Nutrient Assimilation by First-Feeding African Catfish, Clarias gariepinus, Assessed Using Stable Isotope Analysis...

Article *in* Journal of the World Aquaculture Society · April 2013

CITATIONS
0

READS
30

1 author:

Uchechukwu Enyidi
Michael Okpara University of Agriculture, Umudike
12 PUBLICATIONS **5** CITATIONS

SEE PROFILE

JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 44, No. 2
April, 2013

Nutrient Assimilation by First-Feeding African Catfish, *Clarias gariepinus*, Assessed Using Stable Isotope Analysis

Uchechukwu Enyidi[1], Mikko Kiljunen, Roger I. Jones and Juhani Pirhonen

Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FIN-40014 University of Jyväskylä, Finland

Jouni Vielma

Finnish Game and Fisheries Research Institute, Jyväskylä Game and Fisheries Research, Survontie 9, FIN-40500 Jyväskylä, Finland

Abstract

Knowledge of ingredients assimilation and biomass contribution to recipient fish is important in feed formulation. The stable isotopes of ^{13}C and ^{15}N were used to investigate the assimilation and biomass contribution of bambaranut, *Voandzeia subterranea*, meal (BNM), corn, *Zea mays*, meal (CM) and fish meal (FM), in FM substituted diets of first feeding African catfish, *Clarias gariepinus*, during a 30-d experiment. The catfish larvae were stocked at 40 fish/15 L three replicate glass aquaria. Larvae were fed with experimental diets varying FM, BNM, and CM. Proportions of FM : BNM : CM in the experimental diets were: feed 1 (F1) 60:20:20; feed 2 (F2) 40:40:20; feed 3 (F3) 20:60:20; and feed 4 (F4) 20:20:60. Feeds and larval stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ were analyzed. Larval specific growth rates (SGRs) were high, enhancing incorporation of dietary $\delta^{13}C$ and $\delta^{15}N$. The SGR was highest for catfish fed F1 and F2 but significantly lower for those on F3 and F4. Results of $\delta^{13}C$ and $\delta^{15}N$ analyses showed that the biomass contribution from FM was similar to BNM, which were better than CM at equal inclusion levels. The nitrogen contribution per ingredient to larval somatic crude protein was increasing with increases in ingredient inclusion and assimilation. Stable isotope analysis is useful for analyzing assimilation and utilization of nutrients.

The substitution of fish meal and fish oil with plant proteins and oil holds great potential in enhancing the future of aquaculture (FAO 2008). When replacing fish meal (FM) with plant-based diets, it is important to know the assimilation and partitioning of nutrients from the compounded feed by the recipient fish (Beltrán et al. 2009). Feed ingredient utilization and digestibility in aquaculture are traditionally determined by digestibility tests either by using inert chemical markers such as chromium oxide (Glencross et al. 2003; Gaylord and Barrows 2008; Oliveira et al. 2008) or with radioactive ^{14}C (Hovde et al. 2005). Radioactive markers have also been used for measuring nutrient digestibility and assimilation in fish larvae (Conceição et al. 2001; Izquierdo et al. 2001; Morais et al. 2006), but because they pose risks to users their use is subject to strict regulations (Preston et al. 1996; Schlechtriem et al. 2004).

Ratios of naturally occurring stable isotopes of carbon ($^{12}C/^{13}C$) and nitrogen ($^{14}N/^{15}N$), usually expressed as $\delta^{13}C$ and $\delta^{15}N$ values (‰), could provide an alternative approach to measuring nutrient assimilation and retention in fish. For the approach to be successful, feed ingredients used in compounding diets for stable isotope studies should be designed so that they have different isotopic signatures (Post 2002; Beltrán et al. 2009; Redmond 2010). Variable isotope signatures of the ingredients enable analyses of routing and assimilation using stable isotope ratios of carbon and nitrogen (Schlechtriem et al. 2004; Jomori et al. 2008; Beltrán et al. 2009). In animals, stable isotope ratios of carbon and nitrogen reflect their food isotopic ratios with a

[1] Corresponding author.

small fractionation or trophic shift (DeNiro and Epstein 1981). The lighter isotopes (^{12}C and ^{14}N) are more readily used in body metabolism (DeNiro and Epstein 1981; Fry 2006), making the body enriched with heavier isotopes (^{13}C and ^{15}N) (DeNiro and Epstein 1981; Olive et al. 2003; Fry 2006; Dubois et al. 2007).

Most studies using stable isotopes have examined nutrient assimilation from two protein sources and biomass contribution using simple stable isotope linear mixing models (Gamboa-Delgado et al. 2008; Gamboa-Delgado and Le Vay 2009; Beltrán et al. 2009; Martínez-Rocha et al. 2012). Such models assume no variations in input variables such as isotope signatures of dietary sources or consumers (Phillips and Gregg 2001; Phillips 2001), and do not yield reliable results when the food sources have variable C : N ratio and concentration (Robbins et al. 2002; Parnell et al. 2010). Moreover, when the number of diet sources exceeds by more than 1 the number of analyzed isotopes, there can be no definite solution to the linear mixing model (Phillips and Gregg 2003; Parnell et al. 2010). Recently introduced Bayesian-based mixing models (Moore and Semmens 2008; Parnell et al. 2010) can handle multiple source contributions by generating probability ranges for the contribution of different diet sources.

Some previous studies have used $\delta^{13}C$ and $\delta^{15}N$ analyses to examine contributions of ingredients in FM-substituted diets for shellfish (Gamboa-Delgado and Le Vay 2009; Redmond et al. 2010) or to trace dietary protein source in fin fishes (Beltrán et al. 2009) or replacement of sulphur, carbon, and nitrogen due to diet change trace by $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotopes (Hesslein et al. 1993). However, we had noted that such methods have not been applied in multiprotein source nutrient partitioning and contribution to biomass of cultured fin fishes, nor have Bayesian mixing models been used for analyzing nutrient partitioning and assimilation from multiple protein sources. This research was designed to examine larval African catfish nutrient assimilation from compounded dry diets and biomass contribution of ingredients using stable isotopes. Three protein sources with different $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures were selected for this experiment. This enabled their contribution to fish biomass and their nutrient partitioning and nutrient routing to be estimated more accurately from each component. The three ingredients selected were bambaranut, *Voandzeia subterranea*, meal (BNM), corn, *Zea mays*, meal (CM), and FM. The feed ingredient isotope signatures were traced to the fish biomass that made it is possible to quantify the amount of nutrient assimilated per ingredient, while differences in the $\delta^{15}N$ and $\delta^{13}C$ of the fish and feed revealed the catfish fractionation of the diets and showed their contribution to somatic crude protein.

