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ARTICLE

The Potential for Less Invasive Inference of Resource Use: Covariation in Stable Isotope Composition between Females and Their Eggs in Bluegill

Scott F. Colborne[1] **and Timothy J. A. Hain**
Department of Biology, University of Western Ontario, Biological and Geological Sciences Building, 1151 Richmond Street, London, Ontario N6A 5B7, Canada

Fred J. Longstaffe
Department of Earth Sciences, University of Western Ontario, 1151 Richmond Street, London, Ontario N6A 5B7, Canada

Bryan D. Neff*
Department of Biology, University of Western Ontario, Biological and Geological Sciences Building, 1151 Richmond Street, London, Ontario N6A 5B7, Canada

Abstract

Stable isotope analysis is frequently used to examine resource use in wild populations, but it often involves invasive or lethal methods of collecting tissue samples. The development of less invasive or nonlethal sampling techniques will expand the possible uses of stable isotopes. We examined whether fish eggs meet three basic requirements for inferring female resource use from them: (1) the isotope composition of the eggs is correlated with that of other maternal tissues for which isotope composition is known to be related to diet; (2) the isotope composition remains constant over the egg development period; and (3) dietary inferences using eggs are similar to those for other maternal tissues. Using artificial crosses, we tested the relationship between eggs and two commonly sampled maternal tissues (white muscle and liver) in wild-caught Bluegills *Lepomis macrochirus*. We found that egg isotope composition was strongly correlated with that of other maternal tissues, particularly liver, and remained constant from prefertilization to the day of hatch, with no change in $\delta^{13}C$ and an increase of only 0.3‰ in $\delta^{15}N$. Furthermore, the results of SIAR (Stable Isotope Analysis in R) mixing models indicated a large degree of overlap in diet estimates between eggs and the other maternal tissues. Overall, eggs can be reliably used to infer the prebreeding foraging ecology of female Bluegills throughout the egg development period.

Ecologists commonly use stable isotope analysis to study the foraging patterns of wild populations. Isotopic composition has been used to provide insight into resource use (MacAvoy et al. 2008; Derbridge et al. 2012; Ravinet et al. 2013), migration patterns (Hobson 1999; Rubenstein and Hobson 2004), and trophic relationships (Hobson et al. 1994; Vander Zanden and Rasmussen 1999). An important attribute of stable isotope analysis is that it permits inferences about resource use over long periods of time, from days to years depending on the tissues sampled (reviewed by Boecklen et al. 2011). When stable isotopes are applied to fish foraging ecology, samples of white muscle, liver, or blood are typically used, which reflect diet over time scales of several months (white muscle), weeks (liver and blood cellular components), or days (blood plasma;

*Corresponding author: bneff@uwo.ca
[1]Present address: Great Lakes Institute for Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, Ontario N9B 3P4, Canada.
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Perga and Gerdeaux 2005; Guelinckx et al. 2007; Boecklen et al. 2011). However, collection of these tissue types is often lethal or increases the risk of mortality following sampling, which can limit the applicability of stable isotope analysis. Thus, there is interest in developing less invasive and nonlethal sampling techniques to obtain tissues for stable isotope analysis.

Nonlethal sampling techniques are increasingly being used to investigate a variety of research questions. For example, samples of nails (Hobson et al. 1996; Cherel et al. 2007), scales (Wainright et al. 1993), feathers (Bearhop et al. 2002), and hair (Hobson et al. 1996; Derbridge et al. 2012) have all been used to examine diet during the growth period of those tissues. More recently, fish eggs have been collected to infer maternal resource use because they can be stripped from gravid females (e.g., Acolas et al. 2008) or retrieved after spawning (e.g., Snowberg and Bolnick 2008, 2012). Because in most fishes a single female typically produces hundreds or thousands of eggs, the impact on individual fitness of sampling a few eggs is minimal. Certainly different tissue types have expanded the potential use of stable isotope analysis in foraging ecology.

Prior to using a tissue for stable isotope analysis, the relationship between that tissue and diet must be understood. For example, it is well known that the isotopic composition of white muscle reflects diet over a longer period than that of liver due to the greater metabolic activity of liver (Perga and Gerdeaux 2005; Buchheister and Latour 2010). In the same vein, keratin-based tissues, such as hair and nails, can be used to infer past resource use because these tissues are isotopically inert following the growth period (Hobson 1999; Bearhop et al. 2002). The relationship between the isotopic composition of eggs and fish diet is still being determined. Previous analyses comparing eggs and other maternal tissues in two fish species have found correlations between the isotopic composition of eggs and that of both white muscle (e.g., Grey 2001; Snowberg and Bolnick 2008) and liver (e.g., Snowberg and Bolnick 2012). In addition to examining this correlation in additional fish species, researchers need to ascertain whether development during embryogenesis affects the isotopic composition of eggs. Because eggs are closed to the entry of new nutrients during development (Kamler 2008), we expected the isotopic composition of whole eggs to reflect female diet over the period leading up to breeding and to remain constant throughout egg development. To our knowledge, the isotopic composition of fish eggs has not been examined over their postfertilization development period.

