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Sarah Nahon, Sarah Seite, Sébastien Lefebvre, Joanna Kolasinski, Pierre Aguirre, et al.. How protein quality drives incorporation rates and trophic discrimination of carbon and nitrogen stable isotope ratios in a freshwater first-feeding fish. *Freshwater Biology*, 2020, 14 p. 10.1111/fwb.13578 . hal-02957498

HAL Id: hal-02957498

<https://hal.inrae.fr/hal-02957498>

Submitted on 24 May 2024

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How protein quality drives incorporation rates and trophic discrimination of carbon and nitrogen stable isotope ratios in a freshwater first-feeding fish

Nahon Sarah ^{1,*}, Séité Sarah ¹, Lefebvre Sebastien ², Kolasinski Joanna ³, Aguirre Pierre ¹, Geurden Inge ¹

¹ INRAE, UPPA UMR 1419, Nutrition, Métabolisme, Aquaculture Université de Pau et des Pays de l'Adour Saint Pée sur Nivelles, France

² LOG Laboratoire d'Océanologie et Géosciences UMR 8187 Univ. Lille CNRS Univ. Littoral Côte d'Opale Wimereux, France

³ UMR ENTROPIE CNRS 9220 IRD 450 Université de La Réunion St Denis, France

* Corresponding author : Sarah Nahon, email address : sarah.nahon@inrae.fr

Abstract :

1. Using stable isotope ratios to explore the trophic ecology of freshwater animals requires knowledge about effects of food quality on isotopic incorporation dynamics. The aim of this experimental study was to: (1) estimate carbon and nitrogen isotopic incorporation rates and trophic discrimination factors (TDFs) of a freshwater first-feeding fish (i.e. salmonid fry) fed three diets that differed only in protein quality (animal or plant or a blend of both); (2) investigate effects of fasting and; (3) evaluate the proportion of each source assimilated when fry were fed a 50:50 animal:plant-based diet.

2. For each diet, incorporation rates of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were estimated using a time or growth-dependent isotopic incorporation model. Effects of fasting on isotope ratio values were measured regularly until the death of fry. Bayesian stable-isotope mixing models were used to estimate the contribution of animal and plant material to fish fed a blend of both food types.

3. Our results show that incorporation rates were lower for fry fed a plant-based diet than for those fed an animal-based diet as growth rate decreased. Time- and growth-dependent models indicated that growth was solely responsible for isotopic incorporation in fry fed an animal-based diet, whereas catabolism increased in fry fed a plant-based diet. After lipid extraction, carbon TDFs were similar regardless of the diet, whereas nitrogen TDFs increased for fry fed a plant-based diet. Long-term fasting induced an increase of 0.63‰ in $\delta^{13}\text{C}$ values of fry in 23 days, whereas $\delta^{15}\text{N}$ values did not vary significantly. Proportions of food sources assimilated by fry fed an animal:plant-based diet were similar to those consumed when using a mixing model with the estimated TDFs, while proportions were unrealistic when using mean TDFs extrapolated from the literature.

4. The results of our study indicate that the quality of food must be considered to use an appropriate timescale to detect changes in fry diets in the field. Moreover, we recommend using different carbon and

nitrogen TDFs, one for animal-derived sources and one for plant-derived sources, to increase the accuracy of mixing models.

Keywords : animal-based diet, metabolic pathway, mixing model, *Oncorhynchus mykiss*, plant-based diet

1 Introduction

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope analysis is a common method for studying ecological features of animals in freshwater ecosystems, including migration and movement (Jones & Mackereth, 2016), ecophysiological processes (Gannes, del Rio & Koch, 1998; Chappuis et al., 2017), nutrient flows, structure of food webs and partitioning of resources (Jackson et al., 2017; Taylor et al., 2017) and bioaccumulation of contaminants (Thomas et al., 2016). In order to successfully infer a diet to an organism by means of stable isotope analysis, it is necessary to have prior reliable estimations about carbon and nitrogen incorporation rates and trophic discrimination factors (TDF) which are respectively defined as the time necessary for organism tissues to acquire the isotopic composition of the diet and the isotopic discrimination between a consumer and its diet (Martínez del Rio & Carleton, 2012).

First, estimation of isotopic incorporation rate (also called turnover rates) is essential to give a temporal dimension of stable isotope data while using an inappropriate time-scale can lead to erroneous interpretation (O'Reilly et al., 2002). For example, Perga & Gerdeaux, 2005 showed during a field-study on the Whitefish (*Coregonus lavaretus*) that the seasonal amplitude of isotope variation was two to three times higher in liver compared to muscle tissue and that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of muscle tissue only reflected the food consumed during the spring and summer growth period. Turnover rates depend on both growth rate (i.e., anabolism or adjunction of tissue) and catabolic rate (i.e., replacement of tissue, Fry & Arnold, 1982; Hesslein, Hallard & Ramlal, 1993). It is now well established that isotopic incorporation rates are faster in species, organisms or tissues which are metabolically active (Vander Zanden et al., 2015; Thomas & Crowther, 2015). Second, the application of stable isotopes relies upon the assumption that isotope signatures of consumers reflect those of assimilated dietary sources. TDF results from transformations of carbon and nitrogen-containing molecules during the processes of ingestion and assimilation of food sources (Fry, 2006). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of

fish have been estimated to be enriched by 1.5‰ and 2.79‰ in ^{13}C and ^{15}N , respectively, relative to the diet (Sweeting et al., 2007a b).

Isotopic incorporation rates and TDF are linked to multiple nested factors including the fish species and the tissues considered (Madigan et al., 2012; Heady & Moore, 2013; Vander Zanden et al., 2015; Thomas & Crowther, 2015; Sacramento, Manetta & Benedito, 2016; Franssen et al., 2017), the physiological state (e.g., stress, ontogeny, Hertz et al., 2016; Busst & Britton, 2018) and the environmental conditions (e.g. temperature, salinity, food quantity and quality, Ankjærø, Christensen & Grønkjær, 2012; Busst & Britton, 2016; Mohan et al., 2016; Mont'Alverne et al., 2016; Colborne, Fisk & Johnson, 2017). In fast-growing juveniles, studies have shown that organisms fed with a high food quantity and/or quality have faster carbon and nitrogen isotopic incorporation rates than organisms fed with low food quantity and/or quality as they grow more rapidly (Ankjærø et al., 2012; Martínez-Rocha et al., 2013; Mohan et al., 2016). In fact, isotopic incorporation rates of juveniles are largely driven by the rate of new tissue adjunction (i.e. growth) rather than the catabolic tissue replacement rate. Moreover, carbon and nitrogen isotopic TDF are known to vary with the quantity (*protein quantity hypothesis*, Gaye-Siessegger et al., 2004; Martínez del Rio et al., 2009) or the quality (*protein quality hypothesis*, Roth & Hobson, 2000; Florin, Felicetti & Robbins, 2011; Busst & Britton, 2016) of protein. The first hypothesis is based on an increase of excretion with high protein quantity leading to the preferential retention of ^{15}N that elevate $\delta^{15}\text{N}$ value of organisms (Martínez del Rio et al., 2009). The second hypothesis assumes that when quality of protein decreases, higher nitrogen fractionation occurs as amino acids are catabolized and rearranged to meet nutritional requirement (Roth & Hobson, 2000). To our knowledge, data available in literature most focussed on juvenile stage while ontogeny have been shown to be a major factor influencing TDF and isotopic incorporation rates in fish (Busst and Britton, 2018; Hertz et al.,

2016). As first-feeding fish displays a high growth potential and are very responsive to changes in dietary supply, our knowledge must be reinforced for stable isotope field-based application.

In field studies, the nutritional status of organisms (food quality and fasting) is difficult to evaluate and its effect on isotopic ratios of organisms are often ignored. However, when source-specific TDF are used, the accuracy of trophic trophic position and resource assimilation is improved (Bastos et al., 2017). In fact, the number of experimental studies remains rather low compared with field studies (Martínez del Rio et al., 2009) and a recent review on isotopic incorporation rates in animal tissues only reports on the effect of temperature and body mass (Vander Zanden et al., 2015). Twenty years after the call for more laboratory experiments by Gannes, del Rio & Kock (1998), the effects of food quantity and quality on isotopic incorporation rate and TDF have still to be further explored. Moreover, there is little consensus in the literature on how the isotopic values of organisms change with fasting (Hatch, 2012; Hertz et al., 2015).

The aim of this study was to estimate during controlled laboratory experiments carbon and nitrogen isotopic incorporation rates and TDF of rainbow trout (*Oncorhynchus mykiss*) first-feeding juvenile fish (namely fry in salmonids) fed with diets which differed by the quality of the proteins (animal-based diet, vegetal-based diet and 50:50 animal: plant-based diet). Vegetal-based diets were supplemented with phosphorous and essential amino acids (lysine and methionine) in order to fulfill the nutritional requirements of fish known to occur in a 100% plant based diet and prevent fish death (Kaushik et al., 2004; Gatlin et al., 2007). Rainbow trout displays diverse and complex life stories but the processes shaping their behavior are not completely understood (Kendall et al., 2015). Whereas all individuals spawn and reared in freshwater, some individuals undergo marine migration and others complete their entire life cycle in freshwater. Stable isotope ratio analysis is known to be a useful tool to track geographical movement of fish but the estimation of incorporation rate and TDF are often the

missing links for accurate interpretation (Trueman et al., 2012). We hypothesised that fry fed on plant-based diets will have a lower growth rate and thus lower carbon and nitrogen isotopic incorporation rates than fry fed on animal-based diet (Gomes, Rema & Kaushik, 1995; Vilhelmsson et al., 2004). Moreover, we estimated the catabolic tissue replacement rate to test the hypothesis that protein turnover rate will increase for fish fed with the plant-based diet (Wacyk et al., 2012). We also hypothesized that nitrogen TDF will be higher for fry fed with the plant- than the animal-based diet which better matches rainbow trout fry natural feeding habits. We estimated the proportion of animal-based diet and vegetal-based diet assimilated by fry fed on 50:50 animal:plant-based diet using a two-source and two-isotopes mixing model. Finally, we investigated the effect of fasting on carbon and nitrogen isotopic values of first-feeding fry as in field the most important cause of fry mortality is probably starvation.