Materials and Methods

Experimental Fish

African catfish larvae were stocked in three replicate 15-L flow-through glass aquaria per diet at 40 larvae/aquarium for all treatment diets. The larvae in tanks fed with FM, BNM, or CM were stocked at 70 larvae/aquarium, because larvae were taken every 5 d from each of them during sampling as will be described below. The survival rate was not analyzed for these fishes. Water flow rate was adjusted to 400 mL/min, light intensity at the tank surface was approximately 15 lx (HD 9221 lux meter, Delta OHM, Padua, Italy), and photoperiod was D16 : L8.

Diet Formulation and Feeding

Experimental diets were produced by substituting FM with BNM and CM at four different levels. There was no vitamin or mineral premix added to the diets as the larval catfish growth and assimilation of the ingredients were examined in their raw form. Experimental diets were labeled as feed 1 (F1) to feed 4 (F4). The % inclusion levels of FM : BNM : CM ranged from 20 to 60%. In addition, three more diets (FM, BNM, and CM) were produced based entirely on the individual ingredients (Table 1).

Appropriate levels of the ingredients were measured and 0.2 L of water was added to the dough and mixed with an electric mixer.

TABLE 1. *Composition and proximate values of diets used in feeding first-feeding African catfish larvae for 30 d.*[a]

Ingredients	F1	F2	F3	F4	FM	BNM	CM
Bambaranut meal	20	40	60	20		100	
Corn meal	20	20	20	60			100
Fish meal	60	40	20	20	100		
Total (%)	100	100	100	100	100	100	100
(%) Proximate composition							
Moisture	7.13	6.22	5.61	5.07	6.70	5.50	6.00
Crude protein	47.80	38.08	28.20	23.00	65.50	21.00	8.00
Lipids	9.50	7.89	6.90	4.30	12.00	8.00	1.98
Ash	6.68	8.43	8.45	8.34	5.89	0.23	1.00

[a]Ingredients levels for compounding feed (F1–F4) are in percentages (%).

There was no fish oil added to mixture. Mixed ingredients were preconditioned at 100 C for approximately 20 min. Preconditioned dough was pelleted using a kitchen meat mincer. Pelleted feeds were oven dried at 70 C for 18 h and dried pellets were ground to dust and stored at −20 C. After formulation, samples were taken of each feed patch for proximate and isotope analyses. The catfish were fed by hand four times per day *ad libitum* for 30 d with the experimental diets. Precautions were taken to avoid over feeding of the larvae and escape of food particles with outflow water.

Sampling

At the beginning of the experiment, five larvae were placed individually into separate glass vials and stored at −80 C until analyses. Thereafter every 5 d, five fish larvae (or 4 on the last sampling day) were taken from each of the aquaria where larvae were fed with single ingredient diets (BNM, CM, and FM). The larvae were weighed and individually placed in a glass vial and stored under −80 C till analyzed. Three replicate samples were later analyzed as described below. The fish were always starved for 18 h before collection to ensure evacuation of the feed and fecal matter from their guts. Larval African catfish can evacuate the gut in less than 11 h, especially with continuous feeding (culture temperature 28 C) (García-Ortega et al. 2010). The following parameters were calculated for each aquarium: Specific growth rate (SGR, %/day) of the larvae was calculated as 100 $(\text{Ln } W_2 - \text{Ln } W_1)/t$, where W_1 and W_2 were average weights in grams at the start and the end of the experiment and t was the length of the experiment in days (i.e., 30).

$$\text{Survival} = 100 \times \text{Final number of fish} / \text{Initial number of fish}$$

$$\text{Average weight gain (AWG)} = \text{Initial average weight (g)} - \text{final average weight (g)}$$

Feed samples were dried at 70 C in an oven to a constant weight and moisture content was measured as the difference between wet and dry samples. The crude protein value of the diets were derived from the formula N × 6.25, where 6.25 is the conversion factor of total nitrogen to protein (this assumes all nitrogen were coming from fish sample analyzed) (Levey et al. 2000). The nitrogen value (N) was obtained during the isotope analyses. Lipid analysis of the samples was done by chloroform–methanol extraction at a ratio of 2:1, a modification of the method described by Kainz et al. (2004).

Stable Isotope Analyses

Feed samples and the catfish larvae were freeze-dried and ground to powder, and a sample of 0.4–0.6 mg was precisely weighed into tin cups (D4057 Elemental Microanalysis, Devon, UK). Three replicate samples were analyzed at the stable isotope laboratory of the University of Jyväskylä using a Carlo Erba Flash EA1112 elemental analyzer coupled to

a Thermo Finnigan DELTAplusAdvantage continuous flow stable isotope-ratio mass spectrometer.

The stable isotope ratio output results are expressed in standard delta (δ) notation as ppt (‰) relative to the international standard of Vienna Pee Dee belemnite for carbon and atmospheric N_2 for nitrogen. Two replicate internal laboratory standards (pike muscle) were placed after every 10 samples. The analysis also yielded %C and %N values and hence also C : N ratios for samples.

The observed nitrogen contribution from FM, BNM, and CM to the somatic crude protein content of the fish was estimated by first determining the crude protein of fish. The crude protein values per mean assimilation of the specific ingredient (from SIAR results) estimates the nitrogen contribution. The sum total of the ingredients contribution to the crude protein equals the fish crude protein content. This was calculated as follows:

$$\text{Somatic crude protein} = \%\text{N} \times 6.25$$

where %N is from the isotope analyses. Observed nitrogen contribution to crude protein (%) $\text{N}_{(\text{FM, BNM,CM})}$ was calculated as, for example, for FM,

$$\begin{aligned}(\%)\,\text{N}_{(\text{FM})} &= (N\,(\%) \times 6.25) \\ &\times \left(X_{\text{ass(FM)}} \times \%\text{nitrogen of (FM)}\right) \\ &/ \left(X_{\text{ass(FM)}} \times \%\text{nitrogen of (FM)}\right) \\ &+ X_{\text{ass(BNM)}} \times \%\text{nitrogen of (BNM)} \\ &+ X_{\text{ass(CM)}} \times \%\text{nitrogen of (CM)}\end{aligned}$$

where FM, BNM, and CM are the ingredients used for the feeds.

$$\begin{aligned}(\%)\,\text{N}_{(\text{FM,BNM,CM})} &= \%\text{Nitrogen from either FM,} \\ &\text{BNM, or CM in the diet (F1} - \text{F4)}\end{aligned}$$

$$N\,(\%) = \%\text{Nitrogen of fish}$$

$$X_{\text{ass}} = \text{Mean assimilation of ingredients from SIAR model.}$$

The multiplier 6.25 is a constant for estimating crude proteins from determined sample nitrogen value. The whole N is assumed to come from fish sample analyzed (Levey et al. 2000).