We used artificial crosses of Bluegills *Lepomis macrochirus* to determine the suitability of sampling eggs for foraging studies. Bluegills are members of the Centrarchidae family, which includes some of the most common freshwater species in North America (Scott and Crossman 1998). We studied a northern population of Bluegills that spawn within a few months after foraging resumes in the spring (Keast 1978). During this prebreeding period, female Bluegills synthesize yolk that is deposited into the eggs and used to sustain the developing embryo until exogenous feeding by larvae commences (Wiegand 1996; Kamler 2008). Due to the depletion of their body reserves over the winter and energy allocation to gonads during the spring months, Bluegills have been identified as income breeders, i.e., as a species that produces egg yolk directly from the resources consumed during the spring prebreeding period (Acolas et al. 2008; Beuchel et al. 2013); therefore, we expected the isotopic composition of their eggs to reflect the diet of females during the period leading up to reproduction.

If the isotopic composition of eggs can be reliably used to infer maternal diet, three requirements must be met: (1) there must be a relationship between the isotopic composition of eggs and that of other maternal tissues for which isotopic composition is known to reflect diet; (2) the isotopic composition of eggs must remain constant over the development period; and (3) inferences of resource use based on eggs must be similar to those for other maternal tissues.

METHODS

By means of daily snorkel surveys of the littoral habitat of Lake Opinicon, Ontario (44°34′N, 76°19′W), 40 Bluegills (20 males and 20 females) were collected using a dip net on the day of spawning. Collections were made from two separate colonies on June 7 and 8, 2011. Spawning pairs were identified when in a nest together and the female began "dipping" behaviors indicating spawning (Gross 1982). Within 1 h of capture, the males and females were transported to the Queen's University Biological Station on the shore of Lake Opinicon and housed in an on-site aquarium facility for no longer than an additional 3 h.

Artificial crosses of males and females were completed using the in vitro fertilization technique described by Neff and Lister (2007). Each male and female collected was used once to make an artificial cross, for a total of 20 crosses. Briefly, eggs were obtained from each female by applying gentle pressure to the abdomen and collecting the eggs in a 500-mL glass jar containing 50 mL of lake water drawn directly from Lake Opinicon. A subsample of the eggs from each female was also placed in a 1.5-mL microcentrifuge tube and stored at −20°C for subsequent isotopic analysis. Sperm was then collected from each male by applying gentle pressure to the abdomen and gathering the released sperm in a 2-mL syringe. It was then gently mixed with the eggs, following which each jar was filled with lake water. Air stones were attached to the top of each jar to maintain oxygen levels in the water. Each day, 50% of the water was replaced with freshly drawn lake water.

After egg collection, the females were euthanized with an overdose of clove oil and a sample of white muscle tissue was removed from below the posterior portion of the dorsal fin on the right side of the fish. The liver was also removed and both tissue samples were stored at −20°C. The males were then

released at the site of their original collection. Subsamples of the eggs were taken at 24, 48, and 72 h postfertilization, representing the entire period of egg development (Gross 1982; Neff 2003). These eggs were collected using a plastic pipette to gently loosen them from the jar, followed by the retrieval of 50–75 eggs. The eggs were stored in 1.5-mL microcentrifuge tubes, drained of excess water, and frozen at −20°C. The overall health of the eggs in each cross was monitored; if the majority of the eggs in a jar changed from their usual grey coloration to white and lost their attachment to the jar surface, they were considered unhealthy or dead and collections were stopped for that cross.

Maternal tissue and egg samples were prepared for isotopic analysis by freeze-drying at −50°C for 24 h followed by manual grinding with a mortar and pestle. The stable isotope ratios of carbon ($^{13}C : {}^{12}C$) and nitrogen ($^{15}N : {}^{14}N$) were determined using a continuous-flow mass spectrometer (Costech elemental analyzer coupled with a Thermo Finnigan Delta^{plus} XL mass spectrometer) in the Laboratory for Stable Isotope Science at the University of Western Ontario in London. Isotope ratios were calculated as the per mil difference between the measured sample and international standard reference material, that is,

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1),$$

where X is ^{13}C or ^{15}N, R is the ratio $^{13}C : {}^{12}C$ or $^{15}N : {}^{14}N$, and δ is the ratio of heavy to light isotopes in the sample relative to the same ratio in the standard.

