

# Diet discrimination factors are inversely related to $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of food for fish under controlled conditions

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A recent literature review reported negative relationships between diet discrimination factors (DDFs =  $X_{\text{fish}} - X_{\text{food}}$ ;  $X = \delta^{15}\text{N}$  or  $\delta^{13}\text{C}$ ) and the values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in the food of wild organisms but there has been no laboratory-based confirmation of these relationships to date. Laboratory reared guppies (*Poecilia reticulata*) fed a series of diets with a range of  $\delta^{13}\text{C}$  (–22.9 to –6.6‰) and  $\delta^{15}\text{N}$  (6.5 to 1586‰) values were used to magnify diet-tissue dynamics in order to calculate DDFs once the fish had achieved equilibrium with each of the diets. Values of DDFs range widely for  $\delta^{15}\text{N}$  (7.1 to –849‰) and  $\delta^{13}\text{C}$  (1.1 to –7.0‰) and showed a strong negative correlation with the stable isotope value in the food for  $\delta^{15}\text{N}$  (slope =  $-0.59 \pm 0.02$ ,  $r^2 = 0.95$ ) and  $\delta^{13}\text{C}$  (slope =  $-0.56 \pm 0.02$ ,  $r^2 = 0.94$ ). Based on these relationships, the magnitude of DDF change over environmentally relevant values of  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  would be significant and could confound the interpretation of stable isotopes in the environment. Using highly enriched experimental diets, our study adds to a growing number of studies that undermine the consistent trophic enrichment paradigm with results that demonstrate the currently poor mechanistic understanding of how DDFs arise. The results of our study highlight that the magnitude of the stable isotope values in prey must be considered when choosing DDF values. Future laboratory studies should therefore be directed at uncovering the mechanistic basis of DDFs and, like others before, we recommend the determination of diet-dependent DDFs under laboratory conditions before modeling dietary proportions or calculating trophic positions. Copyright © 2010 John Wiley & Sons, Ltd.

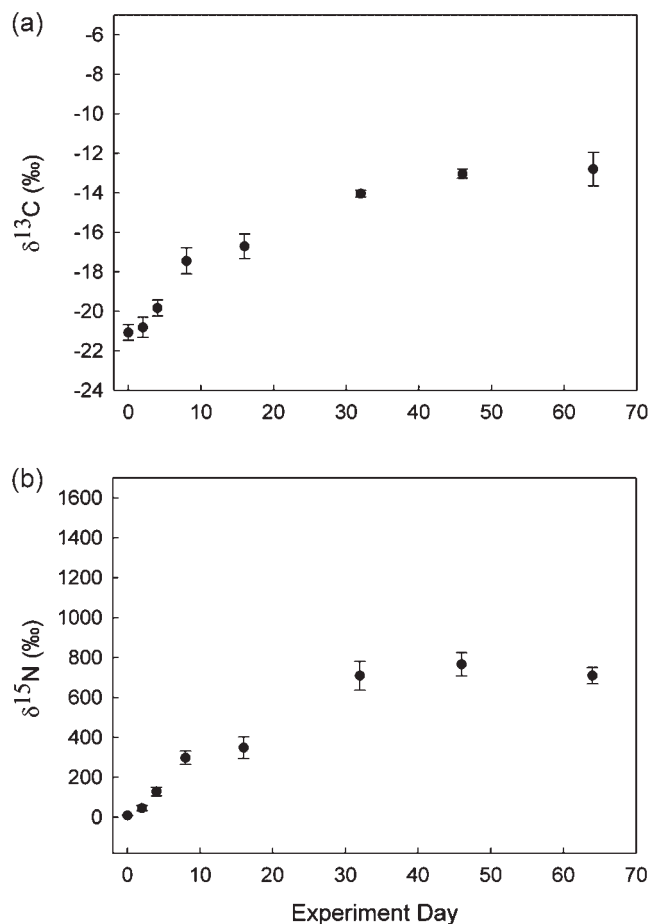
Carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope values in fish can be used to evaluate trophic relationships<sup>1,2</sup> and assess carbon and nutrient sources.<sup>2,3</sup> Although the use of stable isotopes as chemical tracers has become a common and powerful tool in ecological research, literature relating to this subject has identified many caveats to their use and has called for controlled calibration of these tracers.<sup>4–6</sup> Specifically, there is currently limited mechanistic understanding of diet discrimination factors ( $\Delta\delta X = \delta X_{\text{consumer}} - \delta X_{\text{food}}$ ;  $X = \delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ ). Diet discrimination factors (DDFs) are required for a variety of stable isotope studies including those that employ isotopic modeling in order to estimate the proportional contribution of different diet items to the isotopic composition of the tissue under consideration<sup>7–9</sup> and those that estimate trophic position following the logic that the isotopic value of an organism will increase by one DDF

each trophic level as you move up the food chain.<sup>2,10</sup> For these studies, a single DDF is often selected based on published reviews despite the fact that these factors have been shown to vary across species<sup>11</sup> and taxonomic classes,<sup>12</sup> within species across different temperature regimes,<sup>13</sup> and among different tissues within the same organism.<sup>14</sup>