2 Methods

2.1 Ethics statement

Animal experiments and sampling procedures were conducted following the Guidelines of the National Legislation on Animal Care of the French Ministry of Research (Décret 2001-464, May 29, 2001) and in accordance with the boundaries of EU legal frameworks, relating to the protection of animals used for scientific purposes (*i.e.* Directive 2010/63/EU). The INRA experimental facilities are certified under the permit number A40.228.1 and A64.495.1 for animal services and permit number FR40090951-40090002 for animal feed production by the French veterinary services.

2.2 Experimental diets

The three experimental diets used to feed rainbow trout fry, namely fish meal (FM), corn gluten meal (CGM) and a blend of both (MixM), were manufactured using a twin-screw extruder (BC 45, Cleextral, France) at the fish farm of Donzacq (INRA, France). Pellets with a diameter of 400-600 μm , 600-800 μm and 800-1500 μm were dried and stored at -20°C until used. The pellet diameter was adjusted to fish size throughout the experiment. Diet FM contained fish meal as protein source, diet CGM contained corn gluten meal and diet MixM had a combination of 50% FM and 50% CGM (Table 1). The three diets were formulated to meet the nutritional requirements of the fish and to have similar levels of crude proteins, lipids and energy. For this, diets CGM and MixM were supplemented with phosphorous and with the essential amino acids, lysine and methionine, in order to fulfill the nutritional requirements. Added levels of fish oil and starch varied in order to compensate for the residual levels of fat and carbohydrates present in fish meal and plant meal, respectively. The biochemical composition of the experimental diets was analyzed according to the following procedures: dry matter and ash content were weighted after respectively drying at 105°C for 24 h and incineration at 550°C for 6 h ; C and N contents were determined using an Elementar Vario Pyrocube elemental analyzer (Elementar, Langenselbold, Germany), crude protein was determined using Kjeldahl method (Kjeldahl, 1883), total lipids by petroleum ether extraction using Soxhlet method (Soxtherm[®], C. Gerhardt GmbH & Co. KG, Germany) and gross energy content in an adiabatic bomb calorimeter (IKA[®], Heitersheim Gribheimer, Germany). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as well as C and N contents of each diet were measured once a week as described in the sub-section C and N stable isotope analysis. Due to their different origin, diets FM and CGM showed very contrasting $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Table 1, Figure 1).

2.3 Experimental design, sampling and estimation of growth rates

Alevins of rainbow trout *Oncorhynchus mykiss* were produced at the INRA experimental fish farms of Lee-Athas, France. 4800 alevins were then transferred to the experimental fish rearing facilities of Saint-Pée-sur-Nivelle (Aquapole INRA, France), randomly distributed in 13 separate tanks of 60 l and acclimated during 4 days to their new environment. Water temperature was stabilized around 14°C and quality (ammoniac, nitrite, nitrate and oxygen) was monitored every three days to ensure animal welfare. Water was renewed in tanks every two hours and recycled throughout a series of filters in a recirculating system. An automatic photoperiod was set up to provide a light-dark ratio of 12:12 hour.

The experiment started once at least 90% of alevins have reached the swim-up fry stage. We assessed the transformation from alevin to fry by a reduction of yolk sac and the ability of fry to leave the bottom of tanks to feed on exogenous food sources. From the first meal, fry contained in 4 different tanks were fed *ad libitum* and continuously during 8 hours per days either with the diet FM, or the diet CGM, or the diet MixM. One fry tank was kept without any food supply. At the end of each feeding period, uneaten feed and faeces were removed from the tanks. Following 16 hours of fasting (time necessary to clear the food from fry gut), 6 fry were randomly sampled in each tank, carefully rinsed ultra-pure water (milli-Q[®], Merck-Millipore, Molsheim, France), killed by immersion in ice-slurry, freeze-dried and then stored at -80°C until analysis (Nahon et al., 2017). Samplings were performed daily from day 0 to day 15, every second day from day 15 to day 31 and every week from day 31 to day 36. Mortality was recorded daily in all tanks.

Dry weight of 1980 sampled fry was measured and growth rate constants (K_g) were estimated for each diet by fitting an exponential growth model to measured data of fry weight as following:

$$W_t = W_0 e^{K_g t} \quad (1)$$

Where W_t and W_0 are respectively the fry weight at time t and time 0. K_g is expressed in day^{-1} .

2.4 Sample preparation for carbon and nitrogen stable isotope ratio analysis

For carbon and nitrogen stable isotope analysis, one fry per tank (*i.e.*, 4 fry per experimental diet) were selected at 12 different times from day 0 to day 36 according to the fry growth curve and isotopic equilibrium with diet. Whole dried fry were then ground to a fine homogeneous powder using a Precellys[®] grinder mill (Bertin Technologies, Montigny-le-Bretonneux, France). In order to remove naturally ^{13}C -depleted lipids (DeNiro & Epstein, 1977), diet and fry tissue were treated with cyclohexane (Merck, Darmstadt, Germany) as described by Chouvelon et al. (2014). Approximately 20 mg of powder were weighted in glass vials. 4 ml of cyclohexane were added and after 1 hour, samples were centrifuged (4000g, 10 min, 10°C). The supernatant was discarded and the procedure was repeated. Samples were then dried in a dry bath at 45°C before isotopic analysis. This method has been chosen as it presents the advantage of not impacting $\delta^{15}\text{N}$ values (Chouvelon et al., 2014), compared to commonly used chloroform-methanol or dichloromethane-methanol mixtures (Schlechtriem, Focken & Becker, 2003; Post et al., 2007). In this study, this assumption has been confirmed on rainbow trout fry (Supplementary Figure 1, A and B). Preliminary isotopic analysis have also been realized to test the influence of carbonate on $\delta^{13}\text{C}$ values of whole fry. As previously observed by (Pinnegar & Polunin, 1999), the influence of acid washing on $\delta^{13}\text{C}$ was neglectable on rainbow trout fry despite the presence of bones and scales (Supplementary Figure 1, C and D). Approximately 1 mg of each dried sample was weighed and packed into a tin capsule for simultaneous C and N stable isotope analysis.

2.5 Carbon and nitrogen stable isotope ratio analyses

Isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) composition of all samples as well as C and N content of diets were analyzed by elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) in continuous-flow mode using an Isoprime 100 isotope ratio mass spectrometer interfaced with and Elementar Vario Pyrocube elemental analyzer (Elementar, Langensfeld, Germany). The $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratios are expressed in conventional delta notation in per mil (‰) relative to the levels of ^{13}C in Vienna Pee Dee Belemnite and ^{15}N in atmospheric air, according to the following equation:

$$\delta X = \left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right)$$

where X is ^{13}C or ^{15}N and R is the ratio of heavy to light isotope ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$). Repeated measurements on glycine exhibited a precision of $\pm 0.16\text{‰}$ for $\delta^{13}\text{C}$ value, $\pm 0.17\text{‰}$ for $\delta^{15}\text{N}$ value, $\pm 1.05\%$ for C and $\pm 1.12\%$ for N. Commercial standards, alanine, wheat flour and corn flour from IsoAnalytical Lab (Crew, United Kingdom), IAEA-N-2 and IAEA-600 caffeine from National Institute of Standard and Technology (Gaithersburg, USA) and USGS66 glycine from Reston Stable Isotope Laboratory (Reston, USA) were used for a multipoint calibration.

2.6 Estimation of carbon and nitrogen incorporation rates, catabolic tissue replacement rates and trophic discrimination factors

Animals are assumed to change their isotopic signature according to the isotopic signature of their diet. Isotopic shifts observed in animal tissues are due to the incorporation of new isotopes from the diet. For each diet, incorporation rates of C and N were estimated using a single-compartment first-order kinetic time-dependent model (Hobson & Clark, 1992; O'Brien, Schrag & Del Rio, 2000; Carleton & Martinez del Rio, 2010):

$$\delta X_t = \delta X_\infty + [\delta X_{t0} - \delta X_\infty]e^{-\lambda_x t} \quad (2)$$

where X is ^{13}C or ^{15}N , δX_t and δX_{t0} are respectively isotopic value of fry at time t and time 0, δX_∞ is the estimated asymptotic value that fry reaches at the steady-state with their new diet, and λ_x is the isotopic incorporation rate expressed in day^{-1} .

Multi-compartment models can provide better insight into turnover dynamic than first-order one compartment models. When experimental data were transformed and linearized using the reaction-progress diagnostic procedure described by Cerling et al. (2007) straight lines were obtained whatever the diet considered (Supplementary Figure 2). Thus, a single-compartment model has been chosen as using more than one compartment did not increase the accuracy of the model.

The time period necessary for half of the fry carbon or nitrogen to be replaced by new atoms after the consumption of a new diet (isotopic half-life) was calculated as following:

$$t_{50X} = \frac{\ln(2)}{\lambda_x} \quad (3)$$

where t_{50} is the isotopic half-life, X is C or N and λ is the estimated value of turnover. t_{50} is expressed in day.

Isotopic half-life estimated with the equation 3 were compared those obtained with the equation proposed by Weidel et al. (2011) for ectodermic vertebrate which negatively correlates fish size and the rate of isotopic change regardless the origin of food sources.

$$\ln(t_{50X}) = 0.20\ln(\text{mass}) + 3.65 \quad (4)$$

where t_{50} is the isotopic half-life, mass is fish size. t_{50} is expressed in day.

For each diet, the relative contribution of growth and catabolism to change in carbon and nitrogen isotopic values of fish were estimated using both a time-dependent model (model 5, Hesslein, Hallard & Ramlal, 1993) and a growth-dependent model (model 6, Fry & Arnold, 1982; Maruyama et al., 2001). The time-dependent model was a modification of the model (2)

where isotopic incorporation (λ_X) is the result of the joint contribution of growth (K_g) and catabolism (K_c). For each diet, catabolic tissue replacement rates of carbon or nitrogen were determined as following:

$$\lambda_X = K_g + K_c \quad (5)$$

where X is C or N and K_g is estimated using the model (1). K_c is expressed in day^{-1} .

