Wetlands (2015) 35:349–356
DOI 10.1007/s13157-015-0624-8

ORIGINAL RESEARCH

Efficacy of Labeling Wetlands with Enriched ^{15}N to Determine Amphibian Dispersal

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Received: 5 August 2014 / Accepted: 6 January 2015 / Published online: 17 January 2015

Abstract Stable isotope enrichment techniques can aid in understanding dispersal of animals. Pond-breeding amphibians often have spatially disjunct populations that depend on immigration for persistence, yet obtaining direct estimates of dispersal rates among wetlands is challenging. We enriched aquatic mesocosms with ^{15}N to "mark" amphibian larvae and determine the feasibility of using enrichment techniques to study dispersal in pond-breeding amphibians. Because newly metamorphosed amphibians in mark-release-recapture studies may not be recaptured until adulthood, we estimated persistence of the ^{15}N enrichment signature up to 3 years post-metamorphosis. We reared larval marbled salamanders (*Ambystoma opacum*) in artificial mesocosms dosed with $^{15}NH_4Cl$, and maintained metamorphs on unlabeled prey for 7 months to estimate the biological half life (*BHL*) of ^{15}N in tissue. Metamorphs in spiked treatments attained $\delta^{15}N$ levels >1000 times higher than reference animals (5±1‰), and levels remained ~225 times higher than controls after 7 months. The average ^{15}N *BHL* was 2.49±0.24 months, indicating that the elevated signature should be discernible for a minimum of 20–28 months after metamorphosis. Our results suggest that ^{15}N enrichment is feasible for field studies of amphibian dispersal, as metamorphs will retain isotope-enriched tissues that persist until at least the second year of breeding.

Keywords *Ambystoma* · Connectivity · Isolated wetland · Metapopulation · Spiked nitrogen isotopes

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Introduction

Data that characterize movement and dispersal patterns of animals at different spatial and temporal scales are essential to understand their ecology, and such data are prerequisites for effective conservation and management efforts (Rubenstein and Hobson 2004). Metapopulation processes appear to describe the dynamics of a variety of taxa that are spatially subdivided among discrete habitat patches. At a landscape scale, movements among local populations are essential for recolonization after extinction, and ultimately the persistence of the metapopulation (Levins 1969; Hanski and Gaggiotti 2004). Thus, estimating immigration/dispersal rates facilitates an understanding of broad spatial patterns and is a primary goal of metapopulation studies (Hanski et al. 1994; Marsh and Trenham 2001). As most habitats become more fragmented, knowledge of the factors influencing population connectivity for taxa with varied life histories is crucial for their conservation (Zamudio and Wieczorek 2007). Although many metapopulation studies to date have focused on invertebrates (see Hanski and Gaggiotti 2004), some recent studies have examined amphibians in a metapopulation context (e.g., Trenham et al. 2001; Greenberg and Tanner 2005; Gamble et al. 2007).

Pond-breeding amphibian populations are both spatially disjunct and highly dynamic, with local populations

exhibiting large fluctuations (Semlitsch et al. 1996; Gamble et al. 2007); they are generally thought to function as metapopulations (Alford and Richards 1999; but see Smith and Green 2005). Although terrestrial juveniles and adults are capable of long-distance movements, many amphibians appear to have limited dispersal and high natal site fidelity (Gamble et al. 2007; Scott et al. 2013). Despite their relative philopatry, small percentages of juvenile and adult amphibians do disperse to new sites (Scott 1994; Gamble et al. 2007). In ambystomatid salamanders, new immigrants may be necessary for population persistence given the observed levels of larval mortality, density dependence, adult survival rates, and breeding frequencies (Taylor and Scott 1997; Taylor et al. 2006). Although advances have been made in describing amphibian metapopulation dynamics, little is known about the underlying processes that govern it (Gamble et al. 2007), in part because of challenges associated with obtaining direct estimates of dispersal rates among wetland breeding sites.