The $\delta^{13}C$ values were calibrated to Vienna Pee Dee Belemnite using a two-point curve calibrated using the international standards USGS-40 (accepted value, −26.4‰) and USGS-41 (accepted value, +37.6‰). Internal standards for keratin (accepted value, −24.0‰) and ANU-sucrose (IAEA-CH-6; accepted value, −10.5‰) were used to monitor the precision and accuracy of the isotope measurements. The $\delta^{13}C$ values of the keratin standard were -24.1 ± 0.1‰ ($n = 34$), and IAEA-CH-6 has a mean measured value of -10.4 ± 0.1‰ ($n = 15$). The mean $\delta^{13}C$ sample reproducibility of the fish and resource samples was ±0.1‰ ($n = 12$). These measurements for the $\delta^{13}C$ values of the standards and sample reproducibility were within the acceptable ±0.2‰ range. The international standards USGS-40 (accepted value, −4.5‰) and USGS-41 (accepted value, +47.6‰) were also used to calibrate the $\delta^{15}N$ values for atmospheric nitrogen. Internal keratin (accepted value, +6.4‰) and the international IAEA-N2 (accepted value, +20.3‰) reference standards were used to verify measurement precision and accuracy. The keratin standards had a $\delta^{15}N$ of $+6.4 \pm 0.2$‰ ($n = 34$) and IAEA-N2 had a $\delta^{15}N$ of +20.5 ± 0.4‰. Additionally, the reproducibility of fish and resource samples was ±0.2‰ across 12 replicate samples. Therefore, the $\delta^{15}N$ standards and sample replicates were also generally within the acceptable ±0.2‰ range for accuracy and reproducibility.

Atomic carbon to nitrogen ratios greater than 4 are considered to indicate high levels of lipids within a given tissue, which may decrease $\delta^{13}C$ to values below those of pure tissue protein (Pörtner 2002; Fry et al. 2003; Boecklen et al. 2011). This "lipid effect" can be compensated for mathematically with a mass balance correction (e.g., McConnaughey and McRoy 1979; Fry et al. 2003). We corrected our $\delta^{13}C$ values using the lipid normalization equation developed by McConnaughey and McRoy (1979) and refined by Kiljunen et al. (2006) for aquatic organisms:

$$\delta^{13} = \delta^{13}C' + D \times \left(I + \frac{3.90}{1 + 287/L}\right),$$

where

$$L = \frac{93}{1 + (0.246 \times C : N - 0.775)^{-1}}.$$

In these equations, $\delta^{13}C'$ is the lipid-corrected value of a sample, $\delta^{13}C$ is the measured value of a sample, D is the isotopic difference between lipid and pure protein (7.02‰; Kiljunen et al. 2006), I is a constant (0.05; Kiljunen et al. 2006), and L is the estimated lipid content of the sample based on its measured atomic ratio of carbon to nitrogen (C : N).

To compare the $\delta^{13}C$ or $\delta^{15}N$ values of eggs over the four sampling periods (days 0, 1, 2, and 3), one-factor repeated-measures analysis of variance (ANOVA) was used (dependent factor: $\delta^{13}C$ or $\delta^{15}N$ value; within-subjects factor: collection day; random effect: individual female) with subsequent Tukey's post hoc comparisons when appropriate. The repeated-measures ANOVA included only crosses for which viable eggs were collected at all four sampling points. This criterion removed three samples from the analysis due to egg mortality on day 2 or day 3. If the assumption of sphericity was violated for either of the repeated-measures ANOVAs, the Greenhouse–Geisser correction was applied (Greenhouse and Geisser 1959). One-factor ANOVAs were then used to compare the three tissues (white muscle, liver, or eggs) collected from females on day 0 (dependent factor: $\delta^{13}C$ or $\delta^{15}N$ value; independent factor: tissue type; random effect: individual female). We then used analysis of covariance (ANCOVA) to test whether the correlation between the isotopic compositions of the maternal tissues and the eggs differed across the sampling days (dependent factor: egg isotopic composition; covariate: maternal tissue isotopic composition; independent factor: sampling point). To determine whether there was a relationship between maternal tissues (white muscle or liver) and the egg values, we developed linear regression models for each sampling day.

The linear regression analyses from above were used to generate tissue-specific correction formulas for both $\delta^{13}C$ and $\delta^{15}N$ to estimate the values of maternal tissues based on the

isotopic compositions of the eggs, that is,

$$\delta X_{\text{maternal}} = a + \delta X_{\text{nonlethal}} \times b,$$

where $\delta X_{\text{maternal}}$ is the estimated isotope value (δ^{13}C or δ^{15}N) for the maternal tissue of interest (white muscle or liver), $\delta X_{\text{nonlethal}}$ is the measured isotope value of the eggs, a is the intercept and b is the slope of the tissue- and isotope-specific regression analysis (Jardine et al. 2011; Fincel et al. 2012; Hette-Tronquart et al. 2012).

