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## Short-term accumulation and elimination of carbon-14 in the common carp *Cyprinus carpio* under laboratory conditions

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**Abstract**

This study examined the short term transfer of carbon-14 ( $^{14}\text{C}$ ) in the common carp *Cyprinus carpio* under laboratory conditions. Various experiments were achieved in order to investigate direct or trophic transfer for 4 days, using waterborne  $^{14}\text{C}$ -labelled arginine or  $^{14}\text{C}$ -labelled food pellets respectively. Radiolabelled food was prepared with  $^{14}\text{C}$ -labelled arginine or glucose in order to test how transfer kinetics might vary with the biochemical form of  $^{14}\text{C}$ . Elimination experiments were achieved using fish fed for 5 days on radiolabelled food and then placed under starvation for 4 days. In all experiments, water, food and fish activities were monitored every day. Different fish fractions (whole body, muscle) were sampled in order to elucidate the role of muscle as a potential storage. Results suggested that direct water-to-fish absorption rate was  $20\% \text{ d}^{-1}$  per fish. Carps incorporated 14.3% of the absorbed  $^{14}\text{C}$ . Fish activity did not increase over days, due to a strong decrease in  $^{14}\text{C}$  concentration in the water (resulting from aquarium sorption). During trophic transfer experiments, food was entirely ingested and  $^{14}\text{C}$  sources rapidly assimilated. For either arginine or glucose, results suggested that 19-20% of ingested  $^{14}\text{C}$  was incorporated, yielding a significant increase in fish activity over days. No difference in mass-specific activity was observed among muscle and whole body. Total activity in the muscle represented 29%-32% of whole body activity, this proportion reflecting the contribution of muscle to whole body weight. During elimination experiments, results showed a significant decrease in whole body total activity and mass-specific activity with arginine. The decrease was not significant with glucose due to a great variability among fish. Results suggested that an essential amino-acid like arginine can be used as an energy source under starvation and that muscles can act as a storage for essential amino-acids.

*Keywords:*  $^{14}\text{C}$  transfer kinetics; freshwater fish; arginine and glucose metabolism.

## 1 1. Introduction

2 The radioactive isotope  $^{14}\text{C}$ , also known as carbon-14 or C-14, is present in the environment  
3 as an infinitesimal proportion ( $10^{-12}$ ) of total carbon. In fact, carbon is almost entirely  
4 represented by its two stable isotopes,  $^{12}\text{C}$  and  $^{13}\text{C}$ , contributing 98.9 and 1.1% of the total  
5 respectively. The main source of  $^{14}\text{C}$  in the environment is natural, resulting from the reaction  
6 of cosmic neutrons with nitrogen atoms in the high atmosphere. The atmospheric pool of natural  
7  $^{14}\text{C}$  is estimated at  $1.4 \times 10^{17}$  Bq, with a half-life of 5730 years and a production rate of  $1.4 \times$   
8  $10^{15}$  Bq per year (UNSCEAR, 2008). However,  $^{14}\text{C}$  also has several anthropogenic origins.  
9 First, nuclear weapon testing caused a rapid doubling of  $^{14}\text{C}$  proportion in all environmental  
10 compartments during the second half of the 20<sup>th</sup> century. This proportion gradually decreased  
11 after the end of testing, to return to its initial level during the 2010 decade (McGee et al., 2004;  
12 Otlet et al., 1997; Roussel-Debet et al., 2006; Roussel-Debet, 2014). Second,  $^{14}\text{C}$  is the  
13 radionuclide released in greatest amount by nuclear reprocessing plants and nuclear power  
14 plants (NPP) in normal operation (Florence & Hartmann, 2003; Garnier-Laplace et al., 2009).  
15 In France for example, an average activity from  $1.5$  to  $3.0 \times 10^{11}$  Bq is released every year by  
16 a pressurized water reactor (Florence & Hartmann, 2003). A major part of this radioactivity  
17 (approximately 85–95%) is released into the atmosphere as  $\text{CO}_2$  and  $\text{CH}_4$ , while 5–15% is  
18 released into the rivers as dissolved  $\text{CO}_2$  with liquid effluents (EDF, 2014).

19 Following the cycle of carbon, both anthropogenic and natural  $^{14}\text{CO}_2$  enter aquatic food  
20 webs through photosynthesis and primary production and are transferred towards higher trophic  
21 levels. In order to assess the consequences for the environment and for human health, transfer  
22 models are used to predict  $^{14}\text{C}$  activity in fish and the associated dose for human consumers,  
23 based on average amounts released over the year (Avetisov et al., 2001; Boyer & Beaugelin-  
24 Seiller, 2002; Galeriu et al., 2007; Le Dizès et al., 2012). These models assume that  $^{14}\text{C}$   
25 behaviour is similar to that of stable carbon and that a common isotopic ratio is shared among  
26 water and aquatic organisms. Predictions suggest that anthropogenic  $^{14}\text{C}$  is the main contributor  
27 to the absorbed dose of  $1 \mu\text{Sv}$  per year, approximately estimated for generic fish consumers  
28 living in the vicinity of NPP (Mourlon & Vermorel, 2014; Siclet, 2001; Siclet et al., 2002;  
29 UNSCEAR, 2000). This should be noted that this dose remains largely lower than the annual  
30 legal limit of  $1 \text{ mSv}$  per year for the public. However, the estimated dose is calculated for  
31 equilibrium  $^{14}\text{C}$  concentrations and does not consider time variations observed in the field. In

32 fact, aquatic discharges by NPP generally occur 2 or 3 times per month, depending on operating  
33 requirements, causing a temporal variability in  $^{14}\text{C}$  activity in the water (Siclet et al., 2002). As  
34 a result, mass-specific  $^{14}\text{C}$  activity shows a greater variability over time in water in the aquatic  
35 systems than in the terrestrial environments, with bicarbonate varying from 150 to 250 Bq  $^{14}\text{C}$   
36 per kg C (Florence & Hartmann, 2003). Finally, mass-specific  $^{14}\text{C}$  activities in fish range from  
37 200–240 Bq  $^{14}\text{C}$  per kg C (in areas outside NPP influence) to 300–1000 Bq  $^{14}\text{C}$  per kg C (in  
38 areas under NPP influence), but this variability is not clearly understood (EDF, 2014).

39 In order to address variations in  $^{14}\text{C}$  concentrations in the water over time, dynamic transfer  
40 models have been established (Sheppard et al., 2006; Smith, 2006). However, these models do  
41 not take account of differences in fish physiology among species (ingestion rate, growth rates,  
42 etc.) varying with age and with environmental factors such as temperature or resource  
43 availability. In this context, physiology-based models such as those based on the Dynamic  
44 Energy Budget (DEB) Theory (Kooijman, 2010) appear as an interesting alternative. For more  
45 than two decades, DEB models have been used to describe, in a generic way, how organisms  
46 take their energy from food and allocate it to major biological functions in a growing range of  
47 animal species (Add-my-pet collection, 2018). This modelling approach offers a unique  
48 conceptual framework to predict time-varying metabolic responses as a function of temperature  
49 and food (Kooijman, 2010). One particularly interesting extension of the DEB theory, the  
50 Dynamic Isotope Budget (DIB) model (Pecquerie et al., 2010) was developed to study the  
51 fluxes of C, N, H and O isotopes within organisms. DIB models are used to analyse the  
52 composition in stable isotopes ( $^{13}\text{C}$  and  $^{15}\text{N}$ ), which reflects species trophic position in food  
53 webs. The approach, which was applied to  $^{13}\text{C}$  and  $^{15}\text{N}$  in the pacific oyster *Crassostea gigas*  
54 only (Emmery et al., 2011), might be highly useful to predict  $^{14}\text{C}$  transfer in aquatic organisms.  
55 The approach requires adequate experimental data which are too scarce in the literature. In this  
56 context, the present study aims to investigate direct and trophic  $^{14}\text{C}$  transfer in the common carp  
57 *Cyprinus carpio*, as a representative species inhabiting French rivers. Furthermore,  
58 accumulation and elimination kinetics might vary depending on the biochemical form of  $^{14}\text{C}$   
59 and this hypothesis is tested using various  $^{14}\text{C}$  sources, including arginine (an essential amino  
60 acid for carps) and glucose (an important source of energy in a majority of animal species)  
61 (Bouche & Vellas, 1975; Murai et al., 1986; Nagai & Ikeda, 1971a, 1971b; Ranson, 2003).