Diet isotopic composition has recently been shown to affect DDFs. An extensive review of 66 publications concluded that diet isotopic value had a significant impact on DDFs and recommended the use of diet-dependent discrimination factors.<sup>15</sup> In addition, DDFs were shown to decrease linearly with increasing  $\delta^{15}\text{N}$  in the black fly, *Simulium vittatum* IS-7.<sup>16</sup> However, this study stated that the negative  $\delta^{15}\text{N}$  DDFs could be due to elimination processes such as molting or elimination of feces, lower assimilation of food due to high metabolism and growth rates, and differential assimilation of diet components.<sup>16</sup> Therefore, there is a need to perform controlled laboratory studies to determine the relationship between diet isotopic value and diet discriminations.

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The objective of this study was to estimate DDFs across a range of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in food for a fish under controlled conditions. To quantify this relationship, we developed a series of experimental foods of consistent composition over an exceptionally wide range of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  that would unambiguously determine whether DDFs are concentration-dependent. The  $\delta^{13}\text{C}$  values of the food in this study were in the range of values ( $-22.9$  to  $-6.6\%$ ) seen naturally in the environment but the  $\delta^{15}\text{N}$  of the food ranged from values seen in the environment ( $6.5\%$ ) to much higher values ( $1586\%$ ). This range of stable isotope values permitted strong inferences to be made about the relationship between diet stable isotope value and DDF, and avoided the difficulty in selecting suitable diets with variable  $\delta^{15}\text{N}$ . Using enriched  $\delta^{15}\text{N}$  has not biased results in other studies,<sup>17,18</sup> and the  $\delta^{15}\text{N}$  values in this study are well within the range where the relationship of  $\delta^{15}\text{N}$  with  $\%^{15}\text{N}$  remains linear.<sup>19</sup> The guppy (*Poecilia reticulata*) is an ideal organism to use for this study due to its small size, which allows it to come into equilibrium with the stable isotope values of a new diet relatively quickly (see Fig. 1).



**Figure 1.** Treatment F stable isotope values for (a)  $\delta^{13}\text{C}$  and (b)  $\delta^{15}\text{N}$  in guppies (each point is the mean  $\pm$  SE,  $n = 3$ ) fed a maggot diet ( $\delta^{13}\text{C} = -6.6 \pm 0.4$  and  $\delta^{15}\text{N} = 1586 \pm 39.0$ ). Dashed lines indicate the (a)  $\delta^{13}\text{C}$  and (b)  $\delta^{15}\text{N}$  values of the food.

## EXPERIMENTAL

### Fish and aquarium

A total of seven treatments were used for this study. All guppies were held in quarantine for a minimum of 4 weeks prior to the start of the experiment and were fed TetraMin Tropical Flakes in order to establish a common dietary baseline and to allow the fish to acclimate to their surroundings. The aquaria, 20 L, were exposed to a 12-h light and 12-h dark cycle, the dechlorinated water was held at a constant temperature of  $25^\circ\text{C}$  and the aquaria were thoroughly cleaned biweekly with approximately one-third of the water being replaced. Approximately 10 guppies were held in each aquarium at the start of the experiment and multiple aquaria were assigned to each treatment.

### Food preparation

Two types of basic food were used for this study, TetraMin, a standard commercial fish food, and pulverized maggots. Maggots, *Ophyra aenescens*, were selected as a food source because their stable isotope values can be manipulated without creating biases in amino acid composition, which can influence stable isotope dynamics.<sup>20</sup> An adult fly colony was established using wild caught flies from the Windsor-Essex area of Ontario, Canada. Flies were maintained in the lab with a diel cycle, 16(on) eight (off), at a humidity of 50–60% and temperature of  $21\text{--}22^\circ\text{C}$ . The flies were fed water and sugar *ad libitum* and held until gravid, at which time they were given a small amount of No Name Meat Mix (Loblaws Companies Ltd., Brampton, Canada) dog food with no chemicals added on which to lay eggs. The maggot eggs were transferred into 1-L glass mason jars containing paper towels and one of three different rearing treatments.