Direct and indirect approaches have been used to study dispersal-related questions in a variety of taxa (Slatkin 1985; Rubenstein and Hobson 2004). Direct techniques to estimate dispersal, such as mark-release-recapture (MRR) studies, require simultaneous sampling at multiple sites, and often have low recapture rates. In the extreme, some studies of bird migrations with more than one million birds banded on breeding grounds have had fewer than ten recaptures on wintering grounds (Webster et al. 2002). In amphibians, MRR studies typically employ drift fences that completely encircling breeding sites (*sensu* Gamble et al. 2007); these studies are labor-intensive, and may be made more difficult by the boom-or-bust nature of amphibian recruitment, in which juvenile production is very low in many years (Semlitsch et al. 1996), but exceptionally high under certain environmental conditions (e.g., Gibbons et al. 2006). Additionally, standard MRR studies tend to underestimate rare events such as long-distance dispersal unless all possible dispersal sites are monitored (Porter and Dooley 1993). Studies that rely solely on extrinsic markers (e.g., banding, toe-clipping) to track movements have a low probability of finding long-distance dispersers, and in some instances the technique itself may affect dispersal behavior (Rubenstein and Hobson 2004). Both genetic and biogeochemical techniques offer valuable complements to large-scale MRR studies.

Stable isotope signatures of organism tissues have been used in a variety of systems to track animal movements (Rubenstein and Hobson 2004). In most isotope studies at regional or landscape scales, natural variation in the isotopic signatures of different habitats enables one to determine the origin of an individual, thereby documenting movement/migration between sites with different isotopic signatures (e.g., Graves et al. 2002; Hobson et al. 2012). Even at a local scale, one may be able to distinguish organisms from ephemeral wetlands vs. permanent lakes (France and Schlaepfer 2000). However, for the purpose of tracking fine-scale movements among a suite of nearby (<10 km) isotopically similar isolated wetlands, the natural variation in $^{13}C/^{12}C$, $^{15}N/^{14}N$, and other isotope ratios can be insufficient to distinguish the wetland of origin for animals that have been out of the aquatic habitat for several months or longer, such as juvenile and non-breeding adult amphibians residing in terrestrial habitat.

Several studies of invertebrate dispersal have used isotope enrichment techniques in aquatic habitats to artificially elevate levels of a stable isotope, which becomes incorporated in the food chain and alters the natural range of stable isotopes of organisms in the habitat. The technique has worked well for aquatic insects (Caudill 2003; Briers et al. 2004), because millions of aquatic invertebrates can be marked, and their dispersal patterns determined via subsequent isotope analysis of adults captured at other (non-enriched) sites. For example, the use of an artificially enriched nitrogen stable isotope (where $^{15}N/^{14}N$ is greater than known natural abundance patterns) to mark large numbers of stoneflies resulted in the discovery of low levels of long-range dispersal among populations, which would otherwise have been undetectable using direct trapping methods (Briers et al. 2004).

In this study we investigated the efficacy of using an isotope spiking technique to mark amphibians (as aquatic larvae) to determine among-pond movements in the post-metamorphic terrestrial stage. In contrast to invertebrates—such as mayflies and stoneflies—that are recaptured as adults within weeks of leaving the spiked aquatic habitat, newly metamorphosed amphibians may not be recaptured until adulthood one or more years later. Thus, for the technique to be useful in understanding the source of immigrants to a focal wetland, recently metamorphosed animals must leave isotopically enriched sites with a stable isotope signature that will persist for at least 1 year. Our objective in this study was to use aquatic mesocosms to test the feasibility of isotope marking in large-scale field studies of dispersal.

Materials and Methods

Experimental ^{15}N Enrichment

In October 2004 we collected 20 pairs of immigrating adult marbled salamanders (*Ambystoma opacum*) at a natural wetland (Ginger's Bay) on the Department of Energy's Savannah River Site (SRS) in Aiken County, South Carolina. We placed adults in outdoor soil-filled tanks, where females laid eggs terrestrially in early November. On 13–15 February 2005 we created aquatic communities in nine 1.73-m diameter (1300-L volume) polyethylene tanks by adding 1000 L of well water, 1.5 kg of leaf litter from a mixed deciduous forest, and zooplankton stock from three seasonal wetlands. We collected salamander eggs in late February 2005, mixed clutches to

homogenize genetic variation, and hatched the embryos in mesocosm water on 25–27 February. We added salamander hatchlings to the nine tanks on 5 March (n=27 per tank); zooplankton communities were well established when we added the hatchlings. We added newly hatched southern leopard frogs (*Lithobates (Rana) sphenocephalus* Cope; n=70) from Bay 58 (Barnwell County SC) as an additional food resource to each tank on 25 March. We housed our experimental tanks at the Savannah River Ecology Laboratory (SREL) mesocosm facility on the SRS.

