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Abstract

This study examined the short term transfer of carbon-14 (^{14}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}C-labelled arginine or ^{14}C-labelled food pellets respectively. Radiolabelled food was prepared with ^{14}C-labelled arginine or glucose in order to test how transfer kinetics might vary with the biochemical form of ^{14}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% d^{-1} per fish. Carps incorporated 14.3% of the absorbed ^{14}C. Fish activity did not increase over days, due to a strong decrease in ^{14}C concentration in the water (resulting from aquarium sorption). During trophic transfer experiments, food was entirely ingested and ^{14}C sources rapidly assimilated. For either arginine or glucose, results suggested that 19-20% of ingested ^{14}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}C transfer kinetics; freshwater fish; arginine and glucose metabolism.

1. Introduction

The radioactive isotope ^{14}C, also known as carbon-14 or C-14, is present in the environment as an infinitesimal proportion (10^{-12}) of total carbon. In fact, carbon is almost entirely represented by its two stable isotopes, ^{12}C and ^{13}C, contributing 98.9 and 1.1% of the total respectively. The main source of ^{14}C in the environment is natural, resulting from the reaction of cosmic neutrons with nitrogen atoms in the high atmosphere. The atmospheric pool of natural ^{14}C is estimated at 1.4 x 10^{17} Bq, with a half-life of 5730 years and a production rate of 1.4 x 10^{15} Bq per year (UNSCEAR, 2008). However, ^{14}C also has several anthropogenic origins. First, nuclear weapon testing caused a rapid doubling of ^{14}C proportion in all environmental compartments during the second half of the 20th century. This proportion gradually decreased after the end of testing, to return to its initial level during the 2010 decade (McGee et al., 2004; Otlet et al., 1997; Roussel-Debet et al., 2006; Roussel-Debet, 2014). Second, ^{14}C is the radionuclide released in greatest amount by nuclear reprocessing plants and nuclear power plants (NPP) in normal operation (Florence & Hartmann, 2003; Garnier-Laplace et al., 2009). In France for example, an average activity from 1.5 to 3.0 x 10^{11} Bq is released every year by a pressurized water reactor (Florence & Hartmann, 2003). A major part of this radioactivity (approximately 85–95%) is released into the atmosphere as CO_2 and CH_4, while 5–15% is released into the rivers as dissolved CO_2 with liquid effluents (EDF, 2014).

Following the cycle of carbon, both anthropogenic and natural $^{14}CO_2$ enter aquatic food webs through photosynthesis and primary production and are transferred towards higher trophic levels. In order to assess the consequences for the environment and for human health, transfer models are used to predict ^{14}C activity in fish and the associated dose for human consumers, based on average amounts released over the year (Avetisov et al., 2001; Boyer & Beaugelin-Seiller, 2002; Galeriu et al., 2007; Le Dizès et al., 2012). These models assume that ^{14}C behaviour is similar to that of stable carbon and that a common isotopic ratio is shared among water and aquatic organisms. Predictions suggest that anthropogenic ^{14}C is the main contributor to the absorbed dose of 1 µSv per year, approximately estimated for generic fish consumers living in the vicinity of NPP (Mourlon & Vermorel, 2014; Siclet, 2001; Siclet et al., 2002; UNSCEAR, 2000). This should be noted that this dose remains largely lower than the annual legal limit of 1 mSv per year for the public. However, the estimated dose is calculated for equilibrium ^{14}C concentrations and does not consider time variations observed in the field. In

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

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

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 (± 1°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 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 (± 1°C), oxygenated with constant air bubbling and partly renewed
74 (20%) every day.

75 **2.2. Food contamination and desorption**

76 ^{14}C-labelled solutions of arginine and glucose (arginine L-[^{14}C(U)] and glucose D-[^{14}C(U)],
77 97% purity, 1.85 MBq) were purchased from PerkinElmer (Courtaboeuf, France). ^{14}C-Arginine
78 and ^{14}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 µL of ^{14}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}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}C activity in the food by liquid
85 scintillation.