Isotopically distinct control and treatment maggots were created by raising maggots on dog food that either had no chemicals added (called *control* maggot treatment) or approximately 0.25g each of non-labeled sodium acetate (99.0%, Sigma-Aldrich Inc., St. Louis, MO, USA) and ammonium chloride (99.9%, J.T. Baker, Phillipsburg, NJ, USA) added to 624 g of dog food (called *low* maggot treatment); or 0.25 g  $^{15}\text{N}$ -enriched (99%) ammonium chloride and  $^{13}\text{C}$ -enriched (99%) sodium acetate (Cambridge Isotope Laboratories, Andover, MA, USA) (called *high* maggot treatment). The mixtures were left to stand for a minimum of 6 days prior to adding the maggots, to allow bacteria present in the mixture to absorb the chemicals that had been added. The maggots were allowed to feed *ad libitum* on the dog food until the pre-pupal stage of development. During this time, the maggots were monitored to ensure an adequate moisture level, food supply, and maggot density. Once they reached the pre-pupal stage, the maggots were collected and allowed to wander without food until the contents of their gut had emptied.

After emptying their guts, the maggots were frozen for 24h, washed and freeze-dried for 48h in a VLP200 ValuPump freeze drier (Thermo Savant Instruments Inc., Holbrook, NY, USA). The maggots were pulverized using a mortar and pestle and frozen until fed to the fish. Seven different foods were used that consisted of six maggot diets with varying stable isotope values and a single TetraMin diet (see Table 1). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the different foods

**Table 1.** DDFs and values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the food for treatments A through G (mean  $\pm$  SE). See Experimental section for details

Treatment	Food composition	Isotope	Food $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$	n	DDF
<i>Maggots only</i>					
A	Control	$\delta^{15}\text{N}$	$6.5 \pm 0.2$	6	$6.02 \pm 0.6$
		$\delta^{13}\text{C}$	$-20.0 \pm 0.1$		$0.1 \pm 0.1$
B	Low	$\delta^{15}\text{N}$	$8.2 \pm 0.2$	7	$7.1 \pm 2.2$
		$\delta^{13}\text{C}$	$-19.5 \pm 0.04$		$0.03 \pm 0.1$
C	10% High, 90% Control	$\delta^{15}\text{N}$	$151.7 \pm 68.7$	9	$-54.4 \pm 7.4$
		$\Delta^{13}\text{C}$	$-18.6 \pm 0.6$		$-0.4 \pm 0.1$
D	50% High, 50% Control	$\delta^{15}\text{N}$	$747.1 \pm 151.8$	10	$-491.3 \pm 20.4$
		$\delta^{13}\text{C}$	$-14.2 \pm 1.0$		$-3.6 \pm 0.3$
E	90% High, 10% Control	$\delta^{15}\text{N}$	$1154.8 \pm 65.9$	8	$-729.3 \pm 26.2$
		$\delta^{13}\text{C}$	$-10.7 \pm 0.5$		$-5.3 \pm 0.2$
F	High	$\delta^{15}\text{N}$	$1586 \pm 39.0$	6	$-849 \pm 43.6$
		$\delta^{13}\text{C}$	$-6.6 \pm 0.4$		$-7.0 \pm 0.3$
<i>TetraMin only</i>					
G	TetraMin	$\delta^{15}\text{N}$	$8.2 \pm 0.8$	9	$2.9 \pm 0.4$
		$\delta^{13}\text{C}$	$-22.9 \pm 0.05$		$1.1 \pm 0.1$

were measured two to three times throughout the experiment and these values did not differ significantly for  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  for any of the food types (data not shown).

### Protocol

Of the seven treatments (see Table 1) used for this study, six involved a diet consisting of only maggots (treatments A–F) and one involved a diet consisting of only TetraMin Tropical Flakes (treatment G). Treatments A, B and F represent the control, low, and high maggot diets while treatments C, D and E represent mixtures of the control and high maggots (proportions of each mixture are given in Table 1). All fish were fed a consistent amount of food six days a week and the treatments were maintained until the stable isotope values in the fish came into equilibrium with their diet. The stable isotope values were monitored for the fish throughout the experiment to determine when they had reached an apparent steady state (isotope values remained constant across multiple sampling days) with the diet (see Fig. 1 as an example). Six to ten fish from each treatment were sampled over multiple days to calculate DDFs once the fish had come into equilibrium with their diet.

The guppies were sacrificed using a lethal dose of MS-222 (Finquel, Redmond, WA, USA), and weight, standard and total length measurements were recorded. The gastrointestinal tract of each fish was removed under a dissecting microscope, in order to ensure that undigested food would not interfere with the stable isotope values recorded.