We measured the proportion of littoral and pelagic resource use based on the tissues collected using two-source mixing models in SIAR (Stable Isotope Analysis in R; R version 3.0.1; R Development Core Team, Vienna) to provide estimates based on the isotopic composition of the two maternal tissues, the measured isotopic composition of the eggs, and the tissue-specific corrected egg values for each sampling day (Parnell et al. 2010). "Consumer" values were represented in the model using the tissue δ^{13}C and δ^{15}N measurements for each female. The "source" values for the mixing model were represented by the mean measured δ^{13}C and δ^{15}N values $\pm$ 1 SD of snails (Gastropoda) and zooplankton (Cladocerans and Copepoda) to represent the littoral and pelagic resource pools, respectively (Post 2002; also see the Supplement in the online version of this article). Mean trophic enrichment factors of 0.5 ± 1.2‰ (δ^{13}C) and 5.0 ± 1.5‰ (δ^{15}N) used in other studies of temperate freshwater fishes (Vander Zanden and Rasmussen 2001; Locke et al. 2014) were used as the enrichment factors in the mixing model because Bluegill-specific trophic enrichment factors are unavailable. We report the results of the SIAR mixing model in terms of 95% Bayesian credibility intervals.

We estimated the additional error introduced into the isotope measures by correcting the isotopic composition of eggs to that of the maternal tissues by means of the analytical error obtained using replicate tissue samples following the procedure outlined by Jardine et al. (2011). Briefly, we used the difference between the standard deviation of the mean difference in isotopic composition between tissues (i.e., eggs and white muscle or eggs and liver) and the standard deviation of tissue samples analyzed in duplicate (reported as sample reproducibility above) and report this as the additional error associated with the correction of egg isotopic composition.

Statistical analyses were completed using JMP version 10.0.0 (SAS Institute, Cary, North Carolina) or R software (R version 3.0.1; R Development Core Team, Vienna) with $\alpha = 0.05$ for all tests. Means are reported plus or minus one standard deviation.

RESULTS

Across the four egg collection times, there was no change in the δ^{13}C values of the eggs (repeated-measures ANOVA; $F_{1.31,\ 19.66} = 3.07$, $P = 0.09$; Figure 1a). In contrast, there was a significant, albeit slight, change in the δ^{15}N values ($F_{1.87,\ 28.00} = 8.43$, $P = 0.002$; Figure 1b); post hoc comparisons indicated that the values on days 0, 1, and 2 were not different from each other (Tukey's test; all $P > 0.05$) but that all were statistically different from those on day 3 (all $P < 0.05$). (Full details of the isotopic composition measures for females and their eggs are available in Supplementary Table S.1)

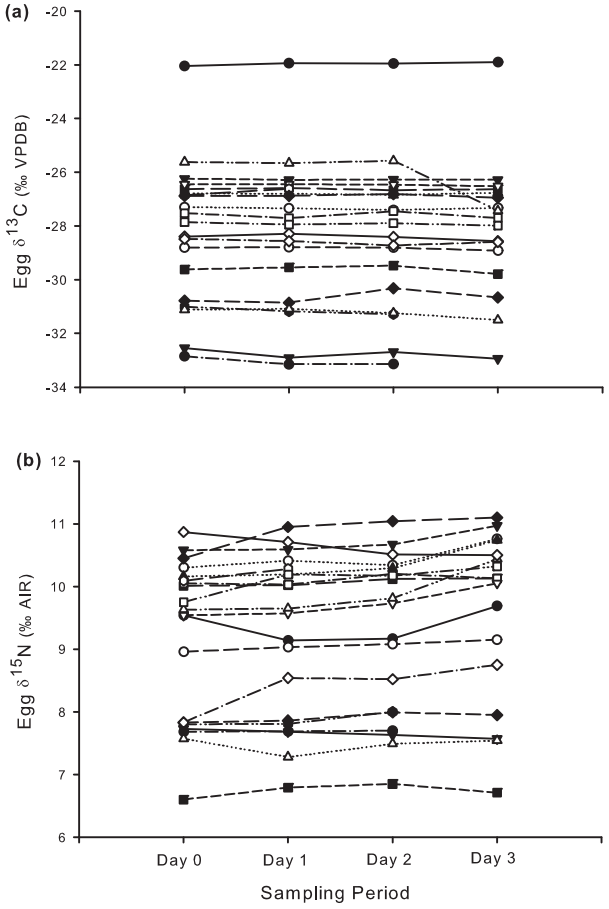

FIGURE 1. Stable isotopic composition of Bluegill eggs during embryo development. Shown are measurements of **(a)** δ^{13}C and **(b)** δ^{15}N for eggs sampled prior to fertilization (day 0) and daily until hatching (days 1, 2, and 3). Each line represents the data for one female ($n = 20$). Carbon isotope values were calibrated to Vienna Pee Dee Belemnite (VPDB) and nitrogen values were calibrated to atmospheric nitrogen (AIR).