86 ^{14}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}C-labelled food was placed in a vial containing 5 mL of water. Working with a small
89 volume was necessary to avoid ^{14}C dilution and keep water activity above the detection limit
90 by liquid scintillation. Five replicate vials were used to monitor ^{14}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}C transfer. To do so, 30 000 Bq of ^{14}C-labelled arginine were added to each aquarium containing a fish in 8 L of water (yielding a ^{14}C concentration of 3750 Bq L^{-1} on Day 0). A solution of ^{14}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}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}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}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}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}C-labelled food pellets. Food was left in water for 10 min maximum, in order to minimize ^{14}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}C supplied with food entered fish digestive tracts;
- assimilation, where a fraction of ingested ^{14}C entered fish organisms through the intestinal epithelium;

- incorporation, where the fraction of assimilated ^{14}C became part of the fish body (as opposed to assimilated ^{14}C which was immediately used as an energy source and released as $^{14}CO_2$ within 24 h).

2.5. Elimination experiments

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

2.6. Sample treatments and ^{14}C analyses

Each carp was euthanized immediately and its body size and wet weight (ww) were measured. Different fractions (muscles, rest of the body) were dissected, weighed and homogenised with a blender. Three replicate samples of 1 mL approximately were collected from each homogenised fraction. Collected faeces and food samples were dried at 60°C overnight. All samples (including water samples of 1 mL) were transferred into scintillation vials and weighed.

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

2.7. Data analyses

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

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

3. Results

3.1. Direct ^{14}C transfer from water

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

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

3.2. Food activity

Radiolabelled food showed different mass specific activities depending on the ^{14}C source, with values of ~590 ± 100 Bq g^{-1} with glucose and 5950 ± 470 Bq g^{-1} with arginine. When food

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

3.3. Trophic transfer

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

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

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

whole body and muscle (Figures 5B and 5D, SI Table S4B). Average values ranged from 7.0 ± 1.5 to 32 ± 5.2 Bq g^{-1} ww with arginine and from 0.5 ± 0.1 to 1.7 ± 0.4 Bq g^{-1} ww with glucose. Associated trophic transfer factors (TTF, as Bq g^{-1} ww per Bq g^{-1} water) increased over time, from 1.3% on Day 1 to 6.2% on Day 4 with arginine and from 1.0% on Day 1 to 3.4% on Day 4 with glucose.

3.4. Elimination

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

With arginine, whole body total activity significantly varied among days of elimination (Figure 7A, SI Table 5), from 2220 ± 300 to 1540 ± 580 Bq per fish. Value on Day 2 represented ~41% of ^{14}C activity supplied with food over the 5 days. With glucose, whole body total activity did not significantly decrease over days of elimination, varying from 105 ± 40 to 85 ± 4 Bq per fish (Figure 7C, SI Table 5). With glucose, whole body total activity represented ~15% of activity supplied with food, suggesting a much greater ^{14}C incorporation with arginine than with glucose. Again, considering an absorption rate of 20% per day and an incorporation rate of 14.3% per day for absorption and incorporation respectively (as estimated from direct transfer), observed water ^{14}C activity might result in a direct incorporation of 220 and 80 Bq per fish, with arginine and glucose respectively, approximately estimated over 5 days of elimination. These activities accounted for 10% and 72% of whole body total activity, and for 4 and 10% of supplied food activity, with arginine and glucose respectively.

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

not differ significantly between the muscle and whole body (Figures 7B and 7D, SI Tables 5). With arginine, values varied significantly among days of elimination, from 50 ± 9.0 to 26 ± 17 Bq g^{-1} ww. With glucose, values did not differ significantly among days of elimination, ranging from 0.6 ± 0.2 to 1.2 ± 0.6 Bq g^{-1}. Associated trophic transfer factors (TTF) ranged from 7.3% to 14% with arginine and from 1.2% to 2.4% with glucose. With glucose, estimated TTF values were similar between the elimination and the feeding experiments. With arginine, estimated TTF values were twice greater during the elimination experiment than during the feeding experiment.