### Stable isotope analysis

Prior to stable isotope analysis, samples were freeze dried for 48 h and pulverized with a stainless steel spatula. The samples were weighed into 0.5 mg tin capsules and analyzed with a Delta V Advantage isotope ratio mass spectrometer (Thermo Electron Corporation, Bremen, Germany) and 4010 elemental combustion system (Costech Instruments, Valencia, CA, USA). Every tenth sample was run in triplicate and lab and National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) reference standards were used for quantification. The analytical precision based on the

standard deviation of an internal lab (fish muscle) and NIST standard 8414 (bovine liver) for  $\delta^{15}\text{N}$  ranged from 0.14‰ to 0.21‰, respectively, and for  $\delta^{13}\text{C}$  ranged from 0.05‰ to 0.08‰, respectively, during the analysis of these samples. The analysis of NIST standards (sucrose and ammonia sulphate;  $n=3$  for each) during the sample analysis generated values that were within 0.01‰ and 0.07‰ of certified values for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , respectively. The stable isotope values are conveyed in  $\delta$  notation using the following equation:

$$\delta X = [R_{\text{sample}}/R_{\text{standard}} - 1] \times 1000$$

where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is the ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ . The lipid contents were normalized mathematically using the equation suggested by Post:<sup>21</sup>

$$\delta^{13}\text{C}_{\text{normalized}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times C : N$$

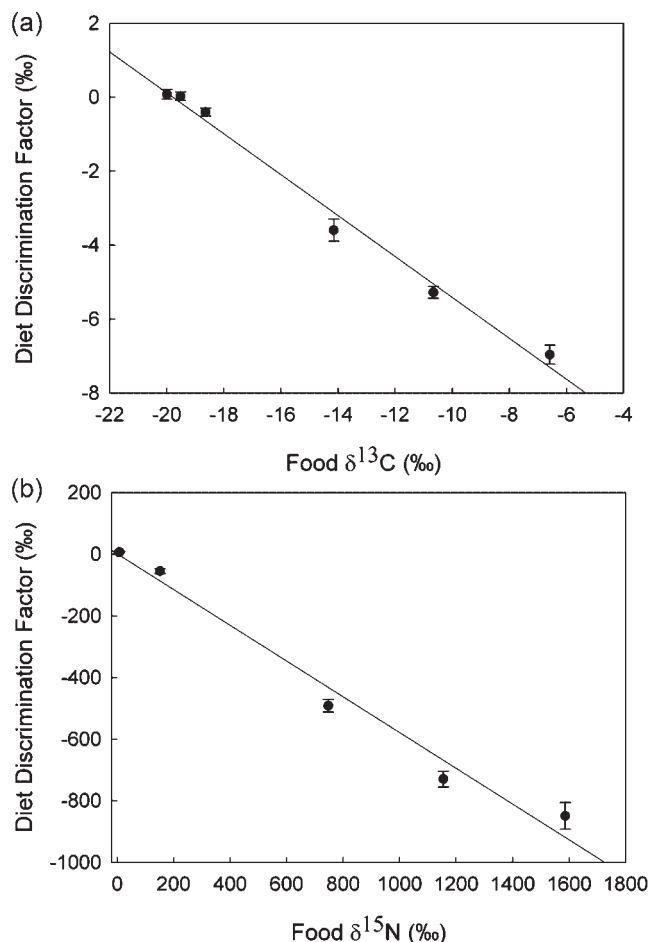
### Statistical analyses

The growth rate was calculated as  $g = \ln(W_f/W_o)/t$ , where  $W_f$  is the weight at the time of sampling (g),  $W_o$  is the initial weight at the start of the experiment (g) and  $t$  is time (days). All statistics were calculated using Sigmapstat 3.5 (Systat Software Inc., Point Richmond, CA, USA). A simple least-squares linear regression was applied to a plot of food isotopic composition against DDF in order to determine the relationship between these two variables. A t-test was used to compare DDFs of guppies fed different food types.

## RESULTS

### General health and growth

Fish grew throughout the experiment at a rate of 0.01 g day<sup>-1</sup> across all treatments. Although most of the fish appeared to be in good health throughout the experiment, approximately 20% of the fish died of natural causes, which is a normal rate for aquarium-held guppies (unpublished data), and had to be removed. Differences in mortality among aquaria were very minor, differing only by approximately 1–2 fish deaths per treatment.



**Figure 2.** Relationship between DDFs (mean  $\pm$  SE) and (a)  $\delta^{13}\text{C}$  and (b)  $\delta^{15}\text{N}$  values in the guppy food for treatments A through F (maggots only). Lines represent linear regression for (a)  $\delta^{13}\text{C}$  ( $\text{DDF} = -11.07 - (0.56 \times \delta^{13}\text{C}_{\text{diet}})$ ;  $r^2 = 0.94$ ,  $p < 0.001$ ) and (b)  $\delta^{15}\text{N}$  ( $\text{DDF} = 1.44 - (0.59 \times \delta^{15}\text{N}_{\text{diet}})$ ;  $r^2 = 0.95$ ,  $p < 0.001$ ).