We randomly assigned tanks to one of three treatments (n= 3 replicates each): no ^{15}N addition (control), a single ^{15}N dose added early in the larval period (29 March), and two half-strength doses added on 29 March and later in the larval period, 19 April. For the ^{15}N addition tanks we added a total of 445 mg of $^{15}NH_4Cl$ (^{15}N 99 %; 0.196 g/m^2) in either one or two doses. Our objective was to achieve a $\delta^{15}N$ value in metamorphosed salamanders that would be recognizable after 6–18 months, the time interval before most are mature (Jensen et al. 2008) and ready to breed (and therefore captured at drift fences as they enter a breeding site); such an endpoint in metamorphosed salamanders would be ~600 times higher than the values observed in nature (~4–8‰) on the SRS (Willson et al. 2010).

Salamanders began to metamorphose from tanks in early May. We euthanized (3 % MS-222 solution) two to five newly metamorphosed *A. opacum* from each tank to estimate $\delta^{15}N$ values at metamorphosis (n=27). We reared the remaining metamorphs from the three aquatic treatments in individual 144-cm^2 containers in the laboratory for up to 7 months, during which time we fed salamanders small unlabeled crickets (~4–6 per week). After 6 weeks feeding on unlabeled prey, we sacrificed a second batch of juveniles (1–10 juvenile *A. opacum* derived from each aquatic tank; n=52), and repeated this procedure again for our final time point at 7 months (n=36). We triple-washed samples in millipore water and placed them in 40-ml vials stored at −20 °C until isotopic analyses were conducted.

Isotopic Analysis

For the isotope analysis of *A. opacum* metamorphs and juveniles we clipped approximately 3 mm of tail tissue and dried the tissue samples for 2 days at 40 °C. Using an ultra-microblalance, we loaded approximately 2 mg (±1 μg) of each dried tissue sample into tin capsules. We sealed capsules and placed them in the autosampler of a Carlo Erba Elemental Analyzer NA 2500 (Milan), attached to a continuous flow isotope ratio mass spectrometer [Finnigan Delta^{PLUS} XL (Finnigan-MAT, San Jose, CA)] for nitrogen isotope analysis. Samples were converted to N_2 in oxidation/reduction furnaces, purified by gas chromatography, and then $^{15}N/^{14}N$ ratios were measured on N_2 using the isotope ratio mass spectrometer. An internal $N_{2(g)}$ working standard was admitted before the introduction of each sample for calibration to AIR (nitrogen) international standard (Mariotti 1983; Coplen 1996). We report our isotopic data using standard delta (δ) notation, where δ values represent the ratio of the rare to common isotopes in a sample normalized to the same ratio in a standard minus 1 and then multiplied by 1000. Thus, delta values are expressed in parts per thousand (‰; Craig 1957). Several in-house working standards of known isotope value vs AIR were placed among samples to determine external precision during the sequential analysis of samples; these standards were reproducible to better than ±0.1‰ (1σ standard deviation) for $\delta^{15}N$ (n=7).

Data Analysis

Using the natural-log transformed values of $\delta^{15}N$ in a two factor analysis of variance (ANOVA) model, the within-group residuals were normally distributed (Shapiro-Wilk test, p>0.19 for all ^{15}N groups) and showed equality of variances (Levene's test, p>0.18). Therefore, we used parametric tests on log-transformed $\delta^{15}N$ values to test the effects of treatment (no ^{15}N addition, one full dose of ^{15}N, or two half doses) and time period (at metamorphosis, 6-week post metamorphosis, 7-month post metamorphosis) on $\delta^{15}N$ values in post-metamorphic *A. opacum* (PROC GLM, SAS 2011). We used the tank(treatment) term as the error term to test for a treatment effect, and *a posteriori* Bonferroni-corrected pairwise comparisons of least squares treatment means to test overall $\delta^{15}N$ differences among groups.