Martínez del Río & Carleton, 2012 noticed that although it is tempting to use the change in total mass of an animal as a proxy to estimate growth, this approximation will be poor if animals change in body composition (i.e., C:N ratio) as they grow. After lipid extraction, the quantity of C and N per 100 mg of fish dry weight were constant throughout the trial whatever the diet considered (43.90 ± 1.88 , 42.91 ± 1.01 , 43.16 ± 4.84 % of carbon and 15.7 ± 2.99 , 12.16 ± 0.92 , 11.92 ± 1.31 % of nitrogen for fish fed with FM, CG and MixM respectively). Thus, K_g expressed in mg of dry weight, in mg of carbon or in mg of nitrogen were similar.

The growth-dependent model used was:

$$\delta X_t = \delta X_\infty + [\delta X_{t0} - \delta X_\infty] \left[\frac{W_t}{W_0} \right]^C \quad (6)$$

where X is ^{13}C or ^{15}N , δX_t and δX_{t0} are respectively isotopic value of fry at time t and time 0, δX_∞ is the estimated asymptotic value that fry reaches at the steady-state with their new diet, W_0 is the weight at time 0, W_t is the weight at the time t and C is the catabolic decay constant. C represents the relative contribution of catabolism to the observed change in the isotopic values of fish. A C-value of -1 indicates only growth (simple dilution) while a C-value less than -1 represents proportionately greater contributions of catabolism to overall isotopic change (Fry & Arnold, 1982).

C and N diet-to-fry trophic discrimination factor (TDF) have been calculated for each diet as following:

$$\Delta \delta X = \delta X_\infty - \delta X_{\text{diet}} \quad (7)$$

where Δ is the TDF, X is ^{13}C or ^{15}N , δX_{∞} is the value at the steady-state between fry and their diet and δX_{diet} is the mean isotopic value of the diet. $\Delta\delta X$ is expressed in ‰.

2.7 Estimation of fish meal and corn gluten meal assimilated by fry fed on a blend of meals

Mixing models SIMMR (Stable Isotope Mixing Models in R, Parnell et al., 2013) were used to determine the likely contribution of FM and CGM to fish fed with MixM using average values from large literature review (1‰ and 3.4‰ for carbon and nitrogen, respectively, Deniro & Epstein, 1978; Post, 2002), or specific average values from fish literature review (1.5‰ and 2.79‰ for carbon and nitrogen, respectively, Sweeting et al., 2007a b). Model without including the percentage of carbon and nitrogen was run as concentration-dependent model gave the same results (Supplementary Table 1). In fact, FM and CGM contained similar proportions of carbon and nitrogen (Table 1).

2.8 Statistical analysis and modelling

Initial and final weight as well as specific growth and survival rates were compared among treatments (fish meal, corn gluten meal and mix meal) using one-way analysis of variance (ANOVA). ANOVA were also used to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of unfed fish among times. Tukey's post hoc tests were used to further explore pairwise differences. The assumption of normality and homoscedasticity of residuals has been tested with Shapiro and Bartlett tests, respectively. For each treatment, Student's t-test compared $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between day 0 and day 36 after checking the normality and homoscedasticity of data using Shapiro and Bartlett tests, respectively. The application of exponential growth model and time-dependent models to the data was completed using iterative non-linear regression. Akaike's information

criterion corrected for small sample size (AICc) scores were calculated to evaluate how models described data. Models with low AICc values are better supported by data than models with high values. Statistical analyses and modelling were done using R software (version 2.15.2).

3. Results

3.1 Growth and survival rates

During the experiment, fish grew exponentially whatever the diet. Growth and survival rates were similar for fry fed with FM and MixM but significantly lower for fish fed with CGM (Figure 1, Table 2). After 36 days of experimentation, fry fed with FM and MixM increased their weight by approximately 23 fold whereas fry fed with CGM slowly grew by approximately 3 fold%. Mortality was very low (below 4%) for fry fed with FM and MixM and high for fry fed with CGM (superior to 95%). Unfed fish did not grow and were dead after 23 days of experimentation (Figure 1).

3.2 Isotopic incorporation rates

From the first feeding, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fry rapidly changed whatever the considered diet and differences measured between day 0 to day 36 were significant for all diets (Figure 2, Tables 3). Carbon and nitrogen incorporation rates of fry fed with CGM and MixM were well estimated using a single-compartment first-order kinetic time-dependent models (Figure 2, Tables 4). For fry fed with FM, only nitrogen incorporation rate was estimated using the model since $\delta^{13}\text{C}$ values of fry at day 0 were already at steady-state with the diet. In fact, $\delta^{13}\text{C}$ value of FM (-21.12‰) was too close to $\delta^{13}\text{C}$ value of fry + TDF at day 0 (-19.90‰). Carbon

incorporation rates were lower for fish fed with CGM than for fish fed with MixM and half-life was 16.1 and 8.7 days, respectively (Table 4). Additionally, 95% of the diet steady-state was predicted to be reached at 11.5 and 21.8 days for fry fed with MixM and CGM respectively. Nitrogen incorporation rates were similar for fry fed with FM and MixM but lower for fish fed with CGM (Table 4). Nitrogen isotopic half-life were 7.1, 8.2 and 11.5 days and 95% of the diet steady-state was predicted to be reached at 11.7, 11.9 and 20.4 days for fry fed with FM, MixM and CGM, respectively. Carbon incorporation rates were similar to nitrogen incorporation rates for fry fed either MixM or CGM. Using the equation 4 proposed by Weidel et al. (2011), carbon and nitrogen half-life were estimated to 15.7 day with a 95% prediction interval ranging from 13.9 to 16.4 day.

The time-dependent model indicated that the contribution of catabolism to carbon and nitrogen isotopic incorporation rates were not different from zero for fry fed with FM and MixM (Table 4). For fry fed with CGM, carbon and nitrogen catabolic tissue replacement rates contributed to 9.3% and 35%, respectively to isotopic incorporation rates (Table 4). Accordingly, carbon and nitrogen C -values estimated using the growth-dependent model were not significantly different from -1 for fry fed with FM and MixM and largely inferior to -1 for fry fed with CGM (Supplementary Table 2, Supplementary Figure 3). AICc values were lower for time-dependent models than growth-dependent models.

3.3 Trophic discrimination factors and effect of fasting

At day 36, fry fed with FM and MixM reached carbon and nitrogen isotopic steady-states with their diet. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fry measured at day 36 were closed to the asymptotic values estimated by the time-dependent model (Tables 4). For fry fed with CGM, $\delta^{13}\text{C}$ value estimated by the time-dependent model at steady-state was ^{13}C -enriched compared to $\delta^{13}\text{C}$ value of fry at

the end of the experimentation (-15.06‰ and -13.38‰, respectively) meaning that fry had not completely reached carbon isotopic equilibrium with their diet. Conversely, $\delta^{15}\text{N}$ values of fry estimated by the time-dependent model at steady-state and measured at day 36 were similar (10.13‰ and 9.94‰, respectively). Carbon and nitrogen trophic discrimination factors (TDF) have been determined in accordance with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fry at steady state with the three different diets. Carbon TDF were similar whatever the origin of the protein sources (around 1.7‰, Table 3 and 4). Conversely, nitrogen TDF were lower for fry fed with FM and MixM compared to fry fed with CGM (1.12‰, 1.62‰ and 5.87‰, respectively, Table 3 and 4).

From day 10, $\delta^{13}\text{C}$ values of fry deprived of food started to significantly increase (Figure 3A, $p \leq 0.01$). $\delta^{13}\text{C}$ values of starved fry slightly increased by 0.63‰ from $-19.90 \pm 0.12\text{‰}$ at day 0 to $-19.27 \pm 0.21\text{‰}$ at day 21. $\delta^{15}\text{N}$ values of starved fry did not show any significant variation over time (Figure 3B, $p = 0.054$) and were $13.39 \pm 0.27\text{‰}$ at day 0 and $12.69 \pm 0.46\text{‰}$ at day 21.

3.4 Estimation of fish meal and corn gluten meal assimilated by fry fed on a blend of meals

MixM was formulated to provide carbon and nitrogen from FM and CGM in ratio of 50:50. The result of bayesian mixing models using carbon and nitrogen TDF estimated during this experiment showed that rainbow trout fry tissues were composed in a ratio of $49 \pm 0.1\%$ of FM and $51 \pm 0.1\%$ of CGM (Supplementary Figure 4A). When using carbon and nitrogen TDF values averaged from literature (1 and 3.4‰ or 1.5 and 2.79‰ for C and N, respectively), mixing model estimated that rainbow trout fry tissues were composed of 31 ± 0.5 or $39 \pm 0.5\%$ of FM and 69 ± 0.5 or $61 \pm 0.5\%$ of CGM (Supplementary Figure 4, B and C).

4. Discussion

4.1 Plant-based diet decreased growth and survival rates of first-feeding rainbow trout fry

The three experimental diet used to feed rainbow trout first-feeding fry were formulated to contain a similar quantity of proteins, lipids and energy (Table 1). The source of proteins varied between the three diets with a plant based diet containing 100% of corn gluten protein (CGM), an animal based diet containing 100% of fish protein (FM) and a mix diet containing 50% of corn gluten protein and 50% of fish protein (MixM). Corn gluten meal was chosen as plant protein source because of its carbon isotopic signature (specific of C4 plants), its low content of anti-nutritional factors and also because it is protein-rich and highly digestible. Diet CGM and MixM were supplemented with lysine and methionine in order to correct for the essential amino acid deficiency known to occur in a 100% plant-based diet (Kaushik et al., 2004; Gatlin et al., 2007). As observed in previous studies, rainbow trout fry fed with the plant-based diet (CGM) exhibited lower growth rate and higher mortality rate than those fed with animal based diet (FM) or 50:50 animal:plant based diet (MixM). Previous studies have shown that 100% plant based diet reduced growth and survival rates of Salmonidae, even if the essential amino acid profile is balanced, due to a decrease of food intake and/or by an increase of protein turnover rate to the detriment of growth (Gomes et al., 1995; Vilhelmsson et al., 2004, Wacyk et al., 2012). Animal protein must represent at least 5% of the diet to maintain good growth and survival of fish (Kaushik et al., 2004) and this is probably why the mix diet performed roughly the same than the animal based diet.