Observed nitrogen contribution to fish biomass from feed was calculated by multiplying the mean assimilation value of the ingredient from SIAR by the %N of diet ingredient and dividing this by sum total of same calculation for FM, BNM, and CM as follows:

$$\begin{aligned}\text{N}_{(\text{FM,BNM,CM})} &= (X_{\text{ass FM}} \times \text{N\%of diet}) \\ &/ (X_{\text{ass FM}} \times \text{N\% of diet}) \\ &+ X_{\text{ass BNM}} \times \text{N\% of diet} \\ &+ X_{\text{ass CM}} \times \text{N\%}g\text{of diet.}\end{aligned}$$

where FM, BNM, and CM are ingredients of the diets

$$X_{\text{ass(FM,BNM,CM)}} = \text{Mean assilimation of ingredient from SIAR model.}$$

The predicted nitrogen contribution is similar to the above but the multiplier $X_{\text{ass (FM, BNM,CM)}}$ (i.e., the assimilation of either FM, BNM, or CM) was changed to $X_{\text{D (FM, BNM, CM),}}$ which was the inclusion level of the ingredient (either FM, BNM or CM) in the diets F1 to F4. The values were calculated as follows:

$$\begin{aligned}\text{N}_{(\text{FM;BNM;CM})} &= \left(X_{\text{D(FM)}} \times \text{N\% of diet}\right) / \\ &\left(X_{\text{D(FM)}} \times \text{N\% of diet} + X_{\text{D(BNM)}}\right. \\ &\left.\times \text{N\% of diet} + X_{\text{D(CM)}} \times \text{N\% of diet}\right)\end{aligned}$$

where D stands for feed (diet), $X_{\text{D(FM)}}$ represents the inclusion level of the ingredient (FM, BNM, CM) in diet.

Statistical Analyses

The possible statistical differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the feeds and the larvae,

as well as differences in growth and survival were analyzed using ANOVA, and in case of significant differences LSD test was used for *post hoc* comparisons. Linear regression analysis was used in analyzing nitrogen contributions and relationship between C : N and SGR, using version 18 PASW statistics. Tank average value was used as the observational unit, and $P < 0.05$ was used as the level of statistical significance.

SIAR Model

The assimilation of nutrients by the catfish was analyzed with the model Stable Isotope Analyses in R (SIAR, Version 4). SIAR is freely downloadable software at the Comprehensive R Archive Network site (CRAN) – http://cran.r-project.org/ (Parnell et al. 2010). R version 15.0 was used.

SIAR is sensitive to isotope fractionation and incorporates the isotopic ratio and concentration of nitrogen and carbon (Parnell et al. 2010; Layman et al. 2012). Fractionation values of diets was initially planned to be from larvae fed single diet (FM, BNM, or CM). However, with some of the single diets the fish isotopic signature did not achieve an asymptotic value with diet (indicating equilibration) by the end of the experiment. Therefore larval fractionation ($\Delta^{13}C‰$ and $\Delta^{15}N‰$) was calculated from the averages of the values from all mixed diet experiments (F1–F4) as $\Delta^{13}C/\Delta^{15}N = \delta K_{\text{consumer}} - \delta K_{\text{diet}}$, where $K = {}^{13}C$ or ${}^{15}N$. The estimated fractionation values were ($\Delta^{13}C \pm SD = -0.18 \pm 0.98$, $\Delta^{15}N \pm SD = 3.3 \pm 0.61$), and these are very close to typical values reported in the literature.

Results

The first-feeding African catfish readily accepted the treatment diets. The mean SGR of the catfish fed F1 was significantly ($P < 0.05$) higher than those fed with F2 as well as those on F3 and F4. However SGR of F2 fed catfish was significantly ($P < 0.05$) higher than those fed F3 and F4 (Table 2). The SGR was negatively related to the C : N ratio of the mixed feeds F1–F4 (Fig. 1). At the end of the experiment, African catfish larvae fed with F1 were significantly larger than those fed with F2, F3, and F4. The fish fed F4 were significantly smaller than those in any other group (Table 2). Average weight gain (AWG) of the larvae followed a similar pattern to SGR (Table 2). However, survival of the larvae was different from growth pattern. The larvae fed with F3 and F4 had higher survival than those fed with F1. Larval survival was not significantly different between larvae fed F3 and F2 (Table 2). The catfish fed single diets (FM, BNM, and CM) did not grow as fast as those fed mixed diets and they were significantly different from each other (Table 2).

The graphical analyses of single diet fractionation (Fig. 2) showed that the catfish $\delta^{15}N$ and $\delta^{13}C$ equilibrated with the diets before the end of the experiment (Day 30) with the exception of $\delta^{15}N$ of BNM and $\delta^{15}N$ of CM. The isotope

TABLE 2. *Initial weight, final weight, specific growth rate (SGR), average weight gain (AWG), and survival of larval African catfish fed for 30 d with diets F1 to F4 varying in fish meal (FM), bambaranut meal (BNM), and corn meal (CM) content.*[1,2]

Feed	Initial weight (g)	Final weight (g)	SGR (%)	AWG (g)	Survival (%)
F1	0.013 ± 0.0^{ns}	0.168 ± 0.02^{a}	8.520 ± 0.46^{a}	0.155 ± 0.02^{a}	36.66 ± 20.05^{b}
F2	0.012 ± 0.0^{ns}	0.114 ± 0.01^{ab}	7.440 ± 0.39^{b}	0.102 ± 0.01^{b}	49.16 ± 12.58^{ab}
F3	0.012 ± 0.0^{ns}	0.086 ± 0.01^{b}	6.590 ± 0.57^{c}	0.075 ± 0.01^{c}	60.00 ± 8.66^{a}
F4	0.014 ± 0.0^{ns}	0.071 ± 0.02^{c}	5.340 ± 0.36^{d}	0.057 ± 0.02^{d}	58.33 ± 18.05^{a}
FM	0.026 ± 0.0^{ns}	0.085 ± 0.04^{A}	3.920 ± 0.02^{A}	0.059 ± 0.01^{A}	n/a
BNM	0.013 ± 0.0^{ns}	0.024 ± 0.02^{B}	2.010 ± 0.01^{B}	0.011 ± 0.03^{B}	n/a
CM	0.012 ± 0.0^{ns}	0.016 ± 0.01^{C}	0.820 ± 0.03^{C}	0.003 ± 0.01^{C}	n/a

[1]Values not followed by the same superscript are significantly different ($P < 0.05$).

[2]The larvae fed with single diets (FM, BNM, and CM) were weighed individually and analyzed separately from the fish fed with mixed diets.

