  and  $0.06 \pm 0.04$  mg L<sup>-1</sup> for N and 309 P (i.e. values in the range of those observed in semi-intensive pond aquaculture; Diana et al., 310 2017). Overall, these results suggest that water quality 1) was not affected by the feeding 311 strategy used and 2) is not likely to explain the observed variations in growth and feed 312 efficiency during the experiment 313

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

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

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

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

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

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

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

compartments are regularly harvested by fish farmers who use snails to feed poultry and other 354 fish (Pouil et al., 2020), and plankton is used to feed young gourami or other fish species 355 (Kristanto et al., 2020). Thus, although the main goal of using commercial pellets is to 356 improve fish growth, it can contribute to the entire food web through direct consumption of 357 food remains as suggested for snail and large plankton (> 200  $\mu$ m) but also through nutrient 358 recycling which include several processes of respiration, oxidation, fermentation and 359 methanogenesis before fixation of the resulting CO<sub>2</sub> into organic carbon by primary producers 360 (Cam et al., 1991; Gamboa-Delgado, 2014; Nahon et al., 2020; Narimbi et al., 2018). 361 Nonetheless, the feeding strategy shall be improved and the alternate-day feeding could be 362 profitable for global performances of semi-intensive farms of giant gourami as in other 363 tropical species (Bolivar et al 2006; Amin and Hossain, 2008). Regular fasting inserted in a 364 feeding strategy may induce more efficient nutrient uptake from fish feed, and promote 365 366 foraging on natural unexploited resources from the pond by fish; Salger et al., 2020). Over the past two decades, several studies showed that raising the carbon: nitrogen (C:N) ratio to 15-367 368 20 can be efficient to enhance natural productivity of aquaculture ponds, and its subsequent use by the farmed aquatic animals (Asaduzzaman et al., 2008; 2010; Hari et al., 2004; 2006). 369 Similarly, Kabir et al (2019) showed that the same effect can be achieved by altering the feed 370 composition and reducing the dietary protein to energy ratio (P:E) from the common 371 commercial levels of 19 g MJ<sup>-1</sup> to 14 g MJ<sup>-1</sup>. In the present study, we use a commercial pellet 372 with a P:E ratio of 21 g MJ<sup>-1</sup>, leading to a C:N of 8.2 and fish were fed at satiation level. 373 Under these production conditions that mimic the current farmers' practices, the system is 374 overruled by a high quantity of high protein exogenous feed. Under such circumstances, the 375 system may turn into a hypertrophic heterotrophic ecosystem, dominated by bacterial nutrient 376 cycles. Chlorophyll-a measured in the ponds were  $344 \pm 223 \ \mu g \ L^{-1}$  (i.e., on average, 18-fold 377 the levels measured in the inlet water), similarly to concentrations found by Wang et al. 378

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

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

Although full trophic niche overlap was recorded for fish collected months 0 and 3, fish collected in month 5 did not overlap with the samples from the other months (Fig. 2A). Also, the SEAc value of fish at month 5 was lower than the SEAc value of fish at month 3 (Fig. 2B), suggesting slight differences in the trophic niche of giant gourami over time. Nonetheless, the turnover time of tissues under different diets has been demonstrated to reach a half-life of 84 d for muscle (95% turnover: 371 d) in a cyprinid (*Barbus barbus* L., Busst and Britton. 2018). A similar half-life of 88 d was reported by Zanden et al. (2015). Based on this latter finding, our results suggest that the isotopic signature of fish from month 3 had not
reached equilibrium with the commercial pellets used throughout the experiment. Overall, our
results confirm the preferential consumption and retention of high-quality dietary sources
(such as formulated feeds) over natural sources in giant gourami, as previously reported for
the common carp (Schultz et al., 2012).

409

#### 410 **4. Conclusion**

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

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#### 655 **Captions to figures**

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

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Figure 2. A) Standard ellipse area (SEAc) and B) density plot showing 95%, 75% and 50% confidence intervals (from dark to light grey boxed areas) for giant gourami collected at the 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

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

Chemical composition	%
Humidity	$7.9 \pm 0.5$
Crude proteins	$32.7\pm0.2$
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

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

Sample	n	δ <sup>13</sup> C (‰)	δ <sup>15</sup> N (‰)	<b>SEAc</b> (‰ <sup>2</sup> )	
Giant gourami					
Fish month 0	5	$-23.91 \pm 0.24^{a}$	$6.41\pm0.08^{a}$	0.08	
Fish month 3	4	$-23.91 \pm 0.31^{a}$	$6.48\pm0.19^{a}$	0.28	
Fish month 5	4	$-23.65 \pm 0.09^{a}$	$6.88\pm0.17^{b}$	0.07	
Other samples				·	
Bivalves	8	$-27.08 \pm 1.53^{a}$	$8.00 \pm 1.03^{de}$	-	
Commercial pellets	4	$-25.44 \pm 0.24^{ab}$	$2.84\pm0.37^{b}$	-	
Insects	4	$-23.14 \pm 0.82^{b}$	$8.93 \pm 0.49^{e}$	-	
Periphyton	4	$-23.07 \pm 1.40^{b}$	$10.78\pm0.77^{\rm f}$	-	
Plankton < 40µm	3	$-27.65 \pm 0.55^{a}$	$0.66\pm0.27^{\rm a}$	-	
Plankton < 200µm	4	$-23.62 \pm 1.85^{b}$	$4.51\pm0.49^{bc}$	-	
Plankton > 200µm	4	$-23.84 \pm 0.80^{b}$	$7.01 \pm 1.00^{d}$	-	
Sediment	8	$-23.63 \pm 1.46^{b}$	$4.63 \pm 0.96^{\circ}$	-	
Snail	4	$-22.76 \pm 0.67^{b}$	$6.89 \pm 0.44^{d}$	-	

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