We plotted the log-transformed values of $\delta^{15}N$ for the ^{15}N-spiked treatments versus the sample time (month). Visual inspection of the plot showed the relationship was linear, and regression analysis produced a high R^2 (0.91), so we modeled the biological elimination rate constant (i.e., *BER*, the loss rate of $\delta^{15}N$) as an exponential decay using a non-linear regression model (PROC NLIN, SAS 2011). We estimated the *BER* for each of the ^{15}N-spiked tanks, and calculated the biological half-life (*BHL*) of $\delta^{15}N$ for salamanders from each tank as $[ln(2)]/BER$.

The exponential rate of elimination of ^{15}N in the spiked treatments is due to both growth (i.e., new tissue) and metabolic tissue replacement (Hesslein, Hallard, and Ramlal 1993). We determined growth rate (k) of the newly metamorphosed salamanders from each tank using an exponential model (PROC NLIN, SAS 2011), and estimated metabolic turnover (m) as the difference $BER - k = m$. Due to the small sample size in each treatment group (n=3 tanks per group), we used a Kruskal-Wallis nonparametric ANOVA to compare *k, m, BER* and *BHL* differences between the single-dose and two-dose treatments (PROC NPAR1WAY, SAS 2011).

Results

At metamorphosis, $\delta^{15}N$ values in *A. opacum* from the control treatment (no ^{15}N added) averaged 5±1.0‰ (n=13; Fig. 1), which is in the range of values of wild-caught pond-breeding salamanders in the study area (Willson et al. 2010). The $\delta^{15}N$ values at metamorphosis in the two spiked treatments were significantly elevated compared to controls (Table 1), averaging 9452±951‰ (n=10) in the single-dose treatment and 7351±1191‰ (n=4) in the two-dose treatment. At 6 weeks post-metamorphosis, juvenile *A. opacum* from ^{15}N-addition treatments averaged $\delta^{15}N$ values that were nearly 1000 times greater than controls (5229‰ compared to 5.5‰). Seven months after metamorphosis, $\delta^{15}N$ values remained extremely high (1767‰) in juveniles from the spiked tanks, averaging approximately 225 times greater than in controls.

Modeled as an exponential decay function, the biological elimination rate of ^{15}N averaged −0.2788±0.0279 month^{-1} in the ^{15}N-spike treatments; the *BER* was faster in the single-dose treatment (−0.3198±0.0069 month^{-1}) than in the two-dose treatment (−0.2000±0.0127 month^{-1}; χ^2=3.86, df=1, P<0.05). The faster *BER* in the single-dose treatment corresponded to a shorter biological half-life (2.17±0.05 month) compared to the *BHL* in the two-dose group (3.49±0.23 month). Post-metamorphic salamanders from the single-dose treatment would have a $\delta^{15}N$ value of ~18‰ after nine half-lives (19.5 month post-metamorphosis). The slower *BHL* associated with the two-dose treatment would result in a $\delta^{15}N$ value of ~15‰ after 30.5 month. An elevated $\delta^{15}N$ signature that remains discernibly above the normal level is likely to persist for at least 20 to 30 month after metamorphosis.