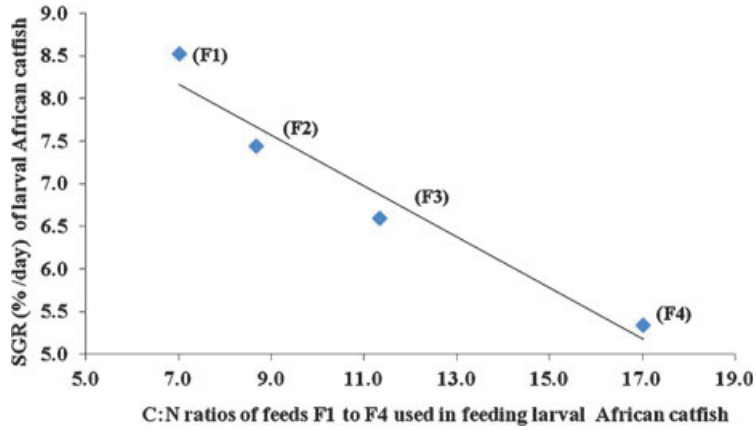

FIGURE 1. *Relationship between specific growth rate of African catfish larvae and the carbon: nitrogen (C : N) ratio of the experimental diets. (*$y = -0.30x + 10.26$, $R^2 = 0.95$, $P = 0.03$*).*

fractionation values obtained from calculations using averages of all mixed diets were very close to the values for catfish fed FM that equilibrated in the single diet experiments ($\Delta^{13}C$ -1.10 ± 0.55 and $\Delta^{15}N$ 3.0 ± 0.06) (Fig. 2).

The three feed ingredient sources (BNM, CM, and FM) had divergent isotope signatures (Table 3; Fig. 3). CM had typical $\delta^{13}C$ values for a C4 plant source ($-10‰$ to $-14‰$) whereas BNM values reflected a C3 plant source ($-22‰$ to $-30‰$) (Fig. 3). Larval catfish isotope signatures resembled that of their respective diet plus some fractionation (Fig. 3). Consequently the isotope signatures of the larvae fed with F1 orientated toward FM while those on F3 were more toward BNM and on F4 more toward CM (Fig. 3). The C : N ratios of the feeds showed an increase from F1

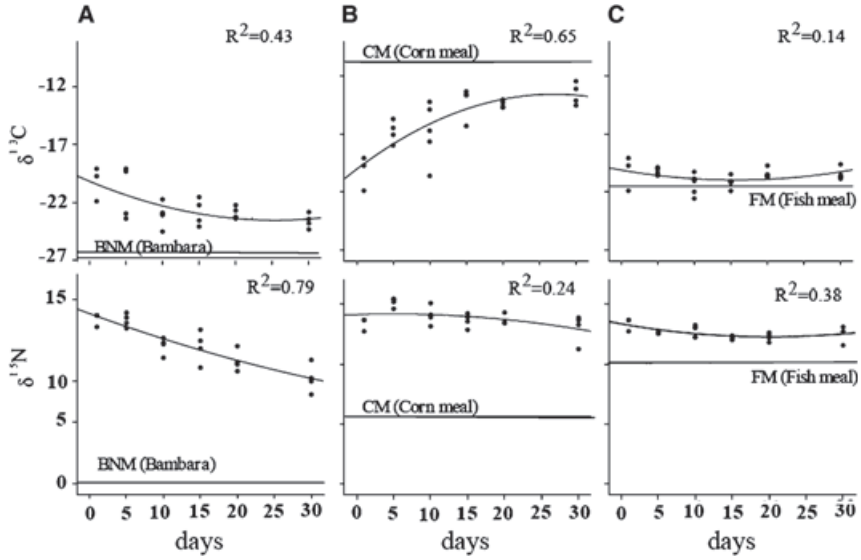

FIGURE 2. *Stable isotope signatures of five replicate African catfish larvae fed with single diets of fish meal (FM), bambaranut meal (BNM), or corn meal (CM) for 30 d. Horizontal lines represent isotope values of feeds: (A)* $\delta^{13}C$ *BNM* $R^2 = 0.43$, $\delta^{15}N$ *BNM* $R^2 = 0.79$, *(B)* $\delta^{13}C$ *CM* $R^2 = 0.65$, $\delta^{15}N$ *CM* $R^2 = 0.24$, *(C)* $\delta^{13}C$ *FM* $R^2 = 0.14$, $\delta^{15}N$ *FM* $R^2 = 0.38$.

TABLE 3. *$\delta^{13}C$(‰) and $\delta^{15}N$ (‰) values, percentage carbon (%C), percentage nitrogen (%N) and carbon : nitrogen concentration ratios (C : N) of pelleted diets (F1–F4) with varying proportions of fish meal (FM), bambaranut meal (BNM), and corn meal (CM) and the single diets FM, BNM, and CM used in feeding larval African catfish for 30 d.*[1]

Feed	δ^{13}C(‰)	δ^{15}N(‰)	%C	%N	C : N
F1	−20.72	9.01	48.08	6.86	7.01
F2	−21.50	7.36	46.08	5.30	8.68
F3	−22.37	4.93	45.98	4.06	11.33
F4	−17.07	7.48	44.60	2.62	17.02
FM	−21.50	10.14	49.32	10.48	4.70
BNM	−26.78	0.08	42.94	3.10	13.82
CM	−10.79	5.62	43.10	1.42	30.20

[1] See Table 1 for details of feed compositions.

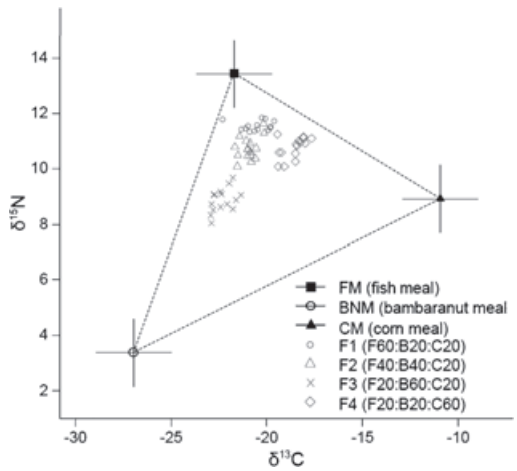

FIGURE 3. *Isotope signature bi-plot for fish meal (FM), bambaranut meal (BNM), and corn meal (CM), and for larval African catfish at the end of a 30-d feeding experiment fed four experimental diets (F1 to F4) varying in FM, BNM, and CM inclusion levels. See Table 1 for details of feed composition. F1 to F4 represent feed 1 to feed 4 and the inclusion percentage are inset per feed type. Note that isotope values for feed ingredient have been corrected for trophic fractionation ($\Delta^{13}C \pm SD = -0.18 \pm 0.98$, $\Delta^{15}N \pm SD = 3.3 \pm 0.61$).*

to F4 and this was also reflected in the larvae (Table 4). The catfish showed an increase in percentage carbon content (%C) from feed F1 to feed F4 (Table 4).