## 62 2. Materials and methods

63        **2.1. Fish maintenance**

64        Juvenile common carps (*Cyprinus carpio*) were obtained from Saint-Julitte fish farm (Saint-  
65 Flovier, France). Fish were kept in the laboratory in 400 L-tanks. Water was maintained at 18°C  
66 ( $\pm 1^\circ\text{C}$ ), in agreement with average temperature in La Vienne river (France), continuously  
67 oxygenated and filtered, and partly renewed (50%) every day. Water quality was monitored on  
68 a daily basis (pH = 8 and nitrite concentration  $<50 \text{ mg L}^{-1}$ ). Fish were fed at a daily ratio of 3%  
69 w/w with sinking food pellets for carps (« T-Etang » 5-mm pellets, composition in SI Table S1)  
70 purchased from Le Gouessant (Lamballe, France).

71        Before experiments, carps were acclimated to experimental conditions for 3 weeks. Each  
72 carp was kept in an individual aquarium of 8 L and fed a daily ration of 0.3 g per fish. Water  
73 was maintained at 18°C ( $\pm 1^\circ\text{C}$ ), oxygenated with constant air bubbling and partly renewed  
74 (20%) every day.

75        **2.2. Food contamination and desorption**

76         $^{14}\text{C}$ -labelled solutions of arginine and glucose (arginine L- $^{14}\text{C}(\text{U})$ ] and glucose D- $^{14}\text{C}(\text{U})$ ],  
77 97% purity, 1.85 MBq) were purchased from PerkinElmer (Courtaboeuf, France).  $^{14}\text{C}$ -Arginine  
78 and  $^{14}\text{C}$ -Glucose were stored at 4°C in ethanol solutions of 2% and 90% respectively.  
79 Radiolabelled food was prepared by mixing 15 g of ground food pellets with 17.7 mL of UHQ  
80 water and 292  $\mu\text{L}$  of  $^{14}\text{C}$ -solution (arginine or glucose). The homogenised mixture was divided  
81 into 5-mm pellets which were dried for 2 days at 60 C. In order to reduce the potential loss of  
82  $^{14}\text{C}$  into water during feeding experiments, radiolabelled food pellets were coated with agar-  
83 agar (using 0.08 g of agar in 10 mL of water) and dried for another 2 days at room temperature.  
84 Five samples of 0.1 g were collected in order to estimate  $^{14}\text{C}$  activity in the food by liquid  
85 scintillation.

86         $^{14}\text{C}$  desorption from food to water was investigated in order to determine how long  
87 radiolabelled food pellets should be left in water during the feeding experiments. To do so, 0.1  
88 g of  $^{14}\text{C}$ -labelled food was placed in a vial containing 5 mL of water. Working with a small  
89 volume was necessary to avoid  $^{14}\text{C}$  dilution and keep water activity above the detection limit  
90 by liquid scintillation. Five replicate vials were used to monitor  $^{14}\text{C}$  activity in water after 2, 5,  
91 10, 20, 30 and 60 min. Each time, 1 mL of water was collected from each vial and 1 mL was  
92 added to achieve a volume of 5 mL again.

### 2.3. Direct transfer experiment

This experiment aimed to estimate the magnitude of direct water-to-fish  $^{14}\text{C}$  transfer. To do so, 30 000 Bq of  $^{14}\text{C}$ -labelled arginine were added to each aquarium containing a fish in 8 L of water (yielding a  $^{14}\text{C}$  concentration of 3750 Bq L $^{-1}$  on Day 0). A solution of  $^{14}\text{C}$ -labelled arginine at the same concentration was used on Days 1 and 3, to bring the volume back to 8 L and compensate water evaporation. Direct absorption was estimated by measuring  $^{14}\text{C}$  activity in water and fish after 1, 3 and 4 days. Two fish were collected on each sampling day. Water samples of 1 mL were collected before and after water addition. Control aquaria were also prepared to monitor the background decline in water activity without fish.

Changes in  $^{14}\text{C}$  activity in water were described assuming exponential decay models, with coefficients  $k_f$  and  $k_b$  associated with fish absorption and background aquarium sorption. On this basis,  $^{14}\text{C}$  concentration  $[C_{14}]_t$  in the water at time  $t$  was calculated as:

$$[C_{14}]_t = [C_{14}]_{t_0} \cdot \exp(-k \cdot (t - t_0))$$

where  $[C_{14}]_{t_0}$  is  $^{14}\text{C}$  concentration in the water at time  $t_0$ , and  $k = k_f + k_b$  measured in presence of carps or  $k = k_b$  measured in control aquaria respectively.

### 2.4. Trophic transfer experiments

Feeding experiments were carried out in order to estimate the magnitude of trophic food-to-fish transfer. Before the beginning of experiments, fish were starved for two days, in order to stimulate food intake. During the feeding experiments, carps were fed for 4 days a daily ration of 0.3 g per fish using  $^{14}\text{C}$ -labelled food pellets. Food was left in water for 10 min maximum, in order to minimize  $^{14}\text{C}$  desorption to water and direct absorption by fish. After this time, non-ingested pellets were collected, weighed and analysed by liquid scintillation. In a few cases, fish did not eat their daily ration and were discarded from experiments. Three fish were collected on days 1, 2, 3 and 4 (total of 12 fish). Every day during feeding experiments, faeces were collected and 1 mL of water was sampled before and after feeding.

Trophic transfer was considered following a succession of processes, including:

- ingestion, where a fraction of  $^{14}\text{C}$  supplied with food entered fish digestive tracts;
- assimilation, where a fraction of ingested  $^{14}\text{C}$  entered fish organisms through the intestinal epithelium;

122 - incorporation, where the fraction of assimilated  $^{14}\text{C}$  became part of the fish body (as opposed  
123 to assimilated  $^{14}\text{C}$  which was immediately used as an energy source and released as  $^{14}\text{CO}_2$   
124 within 24 h).

## 125 **2.5. Elimination experiments**

126 Elimination experiments aimed to investigate how fast fish might use incorporated  $^{14}\text{C}$  as  
127 an energy source under starvation. Before experiments, carps were fed for 5 days a daily ration  
128 of 0.3 g per fish using  $^{14}\text{C}$ -labelled food pellets. At the beginning of experiments, half of the  
129 volume was replaced with clean water in order to reduce  $^{14}\text{C}$  concentration in the water and  
130 direct absorption by fish. During the elimination experiments, carps were starved for 4 days.  
131 Three fish were collected on days 2, 3 and 4 (total of 9 fish). Every day during elimination  
132 experiments, faeces were collected and 1 mL of water was sampled in each aquarium.

## 133 **2.6. Sample treatments and $^{14}\text{C}$ analyses**

134 Each carp was euthanized immediately and its body size and wet weight (ww) were  
135 measured. Different fractions (muscles, rest of the body) were dissected, weighed and  
136 homogenised with a blender. Three replicate samples of 1 mL approximately were collected  
137 from each homogenised fraction. Collected faeces and food samples were dried at  $60^\circ\text{C}$   
138 overnight. All samples (including water samples of 1 mL) were transferred into scintillation  
139 vials and weighed.