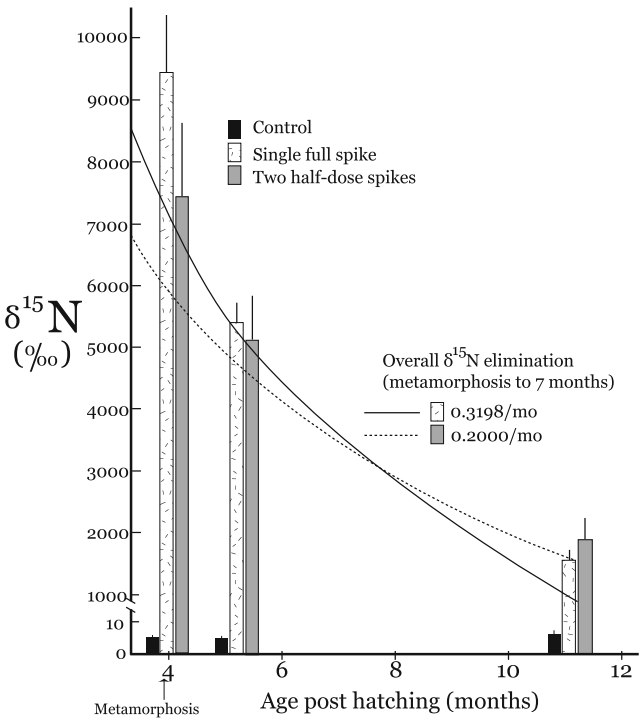

Fig 1 Mean (±1 SE) $\delta^{15}N$ values for three treatment groups of *A. opacum* at metamorphosis (approximately 4 months post-hatching), 6 weeks, and 7 months. Curves represent exponential elimination rates (log $\delta^{15}N$ lost per month) for the single full-dose and two half-dose ^{15}N-addition treatments (note break in *y-axis*). For many pond-breeding amphibian species, large proportions of a juvenile cohort mature and return to breeding sites within 6 month post-metamorphosis

Table 1 Results of analysis of variance of effects of treatment (no ^{15}N added, single full dose, or two half-dose ^{15}N additions in mesocosms) and time (at metamorphosis, 6 weeks, and 7 months post-metamorphosis) on natural log-transformed $\delta^{15}N$ values in post-metamorphic marbled salamanders (*Ambystoma opacum*). Tank(Trt) was used as error term to test for Trt effect; full model R^2=0.99

Effect	df	MS	*F*-value	*P*-value
Treatment	2	455.3	1162.7	<0.0001
Time	2	5.85	122.7	<0.0001
Trt x time	4	4.14	86.7	<0.0001
Tank(Trt)	6	0.39	8.2	<0.0001
Error	95	0.05		

Post-metamorphic growth rates (k) did not differ among the treatment groups: control (0.154±0.006 g month^{-1}), single dose (0.139±0.003 g month^{-1}), and two dose (0.121±0.015 g month^{-1}). However, decreases over time in $\delta^{15}N$ due to metabolic turnover (m) was faster (χ^2=3.86, df=1, P<0.05) in the single-dose (−0.1812±0.0080 month^{-1}) compared to the two-dose treatment (−0.0790±0.0203 month^{-1}).

Discussion

^{15}N-marked Salamanders in a Controlled Study

The elimination rates of spiked ^{15}N that we observed in this pilot study indicate that 1) ^{15}N loss is exponential and follows first order kinetics, 2) multiple partial doses may provide a more effective spike than a single full dose of ^{15}N (especially considering the spread in timing of amphibian oviposition and hatching that occurs in natural wetlands), and 3) stable isotope spikes of natural wetlands to aid in studies of amphibian dispersal are feasible. The exponential loss rate of ^{15}N reflects the switch back to unlabeled prey and the addition of new body mass (i.e., growth) that is in isotopic equilibrium with the lower $\delta^{15}N$ food, coupled with differences in assimilation and turnover rates of ^{15}N among tissues (Hesslein, Hallard, and Ramlal 1993; Warne, Gilman, and Wolf 2010). The initial rapid loss of ^{15}N may be due to loss from the gut and other organs with high turnover. Depending on the initial spike treatment, we estimate that the ^{15}N isotopic signature should persist into the third year (minimum of 20–30 months after metamorphosis) in tail tissue and presumably toe clips of spiked animals. For example, isotopes in human skeletal

tissue reflect diet over a much longer period of time than soft tissues (Hedges et al. 2007).

The stable isotope enrichment technique described here for pond-breeding salamanders has the potential to increase our understanding of dispersal among breeding sites in amphibian metapopulations, much as it has for aquatic invertebrates (Hagler and Jackson 2001). Field studies using stable isotope spiking to estimate dispersal by invertebrates have demonstrated the utility of the technique, but the natural history of the study organisms (e.g., mayflies, stoneflies) may have been key to the success of those studies, because the isotope-labeled adults bred soon after leaving the isotope-spiked aquatic habitat, and did not feed post-emergence (Caudill 2003; Briers et al. 2004). The challenge in adapting the enrichment technique to pond-breeding amphibians such as *A. opacum* is that post-metamorphic individuals dilute the enriched isotope signal by feeding on unspiked prey after leaving the enriched aquatic habitat, and these terrestrial juveniles and sub-adults will not reach maturity for at least 6 months (Scott 1994; Semlitsch et al. 1996).