4. Discussion

Our study addressed ^{14}C accumulation kinetics in *C. carpio* during short term direct or trophic contamination experiments. Results with ^{14}C-labelled arginine showed that carps directly absorbed 20% of waterborne arginine each day, representing approximately the activity in 1.6 L of water (20% of 8 L aquaria). This value was small compared to ventilation volumes expected to be pumped by carps at 18°C (Klyszejko et al., 2003), suggesting that transfer efficiency through gill membranes was below 10%. Results also showed that minor fractions of assimilated ^{14}C-labelled arginine (ranging from 14 to 20%) were finally incorporated to whole body, implying that a major part was catabolized and released as $^{14}CO_2$ via respiration. This result was in good agreement with previous studies on metabolism of ^{14}C-labelled amino-acids (including essential lysine and non-essential glutamate) and glucose in *C. carpio* (Bouche & Vellas, 1975; Nagai & Ikeda, 1971b). Authors reported that a significant fraction (45%) of glutamate activity injected into blood was released as $^{14}CO_2$ within 6 h, while a minor fraction (7% only) were stored as hepatic glycerides. Authors further showed that glutamate incorporation varied depending on diet composition, with a similar level of $^{14}CO_2$ production and no ^{14}C-labelled glyceride storage when carps were fed on a high carbohydrate food (90% starch and 10% casein). In our study, results suggested that arginine incorporation differed between direct and trophic experiments (14.3 and 18.6% respectively), suggesting a greater arginine storage when carps were fed than when they were starved. This interpretation was supported by previous results showing that starved carps stopped storing another essential amino-acid (^{14}C-labelled lysine) (Bouche & Vellas, 1975). The present study reported approximately similar proportions of incorporation with ^{14}C-labelled glucose as with arginine (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 $^{14}CO_2$ production after 6 h was much lower
279 with ^{14}C-labelled glucose than with ^{14}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}C elimination kinetics in *C. carpio* during short term starvation
283 experiments. Results showed that activity of ^{14}C-labelled arginine decreased in starved carps,
284 whereas no similar decrease was observed with ^{14}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}C transfer in an aquatic
296 ecosystem. In the literature, ^{14}C transfer models (Sheppard et al., 2006; Smith, 2006) were based
297 on the common assumption that ^{14}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 nutriments, such as arginine, could directly be absorbed from
300 water, and contribute to a minor fraction of fish ^{14}C intake during feeding experiments (~10%).
301 While waterborne nutriments, 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}C transfer to
305 aquatic organisms (Saito et al., 1994; Shimizu et al., 1978; Tjeerdema & Crosby, 1988; Velisek
306 et al., 2009).

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

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

5. Conclusions

Experimental results showed that common carps can absorb ^{14}C-labelled molecules via direct and trophic routes, with different transfer efficiencies depending on feeding condition and biochemical form of ^{14}C. A major fraction of absorbed ^{14}C was catabolized and released as $^{14}CO2$ on the short term. A minor fraction of ^{14}C was incorporated into organism tissues, contributing to a rapid increase in fish ^{14}C activity. Freshwater ^{14}C transfer models need to consider the influence of ecophysiological conditions on short term ^{14}C kinetics in order to address rapid variations in freshwater fish activity.