According to the outputs of the SIAR model, the biomass of catfish fed with F1 constituted 43.4% nutrient assimilated from FM and 30.2% assimilated from BNM. Although CM was included at same level as BNM, its contribution to biomass was lower at 26.2% (Fig. 4A). The ingredients' somatic nitrogen (%) contribution to the crude protein content of the F1 catfish were: for FM, 42.32%; for BNM, 8.71%; and for CM, 3.46%, respectively (Table 5). Catfish larvae fed with F1 had somatic crude protein of 54.5% and %N of 8.7 (Table 4). The expected nitrogen contribution from the assimilated nutrients and predicted to the catfish biomass were positively correlated $P = 0.001$ (Fig. 5).

The biomass of larvae fed F2 had a similar assimilated nutrient contribution from FM and BNM (Fig. 4B). While catfish assimilated 34.2 % nutrients from FM, from BNM was 39.1%, and CM 26.6% (Fig. 4B). The catfish fed with F2 had somatic crude protein of 55.8% with FM contributing 38.56% while BNM contributed 13.14% and CM 4.10% (Table 5). We observed an increased nitrogen contribution from BNM to the catfish in F2 compared to F1. However FM still contributed more N from F2 even though it was included same amount with BNM (Table 5).

The catfish fed with 60% BNM diet (F3) had high assimilated BNM nutrient contribution to fish biomass (57.4%) while from FM was 20.4% and CM 22.2% respectively (Fig. 4C). The catfish fed with F3 had 54.6% somatic crude protein content of which BNM N contribution was (22.94%), while FM was (27.60%) and CM (4.08%) (Table 5).

Assimilated nutrients from CM constituted 44% of the biomass of larvae fed F4. The biomass contribution from FM and BNM were 27.5 and 28.6% respectively (Fig. 4D). Similarly the somatic (%) nitrogen (N) contribution from CM was 7.72%, while BNM and FM were 10.92 and 35.39% respectively (Table 5). The catfish fed with F4 had 54.0% somatic crude protein and %N of 8.6 ± 0.9 (Table 4).

The nutrient assimilation and biomass contribution were proportional and similar to the nitrogen contribution of the ingredients (Fig. 5). We observed that increase in the assimilated nutrients is followed by an increase

TABLE 4. *The $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) values, %C and %N and C : N ratios of larval African catfish fed diets (F1–F4) varying proportions of fish meal (FM), bambaranut meal (BNM), and CM and single diets FM, BNM, and CM for 30 d.*[1]

Feed	$\delta^{13}C$(‰)	$\delta^{15}N$(‰)	%C	%N	C : N
F1	-20.53 ± 0.72^{a}	11.56 ± 0.74^{a}	43.66 ± 2.21^{c}	8.72 ± 1.31^{a}	5.16 ± 0.60^{a}
F2	-20.99 ± 0.41^{ab}	10.67 ± 0.34^{a}	45.82 ± 1.32^{b}	8.92 ± 0.74^{a}	5.17 ± 0.32^{a}
F3	-22.32 ± 0.52^{b}	8.86 ± 0.43^{b}	47.96 ± 1.64^{ab}	8.74 ± 0.79^{a}	5.53 ± 0.16^{b}
F4	$-18.59 \pm 0.57a$	10.76 ± 0.38^{a}	49.54 ± 2.29^{a}	8.64 ± 0.97^{a}	5.80 ± 0.06^{c}
FM	-20.72 ± 0.16^{B}	12.47 ± 0.58^{B}	43.36 ± 5.86^{A}	9.01 ± 1.66^{A}	4.92 ± 1.40^{A}
BNM	-22.70 ± 1.15^{C}	10.82 ± 0.62^{C}	42.94 ± 6.20^{B}	8.72 ± 2.02^{B}	5.13 ± 0.15^{B}
CM	-15.24 ± 0.38^{A}	13.90 ± 0.96^{A}	43.10 ± 2.63^{A}	7.55 ± 1.02^{C}	5.98 ± 0.87^{C}

[1]See Table 1 for details of feed compositions. Values are averages of three replicates ± SD.

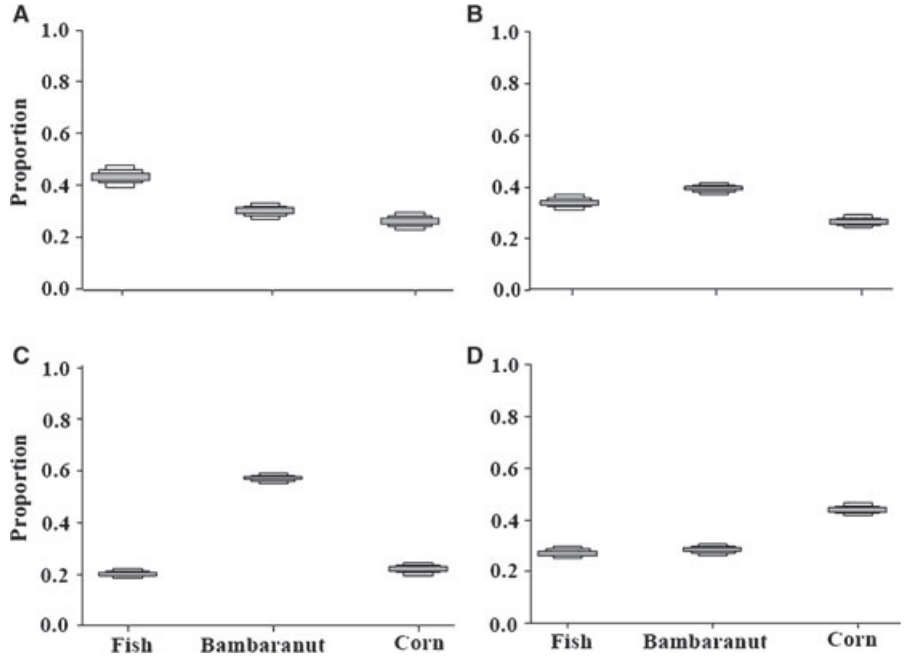

FIGURE 4. *SIAR isotope mixing model results for larval African catfish proportional nutrient assimilation from fish meal (FM), bambaranut meal (BNM), and corn meal (CM) in four different feed mixtures: (A) F1 (FM60 : BNM20 : CM20); (B) F2 (FM40 : BNM40 : CM20); (C) F3 (FM20 : BNM60 : CM20); and (D) F4 (FM20 : BNM20 : CM60). See Table 1 for details of the feeds F1 to F4.*

in N contribution. Similarly, increasing ingredients inclusion amount increases the amount assimilated and contribution to fish biomass (Table 5; Fig. 5).