140 Mineralisation was achieved at  $60^\circ\text{C}$  for 2 days, after addition of 1 mL of NaOH 1 M (dried  
141 faeces, dried food and water samples) or 5 mL of NaOH 1 M (fish samples). One mL of each  
142 mineralised fish sample was transferred into a new scintillation vial. Mineralised fish, faeces  
143 and food samples were diluted with 1 mL of UHQ water to achieve a final volume of 2 mL. All  
144 samples were diluted with 18 mL of Ultima Gold cocktail (PerkinElmer, Boston, USA) and  $^{14}\text{C}$   
145 radioactivity was quantified with a liquid scintillation counter (Quantulus 1220 (Wallac–  
146 PerkinElmer, Finland; detection limit: 30mBq).

## 147 **2.7. Data analyses**

148 All statistical analyses were conducted using the statistical computing software R (RStudio,  
149 2020). Two-factor ANOVA and linear regressions were achieved with the routine *lm*. Validity  
150 assumptions including errors independence and normality were assessed using Durbin and  
151 Watson test and Shapiro test, respectively. Errors homogeneity was assessed using Bartlett test



152 (ANOVA) and Breush-Pagan test (linear regression). All analyses were achieved with alpha  
153 risks  $* < 0.05$ ,  $** < 0.01$ ,  $*** < 0.001$ .

### 154 3. Results

#### 155 3.1. Direct $^{14}\text{C}$ transfer from water

156 With dissolved radiolabelled arginine, water  $^{14}\text{C}$  activity declined significantly over time in  
157 all aquaria (Figure 1). The observed decline was significantly stronger in aquaria with fish than  
158 in control aquaria (SI Table S2A). Linear regression yielded exponential coefficients  
159  $k_b = 0.260 \pm 0.016 \text{ d}^{-1}$  and  $k_f + k_b = 0.552 \pm 0.035 \text{ d}^{-1}$  respectively ( $n = 24$  and  $p.c. < 0.001$  in  
160 both cases). These results suggested a background sorption rate of  $23\% \text{ d}^{-1}$  and a fish absorption  
161 rate of  $20\% \text{ d}^{-1}$ . Estimated  $^{14}\text{C}$  absorption rate per fish decreased over time, as a result of  
162 declining water  $^{14}\text{C}$  activity, with average values ranging from  $4650 \pm 1040 \text{ Bq d}^{-1}$  over 1 day,  
163  $3010 \pm 300 \text{ Bq d}^{-1}$  over 3 days, to  $2600 \pm 150 \text{ Bq d}^{-1}$  over 4 days. Cumulated  $^{14}\text{C}$  absorption  
164 represented total activities per fish of  $4550 \pm 1040$ ,  $9030 \pm 850$  and  $10400 \pm 800 \text{ Bq}$  over 1, 3  
165 and 4 days respectively.

166 Whole body total activity ranged from  $650 \pm 20$  to  $760 \pm 450 \text{ Bq}$  per fish depending on days  
167 (Figure 2A, SI Table S2B). Average mass-specific activities in fish, associated with  $^{14}\text{C}$   
168 incorporation, varied from  $13.6 \pm 0.4 \text{ Bq g}^{-1}$  on Day 1,  $17.7 \pm 11.0 \text{ Bq g}^{-1}$  on Day 3, to  
169  $19.4 \pm 8.5 \text{ Bq g}^{-1}$  on Day 4 (Figure 2B, SI Table S2B). This observed increase over time was  
170 not significant, due to a great individual variability among fish. Accumulation of  $^{14}\text{C}$  in fish  
171 was somehow lessened by the strong decline in arginine concentration observed in water over  
172 time. A minor fraction of absorbed  $^{14}\text{C}$  was incorporated to fish, decreasing over time from  
173  $14.3\%$  on Day 1 to  $7.0\%$  on Day 4 in the whole body and from  $1.9\%$  on Day 1 to  $1.0\%$  on Day  
174 4 in the muscle. This result suggested that a major fraction of absorbed  $^{14}\text{C}$  was likely  
175 metabolized and eliminated through respiration and excretion. Interestingly, water-to-fish direct  
176 transfer (absorption and incorporation) resulted in transfer factors that increased over time, from  
177  $3.9$  to  $10.9$  in whole body and from  $1.7$  to  $4.7$  in muscle.

#### 178 3.2. Food activity

179 Radiolabelled food showed different mass specific activities depending on the  $^{14}\text{C}$  source,  
180 with values of  $\sim 590 \pm 100 \text{ Bq g}^{-1}$  with glucose and  $5950 \pm 470 \text{ Bq g}^{-1}$  with arginine. When food

181 pellets were placed in water, activity of dissolved  $^{14}\text{C}$  significantly increased in water over time  
182 (Figure 3, SI Table S3). This increase was significantly stronger for arginine than for glucose,  
183 with average desorption from food representing respectively 37 and 25% of initial activity after  
184 1 h. On this basis, we decided to remove food pellets from water after 10 min during feeding  
185 experiments, in order to minimize direct  $^{14}\text{C}$  transfer to fish.

### 186 **3.3. Trophic transfer**

187 Each day, 0.3 g of radiolabelled food pellets represented an average supply of 1800 Bq and  
188 180 Bq per aquarium, with arginine and glucose respectively. As a result, water  $^{14}\text{C}$  activity  
189 showed a significant increase over time (Figure 4, SI Table S4A), reaching in 4 days maximum  
190 values of 1260 and 250 Bq, with both arginine and glucose respectively. Each day, water  $^{14}\text{C}$   
191 activity also showed an increase caused by food addition (Figure 4, SI Table S4A). This  
192 increase was statistically significant with arginine only, due to a relatively great variability in  
193 water activity with glucose. Observed values suggested that 250 Bq and 50 Bq approximately  
194 desorbed from food during fish feeding. Desorption from food represented 16% and 26% of  
195 supplied activity, with arginine and glucose respectively.

196 Most fish fed well on radiolabelled food pellets, with only a slight fraction (less than 3%)  
197 of supplied food remaining in aquaria after 10 min. Whole body total activity significantly  
198 increased over time (Figures 5A and 5C, SI Table S4B). With  $^{14}\text{C}$ -labelled arginine, average  
199 values ranged from  $385 \pm 190$  Bq per fish on Day 1 to  $1150 \pm 270$  Bq per fish on Day 4. With  
200  $^{14}\text{C}$ -labelled glucose, average values increased from  $35 \pm 15$  Bq per fish on Day 1 to  $105 \pm 20$   
201 Bq per fish on Day 4. Recorded whole body activity represented 15–16% of supplied activity  
202 in food, and 19–20% of ingested activity (calculated as supplied activity minus desorption from  
203 food). Considering a water absorption rate of 20% per day and an incorporation rate of 14.3%  
204 (as estimated from direct transfer), observed  $^{14}\text{C}$  desorption from food might result in a direct  
205 incorporation of 78 and 12 Bq per fish, with arginine and glucose respectively, approximately  
206 estimated over 4 days. These values accounted for minor proportions (7% and 12%  
207 respectively) of the total activity observed in fish.