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473

7. Figure captions

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

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

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

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

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

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

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

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

Figure

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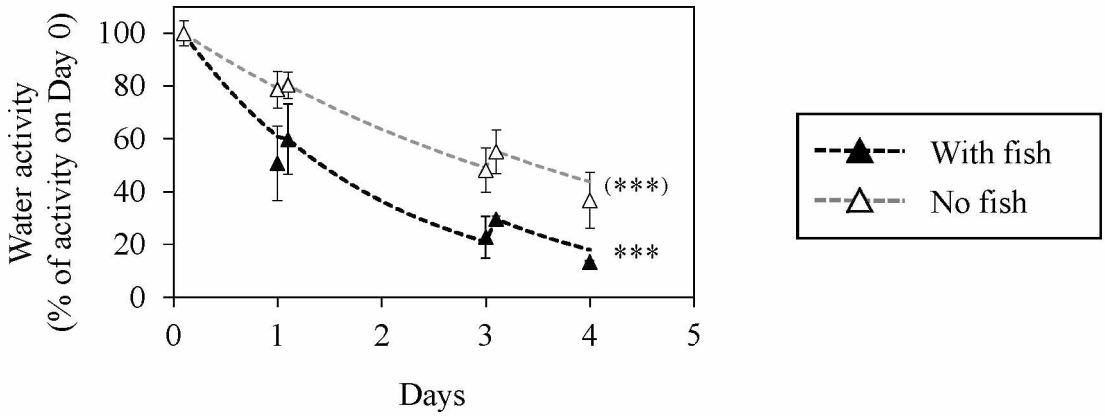

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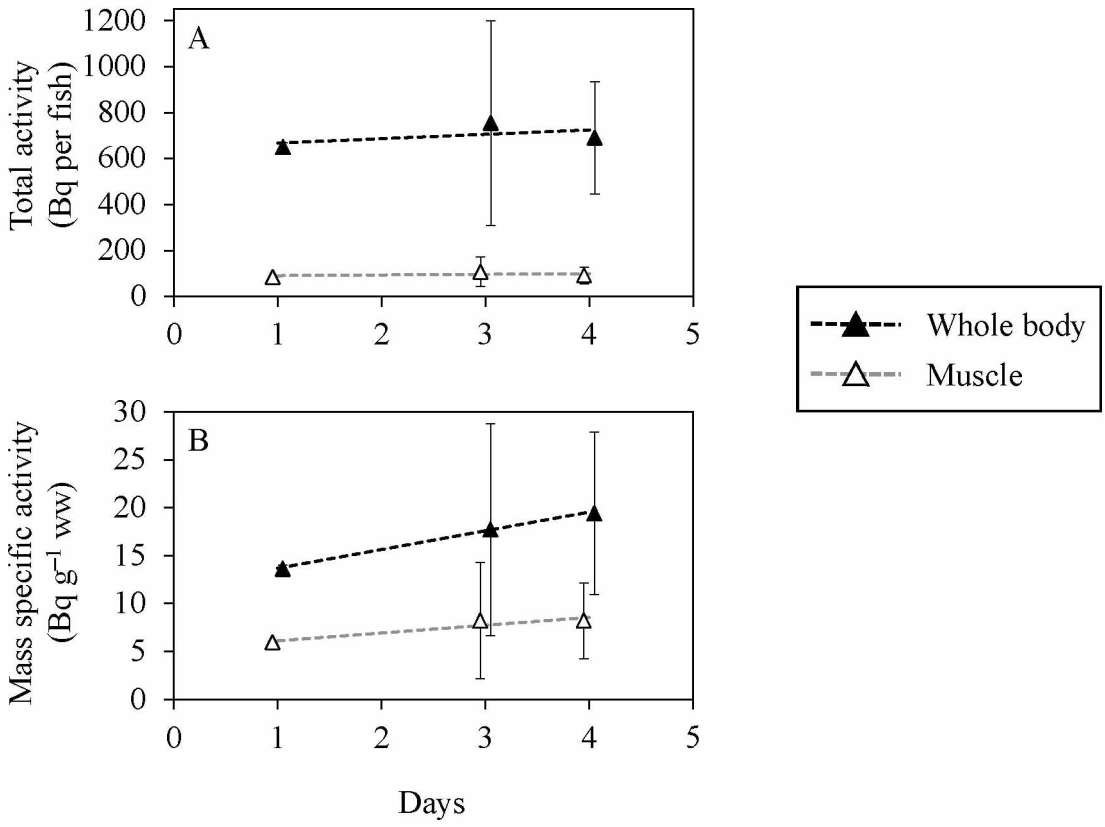