On day 0 there were significant differences between the δ^{13}C values of the maternal tissues and those of the eggs (ANOVA; $F_{2,\ 57} = 4.0$, $P = 0.02$). Based on post hoc comparisons, the δ^{13}C values of white muscle tissue were significantly higher than those of eggs (by 1.9 ± 2.0‰; Tukey's test; $P < 0.05$), but those of the liver and eggs were similar ($P = 0.24$). Similarly, the δ^{15}N values differed significantly among the tissues on day 0 (ANOVA; $F_{1.30,\ 23.36} = 66.1$, $P < 0.001$), with white muscle having significantly higher values than both liver (by 1.9 ± 0.9‰) and eggs (by 0.68 ± 0.9‰; Tukey's test; both $P < 0.001$), but the difference between liver and eggs was nonsignificant ($P > 0.99$).

Using analysis of covariance (ANCOVA) to examine the association between the isotopic compositions of eggs and that of maternal tissues revealed no effect of sampling day ($\delta^{13}C$: both $F_{3,\ 68} \leq 0.08$, $P \geq 0.97$; $\delta^{15}N$: both $F_{3,\ 68} \leq 2.40$, $P \geq 0.08$) or the interaction between sampling day and egg isotopic composition ($\delta^{13}C$: both $F_{3,\ 68} \leq 0.24$, $P \geq 0.87$; $\delta^{15}N$: both $F_{3,\ 68} \leq 0.12$, $P \geq 0.95$). Linear regression models indicated that the $\delta^{13}C$ values of the maternal tissues were related to those of the eggs on day 0 (Table 1; Figure 2a); similarly, the $\delta^{15}N$ values of the maternal tissues were related to those of the eggs on that day (Table 1; Figure 2b). Similar regression results were found for all other sampling periods (see Table 1 for details).

Two-source SIAR mixing models comparing the littoral and pelagic contributions to Bluegill diets indicated that pelagic resources comprised 80% (white muscle) or 91% (liver) of diets based on maternal tissue samples. Mixing model estimates based on the measured values of eggs indicate that pelagic resources represented 94% of diets across the four sampling intervals (Figure 3). Estimates based on tissue-specific corrected egg $\delta^{13}C$ and $\delta^{15}N$ values indicate that on average pelagic resources comprised 78% (white muscle) or 89% (liver) of diets and were within 1–7% of the values reported for the direct tissue estimates for each sampling day (Table 2). Additionally, the 95% Bayesian credibility intervals reveal overlap in the estimated resource group proportions for both maternal tissues and eggs (corrected isotopic compositions), though there was greater overlap for liver and egg estimates (Figure 3; Table 2).

The additional error values associated with the tissue-specific correction formulas for eggs were consistently greater for white muscle than for liver (Table 1). White muscle error rates increased an average of 2.0‰ and 0.7‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, across the four egg sampling periods. Liver error rates increased an average of 0.8‰ and 0.2‰, respectively.

DISCUSSION

In the field of fish ecology there has been interest in using stable isotope analysis of eggs to infer maternal diet. Such inference requires that the isotopic composition of eggs be relatively constant over the egg developmental period and that it be similar to the isotopic composition of other maternal tissues commonly used to infer diet (i.e., white muscle and liver). With Bluegills we found that over the 3-d egg development period (from immediately prior to fertilization to the day of hatching) the stable isotopic composition of the eggs did not change for carbon and increased only slightly for nitrogen (by 0.3‰ between day 2 and day 3). This increase in the $\delta^{15}N$ value is considered inconsequential compared with the

TABLE 1. Summary of linear regression analyses of tissue carbon and nitrogen isotopic composition in tissues and eggs of Bluegills. The regressions relate the stable isotope value ($\delta^{13}C$ or $\delta^{15}N$) of white muscle or liver (dependent variable) to that of eggs (independent variable); df = 1, 18 for all regressions.