208 Muscle total activity similarly increased over time (Figures 5A and 5C), from  $78 \pm 52$  to  
209  $330 \pm 250$  Bq with arginine, and from  $10 \pm 1.3$  to  $34 \pm 5.3$  Bq with glucose. Values represented  
210 approximately 29–32% of whole body total activity, independent of days (SI Table S4B). This  
211 proportion reflected the contribution of the muscle to whole body weight (28–29%). Mass  
212 specific activities (Bq per  $\text{g}^{-1}$  ww) increased over time and did not significantly differ between

213 whole body and muscle (Figures 5B and 5D, SI Table S4B). Average values ranged from  
214  $7.0 \pm 1.5$  to  $32 \pm 5.2$  Bq g<sup>-1</sup> ww with arginine and from  $0.5 \pm 0.1$  to  $1.7 \pm 0.4$  Bq g<sup>-1</sup> ww with  
215 glucose. Associated trophic transfer factors (TTF, as Bq g<sup>-1</sup> ww per Bq g<sup>-1</sup> water) increased  
216 over time, from 1.3% on Day 1 to 6.2% on Day 4 with arginine and from 1.0% on Day 1 to  
217 3.4% on Day 4 with glucose.

### 218 **3.4. Elimination**

219 During the preliminary feeding phase, food supply represented a daily activity of 1070 Bq  
220 and 145 Bq, with arginine and glucose respectively, reaching a total over 5 days of 5350 Bq  
221 and 725 Bq respectively. With arginine, water <sup>14</sup>C activity significantly increased over time,  
222 from 1520 Bq on Day 1 to 1950 Bq on Day 5 (Figure 6A). Due to a large variability in activity  
223 with glucose, water <sup>14</sup>C activity did not increase significantly over time, varying on average  
224 from 350 Bq to 790 Bq among days (Figure 6B). At the beginning of the elimination  
225 experiments, water <sup>14</sup>C activity was diluted by a factor of 2. With both arginine and glucose,  
226 water <sup>14</sup>C activity did not vary significantly over time during the elimination phase (Figures 6A  
227 and 6B).

228 With arginine, whole body total activity significantly varied among days of elimination  
229 (Figure 7A, SI Table 5), from  $2220 \pm 300$  to  $1540 \pm 580$  Bq per fish. Value on Day 2  
230 represented ~41% of <sup>14</sup>C activity supplied with food over the 5 days. With glucose, whole body  
231 total activity did not significantly decrease over days of elimination, varying from  $105 \pm 40$  to  
232  $85 \pm 4$  Bq per fish (Figure 7C, SI Table 5). With glucose, whole body total activity represented  
233 ~15% of activity supplied with food, suggesting a much greater <sup>14</sup>C incorporation with arginine  
234 than with glucose. Again, considering an absorption rate of 20% per day and an incorporation  
235 rate of 14.3% per day for absorption and incorporation respectively (as estimated from direct  
236 transfer), observed water <sup>14</sup>C activity might result in a direct incorporation of 220 and 80 Bq  
237 per fish, with arginine and glucose respectively, approximately estimated over 5 days of  
238 elimination. These activities accounted for 10% and 72% of whole body total activity, and for  
239 4 and 10% of supplied food activity, with arginine and glucose respectively.

240 Muscle total activity showed no statistically significant change over time (Figures 7A and  
241 7C), with values ranging from  $520 \pm 350$  to  $930 \pm 240$  Bq with arginine and, from  $12 \pm 4.9$  to  
242  $25 \pm 9.9$  Bq with glucose. Values represented approximately 34 and 23% of whole body total  
243 activity, for arginine and glucose respectively (SI Table 5). These proportions suggested that  
244 glucose was eliminated more rapidly than arginine in the muscle. Mass-specific activities did

245 not differ significantly between the muscle and whole body (Figures 7B and 7D, SI Tables 5).  
246 With arginine, values varied significantly among days of elimination, from  $50 \pm 9.0$  to  $26 \pm 17$   
247 Bq g<sup>-1</sup> ww. With glucose, values did not differ significantly among days of elimination, ranging  
248 from  $0.6 \pm 0.2$  to  $1.2 \pm 0.6$  Bq g<sup>-1</sup>. Associated trophic transfer factors (TTF) ranged from 7.3%  
249 to 14% with arginine and from 1.2% to 2.4% with glucose. With glucose, estimated TTF values  
250 were similar between the elimination and the feeding experiments. With arginine, estimated  
251 TTF values were twice greater during the elimination experiment than during the feeding  
252 experiment.

#### 253 4. Discussion

254 Our study addressed <sup>14</sup>C accumulation kinetics in *C. carpio* during short term direct or  
255 trophic contamination experiments. Results with <sup>14</sup>C-labelled arginine showed that carps  
256 directly absorbed 20% of waterborne arginine each day, representing approximately the activity  
257 in 1.6 L of water (20% of 8 L aquaria). This value was small compared to ventilation volumes  
258 expected to be pumped by carps at 18°C (Klyszejko et al., 2003), suggesting that transfer  
259 efficiency through gill membranes was below 10%. Results also showed that minor fractions  
260 of assimilated <sup>14</sup>C-labelled arginine (ranging from 14 to 20%) were finally incorporated to  
261 whole body, implying that a major part was catabolized and released as <sup>14</sup>CO<sub>2</sub> via respiration.  
262 This result was in good agreement with previous studies on metabolism of <sup>14</sup>C-labelled amino-  
263 acids (including essential lysine and non-essential glutamate) and glucose in *C. carpio* (Bouche  
264 & Vellas, 1975; Nagai & Ikeda, 1971b). Authors reported that a significant fraction (45%) of  
265 glutamate activity injected into blood was released as <sup>14</sup>CO<sub>2</sub> within 6 h, while a minor fraction  
266 (7% only) were stored as hepatic glycerides. Authors further showed that glutamate  
267 incorporation varied depending on diet composition, with a similar level of <sup>14</sup>CO<sub>2</sub> production  
268 and no <sup>14</sup>C-labelled glyceride storage when carps were fed on a high carbohydrate food (90%  
269 starch and 10% casein). In our study, results suggested that arginine incorporation differed  
270 between direct and trophic experiments (14.3 and 18.6% respectively), suggesting a greater  
271 arginine storage when carps were fed than when they were starved. This interpretation was  
272 supported by previous results showing that starved carps stopped storing another essential  
273 amino-acid (<sup>14</sup>C-labelled lysine) (Bouche & Vellas, 1975). The present study reported  
274 approximately similar proportions of incorporation with <sup>14</sup>C-labelled glucose as with arginine  
275 (20% and 19% of ingested activity), suggesting again that a major fraction of glucose was

276 released via respiration or other metabolic paths during trophic experiments. This observation  
277 was in good agreement with the recognized limited importance of glucose as energy source in  
278 fish (Enes et al., 2009). Other results showed that  $^{14}\text{C}$  production after 6 h was much lower  
279 with  $^{14}\text{C}$ -labelled glucose than with  $^{14}\text{C}$ -labelled glutamate (15% and 45% of activity injected  
280 into blood respectively), although no important storage of radiolabelled glycogen (limited to 7-  
281 8% of injected activity) was observed in fish (Nagai & Ikeda 1971b).

282 Our study also addressed  $^{14}\text{C}$  elimination kinetics in *C. carpio* during short term starvation  
283 experiments. Results showed that activity of  $^{14}\text{C}$ -labelled arginine decreased in starved carps,  
284 whereas no similar decrease was observed with  $^{14}\text{C}$ -labelled glucose. This suggested that carps  
285 stored arginine under favourable food conditions and were able to mobilize it during starvation,  
286 confirming the role of amino-acids (including essential amino-acids, like lysine or arginine) as  
287 an energy source (Bouche & Vellas, 1975; Enes et al., 2009). Other authors pointed the role of  
288 muscle as a storage organ for amino-acids in *C. carpio*. In carps, amino-acids were recognized  
289 as a main precursor for lipids, which were seasonally stored in the hepatopancreas during the  
290 summer (Kminkova et al., 2001; Nagai & Ikeda, 1971b). Lipids were mobilized in priority  
291 under starvation while carbohydrates were consumed when lipid content in hepatopancreas was  
292 depleted (Nagai & Ikeda, 1971a). Our data on mass-specific activities supported the role of  
293 muscle as amino-acid storage, with a greater mass-specific activity in the muscle during feeding  
294 experiments than after starvation.