4.2 Effect of protein quality on isotopic incorporation rates

Our results confirmed that isotopic carbon and nitrogen half-life of first feeding rainbow trout were clearly influenced by the quality of the protein source, animal *versus* vegetal. Our estimated values were comprised between 7 and 16 days, close to reported values for fish of similar age. Zero age bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), and yellow perch (*Perca flavescens*) have carbon half-life comprised between 8 and 18 days (Weidel et al., 2011) whereas larval senegalese sole (*Solea senegalensis*) have carbon half-life estimated at 3.5 days (Gamboa-Delgado et al., 2008). Carbon and nitrogen half-life ranged between 6 and 12 days for red drum larvae (*Sciaenops ocellatus*), chub (*Squalius cephalus*) and roach (*Rutilus rutilus*, Herzka & Holt, 2000; Latli et al., 2017). Carbon and nitrogen isotopic incorporation rates were lower for fry fed with CGM than for fry fed with MixM diet. Carbon half-life of fry fed with CGM was twice that of fry fed with MixM (16.1 and 8.7 days, respectively). For fry fed with FM, carbon half-life could not be estimated as $\delta^{13}\text{C}$ value of diet (-21.12‰) was close to $\delta^{13}\text{C}$ value of fry at day 0 (-19.90‰). Broodstock female rainbow trouts were fed with a commercial diet from Le gouessant (Lamballe, France) composed of a mixt of fish and plant based ingredients with a $\delta^{13}\text{C}$ value of -23.61‰. As eggs are rich in lipids and broodstocks were fed with a ^{13}C -depleted meal compared to FM, fry was expected to have a significant lower $\delta^{13}\text{C}$ value than FM. The originality of this work is that fry were fed from their first meal but this implies that diet must be prepared in advance without measuring $\delta^{13}\text{C}$ value of fry. To anticipate $\delta^{13}\text{C}$ value of fry it would have been interesting to determine how $\delta^{13}\text{C}$ value of broodstock diet influence $\delta^{13}\text{C}$ value of eggs and fry until the first meal, but this is beyond the aim of the present study. Then, the amplitude of carbon isotopic shift was not large enough for the model to fit the data ($0.42 \pm 0.18\text{‰}$). Nitrogen half-life was similar for fish fed with FM and MixM and 1.5 times higher than for fish fed with CGM (7.1, 8.2 and 11.5 days, respectively). These results are similar to those observed on amphipods

where animals fed with the animal-based diet had faster carbon isotopic incorporation rates than animals fed with litter (Remy et al., 2017). As shown before in studies on rapidly growing animals, growth rate is the major factor driving isotopic incorporation rates. Isotopic incorporation rate decreases when growth rate decreases (Gamboa-Delgado et al., 2008; Buchheister & Latour, 2010; Mohan et al., 2016). Using the equation 4 proposed by Weidel et al. (2011), carbon and nitrogen half-life were estimated to 15.7 day with a 95% prediction interval ranging from 13.9 to 16.4 day. These values did not completely match experimental values estimated in this study (ranging from 8 to 16 days) but are of the same order of magnitude.

Carbon and nitrogen half-life were similar for fish fed with MixM. However, for fish fed with CGM, carbon half-life was higher than nitrogen half-life. Some studies have shown that carbon and nitrogen incorporation rates are closely linked despite the different biochemical roles and functions of these elements (Herzka & Holt, 2000; Vander Zanden et al., 2015) whereas others have reported higher carbon half-life than nitrogen half-life (Church et al., 2008) or the opposite (Carleton & Martínez del Rio, 2005). Our results indicate that carbon and nitrogen half-life were similar when fish were fed with MixM but not when fed with CGM. This result agrees with observations in juvenile nectarivorous bats (*Glossophaga soricina*, Miron et al., 2006). When the bats were fed with a high quality diet and exhibited a positive growth rate, carbon and nitrogen metabolic pathways were linked (i.e., similar isotopic incorporation rates). Conversely, when bats were fed with a low quality food diet inducing a low growth, carbon and nitrogen metabolic pathways were not linked as isotopic routing differed (i.e., nitrogen isotopic half-life was lower than carbon isotopic half-life). To summarize, carbon and nitrogen display similar isotopic incorporation rates when nutrition is optimum and fry grow rapidly whereas a decoupling occur when nutrition the process is failing and growth slows.

Isotopic carbon and nitrogen catabolic tissue replacement rates were estimated using both a time-dependent models (Hesslein, Hallard & Ramlal, 1993) and a growth-dependant model (Fry & Arnold, 1982). The results of both models demonstrated that the isotopic carbon and nitrogen catabolic tissue replacement rates were driven by the quality of the diet. Carbon and nitrogen catabolic tissue replacement rates, estimated using the time-dependant model, were negative or close to 0 for fry fed with FM or MixM indicating a negligible contribution of catabolism related to the contribution of growth to isotopic incorporation rates. This result was not surprising as specific growth rates were high and catabolic tissue replacement rate probably too low to be detectable by the model. For fry fed with CGM, carbon and nitrogen tissue replacement catabolic rates increased and contributed to 9.3% and 35%, respectively to isotopic incorporation rates. In fact, growth rate of fry fed on CGM was low and catabolic rates became significant. Studies with shrimp have also reported that nitrogen catabolic tissue replacement rate was higher in shrimp fed with plant-based diet (which also elicited lower growth rates) than in shrimp fed with fish meal (Martinez-Rocha et al., 2013). Isotopic carbon catabolic tissue replacement rate, after lipids are extracted, reflected a total tissue turnover rate (e.g. carbohydrates and proteins) and isotopic nitrogen turnover rate reflected primarily protein turnover rate (MacAvoy, Macko & Arneson, 2005). Whereas the reason why 100% plant-based diet negatively impact Salmonidae fish growth rate is still under discussion even when micro-nutrients meet nutritional fish requirements (Wacyk et al., 2012), results of this study indicated that carbon and nitrogen catabolic tissue replacement rates increased in fish fed with 100% plant-based diet. Carbon and nitrogen atoms from plant-based diet are used to replace those already present in fish tissue rather than to add new atoms for growth. In this study, the high mortality and the low growth rates of fish fed with plant-based diet can be explained by an increase of catabolic tissue replacement.

The results of this study indicate that the origin of food (animal *versus* vegetal) must be considered as an indispensable parameter to use appropriate time-scale to detect change in fry diets in field. Salmonid first-feeding fry feed on most available items such as prey organisms and plant material within the size range that is possible to ingest (Skoglund & Barlaup, 2006). As fry are certainly more carnivorous and omnivorous than herbivorous in field, growth rate should be optimum whereas catabolism negligible. Thus, only 12 days should be necessary for fry to be at steady-state with a new diet after a switch. However, if fry only ingest plant material, the time necessary to identify a food sources after a switch must be almost double. Moreover, carbon and nitrogen stable isotope values must be considered separately.

4.3 Trophic discrimination factors and effects of fasting

Carbon trophic discrimination factors (TDF) of fry were unaffected by the quality, animal *versus* vegetal, of the ingredients (around 1.70‰). These values fit within the range of data in others studies in fish (from -0.8 to 3.7‰, Caut, Angulo & Courchamp, 2009). In general, animals tend to be slightly enriched in ^{13}C compared to their diet due to a preferential excretion of ^{12}C . However, there is little consensus on how carbon TDF varies with the quality or quantity of diet. Some studies report that carbon TDF decreases and may even reach negative values when the organism is fed with a low compared to a high quality diet as the organism catabolizes its ^{13}C depleted fatty acid reserves (Webb, Hedges & Simpson, 1998; Oelbermann & Scheu, 2002; Gamboa-Delgado et al., 2011). On the opposite, others report an increase of carbon TDF with the lack or the deficiency of specific dietary nutrients (Martinez del Rio & Wolf, 2005; Gamboa-Delgado & Le Vay, 2009; Busst & Britton, 2016). In fact, nutritional deficiencies induce high atom rearrangements to meet tissue requirement and isotopic fractionation increases during both biosynthesis and catabolism process. Recent studies on fish have showed

that carbon TDF increases when fish were fed with a low quality 100% plant-based diet compared to a high-quality 100% animal-based diet (McMahon et al., 2010; Busst & Britton, 2016). Yet, in their studies, the 100% animal-based diet had a higher lipid level than the 100% plant-based diet. As lipids are strongly depleted in ^{13}C , if they are catabolized as a main source of energy, they would reduce the consumers' $\delta^{13}\text{C}$ value and thus the carbon TDF. In our study, the amount of proteins, lipids and energy between diets were similar which might explain the absence of differences in carbon TDF of first-feeding fry fed with vegetal- and animal-based diets.

Our results showed a clear effect of the diet on the nitrogen TDF which was lower for fry fed with 100% animal-based diet and 50:50 animal:vegetal based diet than for fry fed with 100% plant-based diet (1.12, 1.62 and 5.87‰, respectively). These values are within the range of nitrogen TDF values reported in fish literature (from 0.1 to 5.3‰, reviewed in Caut et al., (2009)) and confirm the variation due to the quality of the diet. In line with our data, it is generally accepted for many species that nitrogen TDF increased under nutritional stress when growth rates are lower (Hobson, Alisauskas & Clark, 1993; Fantle et al., 1999; Adams & Sterner, 2000; Gaye-Siessegger et al., 2004, Lefebvre an Dubois, 2016). Vanderklift & Ponsard, (2003) showed a weak positive relationship between nitrogen TDF and the nutritional quality of the food as measured by the C:N ratio of diet. In our study, despite the supplementation of both missing essential amino acids (lysine and methionine) in the 100% plant-based diet, the increase of nitrogen TDF suggests an imbalance in the dietary amino acid profile. This probably induces the metabolic amino acid handling (biosynthesis and catabolism) to meet fry muscle demand increasing nitrogen TDF.