Discussion

The isotopic changes in a consumer fed a new diet can arise from turnover (i.e., metabolic breakdown of old tissues resulting from previous diets and replacement with fresh tissues from the new diet) or from fast growth rate (i.e., biomass accretions as a result of intake from the new diet). Nongrowing organisms or very slow growers will reflect metabolic breakdown but fast growing fish incorporate new isotopes through their growth rate (Hesslein et al. 1993; Herzka and Holt 2000). The benefits of fast growth to dietary isotope incorporation had been previously noted (Fry and Arnold 1982; Herzka and Holt 2000; Sakano et al. 2005; Redmond et al. 2010).

TABLE 5. *Nitrogen contribution from BNM, CM, and FM to the somatic crude protein of larval African catfish fed diet mixes F1–F4.*[1]

	F1 (%)	F2 (%)	F3 (%)	F4 (%)
BNM	8.71	13.14	22.94	10.92
CM	3.46	4.10	4.08	7.72
FM	42.32	38.56	27.60	35.39
Total	54.49	55.79	54.63	54.02

[1]See Table 1 for diet compositions.

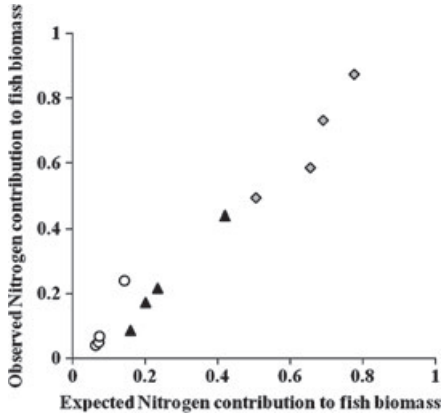

FIGURE 5. *Expected and observed nitrogen contribution from the three major feed ingredients, fish meal (FM), bambaranut meal (BNM), and corn meal (CM), to fish biomass* $R^2 = 0.89$, *MAE* $= 3.001$. *The open circle represents contributions from CM, the dark triangle represents contribution from BNM, and the filled diamond represents contributions from FM.*

We utilized first-feeding catfish that had not been feeding on any other diets after yolk sac absorption and therefore only the experimental diets were reflecting the possible differences in growth and isotopic signatures of the fish. The SGR was clearly high enough during our experiments for somatic incorporation of dietary $\delta^{13}C$ and $\delta^{15}N$ to determine larval isotope signatures. The growth rates of the larvae fed single ingredient diets also enabled incorporation of $\delta^{13}C$ and $\delta^{15}N$ dietary isotopic signatures and attainment of equilibrium with the diets as at Day 30, except $\delta^{15}N$ of BNM and CM. Somatic growth rate was also responsible for the incorporation of $\delta^{15}N$ in posthatch age-0 smallmouth bass, *Micropterus dolomieu* (Vander Zanden et al. 1998), in brown shrimp, *Penaeus aztecus* (Fry and Arnold 1982) and in red drum, *Sciaenops ocellatus* (Herzka and Holt 2000). Conversely, in *Pterygoplichthys disjuncitivitus* (a siluriformis catfish related to African catfish), fed wood detritus, SGR was negligible (0.0017 %/d) and incorporation of $\delta^{13}C$ and $\delta^{15}N$ occurred rather by tissue turnover (German and Miles 2010).

Our results are representative of true larval isotopic assimilation of the diets because we used whole larvae in the analyses. This reduces differential $\delta^{13}C$ and $\delta^{15}N$ isotopic routing effects and incorporation into different tissues. $\delta^{13}C$ and $\delta^{15}N$ isotopic values may vary between tissues, due to differences in tissue growth rate, turnover, or type of tissue. Fish dietary isotope incorporation into tissues can also vary due to different tissue metabolic activities, for example in digestive and mantle tissues (Redmond et al. 2010), blood and fin tissues (German and Miles 2010), muscle tissues (Maruyama et al. 2001), but also in organs such as ctenidia and viscera of Sydney rock oyster, *Saccostrea glomerata* (Piola et al. 2006).

Larvae fed single plant diets, especially CM and BNM, showed elevated fractionation of C and N ($\Delta^{13}C$, $\Delta^{15}N$). This could have been due to the lower nutritional value of these plant feeds compared to FM, particularly in crude protein contents. Previous related research, including that by Beltrán et al. (2009) for rainbow trout, *Oncorhynchus mykiss*, and gilthead sea bream, *Sparus aurata*, fed with mixtures of plant diets, and Gamboa-Delgado and Le Vay (2009) for Pacific white shrimps, *Litopenaeus vannamei*, fed soy protein isolates, has shown increased fractionation for the plant diets. Furthermore CM and BNM had higher C : N ratios than FM. High C : N ratio has been noted to be inversely proportional to feed nutritional value (Burns and Walker 2000; Piola et al. 2006). Furthermore, high dietary C : N ratio has been noted to raise the isotope fractionation by consumers (Fantel et al. 1999; Piola et al. 2006; Gamboa-Delgado and Le Vay 2009). Thus, it is possible that the lower protein value and higher C : N ratios of the F4, BNM and CM feeds contributed to the reduced SGR in these groups.

Fish are known to route essential and nonessential amino acids directly from the diets (Conceição et al. 2007; McMahon et al. 2010). Consumption of low protein diets could lead to accumulation of ^{13}C and ^{15}N following depletion of ^{12}C and ^{14}N (McMahon et al. 2010). Amino acids assimilated by fish are also used for energy provision (Rønnestad et al. 2001; Morais et al. 2004) and for biosynthesis of proteins (McMahon et al. 2010). Biosynthesis can be from other assimilated nutrients and not from body tissues (turnover), and proteins synthesized from other assimilated nutrients will bear their isotope signatures. Consequently, catfish larvae fed with the single diets and F4 could have had lower SGR than those fed with other mixed diets due to high fractionation. The catfish fed F4 had higher N contribution from FM but at same time FM inclusion level was low and inadequate to support faster growth rate. A high degree of biosynthesis has been noted in fish fed protein-deficient diets (McMahon et al. 2010) leading to reduced weight and SGR (Gaye-Siessegger et al. 2004a) and elevated fractionation in Nile tilapia, *Oreochromis niloticus*, and carp, *Cyprinus carpio* (Gaye-Siessegger et al. 2004a, 2004b).