295 Data reported in the present study aimed to be used to model  $^{14}\text{C}$  transfer in an aquatic  
296 ecosystem. In the literature,  $^{14}\text{C}$  transfer models (Sheppard et al., 2006; Smith, 2006) were based  
297 on the common assumption that  $^{14}\text{C}$  activity in the various biotic compartments entered the  
298 aquatic system through photosynthesis and primary producers only. However, our results  
299 showed that highly assimilable nutrients, such as arginine, could directly be absorbed from  
300 water, and contribute to a minor fraction of fish  $^{14}\text{C}$  intake during feeding experiments (~10%).  
301 While waterborne nutrients, such as valuable amino-acids, might be most likely kept at  
302 extremely low concentrations due to biotic activity in the river, any other assimilable molecules,  
303 including xenobiotic contaminants or compounds of the degraded organic matter (Eyrolle et al.,  
304 2018; Jean-Baptiste et al., 2019), might also be involved in a direct and trophic  $^{14}\text{C}$  transfer to  
305 aquatic organisms (Saito et al., 1994; Shimizu et al., 1978; Tjeerdema & Crosby, 1988; Velisek  
306 et al., 2009).

307 In dynamic models of aquatic  $^{14}\text{C}$  transfer (Sheppard et al., 2006; Smith, 2006), specific  
308 activity in fish were assumed to vary over time as a function of  $^{14}\text{C}$  intake from food and C  
309 turnover rates measured at an organism or population level. Intake from food was calculated  
310 based on food activity, ingestion rate (varying with food availability) and associated  
311 assimilation efficiency (or digestibility, i.e. the fraction of ingested food which can enter the  
312 organism through the intestinal epithelium). Turnover rates at the organism level included  
313 metabolic turnover and dilution by somatic growth. Mortality might also be considered when  
314 C turnover was addressed at the population level. In practice, authors acknowledged that a  
315 precise quantification of ingestion, digestion, assimilation and turnover rates were not easy for  
316 wild fish and was complicated by the diversity of their trophic positions in natural food webs.  
317 As a consequence, model parameterization relied most often on indirect estimation methods. In  
318 Smith, (2006), a generic value of assimilation efficiency was estimated for stable carbon using  
319 data on growth rates in Brown trout *Salmo trutta* (Elliott, 1975), assuming that equilibrium  
320 among biotic compartments was reached. Long term turnover rates were addressed in a natural  
321 lake, by analysing changes in  $^{14}\text{C}$  activities over several years (Stephenson et al., 1994),  
322 showing that specific activity in fish was much higher than that measured in their food items at  
323 the time of sampling. In this context, the present study provided valuable new insights in the  
324 short term physiological behaviour of a freshwater fish, showing that rapid fluxes might involve  
325 5 times greater amounts of  $^{14}\text{C}$  than measured in fish tissues. This short term dynamics might  
326 partially explain the great variability in fish  $^{14}\text{C}$  activity observed in samples from  
327 radioecological surveys in the French rivers (Duffa et al., 2007).

328 Addressing both long term and short term changes in  $^{14}\text{C}$  activity in a fish might be difficult  
329 using a single compartment model (Sheppard et al., 2006; Smith, 2006). Other authors  
330 suggested using at least two compartments, one for structural C and another for temporary  
331 storage C (Galeriu et al., 2003), in order to describe more accurately the natural variability in  
332 fish activity. Such models were promoted in the framework of the Dynamic Energy Budget  
333 theory for several decades (Kooijman, 2010), allowing to mechanistically describe metabolic  
334 behaviour of organisms in an increasing range of species in relation with their environment  
335 (Add-my-pet collection, 2018). This approach was further extended to address changes in major  
336 biogenic elements and their isotopes (Pecquerie et al., 2010). One of our future perspectives  
337 should aim to parameterize a DEB model for  $^{14}\text{C}$  in common carps using the data presented in  
338 the present study.

339 **5. Conclusions**

340 Experimental results showed that common carps can absorb  $^{14}\text{C}$ -labelled molecules via  
341 direct and trophic routes, with different transfer efficiencies depending on feeding condition  
342 and biochemical form of  $^{14}\text{C}$ . A major fraction of absorbed  $^{14}\text{C}$  was catabolized and released as  
343  $^{14}\text{CO}_2$  on the short term. A minor fraction of  $^{14}\text{C}$  was incorporated into organism tissues,  
344 contributing to a rapid increase in fish  $^{14}\text{C}$  activity. Freshwater  $^{14}\text{C}$  transfer models need to  
345 consider the influence of ecophysiological conditions on short term  $^{14}\text{C}$  kinetics in order to  
346 address rapid variations in freshwater fish activity.

347 **6. References**

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473

474 **7. Figure captions**

475 Figure 1. Changes in water  $^{14}\text{C}$  activity over time, due to absorption of dissolved radiolabelled  
476 arginine measured in 8 L-aquaria with 1 fish or no fish, and expressed as percent of mean  
477 activity on Day 0. Symbols and error bars represent mean  $\pm$  standard deviation. Dotted lines  
478 represent linear regressions, with statistical significance of slopes ( $n = 24$  and  $p.c. < 0.001^{***}$   
479 each time; brackets indicate that the homogeneity assumption is not validated). Two-factor  
480 ANOVA ( $n = 48$ , see SI Table S2A for details): significant effects of Days ( $p.c. < 0.001$ ), Fish  
481 ( $p.c. < 0.001$ ) and Days  $\times$  Fish ( $p.c. < 0.001$ ) on water activity.

482 Figure 2. Changes in fish  $^{14}\text{C}$  activity over time, due to incorporation of radiolabelled arginine,  
483 measured in different fish fraction (whole body or muscle), and expressed (A) as total activity  
484 (Bq per fish) and (B) as mass specific activity (Bq  $\text{g}^{-1}$  ww). Symbols and error bars represent  
485 mean  $\pm$  standard deviation. Dotted lines represent linear regressions, with no statistical  
486 significance of slopes ( $n = 6$  and  $p.c. > 0.05$  each time). Two-factor ANOVA ( $n = 12$ , see SI  
487 Table S2B for details): significant effects of Fraction on total activity ( $p.c. = 0.0025^{**}$ ) and  
488 mass specific activity ( $p.c. = 0.043^*$ ).

489 Figure 3. Changes in water  $^{14}\text{C}$  activity over time, due to desorption of different radiolabelled  
490 sources (arginine or glucose), and expressed as percent of activity supplied in food at 0 min.  
491 Symbols and error bars represent mean  $\pm$  standard deviation. Dotted lines represent linear  
492 regressions, with statistical significance of slopes ( $n = 30$  and  $p.c. < 0.001^{***}$  each time). Two-  
493 factor ANOVA ( $n = 60$ , see SI Table S3 for details): significant effects of Time ( $p.c. = 0.016^*$ ),  
494 Source ( $p.c. < 0.001^{***}$ ) and Time  $\times$  Source ( $p.c. = 0.0085^{**}$ ) on water activity.