### Diet discrimination factors

All seven treatments achieved an apparent steady state with the diet, based on constant stable isotope values across multiple sampling days (see Fig. 1). The diet discrimination factors ranged from  $-7.0 \pm 0.3$  to  $1.1 \pm 0.1\text{‰}$  for  $\delta^{13}\text{C}$  and from  $-849 \pm 43.6$  to  $7.1 \pm 2.2\text{‰}$  for  $\delta^{15}\text{N}$  depending on food type and stable isotope value of the food (see Table 1), and had a significant negative relationship with the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the food (see Fig. 2).

The food  $\delta^{15}\text{N}$  values were similar for treatment B (maggots) and treatment G (TetraMin) allowing for comparison among DDFs from differing food types. However, the C:N ratio differed for the two food types (See Table 2). The DDFs were significantly higher (see Table 1) for treatment B than for treatment G ( $t = 2.222$ ,  $p = 0.045$ ). The food  $\delta^{13}\text{C}$  values were not similar among treatments; therefore, a comparison of DDFs could not be made.

### Lipid-normalized $\delta^{13}\text{C}$ values

Caution must be used when applying stable isotopes to tissue with high lipid contents because the low  $\delta^{13}\text{C}$  values in lipids compared to other tissues may bias interpretation.<sup>22</sup> In

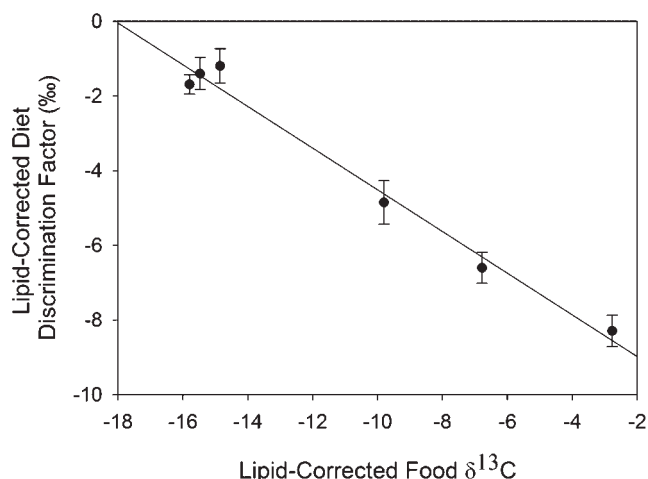
**Table 2.** Lipid-normalized  $\delta^{13}\text{C}$  DDFs and lipid-corrected  $\delta^{13}\text{C}$  of the food (mean  $\pm$  SE) with C:N (mean  $\pm$  SE) for treatments A through G

Treatment	Food $\delta^{13}\text{C}$	Food C:N	DDF	Guppy C:N
A	$-15.8 \pm 0.2$	$7.6 \pm 0.5$	$-1.7 \pm 0.3$	$5.6 \pm 0.2$
B	$-15.5 \pm 0.2$	$7.4 \pm 0.6$	$-1.4 \pm 0.4$	$5.4 \pm 0.2$
C	$-14.9 \pm 0.4$	$7.2 \pm 0.2$	$-1.2 \pm 0.5$	$6.4 \pm 0.4$
D	$-9.8 \pm 0.8$	$7.7 \pm 0.3$	$-4.9 \pm 0.6$	$6.5 \pm 0.5$
E	$-6.8 \pm 0.5$	$7.3 \pm 0.3$	$-6.6 \pm 0.4$	$6.0 \pm 0.4$
F	$-2.8 \pm 0.3$	$7.2 \pm 0.6$	$-8.3 \pm 0.4$	$5.0 \pm 0.2$
G	$-20.8 \pm 0.05$	$5.5 \pm 0.05$	$0.3 \pm 0.1$	$4.2 \pm 0.1$

general it is not necessary to correct for lipid content when the C:N ratio of the tissue being sampled is below 3.5 for aquatic animals.<sup>21</sup> Since the C:N ratio of the guppies ranged from  $4.2 \pm 0.1$  to  $6.5 \pm 0.5$  in this study (see Table 2), the  $\delta^{13}\text{C}$  values were lipid-normalized.<sup>21</sup> A negative linear relationship between lipid-corrected food  $\delta^{13}\text{C}$  and lipid-corrected DDF was also observed (see Fig. 3). The slope of this relationship (slope =  $-0.57 \pm 0.04$ ,  $r^2 = 0.80$ ) was very similar to the slope reported for the non-lipid-corrected data (slope =  $-0.56 \pm 0.2$ ,  $r^2 = 0.94$ ).