In field studies, it is generally considered that fasting has no effect on the isotopic values of organisms but salmonids fry may be exposed to severe fasting when environmental conditions do not match with their nutritional requirements (Kennedy, Nislow & Folt, 2008). In our study,

severe fasting induced an increase of lipid-free $\delta^{13}\text{C}$ values of fry whereas $\delta^{15}\text{N}$ values did not show any significant variations over time. Recent reviews including a wide variety of taxa and tissues showed that fasting causes an average increase of 0.5‰ of $\delta^{15}\text{N}$ values without affecting the $\delta^{13}\text{C}$ values (Hertz et al., 2015; Doi, Akamatsu & González, 2017). During fasting events, organisms generally first catabolize their lipid reserves whereas proteins from tissues are only used when the fasting becomes more severe (Doucett et al., 1999; Hatch, 2012). $\delta^{15}\text{N}$ values of an organism thus start to increase when fasting causes protein rather than lipid catabolism. However, the pattern is not always clear and Doucett et al. (1999) showed that only liver had a consistent enrichment in $\delta^{15}\text{N}$ despite protein losses in all tissues during migration and fasting event of the Atlantic salmon (*Salmo salar*). The result of our study is consistent with previous studies showing no effect of fasting on $\delta^{15}\text{N}$ value of metabolically efficient ectothermic vertebrates adapted to long periods of starvation (McCue, 2007; Castillo & Hatch, 2007; Milanovich & Maerz, 2013). As lipids were removed before $\delta^{13}\text{C}$ analysis, the steady increase of $\delta^{13}\text{C}$ values with fry fasting was certainly due to the use of carbohydrate rather than ^{13}C -depleted lipids. In fact, Kieffer & Tufts (1998) have showed that in juvenile rainbow the levels of glycogen in trout muscle was decreased by approximately 50% following 7 days of food deprivation (at 15 °C). Similarly, Gaye-Siessegger et al. (2007) found enriched $\delta^{13}\text{C}$ values in the lipid-free material of fishes after starvation.

4.4 Estimation of fish meal and corn gluten meal assimilated by fry fed on a blend of meals

A two-source and two-isotopes mixing model have been used to convert both isotopic data ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fish) into estimated proportion of fish meal and corn gluten meal assimilated by fry fed on a blend of meals. Proportions of fish meal and corn gluten meal, estimated using the experimentally measured TDF, were very similar to the expected theoretical

values (50:50 fish meal: corn gluten meal) indicating that rainbow trout fry assimilated with the same efficiency fish meal and corn gluten meal in their tissues when fed with MixM. Thus, reduced growth and survival rates of rainbow trout fry fed with plant-based diet cannot be explained by the hypothesis of a low protein efficiency retention (Gomes et al., 1995; Vilhelmsson et al., 2004) but rather by the increase of catabolic tissue replacement rate. Similar results have been reported for penaeid shrimp (*Litopenaeus vannamei*) fed a diet with different proportions of fish meal and soy protein (Gamboa-Delgado & Le Vay, 2009). This contrasts with a previous results on endotherm adult bats (*Carollia perspicillata*) where ingested vegetal diet were used preferentially to fuel metabolism whereas animal diet were used for tissue synthesis (Voigt et al., 2008). Food quantity impacts incorporation rate (and growth rates) and TDF in opposite ways. When growth rate decreases, incorporation rate decreases and TDF increases (Lefebvre & Dubois, 2016). The effect of food quality is quite similar but could imply also isotopic routing which can blur the response in particular for carbon (Kelly & del Rio, 2010). Our results indicate that fry are adapted to consume a large proportion of vegetal sources as long as the quantity of requested protein is not limiting. Growth rates were similar for fry fed with FM and MixM. Moreover, plant dietary sources are directly incorporated into fry tissues as reflected by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fry fed with MixM: isotopic routing was then probably low at least up to a certain proportion of plant-based source in the diet.

Over the last decade, the use of mixing models in ecological studies to estimate the contribution of different food sources to the tissues' content of animals has considerably increased (Phillips et al., 2014). In the vast majority of studies, species and food-specific TDF are not available, forcing researchers to compromise by using average carbon and nitrogen TDF derived from large literature review (1 and 3.4‰, respectively, Deniro & Epstein, 1978; Post, 2002), or specific average values from fish literature review (1.5 and 2.79‰, respectively, (Sweeting et al., 2007a b). Using these TDF to estimate the proportion of the dietary supply of

FM and CGM for fry fed on MixM, both mixing model indicated a proportion of approximately 30% of FM and 70% of CGM. The use of such TDF clearly over-estimated the proportion of vegetal protein in the rainbow trout fry diet. This finding confirms that TDF are the weakest link in the application of stable-isotope mixing models to study ecological questions of diet reconstruction (Gannes, O'Brien & del Rio, 1997; Wolf, Carleton & Martínez del Rio, 2009). We recommend to use different carbon and nitrogen TDF, one for animal-derived and one for plant-derived sources, to improve the accuracy of mixing model and estimate with precision the proportion of preys *versus* plants in the diet of omnivorous fry. Otherwise, the proportion of vegetal items will be overestimated.

ACKNOWLEDGEMENTS

We acknowledge T. Blasco (Laboratoire d'Océanographie Biologique de Villefranche-sur-mer, UPMC/CNRS) for C and N stable isotope analyses and two referees for their helpful comments. This work has been supported by the Animal Physiology and Livestock Systems (PHASE) department of the French National Research Institute of Agriculture (INRA).

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TABLE 1 Composition of the three diets used to feed rainbow trout fry

	Diets		
	Fish Meal (FM)	Corn Gluten Meal (CGM)	Mix Meal (MixM)
Ingredients (%)			
Fish meal	69.5	-	34.8
Corn gluten Meal	-	75.0	37.5
Soy lecithin	2.0	2.0	2.0
Wheat starch	18.5	5.0	11.8
L-lysine	-	1.5	0.8
L-methionine	-	0.5	0.3
Fish oil	6.0	9.0	7.5
CaHPO ₄ , 2H ₂ O (18% P, 22% Ca)	-	3.0	1.5
Mineral PREMIX [†]	2.0	2.0	2.0
Vitamin PREMIX ^{††}	2.0	2.0	2.0
Biochemical composition			
Dry matter (%)	98.82 ± 0.01	99.39 ± 0.02	98.33 ± 0.03
Ash (%)	17.66 ± 0.02	5.44 ± 0.05	10.79 ± 0.13
C (%)	41.04 ± 1.74	46.39 ± 1.37	42.10 ± 2.26
N (%)	9.06 ± 0.22	9.09 ± 0.23	8.64 ± 0.76
Crude protein (%)	50.30 ± 0.03	51.53 ± 0.44	51.94 ± 0.03
Total lipid (%)	11.67 ± 0.01	12.99 ± 0.09	14.91 ± 0.12
Gross energy (Kj DWg ⁻¹)	20.55 ± 0.07	22.55 ± 0.04	24.33 ± 0.01
Isotopic values			
δ ¹³ C (‰)	-21.12 ± 0.21	-15.04 ± 0.34	-17.98 ± 0.28
δ ¹⁵ N (‰)	11.06 ± 0.12	4.07 ± 0.33	7.48 ± 0.25

[†]Mineral premix (g kg⁻¹ of diet): FeSO₄, 7H₂O (21% Fe, 11.5% S) 25 g; CuSO₄, 5H₂O (25.45% Cu, 12.8% S) 3 g; MnSO₄.H₂O (33% Mn, 19% S) 3 g; ZnSO₄.H₂O (36% Zn, 18% S) 4 g; Na₂SeO₃ (46% Se, 27% Na) 0.03 g and α-cellulose 964.93 g (UPAE, INRA, Jouy-en-Josas, France).

^{††}Vitamin premix (IU or mg kg⁻¹ of diet): 60 IU DL-α tocopherol acetate; menadione sodium bisulphate 5 mg; retinyl acetate 15 000 IU; DL-cholecalciferol 3000 IU; thiamin 15 mg; riboflavin 30 mg; pyridoxine 15 mg; B12 0.05 mg; nicotinic acid 175 mg; folic acid 500 mg; inositol 1000 mg; biotin, 2.5 mg; calcium panthotenate 50 mg; choline chloride 2000 mg (UPAE, INRA, Jouy-en-Josas, France).

TABLE 2 Mean (\pm SD, n = 24) initial and final dry weight (mg) as well as mean (\pm SD, n = 4) specific growth rate (K_g , % day⁻¹) and survival rate (%) of fish reared on three different diets.

Diet	Initial weight	Final weight	Specific growth rate (K_g)	Survival rate
Fish Meal	16.36 \pm 0.91 ^a	339.82 \pm 28.92 ^a	8.98 \pm 0.17 ^a	96.91 \pm 1.36 ^a
Corn gluten meal	15.40 \pm 0.68 ^a	52.06 \pm 2.89 ^b	3.89 \pm 0.31 ^b	5.87 \pm 5.31 ^b
Mix Meal	14.26 \pm 0.93 ^a	350.51 \pm 2.89 ^a	9.29 \pm 0.37 ^a	97.22 \pm 1.07 ^a

Letters indicate significant differences among groups (ANOVA, $p \leq 0.01$)

TABLE 3 Measured $\delta^{13}C$ and $\delta^{15}N$ values at day 0 and day 36 ($\delta^{13}C_{t0}$ and $\delta^{13}C_{t36}$, $\delta^{15}N_{t0}$ and $\delta^{15}N_{t36}$, respectively) as well as trophic discrimination factor ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$). Mean values are given with standard deviation (n = 4).