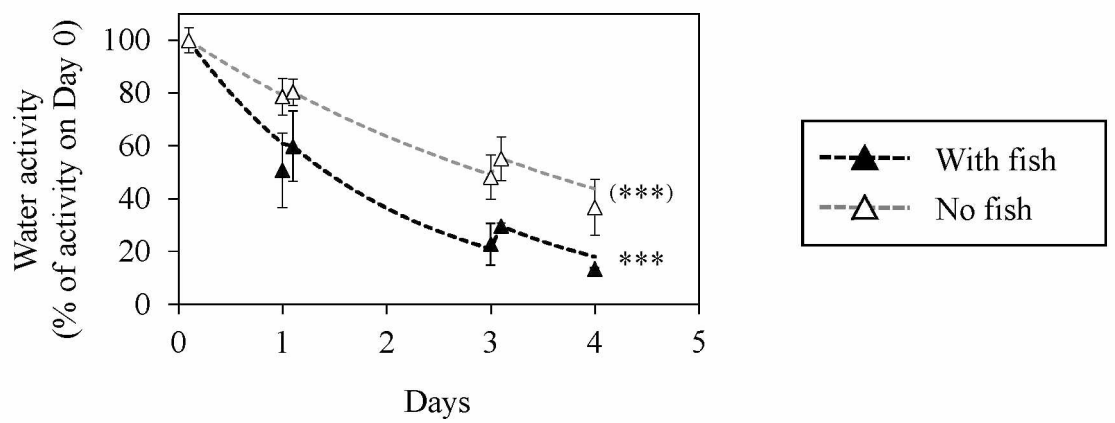
495 Figure 4. Changes in water  $^{14}\text{C}$  activity over time, measured prior or after feeding, using  
496 radiolabelled food with (A) arginine or (B) glucose, and expressed as total activity in 8 L (Bq).  
497 Symbols and error bars represent mean  $\pm$  standard deviation. Dotted lines represent linear  
498 regressions, with statistical significance of slopes ( $n = 24$  and  $p.c. < 0.001^{***}$  each time). Two-  
499 factor ANOVA ( $n = 24$ , see SI Table S4A for details): significant effects of Days ( $p.c. <$   
500  $0.001^{***}$ ) and Feeding ( $p.c. = 0.0019^{**}$ ) on water activity with arginine; significant effect of  
501 Days ( $p.c. = 0.0037^{**}$ ) on water activity with glucose.

502 Figure 5. Changes in fish  $^{14}\text{C}$  activity over time, due to incorporation of radiolabelled arginine  
503 (A and B) or glucose (C and D), measured in different fish fraction (whole body or muscle),

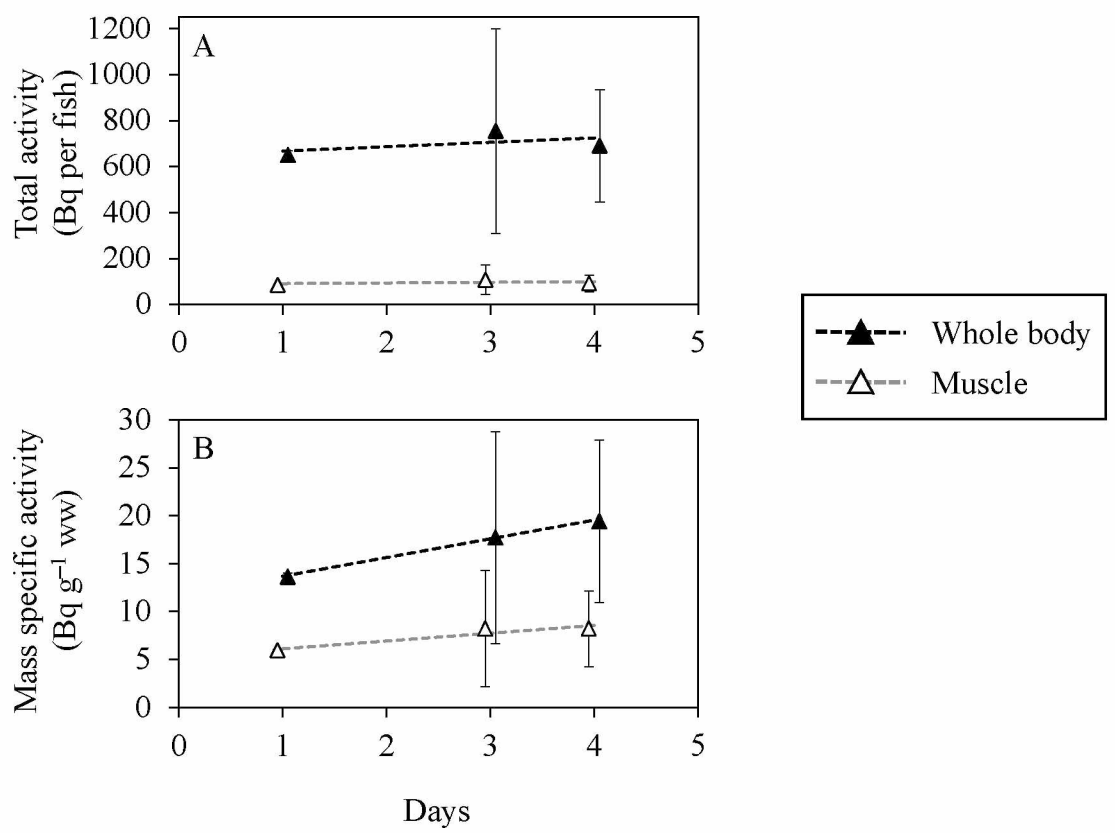
504 and expressed as total activity (Bq per fish) and mass specific activity (Bq g<sup>-1</sup> ww). Symbols  
505 and error bars represent mean ± standard deviation. Dotted lines represent linear regressions,  
506 with statistical significance of slopes ( $n = 12$  and  $p.c. = 0.0016^{**}$  and  $p.c. = 0.046^*$ ,  $p.c. =$   
507  $0.0013^{**}$  and  $p.c. = 0.0011^{**}$ , or  $p.c. < 0.001^{***}$  otherwise; brackets indicate that the  
508 homogeneity assumption is not validated). Two-factor ANOVA ( $n = 24$ , see SI Table S4B for  
509 details): significant effects of Days ( $p.c. = 0.0024^{**}$  or  $p.c. = 0.0043^{**}$ ) and Fraction ( $p.c. <$   
510  $0.001^{***}$  or  $p.c. < 0.001^{***}$ ) on total activity with arginine or glucose; significant effects of  
511 Days ( $p.c. < 0.001^{***}$  or  $p.c. < 0.001^{***}$ ) on mass specific activity with arginine or glucose.

512 Figure 6. Changes in water <sup>14</sup>C activity over time, measured during preliminary feeding or fish  
513 elimination, using radiolabelled food with (A) arginine or (B) glucose, and expressed as total  
514 activity in 8 L (Bq). Symbols and error bars represent mean ± standard deviation. Dotted lines  
515 represent linear regressions, with statistical significance of slopes ( $n = 44$  and  $p.c. < 0.001^{***}$ ;  
516  $n = 14$  to  $29$  and  $p.c. > 0.05$  otherwise; brackets indicate that the independence assumption is  
517 not validated).