### DISCUSSION

The results of this study clearly demonstrate that DDFs are dependent on diet, in terms both of the isotopic composition of the food and of the food type. Although some DDFs were within the range of typically reported values (e.g.,  $2.9\text{‰}$  for  $\delta^{15}\text{N}$  and  $-0.4$  to  $1.1\text{‰}$  for  $\delta^{13}\text{C}$ ), not all treatments followed this trend. Guppies fed a diet that consisted of a highly enriched food displayed DDFs much more negative than previously reported values,<sup>2,11,15</sup> demonstrating that organisms fed a diet with high  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  values will be more depleted in the heavy isotope than their diet. Negative DDFs have also been reported for other organisms including the winter flounder, *Pseudopleuronectes americanus*,<sup>23</sup> black fly



**Figure 3.** Relationship between lipid-corrected DDFs (mean  $\pm$  SE) and lipid-corrected  $\delta^{13}\text{C}$  values in the guppy food for treatments A through F (maggots only). Line represents linear regression ( $\text{DDF} = -10.21 - (0.57 \times \delta^{13}\text{C}_{\text{Lipid Corrected Food}})$ ;  $r^2 = 0.80$ ,  $p < 0.001$ ).

larvae, *Simulium vittatum* IS-7,<sup>16</sup> and the rat, *Rattus rattus*.<sup>24</sup> Therefore, DDFs are dependent on the isotopic composition of the diet, varying more widely among organisms than is currently assumed.

Typically, a single DDF value, based on published reviews, is used in food web studies involving the stable isotopes of carbon and nitrogen.<sup>25,26</sup> However, the DDFs used in those studies did not take account of the influence of diet  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. In this study, the DDFs decreased linearly with increasing  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the food. This negative relationship between DDFs and the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the food has also been reported for black fly larvae<sup>16</sup> and rats.<sup>24</sup> A recent study by Caut *et al.*,<sup>15</sup> which consisted of an analysis of 66 reviewed publications, concluded that diet appears to have a strong influence on DDFs. Our study showed that the relationship between DDFs and  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  of the food is very strong ( $r^2 > 0.94$ ) when the organism is fed a constant food source. The slope of the regression lines for DDF and  $\delta^{13}\text{C}$  in the food ( $-0.56 \pm 0.02$ ) and the slope of the regression line for DDF and  $\delta^{15}\text{N}$  in the food ( $-0.59 \pm 0.02$ ) were similar but more negative than the slopes reported for organisms in the review by Caut *et al.*,<sup>15</sup> which ranged from  $-0.417$  to  $-0.113$  for  $\delta^{13}\text{C}$  and  $-0.311$  to  $-0.141$  for  $\delta^{15}\text{N}$ . The C:N ratio of the maggot diet was consistent among treatments (see Table 2) demonstrating that diet quality was maintained across all treatments used for the regression analysis. For this reason, TetraMin was not used for the regression analysis.

Although some of the  $\delta^{15}\text{N}$  values used in this study were outside the range usually seen in the published literature, high  $\delta^{15}\text{N}$  values in the food were used to establish the relationship between the  $\delta^{15}\text{N}$  value of the diet and DDF and to avoid potential problems with a smaller range and the difficulty of selecting similar diets with variable  $\delta^{15}\text{N}$  values. Even within an environmentally relevant  $\delta^{15}\text{N}$  range, the DDFs were found to vary by over 10‰ according to the regression obtained in this study. To our knowledge there are no studies suggesting that the use of  $^{15}\text{N}$ -enriched foods may influence stable isotope dynamics and the use of enriched values in previous studies has not proved to be a problem.<sup>17,18</sup> In addition, the  $\delta^{13}\text{C}$  values were well within the range found in the environment and the similarity in the regression slopes between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  suggests that similar processes are operating for both isotopes at high concentrations of  $^{13}\text{C}$  and  $^{15}\text{N}$  in the food. Finally, the similarities in the DDF isotope values in the food slopes in our study and in those of Caut *et al.*,<sup>15</sup> which used DDFs from studies in the wild, provides evidence that our enriched  $\delta^{15}\text{N}$  values in the food did not influence the behavior or the DDFs calculated. We can think of no plausible mechanism by which highly enriched foods would be unduly biased relative to natural foods; therefore, our results clearly demonstrate that DDFs are concentration-dependent.