Element and sampling day	R^2	F	P	Egg correction formula	Mean difference (egg – tissue)	Additional error
Carbon						
White muscle						
Day 0	0.43	13.62	0.002	Corrected $\delta^{13}C = 0.40 \times$ egg $\delta^{13}C - 15.0$	-1.9 ± 2.0‰	1.9‰
Day 1	0.43	13.33	0.002	Corrected $\delta^{13}C = 0.39 \times$ egg $\delta^{13}C - 15.5$	-1.9 ± 2.0‰	1.9‰
Day 2	0.43	12.97	0.002	Corrected $\delta^{13}C = 0.40 \times$ egg $\delta^{13}C - 15.1$	-2.0 ± 2.0‰	1.9‰
Day 3	0.39	9.73	0.007	Corrected $\delta^{13}C = 0.42 \times$ egg $\delta^{13}C - 14.1$	-1.8 ± 1.9‰	1.9‰
Liver						
Day 0	0.94	280.33	<0.001	Corrected $\delta^{13}C = 0.75 \times$ egg $\delta^{13}C - 6.0$	-1.1 ± 0.8‰	0.7‰
Day 1	0.93	255.21	<0.001	Corrected $\delta^{13}C = 0.72 \times$ egg $\delta^{13}C - 6.7$	-1.1 ± 0.9‰	0.8‰
Day 2	0.94	258.64	<0.001	Corrected $\delta^{13}C = 0.74 \times$ egg $\delta^{13}C - 6.4$	-1.1 ± 0.9‰	0.8‰
Day 3	0.93	187.68	<0.001	Corrected $\delta^{13}C = 0.77 \times$ egg $\delta^{13}C - 5.2$	-1.1 ± 0.8‰	0.7‰
Nitrogen						
White muscle						
Day 0	0.46	15.28	<0.001	Corrected $\delta^{15}N = 0.40 \times$ egg $\delta^{15}N + 6.2$	-0.7 ± 0.9‰	0.7‰
Day 1	0.51	18.77	<0.001	Corrected $\delta^{15}N = 0.42 \times$ egg $\delta^{15}N + 6.0$	-0.6 ± 0.9‰	0.7‰
Day 2	0.53	18.96	<0.001	Corrected $\delta^{15}N = 0.43 \times$ egg $\delta^{15}N + 5.8$	-0.6 ± 0.9‰	0.7‰
Day 3	0.47	13.43	0.002	Corrected $\delta^{15}N = 0.40 \times$ egg $\delta^{15}N + 6.1$	-0.3 ± 1.0‰	0.8‰
Liver						
Day 0	0.91	179.90	<0.001	Corrected $\delta^{15}N = 1.04 \times$ egg $\delta^{15}N - 1.6$	1.3 ± 0.4‰	0.2‰
Day 1	0.93	251.48	<0.001	Corrected $\delta^{15}N = 1.03 \times$ egg $\delta^{15}N - 1.6$	1.3 ± 0.4‰	0.2‰
Day 2	0.95	302.91	<0.001	Corrected $\delta^{15}N = 1.07 \times$ egg $\delta^{15}N - 2.0$	1.4 ± 0.3‰	0.1‰
Day 3	0.92	177.56	<0.001	Corrected $\delta^{15}N = 0.99 \times$ egg $\delta^{15}N - 1.5$	1.6 ± 0.4‰	0.2‰

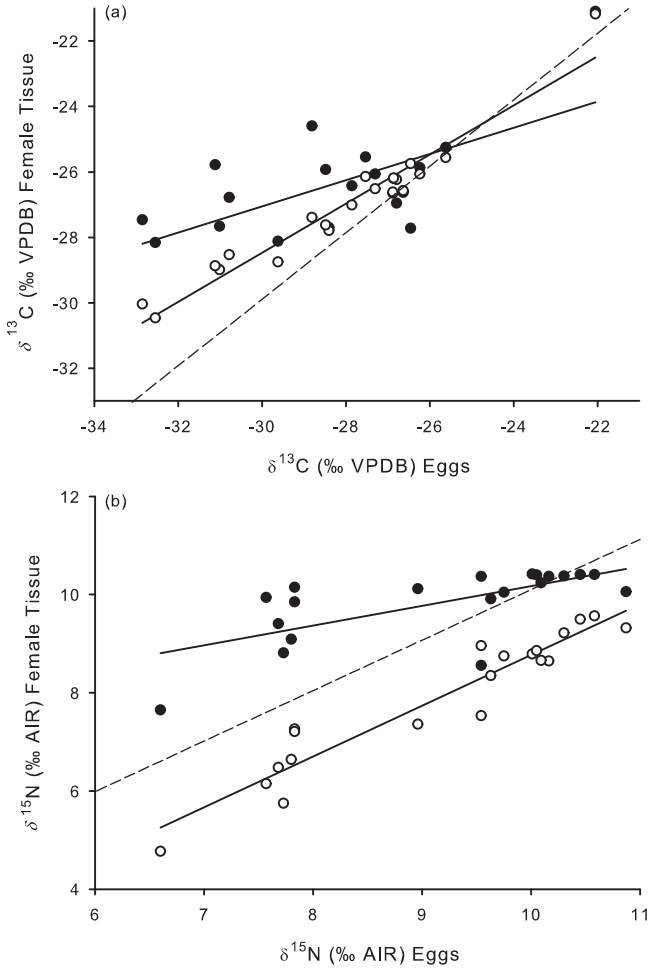

FIGURE 2. Isotopic composition—**(a)** δ^{13}C and **(b)** δ^{15}N—of Bluegill eggs compared with that of two maternal tissues: white muscle (filled circles) and liver (open circles); $n = 20$. The dashed line indicates a 1:1 ratio between the isotopic composition of eggs and maternal tissues.