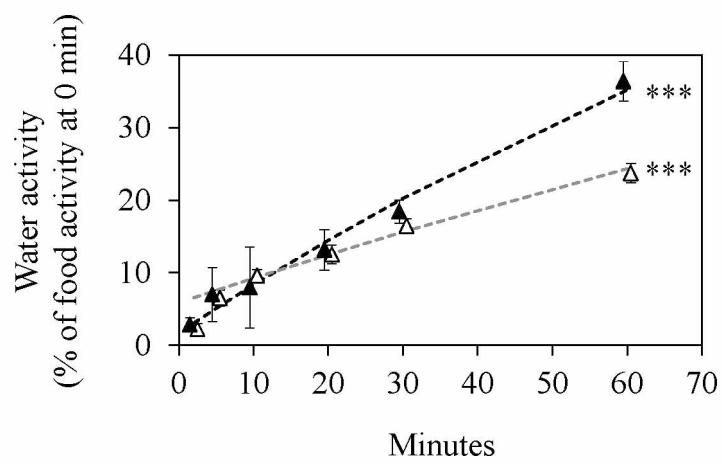
518 Figure 7. Changes in fish <sup>14</sup>C activity over time, due to elimination of incorporated radiolabelled  
519 arginine (A and B) or glucose (C and D), measured in different fish fraction (whole body or  
520 muscle), and expressed as total activity (Bq per fish) and mass specific activity (Bq g<sup>-1</sup> ww).  
521 Symbols and error bars represent mean ± standard deviation. Dotted lines represent linear  
522 regressions, with no significance of slopes ( $n = 9$  and  $p.c. > 0.05$  each time). Two-factor  
523 ANOVA ( $n = 18$ , see SI Table S5 for details): significant effects of Days ( $p.c. = 0.0037^{**}$ ) and  
524 Fraction ( $p.c. < 0.001^{***}$ ) on total activity with arginine; significant effect of Days ( $p.c. =$   
525  $0.0039^{**}$ ) on mass specific activity with arginine; significant effect of Fraction ( $p.c. <$   
526  $0.001^{***}$ ) on total activity with glucose.



Souloumiac et al, Figure 1

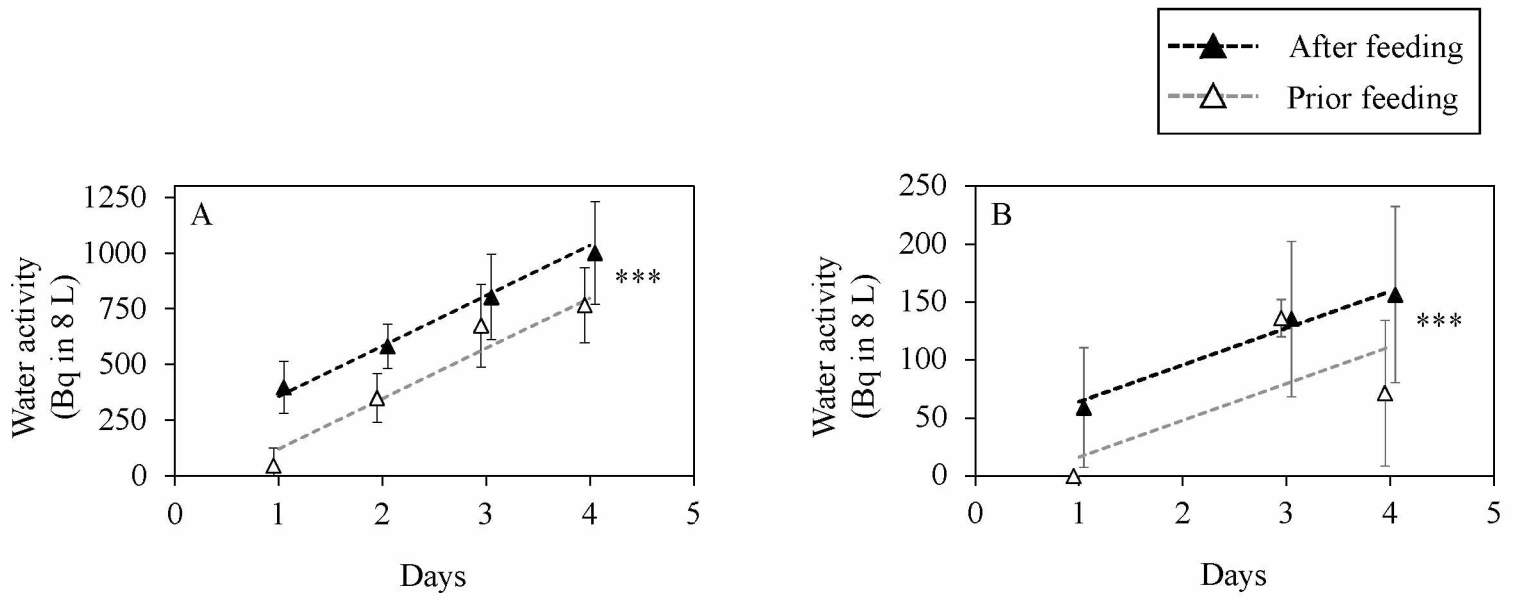


Souloumiac et al, Figure 2

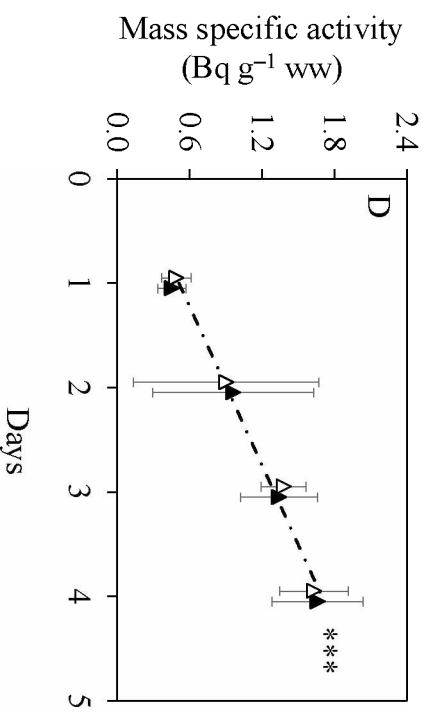
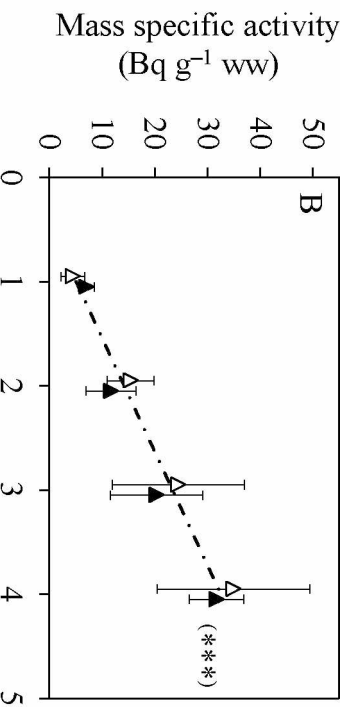
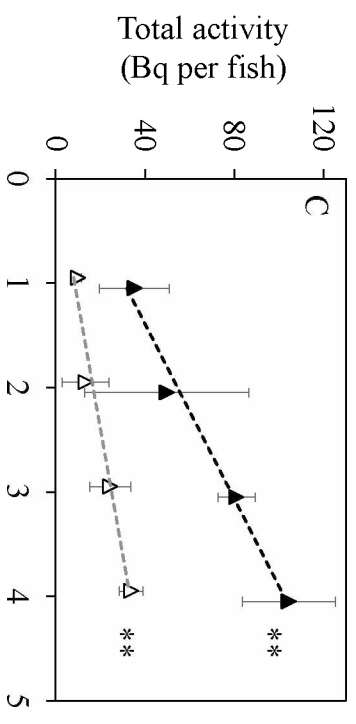
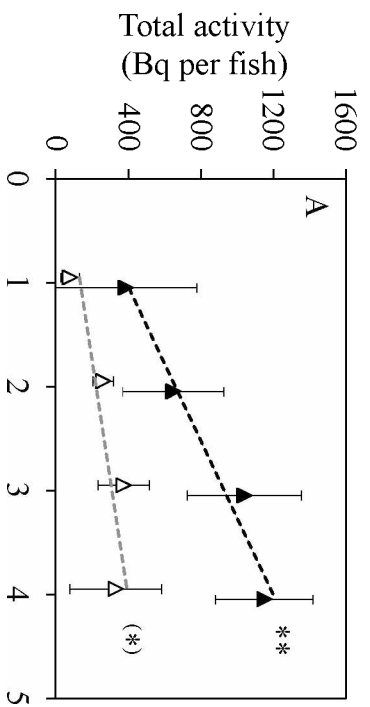