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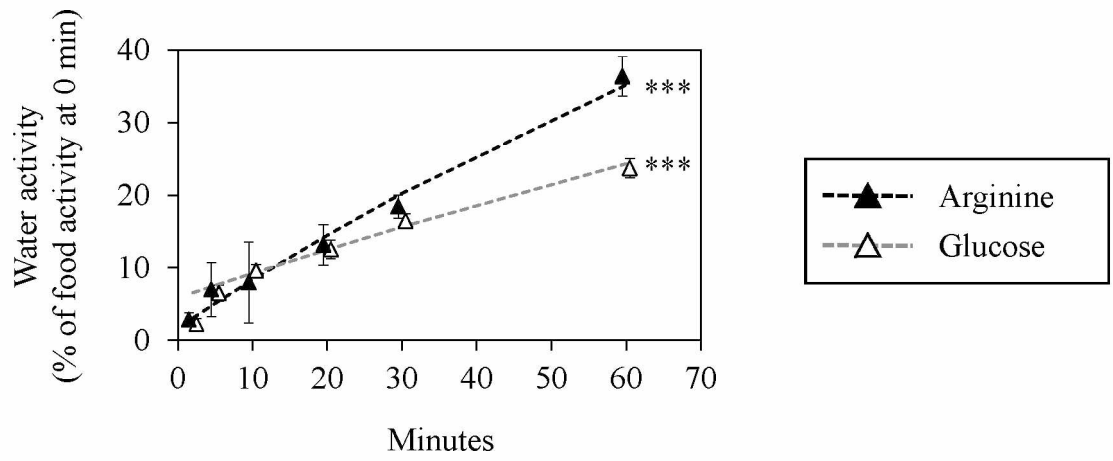

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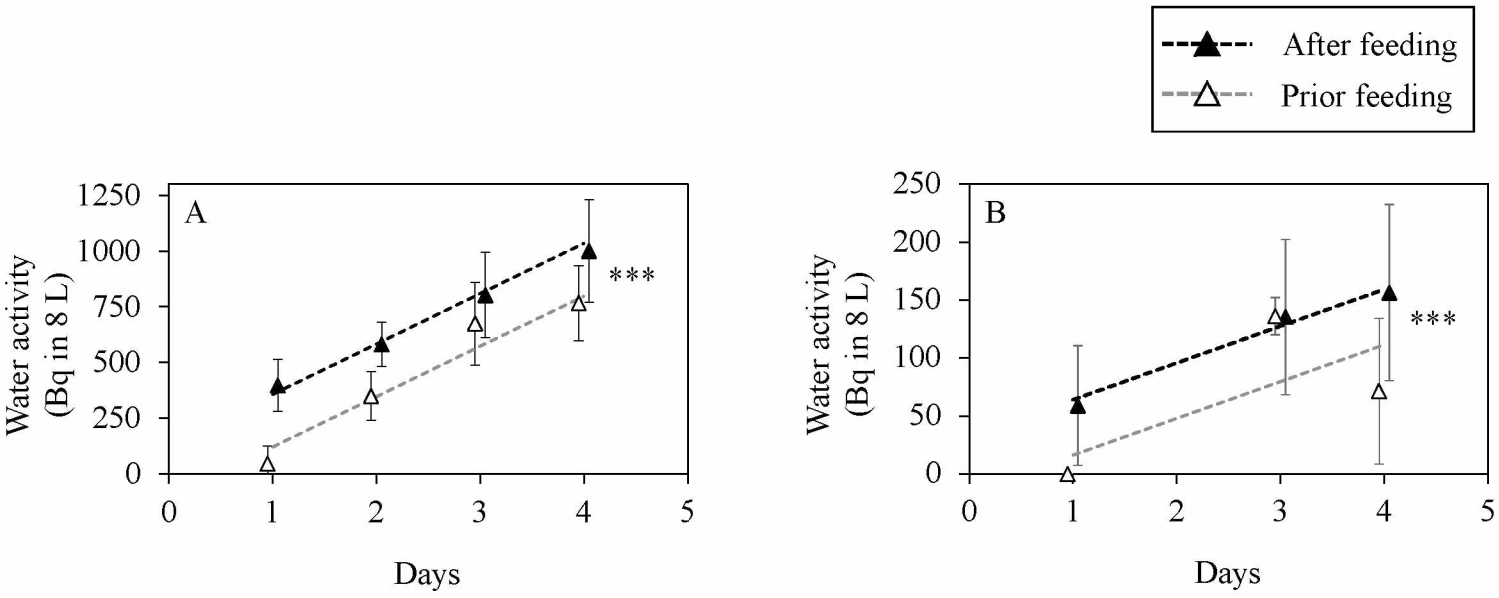