Diet	$\delta^{13}C_{t0}$ (‰)	$\delta^{13}C_{t36}$ (‰)	$\Delta\delta^{13}C$ (‰)	$\delta^{15}N_{t0}$ (‰)	$\delta^{15}N_{t36}$ (‰)	$\Delta\delta^{15}N$ (‰)
Fish Meal		-19.47 \pm 0.07*	1.65 \pm 0.07 ^a		12.24 \pm 0.11*	1.18 \pm 0.11 ^a
Corn gluten meal	-19.90 \pm 0.12	-15.06 \pm 0.26*	0.02 \pm 0.26 ^b	13.39 \pm 0.27	10.13 \pm 1.00*	6.06 \pm 1.01 ^b
Mix Meal		-16.37 \pm 0.21*	1.61 \pm 0.21 ^a		9.31 \pm 0.12*	1.83 \pm 0.12 ^c

$\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ were calculated using $\delta^{13}C_{t36}$ and $\delta^{15}N_{t36}$, respectively. We assumed that fry were at steady-state with their diet at the day 36.

* means a significant difference between $\delta^{13}C$ or $\delta^{15}N$ values at day 0 and day 36 (t-test, $p \leq 0.01$)

Letters indicate significant differences among groups (ANOVA, $p \leq 0.01$)

TABLE 4 Estimated model parameters from equations (1), (2), (3), (5) and (7): $\delta^{13}C$ and $\delta^{15}N$ values of fry at equilibrium with diet ($\delta^{13}C_{t\infty}$ and $\delta^{15}N_{t\infty}$, respectively), carbon and nitrogen incorporation rate (λC and λN , respectively), half-life (T_{50C} and T_{50N} , respectively), growth rate (K_g), catabolic tissue replacement rate (K_cC and K_cN , respectively) and trophic discrimination factor ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$, respectively). Mean values are given with standard deviation. AICc are Akaike's information criterion corrected for small sample size.

Diet	Carbon						
	$\delta^{13}C_{t\infty}$ (‰)	λC (day ⁻¹)	t_{50C} (days)	K_g (day ⁻¹)	K_cC (day ⁻¹)	$\Delta\delta^{13}C$ (‰)	AICc
Fish Meal	n.c.	n.c.	n.c.	0.0898 \pm 0.01	n.c.	n.c.	n.c.
Corn gluten meal	-13.4 \pm 0.5*	0.043 \pm 0.006*	16.1 \pm 2.2*	0.0389 \pm 0.03	0.004 \pm 0.001	1.7 \pm 0.1	66.8
Mix Meal	-16.2 \pm 0.2*	0.080 \pm 0.013*	8.7 \pm 1.4*	0.0927 \pm 0.04	-0.012 \pm 0.002	1.7 \pm 0.1	51.6
Diet	Nitrogen						
	$\delta^{15}N_{t\infty}$ (‰)	λN (day ⁻¹)	t_{50N} (days)	K_g (day ⁻¹)	K_cN (day ⁻¹)	$\Delta\delta^{15}N$ (‰)	AICc
Fish Meal	12.2 \pm 0.1*	0.098 \pm 0.023*	7.1 \pm 3.5*	0.0898 \pm 0.01	0.008 \pm 0.021	1.1 \pm 0.1	36.9
Corn gluten meal	9.9 \pm 0.5*	0.060 \pm 0.015*	11.5 \pm 2.9*	0.0389 \pm 0.03	0.021 \pm 0.004	5.9 \pm 0.3	90.8
Mix Meal	9.1 \pm 0.3*	0.084 \pm 0.012*	8.5 \pm 1.2*	0.0927 \pm 0.04	-0.009 \pm 0.001	1.6 \pm 0.1	95.9

$\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ were determined using $\delta^{13}C_{t\infty}$ and $\delta^{15}N_{t\infty}$, respectively.

n.c. means not calculated since values do not evolve over time (constant)

* means that p value of estimated parameter was ≤ 0.001

Figures

FIGURE 1 Rainbow trout fry growth according to the diet: fish meal (■), corn gluten meal (◆), mix meal (●) and without food supply (▲). Dotted lines indicate the fitted experimental growth model to measured data. Vertical bars indicate standard deviation (n = 24).

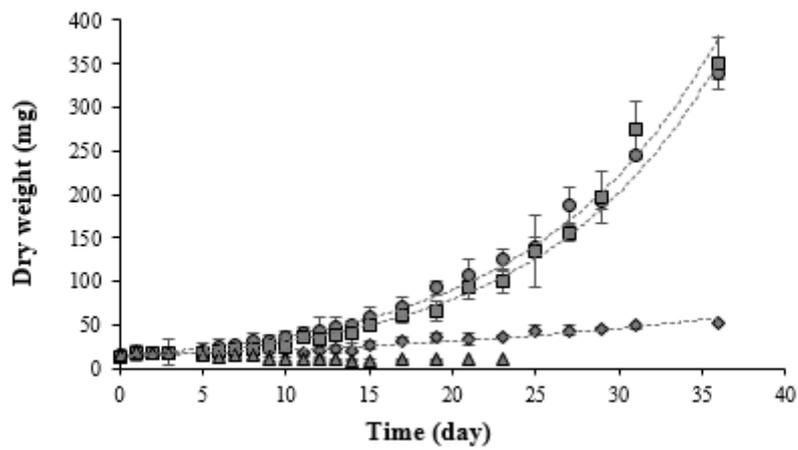


FIGURE 2 Changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of rainbow trout fry fed with fish meal (A-B), corn gluten meal (C-D) and 50:50 fish meal: corn gluten meal (E-F). Straight lines represent $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of diet. Curve lines represent the best single-compartment first-order kinetic time-dependent model fits.