TABLE 2. Summary of SIAR two-source mixing model estimates of Bluegill diet based on δ^{13}C and δ^{15}N values. The results are for directly sampled maternal tissues (white muscle and liver), measured egg values, and tissue-specific corrected egg estimates. The data are mean estimates with 95% Bayesian credibility intervals in parentheses.

Tissue	Proportion littoral	Proportion pelagic
White muscle	0.20 (0.09–0.31)	0.80 (0.69–0.91)
Corrected egg day 0	0.22 (0.12–0.32)	0.78 (0.68–0.88)
Corrected egg day 1	0.17 (0.07–0.27)	0.83 (0.73–0.93)
Corrected egg day 2	0.21 (0.10–0.31)	0.79 (0.69–0.90)
Corrected egg day 3	0.27 (0.16–0.38)	0.73 (0.62–0.84)
Liver	0.09 (0–0.19)	0.91 (0.81–1.0)
Corrected egg day 0	0.10 (0–0.20)	0.90 (0.80–1.0)
Corrected egg day 1	0.11 (0–0.21)	0.89 (0.79–1.0)
Corrected egg day 2	0.08 (0–0.18)	0.92 (0.82–1.0)
Corrected egg day 3	0.15 (0.02–0.28)	0.85 (0.72–0.98)
Eggs		
Corrected egg day 0	0.06 (0–0.15)	0.94 (0.85–1.0)
Corrected egg day 1	0.06 (0–0.16)	0.94 (0.84–1.0)
Corrected egg day 2	0.06 (0–0.17)	0.94 (0.83–1.0)
Corrected egg day 3	0.06 (0–0.15)	0.94 (0.85–1.0)

differences found across trophic levels, which are typically more than 3‰ (Post 2002; Anderson and Cabana 2007). Indeed, the δ^{15}N increase in day-3 eggs did not change mixing model estimates of diet compared with those for the other egg sampling days. We also found strong correlations between the isotopic composition of eggs and that of both white muscle and liver tissues. The strength of these correlations was similar at each sampling point during egg development. And, although there were absolute differences in the stable isotopic compositions of the egg and the other maternal tissue, these differences were relatively small and did not affect the estimates of resource use; the SIAR mixing models produced similar estimates of diet across eggs and maternal tissues, particularly between liver and eggs. Thus, our data support the use of eggs to infer maternal diets during the breeding season.

Tissues with large amounts of lipid stored within them can bias stable isotope analysis of resource use. Lipids typically contain lower levels of ^{13}C than other organic macromolecules (i.e., proteins and carbohydrates), in part because of isotopic fractionation during the initial steps of lipid synthesis (DeNiro and Epstein 1977; Sweeting et al. 2006). This bias may lower the δ^{13}C values of lipid-rich tissues, leading to estimates of resource use that favor the pelagic food groups (e.g., zooplankton). Across fish species, yolk not only constitutes the majority of egg mass but also contains large quantities of lipid that is being stored for use during development (Hemming and Buddington 1988; Kamler 2005). Consistent with past studies of lipid-corrected tissues, we found that Bluegill eggs had δ^{13}C values that were 1–2‰ lower than the maternal white muscle and liver tissues (see Grey 2001; Snowberg and Bolnick 2008). The mathematical correction we applied is based on the total quantity of lipids in a sample. Hence, the postcorrection offset we observed between the δ^{13}C values of maternal tissues and those of eggs is likely the result of metabolic processes within the eggs that utilized ^{13}C-depleted lipids transferred from the female. This altered isotopic composition was then carried forward into the tissues independent of lipid quantity. Even so, the lower δ^{13}C values that we observed in the eggs had little impact on the results of the SIAR mixing models of resource use due to the strength of the linear relationships, which in turn allowed us to make accurate corrections to the egg isotopic compositions. Thus, our data indicate that although eggs have lower δ^{13}C values than maternal tissues, the effect on inferences of resource use is minimal.

Our data show a difference in the strength of the correlations between the isotopic composition of the eggs and those of the other two maternal tissue types, the correlation with