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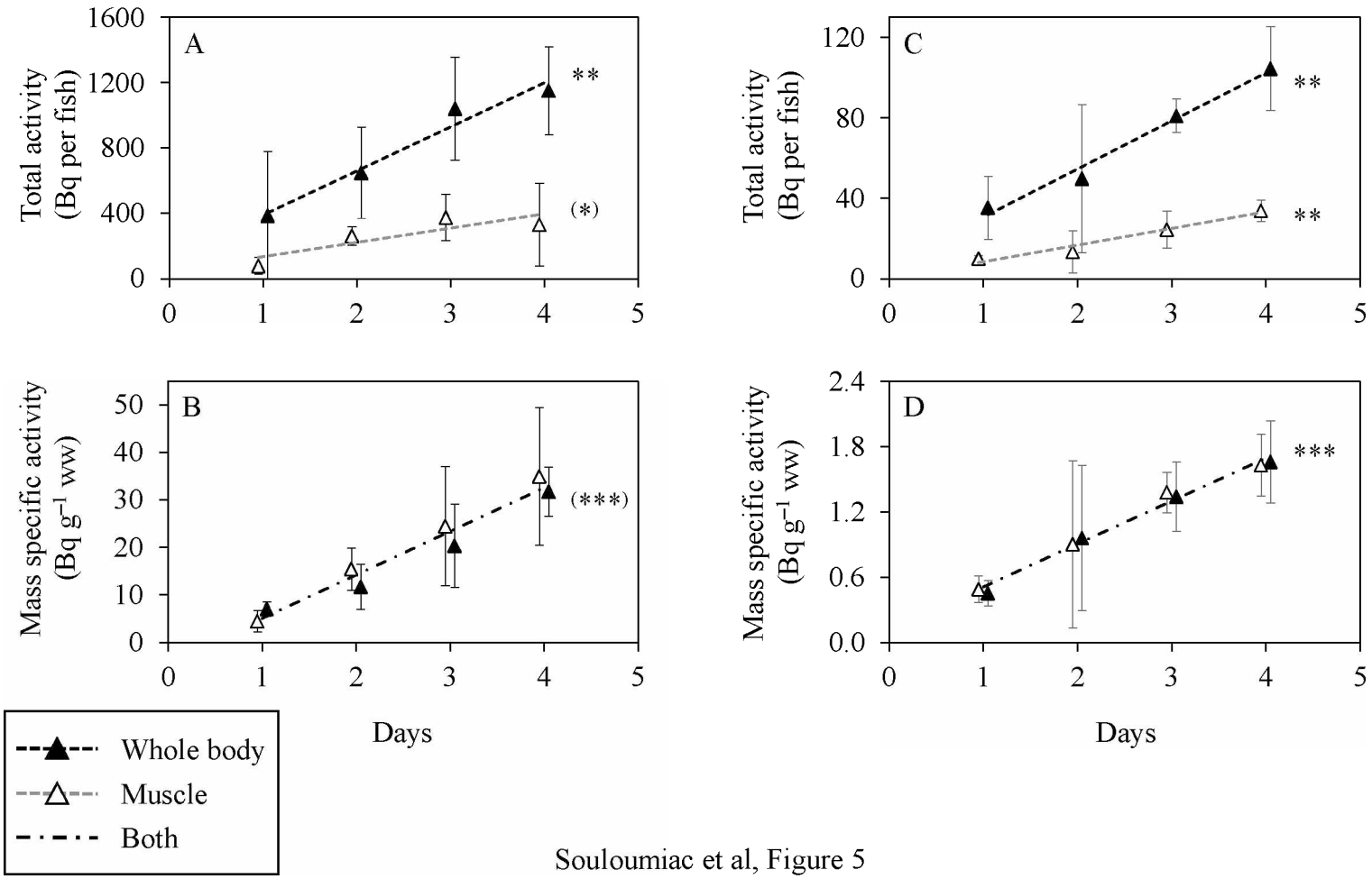