The success of implementing stable isotope spikes in landscape-level field studies may vary depending upon the average age at first reproduction of focal species. Whereas large fractions (66–78 %) of adult mole salamanders (*A. talpoideum*) breed within 6 months after metamorphosis (Semlitsch et al. 1988), most *A. opacum* will not breed until 18 or 30 months after metamorphosis (at 2 or 3 years old; Scott 1994). Importantly, of the 35 species (23 anuran, 12 salamander) in our study region that use isolated seasonal wetlands for breeding, virtually all reach maturity by age three (salamander species accounts in Lannoo 2005; anurans in Dodd 2013), and therefore are candidates for ^{15}N labeling. However, an additional consideration is that females mature on average a year later than males in *A. opacum* and some other species (Scott 1994). For species with extended juvenile stages exceeding 3 years, such as the red-spotted newt (*Notophthalmus viridescens*; Caetano and LeClair 1996) and the California tiger salamander (*Ambystoma californiense*; Trenham et al. 2000), the ^{15}N enrichment technique will require species-specific testing to determine its efficacy for dispersal studies.

Implications for Use in Natural Wetlands

Assuming that spiking natural wetlands with stable isotopes is a feasible method of "marking" entire cohorts of amphibians, under what conditions would the technique be a cost-effective means of estimating dispersal rates among wetlands? The number and sizes of the wetlands targeted for enrichment, as well as population sizes of focal species, will be important determinants of the total cost of the spiking technique.

Many amphibian metapopulation studies—conducted throughout the US and Europe—that used MRR as a direct measure of dispersal were conducted in wetlands that are relatively small (<0.37 ha). In Massachusetts, Gamble, McGarigal, and Compton (2007) estimated dispersal of juvenile *A. opacum* in a system of 14 ponds ranging from 0.03 to 0.35 ha. Greenberg and Tanner (2005) examined the spatial ecology of oak toads (*Anaxyrus* [*Bufo*] *quercicus*) at eight ponds (0.1 to 0.37 ha) in Florida, but were unable to estimate juvenile dispersal because most emigrating juveniles were not marked. Trenham, Koenig, and Shaffer (2001) examined movements of *A. californiense* among 17 ponds (0.036 to 0.37 ha), Hels (2002) studied a Danish metapopulation of spadefoot toads (*Pelobates fuscus*) in five ponds (0.05–0.25 ha), and Perret et al. (2003) estimated dispersal of the alpine newt (*Ichthyosaura* [*Triturus*] *alpestris*) in two French sub-populations (total of nine ponds, 0.004 to 0.01 ha). If we assume the cost per 50 g of the stable isotope (e.g., $^{15}NH_4Cl$) is $2000, which will spike 1.12×10^5 L at the concentration we used in our mesocosms, then costs escalate rapidly as wetland area and volume increase (Fig. 2). However, because the wetland areas are relatively small in all the above studies, the cost of a single-site, single-season ^{15}N spike would be ~$5000 to $10,000 US (Fig. 2). We argue that the isotope cost itself is modest if sites are chosen carefully.

We propose that whole-wetland spiking would be most useful and cost effective in conjunction with studies that use traditional MRR techniques at one to two focal wetlands among a suite of nearby (<4 km) wetlands. After 4–8 years of traditional marking (e.g., toe-clipping) of all individuals—especially large cohorts of juveniles—at a focal wetland, a relatively high proportion of the population will be marked. For example, at Rainbow Bay (RB), an isolated wetland on the SRS where we have conducted MRR studies for 36 years, the proportion of breeding adult *A. opacum* that is marked (i.e., recaptures) from previous years approaches 90 % (Fig. 3), and can be maintained at that level if diligent efforts are made to mark all juveniles that metamorphose from the wetland. Isotope labeling of larvae (and therefore metamorphs) at wetlands within dispersal distance of the focal wetland will obviate the need to install drift fences and physically mark metamorphs at those sites, which is extremely labor intensive. By maintaining a high percentage of marked animals in the focal population, then only the breeding adults of unknown origin (i.e., not toe-clipped) will need to be sampled and analyzed for stable isotope signatures from nearby spiked wetlands. In a system such as the Massachusetts metapopulation of *A. opacum* (Gamble, McGarigal, and Compton 2007) with a primary MRR focal pond (e.g., Pond 4 in their study)—if we assume the per sample analytical cost at a fee-for-service laboratory is $8 US—the analytical costs for samples of unknown origin (i.e., non-toe-clipped animals captured at Pond 4) should be well below $1000.