The mechanism underlying the negative relationship between diet  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values and DDF is not well understood. Fractionation of stable isotopes occurs through a dynamic balance of absorption from the gut and excretion through the formation of excretory products.<sup>27</sup> Since the lighter isotope typically reacts faster chemically,<sup>2</sup> the product

absorbed from the gut will be isotopically lighter than the food. An organism feeding on a food source with a very high  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  value may absorb and break down a lower portion of the compounds containing the heavy isotopes present in an enriched food than in a more depleted food. The product that is assimilated from the gut is then subject to metabolic processes typically responsible for the enrichment in stable isotope values between an organism and its diet but from a substrate pool that is isotopically-depleted relative to the ingested food. If this is the case we would expect the feces to be enriched in  $^{15}\text{N}$ ; Checkley and Entzeroth<sup>28</sup> showed that the feces of copepods was isotopically heavy compared with their diet and Overmyer *et al.*<sup>16</sup> also observed very high  $\delta^{15}\text{N}$  values for the feces of black fly larvae. This is analogous to parasitic organisms that have an abundance of food (the host organism); the parasite is not able to absorb all of the potential food sources and a disproportional amount of the compounds containing the light isotope is absorbed because it is more reactive than the heavy isotope.<sup>29</sup> Alternatively, the differential distribution of heavy and light isotopes in the maggot tissue, due to isotopic routing, followed by the guppy's preferential assimilation of certain tissues could contribute to the difference in DDFs observed.<sup>9,30</sup>

If DDFs are dependent on the food  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, designers of studies applying stable isotopes to natural systems must be sure that the DDFs used reflect the diet  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the food. If this is not done, the results of isotopic modeling and the interpretation of diet reconstruction studies may be biased, particularly where prey stable isotope values span a wide isotopic range. This study also has implications for research employing  $^{15}\text{NH}_4$  as a tracer to study nitrogen cycling within an ecosystem.<sup>31-33</sup> In these studies, very high  $\delta^{15}\text{N}$  values are often observed that could bias both reported results and comparisons among different experiments.<sup>16</sup>

As observed elsewhere, the food type also influenced the DDFs observed for  $\delta^{15}\text{N}$ . When comparing the  $\delta^{15}\text{N}$  DDFs for treatment-fed maggots and the TetraMin diet with similar  $\delta^{15}\text{N}$  values (treatment B compared with treatment G), the treatment-fed maggots had a higher DDF than those typically reported while the  $\delta^{15}\text{N}$  DDF for the treatment involving TetraMin only was very close to the range of 3 to 5‰ commonly used in the literature.<sup>1,2</sup> Using the C:N ratio as an estimate for the protein content of the diet, it appears that the protein content of the diets could account for the discrepancy in DDFs observed for these two treatments since the C:N ratio differed between the maggot and TetraMin diets. Previous studies have shown opposing results for the influence of protein content on DDFs. For example, Focken<sup>34</sup> and Pearson *et al.*<sup>35</sup> found that the  $\delta^{15}\text{N}$  DDFs increased with protein consumption for Nile tilapia, *Oreochromis niloticus*, and wild yellow-rumped warblers, *Dendroica coronata*, respectively. Conversely, Tsahar *et al.*<sup>36</sup> found that yellow-vented bulbuls, *Pycnonotus xanthopygos*, had higher  $\delta^{15}\text{N}$  DDFs when fed a lower protein diet than when fed a higher protein diet and Webb *et al.*<sup>37</sup> found that locusts, *Locusta migratoria*, had higher  $\delta^{15}\text{N}$  DDFs when fed a low-quality maize diet than when fed a higher quality wheat diet. In addition, Robbins *et al.*<sup>38</sup> found no significant relationship between protein content or C:N and DDF.

In the current study, the TetraMin Tropical Flakes are specifically designed to provide fish with a nutritionally balanced diet and are probably more representative of the guppy's natural diet than maggots. Since the TetraMin diet displayed a lower C:N value than the maggot diet, this suggests that the TetraMin diet has a higher protein content. Webb *et al.*<sup>37</sup> attributed the higher DDFs for the low-quality maize diet to substrate recycling. If the maggot diet represents a suboptimal diet for the guppy, substrate recycling may also be responsible for the higher DDFs observed for fish fed the maggot diet. Therefore, the DDFs calculated from the regression analysis obtained in this study may be higher than the DDF for a guppy feeding in the wild. Nevertheless, the negative relationship between DDF and food  $\delta^{15}\text{N}$  values remains.

## CONCLUSIONS

This study has experimentally evaluated the DDFs of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in guppies and has profound implications for the application of stable isotopes in other organisms. DDFs were found to depend both on the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of the diet and on the food type. Although the negative linear relationships between DDF and diet  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values provides an initial estimate of how DDFs may vary through a food web, additional research is warranted to determine this relationship in other species. Reliable DDFs that have been tested under controlled laboratory experiments should be acquired before stable isotopes are applied in the field to investigate diet and food web interaction. Furthermore, the DDFs were found to differ depending on the food type, demonstrating that food protein content may influence DDFs. Overall, our findings highlight the considerable need for more controlled laboratory experiments to interpret stable isotopes dynamics in the field and to understand the mechanism underlying the relationships observed.