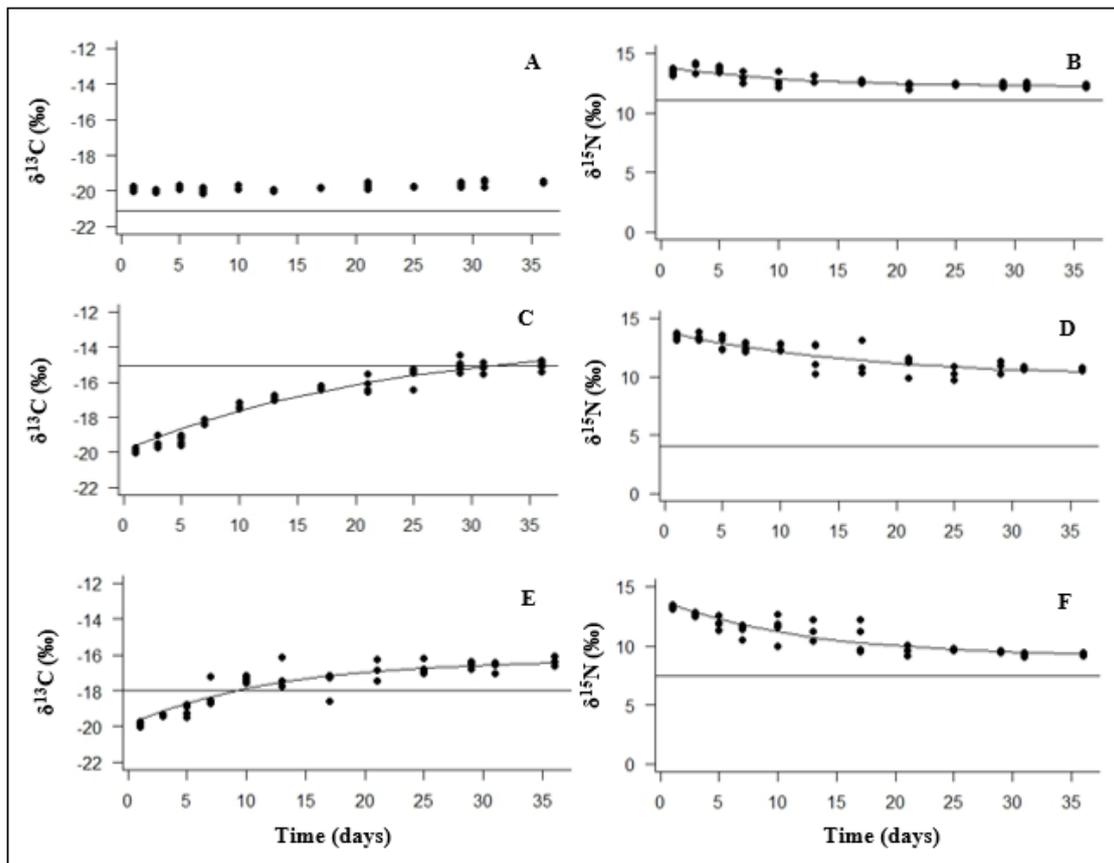
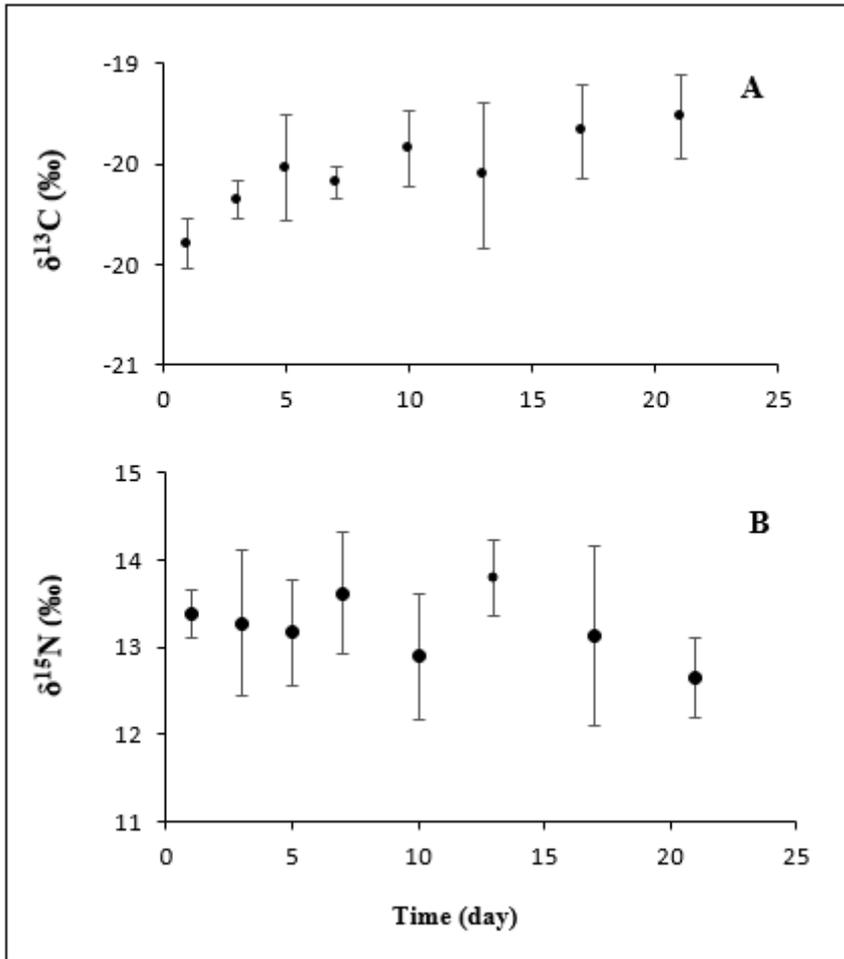
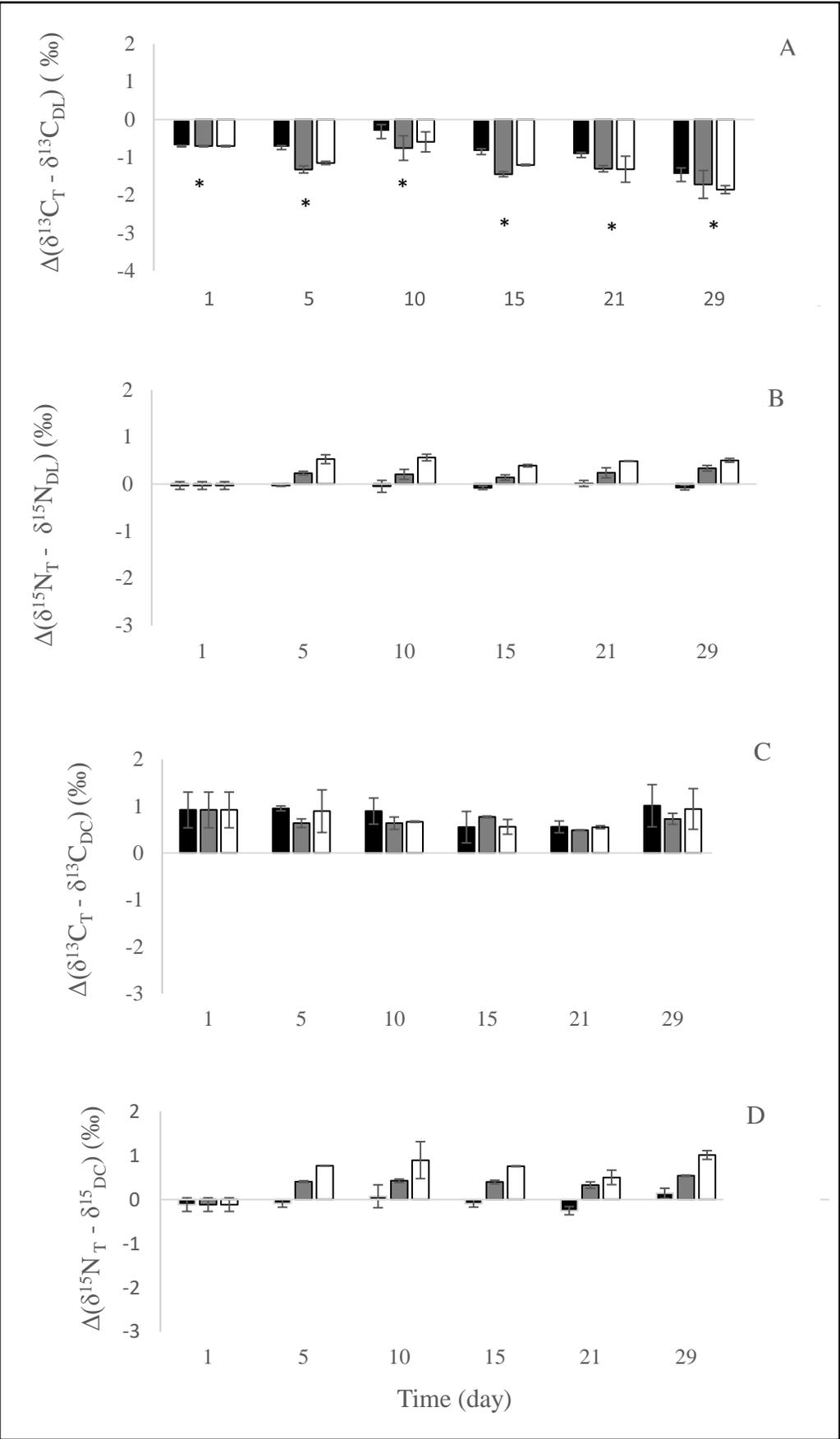


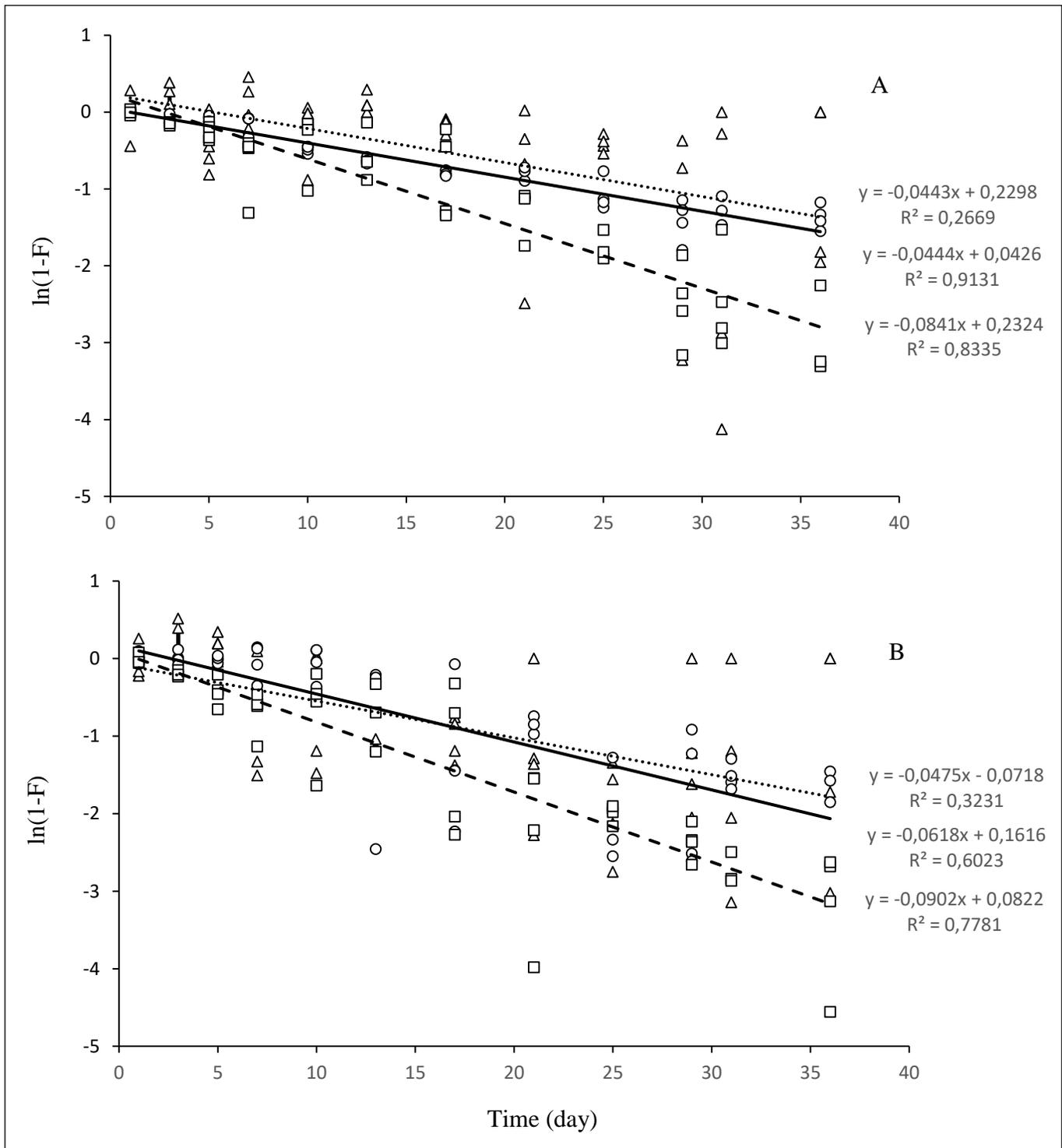
FIGURE 3 Changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of rainbow trout fry unfed (A and B, respectively).

Vertical bars indicate standard deviation (n = 4).