Souloumiac et al, Figure 3

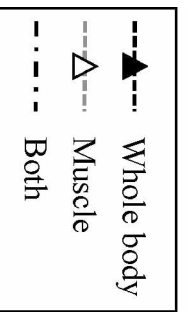


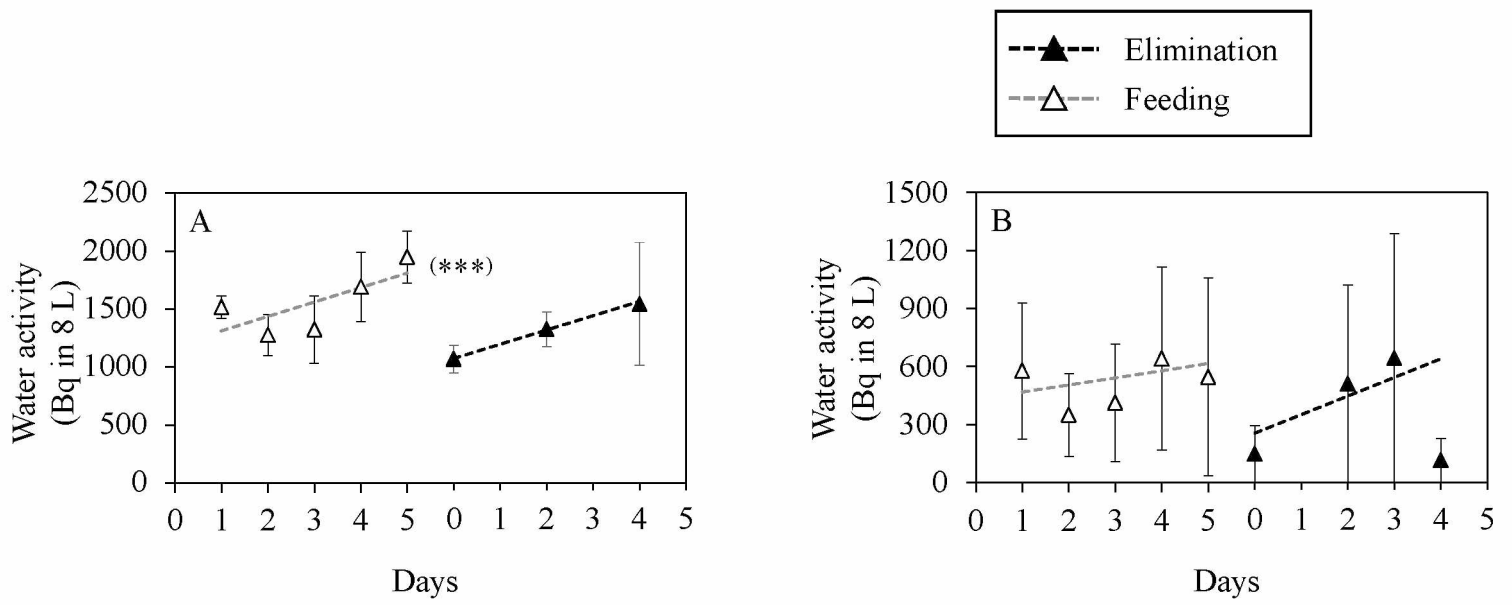


Souloumiac et al, Figure 4

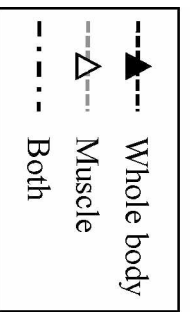
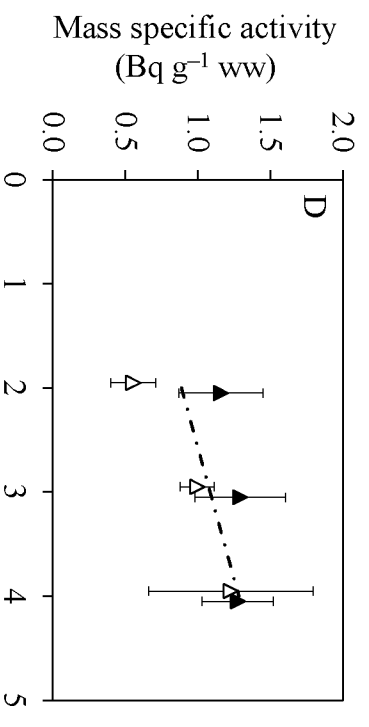
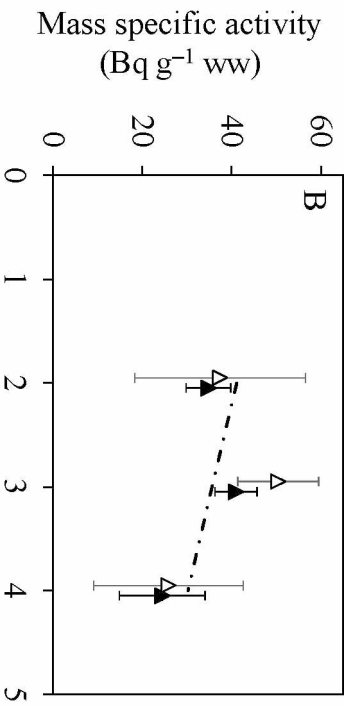
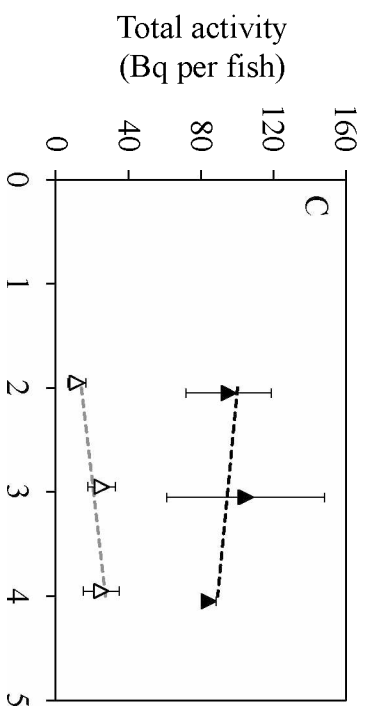
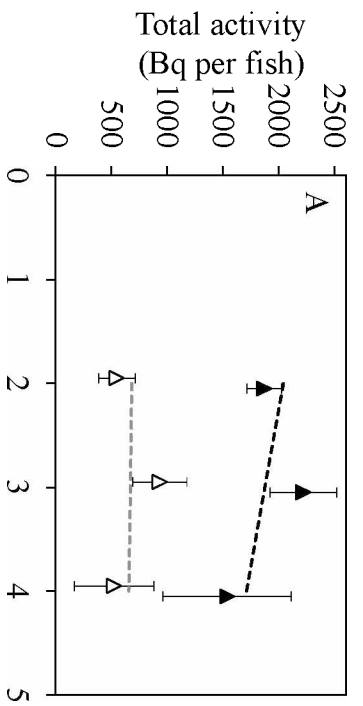


Soulouniac et al, Figure 5





Souloumiac et al, Figure 6



Souloumiac et al, Figure 7