The SIAR model incorporates the variations in fractionation and concentration of C and N. The assimilation of nutrients in the fish feeds suggests a higher assimilation of major ingredient component of diets, probably based on their protein content. We noticed that larvae assimilated more nutrients from the major ingredient component per feed type. This may, as well, be due to the increased level of amino acid pool due to elevated inclusion rate. This holds true for FM in F1, mean assimilation (43.6%), FM and BNM in F2 (34.1%, 39.3%), BNM in F3 (57.4%) and CM in F4 (44.0%). This may be due to more nutrients available for assimilation in active sites of the catfish alimentary canal. The significantly higher C : N ratio and low crude protein of F4 suggests low nutritional quality of that feed. The lower larval assimilation of FM and BNM than CM nutrients in F4 suggests that assimilation and biomass contribution are affected by inclusion levels.

The higher assimilation of BNM and FM than CM in some diets could be due to nutrient interaction and usages. There seems to be no special assimilation preference for FM nutrient at equal inclusion with BNM. FM has very high amount of all essential amino acids. However lysine is most prominent essential amino acid in BNM (Minka and Bruneteau 2000). BNM also have appreciable amount of sulphur-containing amino acids like methionine and good amounts of phenylalanine and histidine. The carbohydrate content of BNM consists of 30% neutral sugars majorly glucose and galactose (Minka and Bruneteau 2000). The amino acids and carbohydrate contents of BNM may have enabled its assimilation and N contributions. Effects of sulphur-containing amino acids on somatic nitrogen accretion have been noted in whiteleg shrimp, *L. vannamei*, fed increasing FM diets (Martínez-Rocha et al. 2012). On the other hand, the greater assimilation of BNM than FM in some feeds can also be because of inclusion amount and because fish can route essential amino acids from diet to tissue.

Conclusions

Stable isotopes analyses elucidated dietary ingredients importance by highlighting biomass contributions to the catfish. The stable isotope analyses also showed the proper utilization of ingredients by means of positive correlation of the observed and expected N contributions to the fish protein and biomass. This was pivotal in understanding growth of the catfish based on different feeds. The nutrient partitioning, assimilation, and growth of the catfish were mainly based on the essential amino acids contents and C : N ratio of the diets. Nutrient interactions of the ingredients seem to have effects on assimilation of ingredients at different inclusion levels. Although it is evident that African catfish larvae grew best at high FM diets they survived better with FM-substituted diets probably due to reduced agnostic behavior. This would, however, not be problematic in a well-managed larviculture where grading will separate shooters from slow growers.

Acknowledgments

We are grateful to T. Sinisalo for helping in allocating time for isotope analysis and Juha Ahonen for help in the water systems and temperature control during the fish culture.

Literature Cited

Beltrán, M., J. Fernández-Borrás, F. Médale, J. Pérez-Sánchez, S. Kaushik, and J. Blasco. 2009. Natural abundance of ^{15}N and ^{13}C in fish tissues and the use of stable isotopes as dietary protein tracers in rainbow trout and gilthead sea bream. Aquaculture Nutrition 15:9–18.

Burns, A. and K. F. Walker. 2000. Biofilms as food for decapods, (Atyidae, Palaemonidae), in the river Murray, South Australia. Hydrobiologia 437:83–90.

Conceição, L. E. C., J. Skjermo, G. Skjåk-Bræk, and J. A. J. Verreth. 2001. Effect of an immunostimulating alginate on protein turnover of turbot, *Scophthalmus maximus* L., we wanted to examine larvae. Fish Physiology and Biochemistry 24:207–212.

Conceição, L. E. C., S. Morais, and I. Rønnestad. 2007 Tracers in fish larvae nutrition: a review of methods and applications. Aquaculture 267:62–75.

DeNiro, M. J. and S. Epstein. 1981. Influence of diets on the distribution of nitrogen in animals. Geochimica et Cosmochimica Acta 45:341–351.

Dubois, S., B. Jean-Loui, B. Bertrand, and S. Lefebvre. 2007. Isotope trophic-step fractionation of suspension-feeding species: implications for food partitioning in coastal ecosystem. Journal of Experimental Marine Biology and Ecology 351:121–128.

Fantel, M. S., A. I. Dittel, S. Schwalm, C. E. Epifanio, and M. L. Fogel. 1999. A food web analysis of the juvenile crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia 120:416–426.

FAO. 2008. FAO Aquaculture production: quantities 1950-2006, Aquaculture production: values 1984-2006; capture production: 1950-2006; Fisheries Department, Fishery Information data and statistical unit. Fishstat plus: universal software for fishery statistical time series. Commodities production and trade: 1950-2006; vers.2.30. FAO, Rome, Italy.

Fry, B. 2006. Stable isotope ecology. Springer Science, New York, New York, USA.

Fry, B. and C. Arnold. 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp, *Penaeus aztecus*. Oecologia 54:200–2004.

Gamboa-Delgado, J. and L. Le Vay. 2009. Nitrogen stable isotopes as indicators of the relative contribution of soy protein and fish meal to tissue growth in Pacific white shrimp, *Litopenaeus vannamei*, fed compounded diets. Aquaculture 291:115–123.

Gamboa-Delgado, J., J. P. Cañavate, R. Zerolo, and L. Le Vay. 2008. Natural carbon stable isotope ratios as indicators of the relative contributions of live and inert diets to growth in larval Senegalese sole, *Solea senegalensis*. Aquaculture 280:190–197.

García-Ortega, A., J. A. J. Verreth, K. Vermis, H. J. Nelis, P. Sorgeloos, and M. W. A. Verstegen. 2010. Laboratory investigation of daily food intake and gut evacuation in larvae of African catfish, *Clarias gariepinus*, under different feeding conditions. Aquaculture International 18:119–134.

Gaye-Siessegger, J., U. Focken, H. Abel, and K. Becker. 2004a. Individual protein balance strongly influence $\delta^{15}N$ and $\delta^{13}C$ values in Nile tilapia, *Oreochromis niloticus*. Naturwissenschaften 91:90–93.

Gaye-Siessegger, J., U. Focken, S. Muetzel, H. Abel, and K. Becker. 2004b. Feeding level and individual metabolic rate affect $\delta^{13}C$ and $\delta^{15}N$ values in carp: implications for web studies. Oecologia 138:175–183.

Gaylord, G. L. and T. F. Barrows. 2008. Apparent digestibility of gross nutrients from feedstuffs in extruded feeds for rainbow trout, *Oncorhynchus mykiss*. Journal of the World Aquaculture Society 39:827–834.

German, D. P. and R. D. Miles. 2010. Stable carbon and nitrogen incorporation in blood and fin tissue of the catfish, *Pterygoplichthys disjunctivus* (Siluriformes, Loricariidae). Environmental Biology of Fishes 89:117–133.

Glencross, B. D., T. Boujard, and S. J. Kaushik. 2003. Influence of oligosaccharides on the digestibility of lupin meals when fed to rainbow trout, *Oncorhynchus mykiss*. Aquaculture 219:703–713.