Alternatively, the focal wetland itself could be spiked. Several studies have noted that capture efficiencies for juvenile amphibians are often less than 90 %; i.e., a relatively large

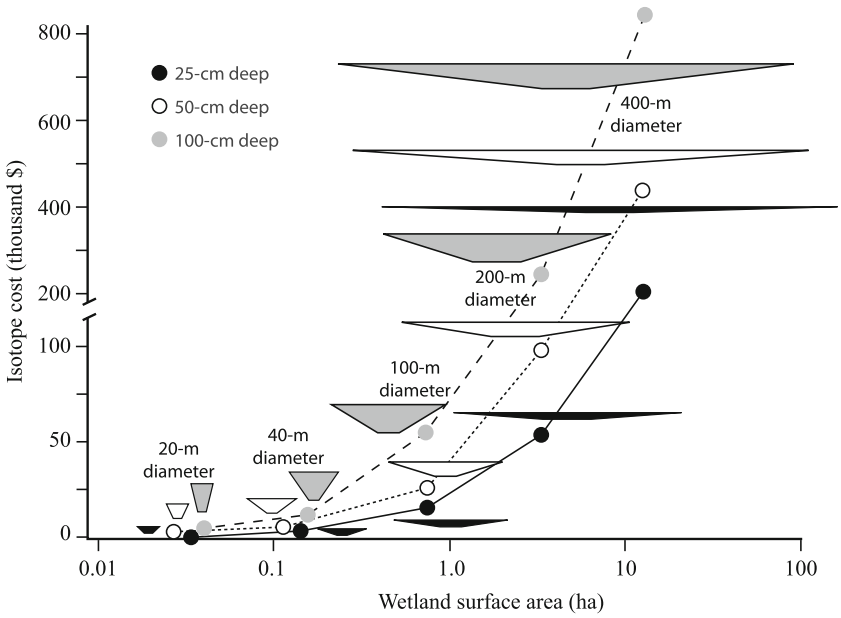

Fig 2 Estimated cost to spike wetlands of varied acreage and depths with ^{15}N levels used in this pilot study, assuming a cost of $2000 per 50 g of isotope and an initial dose of 0.446 mg isotope L^{-1}

number of emigrating new metamorphs may evade capture at the drift fence encircling a wetland (e.g., Trenham et al. 2000; Gamble et al. 2006). Large numbers of unmarked emigrating juveniles, some fraction of which are presumed dispersers (but most of which will return to their natal pond), will cause overestimates of immigration rates from other wetlands. By coupling ^{15}N enrichment with traditional marking methods an independent estimate of juvenile trespass rate could be derived. Additionally, if juveniles were spiked, then over the course of 3 years differential ^{15}N elimination among individuals may reflect variance in growth rates, and this technique could serve as a long-term tracer of the effects of environmental variation on growth.

Even greater potential for the technique exists if our observations for ^{15}N enrichment also hold true for other stable isotope spikes, such as $^{13}C/^{12}C$ and $^{34}S/^{32}S$. Given that metapopulation dynamics involves suites of wetlands in close proximity, a combination of isotopes would allow a unique signature to be administered to several individual wetlands and inhabitants. For example, three different isotopes, alone and in combination, will create unique isotopic signatures for up to seven wetlands at varying distances from a focal wetland. In small wetlands as described above, quantities of isotopes needed may be relatively small. In larger wetlands, if costs preclude isotope additions in quantities sufficient to achieve the same elevated level as in our pilot study, we think elevating $\delta^{15}N$ levels in metamorphs to ~100 times the normal level is more cost feasible, and should remain effective. Even at only 100× normal $\delta^{15}N$ levels (i.e., 400–800‰ in our system), metamorphs from spiked wetlands would conservatively experience 5–6 biological half-lives (17–21 months post-metamorphosis) during which their $\delta^{15}N$ signature will be