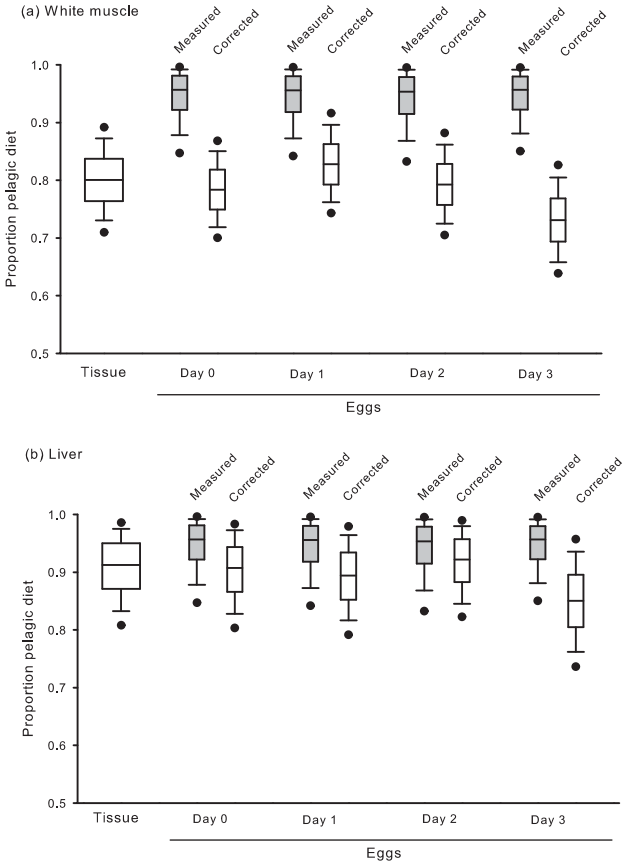

FIGURE 3. SIAR stable isotope mixing model estimates of the pelagic contribution to the diet of female Bluegills based on different tissues. Estimates based on the isotopic composition of **(a)** white muscle or **(b)** liver maternal tissues are presented, along with estimates based on the measured egg $\delta^{13}C$ and $\delta^{15}N$ and tissue-specific corrected egg values based on linear regressions for each sampling day (see Methods). The boxes represent the central 50% of observations, with the means being represented by the lines within each box. The 10th and 90th percentiles are indicated by the whiskers and the 5th and 95th percentiles by the dots.

liver being notably stronger than that with white muscle. This difference may reflect the egg development process. Yolk production, referred to as vitellogenesis, involves the mobilization of lipids from the liver to the eggs that are then used in the production of egg tissues (Wiegand 1996; Dembski et al. 2006). In Bluegills, vitellogenesis occurs from mid-May to June (Keast 1978), which coincides with our time of egg sample collection. Given the important relationship between liver and eggs, strong isotopic correlations between these tissues should be expected. White muscle, in contrast, does not have an active role in providing energy to the eggs, and also reflects diet over a longer period than liver tissue (Buchheister and Latour 2010). Both of these attributes could erode the correlation between the isotopic composition of white muscle and that of eggs, leading to the lower correlation coefficients that we obtained. Overall, our results indicate that with respect to the determination of isotopic composition in Bluegills, eggs are likely best treated as a substitute for liver tissue.

Given that the isotopic composition of eggs reflects female diets during the prespawn period, our results also speak directly to the foraging ecology of Bluegills during the few weeks prior to breeding, when eggs are developed and supplied with yolk (Keast 1978; Dembski et al. 2006). Our analysis of maternal white muscle indicates greater consumption of littoral resources in the period prior to collection, i.e., during the previous fall. Indeed, stomach content analysis of the same population of Bluegills has shown that the consumption of littoral invertebrates is greater during the fall months, probably due to an increase in littoral prey abundance during that period (Keast 1978). By contrast, our analysis of eggs and liver shows that in the spring, just before the onset of breeding, females instead relied more on pelagic resources and that these resources provided the energy required for vitellogenesis. The lower use of pelagic resources indicated by white muscle tissue is likely a product of the longer time period reflected in that tissue.

The results of our specific experiment indicate that eggs can be used as a substitute for maternal tissues in stable isotope studies. In this article, we have suggested a general approach to be used to properly substitute eggs for other tissues. First, knowledge of the energy allocation process to eggs will provide important information about the time frame over which eggs are likely to reflect diet. In Bluegills energy is invested in the eggs as it is gathered in the spring, but other temperate fishes (such as White Crappie *Pomoxis annularis* and Black Crappie *P. nigromaculatus*) are capital spawners that draw on body stores of energy gathered over an extended period of time (Beuchel et al. 2013). Indeed, the eggs of capital breeders may be more similar to muscle in isotopic composition than those of income breeders are. Second, for species in which the relationship between eggs and maternal tissues is unknown, we recommend sampling the standard tissues (e.g., liver) of a subset of females to determine the species-specific relationship between the tissues and the additional error associated with substituting eggs. Third, it is important to consider that foraging can differ within species based on sex, age, and breeding status (e.g., Colborne et al. 2013). For this reason, other tissue sampling techniques may still be required to accurately infer the diet of entire populations and not just that of reproductively mature females. By considering these factors when designing experiments, researchers can reduce their impact on populations by using nonlethal sampling techniques without affecting research quality.

In conclusion, we have shown that female Bluegills rely on pelagic food resources for yolk production during the prebreeding period. Additionally, the results of our experiment indicate that the stable isotopic compositions of fish eggs are a reliable measure of female resource use over a time period similar to that of liver tissue. The nonlethal collection of eggs should reduce the impact on populations associated with lethal tissue-sampling methods.

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