Herzka, S. Z. and G. J. Holt. 2000. Changes in isotopic composition of red drum, *Sciaenops ocellatus*, larvae in response to dietary shifts: potential applications to settlement studies. Canadian Journal of Fisheries and Aquatic Sciences 57:137–147.

Hesslein, R. H., K. A. Hallard, and P. Ramlal. 1993. Replacement of sulfur, carbon and nitrogen in tissue of growing broad whitefish, *Coregonus nasus*, in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Canadian Journal of Fisheries and Aquatic Sciences 50:2071–2076.

Horvàth, L., T. Szab, and J. Burke. 1997. Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species. Polish Archives of Hydrobiology 44:221–226.

Hovde, S. C., M. C. Vidal, I. Opstad, and A. J. Raae. 2005. Design and synthesis of 14C-labelled protein as tools for protein digestion studies in fish larvae. Aquaculture Nutrition 11:395–401.

Izquierdo, M. S., H. Fernández-Palacios, and A. G. J. Tacon. 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197:25–42.

Jomori, R. K., C. Ducatti, D. J. Carneiro, and M. C. Portella. 2008. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes as natural indicators of live and dry food in *Piaractus mesopotamicus*, (Holmberg 1887) larval tissue. Aquaculture Research 39:370–381.

Kainz, M., M. T. Arts, and A. Mazumder. 2004. Essential fatty acids in the planktonic food web and their

ecological role for higher trophic levels. Limnology and Oceanography 49:1784–1793.

Layman, C. A., M. S. Araujo, R. Boucek, C. M. Hammerschlag-Peyer, E. Harrison, Z. R. Jud, P. Matich, A. E. Rosenblatt, J. J. Vaudo, L. A. Yeager, D. M. Post, and S. Bearhop. 2012. Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biological Reviews of the Cambridge Philosophical Society 87:545–562.

Levey, D. J., H. A. Bissell, and S. F. O'Keefe. 2000. Conversion of nitrogen to protein and amino acids in wild fruits. Journal of Chemical Ecology 26:1749–1763.

Martínez-Rocha, L., J. Gamboa-Delgado, M. Nieto-López, D. Ricque-Marie, and L. E. Cruz-Suárez. 2012. Incorporation of dietary nitrogen from fish meal and pea meal, *Pisum sativum*, in muscle tissue of Pacific white shrimp, *Litopenaeus vannamei*, fed low protein compound diets. Aquaculture Research 2012:1–13. DOI: 10.1111/j.1365-2109.2011.03083.x.

Maruyama, A., Y. Yamada, B. Rusuwa, and M. Yuma. 2001. Changes in stable nitrogen isotopes ratio in the muscle tissue of a migratory goby, *Rhinogobius*, sp., in a natural setting. Canadian Journal of Fisheries and Aquatic Sciences 58:2125–2128.

McMahon, K. W., M. L. Fogel, T. S. Elsdon, and S. R. Thorrold. 2010. Carbon isotope fractionation of amino acids in fish muscle reflects biosynthesis and isotopic routing from dietary protein. Journal of Animal Ecology 79:1132–1141.

Minka, S. R. and M. Bruneteau. 2000. Partial chemical composition of bambara pea (*Vigna subterranea* (L.) Verde). Food Chemistry 68:273–276.

Moore, J. W. and B. X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters 11:470–480.

Morais, S., M. Lacuisse, L. E. C. Conceicão, M. T. Dinis, and I. Rønnestad. 2004. Ontogeny of the digestive capacity of Senegalese sole, *Solea senegalenses*, with respect to digestion, absorption and metabolism of amino acids from artemia. Marine Biology 145:243–250.

Morais, S., M. Torten, O. Nixon, S. Lutzky, L. E. C. Conceição, M. T. Dinis, A. Tandler, and W. Koven. 2006. Food intake and absorption are affected by dietary lipid level and lipid source in seabream, *Sparus aurata* L., larvae. Journal of Experimental Marine Biology and Ecology 331:51–63.

Olive, P. J. W., J. K. Pinnegar, N. V. C. Polunin, G. Richards, and R. Welch. 2003. Isotope trophic-step fractionation: a dynamic equilibrium model. Journal of Animal Ecology 72:608–617.

Oliveira, A. C. B., L. A. Martinelli, M. Z. Moreira, and J. E. P. Cyrino. 2008. Determination of apparent digestibility coefficient in fish by stable carbon isotopes. Aquaculture Nutrition 14:10–13.

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using isotopes: coping with too much variations. PLoS ONE 5:e9672.

Phillips, D. L. 2001. Mixing models in analyses of diet using multiple stable isotopes: a critique. Oecologia 127:166–170.

Phillips, D. L. and J. W. Gregg. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia 127:171–179.

Phillips, D. L. and J. W. Gregg. 2003. Source partitioning using stable isotopes: coping with too many sources. Oecologia 136:261–269.

Piola, R. F., S. K. Moore, and I. M. Suthers. 2006. Carbon and nitrogen stable isotopes analysis of three types of oyster tissue in an impacted estuary. Estuarine Coastal and Shelf Science 66:255–266.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods and assumptions. Ecology 83:703–718.

Preston, N. P., D. M. Smith, D. M. Kellaway, and S. E. Bunn. 1996. The use of enriched ^{15}N as an indicator of the assimilation of individual protein sources from compound diet for juvenile *Penaeus monodon*. Aquaculture 147:249–259.

Redmond, K. J., T. Magnesen, P. K. Hansen, Ø. Strand, and S. Meier. 2010. Stable isotopes and fatty acids as tracers of the assimilation of salmon fish feed in blue mussels, *Mytilus edulis*. Aquaculture 298:202–210.

Robbins, C. T., G. V. Hilderbrand, and S. D. Farley. 2002. Incorporating concentration dependence in stable isotope mixing models: a response to Phillips and Koch (2002). Oecologia 133:10–13.

Rønnestad, I., C. R. Rojas-Garca, S. K. Tonheim, and L. E. C. Conceição. 2001. *In vivo* studies of digestion and nutrient assimilation in marine fish larvae. Aquaculture 201:161–175.

Sakano, H., E. Fujiwara, S. Nohara, and H. Ueda. 2005. Estimation of nitrogen turnover rate of *Oncorhynchus nerka*. Environmental Biology of Fishes 72:13–18.

Schlechtriem, C., U. Focken, and K. Becker. 2004. Stable isotopes as a tool for nutrient assimilation studies in larval fish feeding on live food. Aquatic Ecology 38:93–100.

Vander Zanden, M. J., M. Hulshof, M. S. Ridgway, and J. B. Rasmussen. 1998. Application of stable isotopes techniques to trophic studies of age-0 smallmouth bass. Transactions of the Americans Fisheries Society 127:729–739.