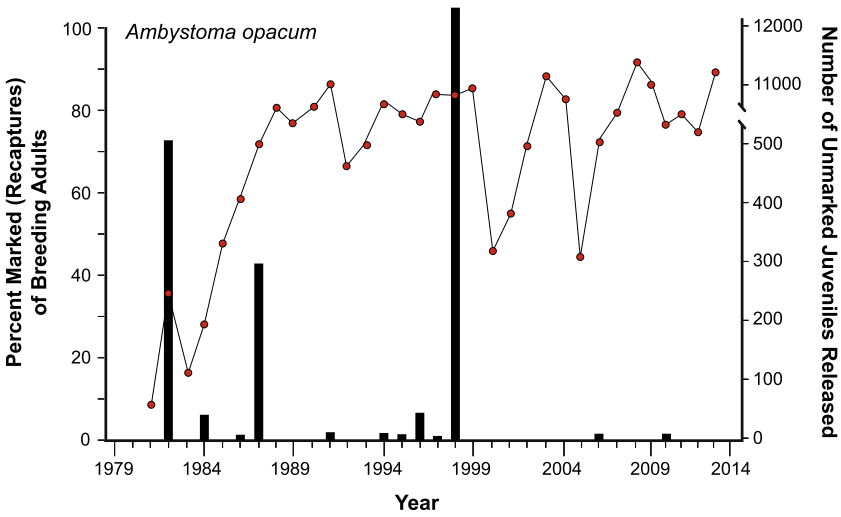

Fig 3 Percent of breeding adults of the marbled salamander, *Ambystoma opacum*, captured each year at Rainbow Bay that are marked (*toe-clipped*) recaptures from previous years (*line and dots*). The number of unmarked juveniles that were released at the site is depicted by bars. Submergence of the entire drift fence in 2003 during the period of metamorphosis may account for low recapture rate in 2005

distinguishable from animals that developed in unspiked systems.

Numerous authors have stated that among-pond dispersal rate is one of the hardest parameters to estimate in amphibian metapopulation models, whether through direct MRR methods (Gamble et al. 2007) or genetic techniques (Zamudio and Wieczorek 2007; Greenwald 2010). Given the importance of understanding dispersal for the conservation and management of amphibians in fragmented landscapes (Semlitsch 2008), stable isotope enrichment of seasonal wetlands may allow estimates of dispersal rates in relation to land use effects on habitat quality, as well as amphibian dispersal responses to climate change impacts on the spatial distribution of wetlands with suitable hydroperiods for juvenile recruitment (Walls et al. 2013).

Stable isotope enrichments have been used in field studies to address numerous ecological questions—we propose adding amphibian dispersal to that list of questions. Whole ecosystem spikes have been used in a variety of wetland types to quantify nitrogen transformations, cycling, and removal (Gribsholt et al. 2007; Wozniak et al. 2008; Harrison et al. 2012; O'Brien et al. 2012), carbon:nitrogen ratios and dynamics (Dodds et al. 2004), and plant niche separation (Clarkson et al. 2009). Many aspects of seasonal wetland ecosystems, including nutrient dynamics and response to pollutants (e.g., Crumpton and Goldsborough 1998), remain understudied despite the importance of these wetlands to regional biodiversity, water quality and ecosystem function (Sharitz 2003; Whigham and Jordan 2003). We strongly encourage collaborative efforts to use whole-ecosystem isotopic labeling techniques to estimate not only the dispersal of amphibians and other taxa (e.g., dragonflies, *sensu* Hobson et al. 2012) away from wetlands, but also describe basic ecosystem processes and food webs (Herman et al. 2000; Lee et al. 2011) of isolated seasonal wetlands.

Acknowledgments We thank Larry Bryan, Stacey Lance, and Jim Beasley for reviews of early drafts of this manuscript, an anonymous reviewer and Robin Warne for additional comments, and Susan Walls and Ken Dodd for insights on age at maturity in southeastern amphibians. Ben Morris and Angie Tucker helped maintain metamorphosed salamanders and weigh samples for isotope analysis, and Heather Brant helped keep the instrumentation up and running. This work was partially supported by the Department of Energy under Award Number DE-FC09-07SR22506 to the University of Georgia Research Foundation.

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