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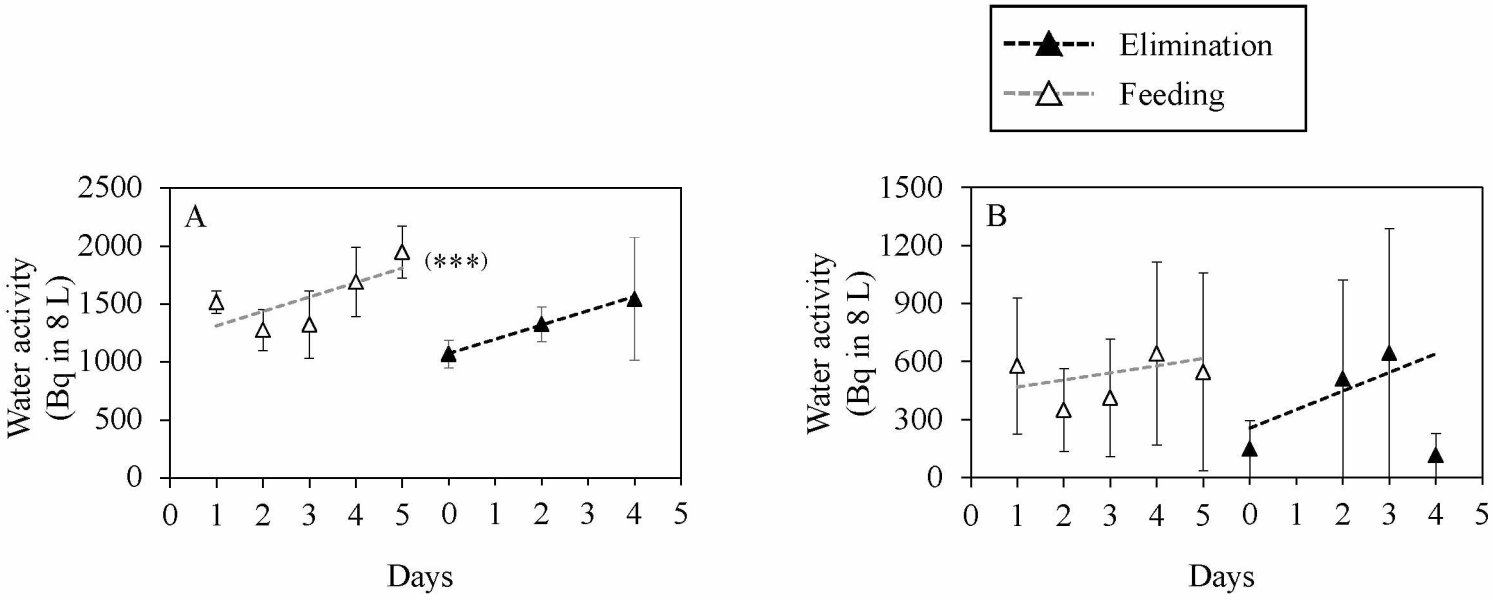