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## REFERENCES

1. Minagawa M, Wada E. *Geochim. Cosmochim. Acta* 1984; **48**: 1135.
2. Peterson B, Fry B. *Annu. Rev. Ecol. Syst.* 1987; **18**: 293.
3. DeNiro MJ, Epstein S. *Science* 1978; **201**: 906.
4. Gannes L, O'Brien D, del Rio CM. *Ecology* 1997; **78**: 1271.
5. Jardine T, Kidd K, Fisk A. *Environ. Sci. Technol.* 2006; **40**: 7501.
6. Wolf N, Carleton S, del Rio CM. *Funct. Ecol.* 2009; **23**: 17.
7. Ben-David M, Flynn RW, Schell DM. *Oecologia* 1997; **111**: 280.
8. Szepanski MM, Ben-David M, Van Ballenberghe V. *Oecologia* 1999; **120**: 327.
9. Phillips DL, Koch PL. *Oecologia* 2002; **130**: 114.
10. Hobson KA, Welch HE. *Mar. Ecol. Prog. Ser.* 1992; **84**: 9.
11. Post D. *Ecology* 2002; **83**: 703.
12. Vanderklift MA, Ponsard S. *Oecologia* 2003; **136**: 169.
13. Power M, Guiguer KRRA, Barton DR. *Rapid Commun. Mass Spectrom.* 2003; **17**: 1619.
14. Hobson KA, Clark RG. *Condor* 1992; **94**: 189.
15. Caut S, Angulo E, Courchamp F. *J. Appl. Ecol.* 2009; **46**: 443.
16. Overmyer JP, MacNeil MA, Fisk AT. *Rapid Commun. Mass Spectrom.* 2008; **22**: 694.
17. MacNeil MA, Drouillard KG, Fisk AT. *Can. J. Fish. Aquat. Sci.* 2006; **63**: 345.
18. Fisk AT, Sash K, Maerz J, Palmer W, Carroll JP, MacNeil MA. *Rapid Commun. Mass Spectrom.* 2009; **23**: 319.
19. Fry B. *Stable Isotope Ecology*. Springer: New York, 2008.
20. Schmidt K, McClelland JW, Mente E, Montoya JP, Atkinson A, Voss M. *Mar. Ecol. Prog. Ser.* 2004; **266**: 43.
21. Post D. *Oecologia* 2007; **152**: 179.
22. DeNiro MJ, Epstein S. *Science* 1977; **197**: 261.
23. Bosley KL, Witting DA, Chambers RC, Wainright SC. *Mar. Ecol. Prog. Ser.* 2002; **236**: 233.
24. Caut S, Angulo E, Courchamp F. *Funct. Ecol.* 2008; **22**: 255.
25. Syvaranta J, Cucherousset J, Kopp D, Crivelli A, Cereghino R, Santoul F. *Aquatic Biol.* 2010; **8**: 137.
26. Schaal G, Riera P, Leroux C. *Estuar. Coast. Shelf S.* 2009; **85**: 565.
27. Ponsard S, Averbuch P. *Rapid Commun. Mass Spectrom.* 1999; **13**: 1305.
28. Checkley DMJ, Entzeroth LC. *J. Plankton Res.* 1985; **7**: 553.
29. Olive PJW, Pinnegar JK, Polunin NVC, Richards G, Welch R. *J. Anim. Ecol.* 2003; **72**: 608.
30. Felicetti LA, Schwartz CC, Rye RO, Haroldson MA, Gunther KA, Phillips DL, Robbins CT. *Can. J. Zool.* 2003; **81**: 763.
31. Hall RO, Peterson BJ, Meyer JL. *Ecosystems* 1998; **1**: 283.
32. Wollheim WM, Peterson BJ, Deegan LA, Bahr M, Hobbie JE. *J. North Am. Benthol. Soc.* 1999; **18**: 199.
33. Dodds WK, Evans-White MA, Gerlanc NM, Gray L, Gudder DA, Kemp MJ, López AL, Stagliano D, Strauss EA, Tank JL, Whiles MR, Wollheim WM. *Ecosystems* 2000; **3**: 574.
34. Focken U. *Isot. Environ. Health Stud.* 2001; **37**: 199.
35. Pearson S, Levey D, Greenberg C, del Rio CM. *Oecologia* 2003; **135**: 516.
36. Tsahar E, Wolf N, Izhaki I, Arad Z, del Rio CM. *J. Exp. Biol.* 2008; **211**: 459.
37. Webb SC, Hedges RE, Simpson SJ. *J. Exp. Biol.* 1998; **201**: 2903.
38. Robbins CT, Felicetti LA, Sponheimer M. *Oecologia* 2005; **144**: 534.