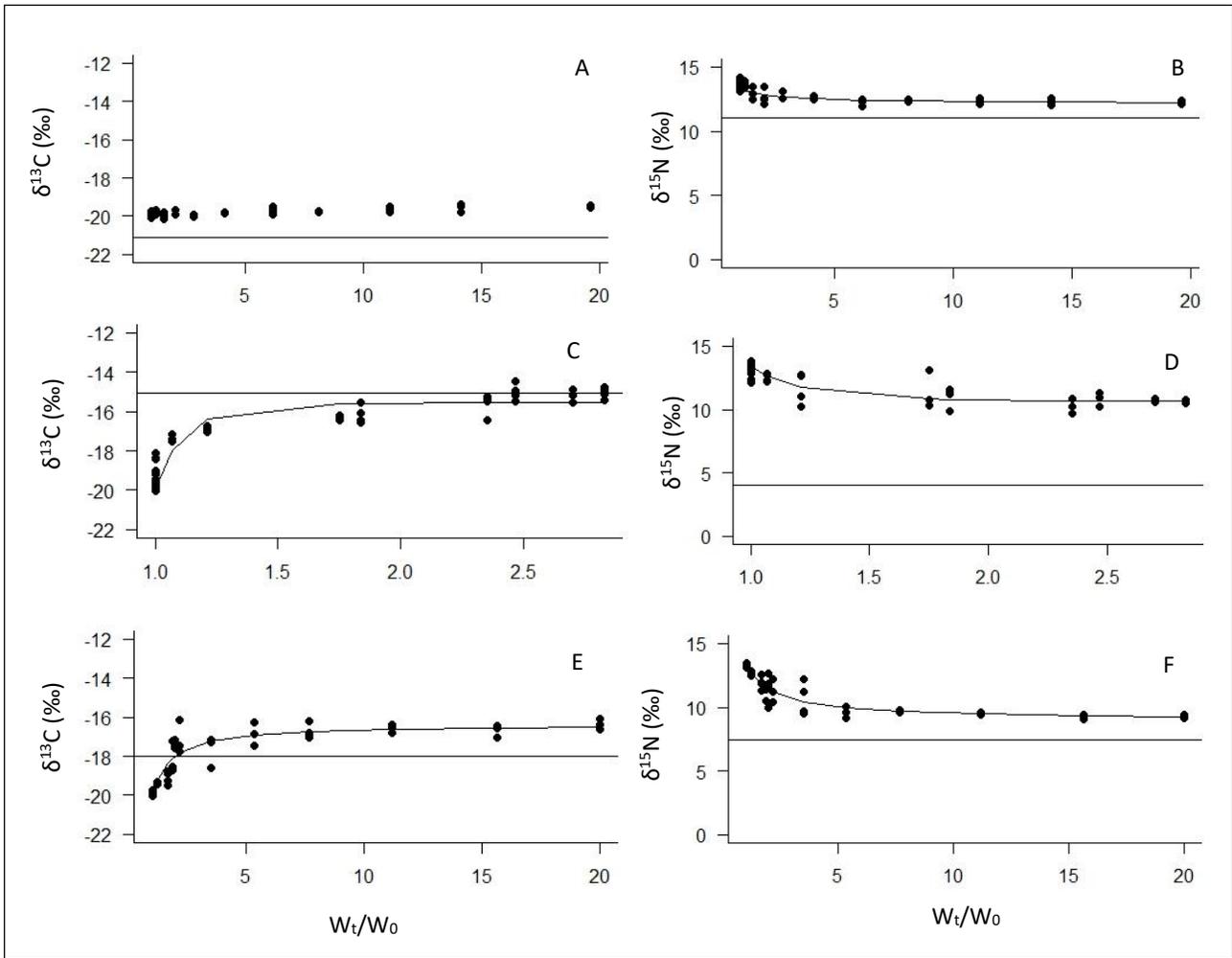




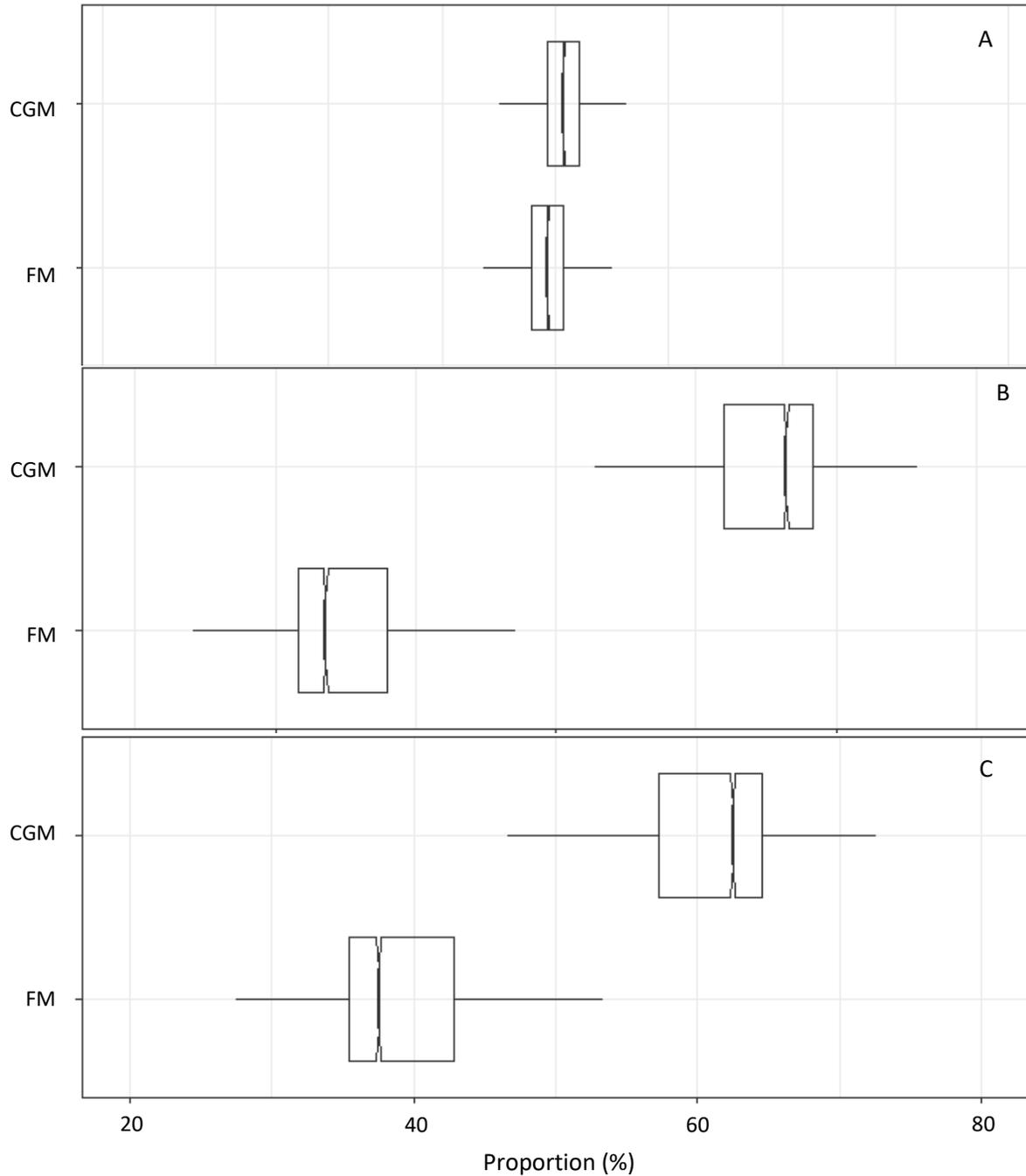
Supplementary Figure 1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ differences (mean \pm standard deviation, $n = 2$) between treated and untreated fry inside each feeding trial (fry fed with fish meal in black, corn gluten meal in grey and mix meal in white). T means fry without traitement, DL means that lipids were removed and DC means acid washing. * means that differences were statistically significant (Wilcoxon-test, $p < 0.001$).



Supplementary Figure 2. Plot of the reaction progress variable $\ln(1 - F)$ against time. A and B have been constructed using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of fry fed with fish meal (Δ with ... lines), corn gluten meal (\square with --- lines) or mix meal (\circ with — lines). Straight lines indicated that a model with a single compartment well describes our data.



Supplementary Figure 3. Changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of rainbow trout fry fed with fish meal (A-B), corn gluten meal (C-D) and 50:50 fish meal: corn gluten meal (E-F). Straight lines represent $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of diet. Curve lines represent the best single-compartment first-order kinetic growth-dependent model fits.



Supplementary Figure 4. Estimated percentage of carbon and nitrogen supplied by fish meal (FM) and corn gluten meal (CGM) for rainbow trout fry fed on a blend of meals. Boxplots are the result of Bayesian mixing model dietary analysis using TDF experimentally estimated (A) or using average TDF from large literature review (B, 1‰ and 3.4‰, respectively), or specific average values from fish literature review (C, 1.5‰ and 2.79‰, respectively) with mean, standard deviation and credible interval ($n = 4$).

Supplementary Table 1. Comparison between a concentration-dependent model and a model that not include the percentage of carbon and nitrogen of fish meal and corn gluten meal. TDF A was those experimentally estimated, TDF B was from large literature review (1‰ and 3.4‰ for C and N, respectively), and TDF C was specific average values from fish literature review (1.5‰ and 2.79‰ for C and N, respectively).

	Proportion of FM (%)		Proportion of CGM (%)	
	Model without concentration	Concentration-dependent model	Model without concentration	Concentration-dependent model
TDF A	49 ± 0.4	49,5 ± 0.46	51 ± 0.4	50.5 ± 0.46
TDF B	31 ± 0.5	31.5 ± 0.62	69 ± 0.5	68.5 ± 0.62
TDF C	39 ± 0.5	40 ± 0.6	61 ± 0.5	60 ± 0.6

Supplementary Table 2. Estimated model parameters from equations (5): $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of fry at equilibrium with diet ($\delta^{13}\text{C}_{t\infty}$ and $\delta^{15}\text{N}_{t\infty}$, respectively), carbon and nitrogen catabolic decay constant (CC and CN, respectively). Mean values of model parameters are given with standard deviation. AICc are Akaike's information criterion corrected for small sample size.

Diet	$\delta^{13}\text{C}_{t\infty}$ (‰)	CC (day ⁻¹)	AICc	$\delta^{15}\text{N}_{t\infty}$ (‰)	CN (day ⁻¹)	AICc
Fish Meal	n.c.	n.c.	n.c.	12.14 ± 0.20*	-0.85 ± 0.33*	34.78
Corn gluten meal	-15.56 ± 0.15*	-8.85 ± 1.77*	109.36	10.64 ± 0.18*	-4.61 ± 1.47*	102.53
Mix Meal	-16.37 ± 0.21*	-1.13 ± 0.18*	78.18	8.93 ± 0.30*	-0.87 ± 0.14*	81.89

n.c. means not calculated since values do not evolve over time (constant)

* means that p value of estimated parameters was ≤ 0.001