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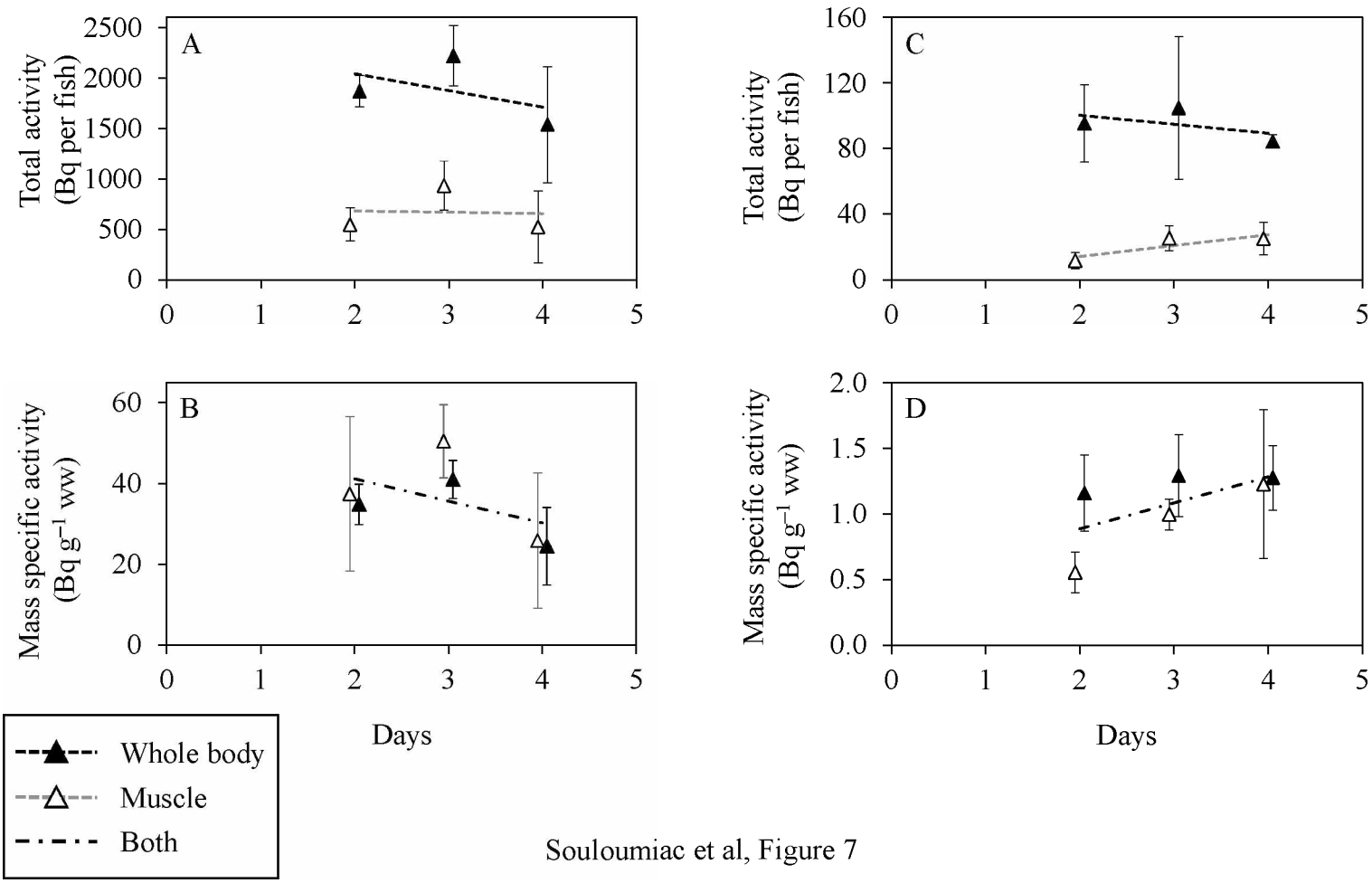

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