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Modeling nutrient transport and transformation by pool-breeding amphibians in forested landscapes using a 21 year dataset

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SUMMARY

1. Migrations of animals can transfer energy and nutrients through and among terrestrial and aquatic habitats. Pool-breeding amphibians, such as the wood frog (*Lithobates sylvaticus*), make annual breeding migrations to ephemeral wetlands in forest habitats in the eastern and midwestern United States and Canada.
2. To model the influence of wood frogs on nutrient transport and transformation through time, we coupled long-term population monitoring data (1985–2005) from a wood frog population with estimates of the elemental composition of wood frog egg masses and emerging juveniles.
3. Over the 21-year study period, 8.8 kg carbon (C), 2.0 kg nitrogen (N) and 0.20 kg phosphorus (P) were transported from the terrestrial to the aquatic habitat and approximately 21 kg C, 5.5 kg N and 1.2 kg P were exported to the surrounding terrestrial habitat by wood frogs.
4. During the study period, the average net flux of C, N and P was from aquatic to terrestrial habitats, but the magnitude and direction of the net flux was element dependent. Thus, the net flux of C, N and P did not always flow in the same direction.
5. Predicting long-term trends in nutrient and energy flux by organisms with biphasic life cycles should rely on long-term population data to account for temporal variability. This is especially true for organisms that are sensitive to long-term shifts in temperature and precipitation patterns, such as amphibians that breed in ephemeral pools.

Keywords: animal migrations, nutrient dynamics, resource subsidies, vernal pool, wood
frog

INTRODUCTION

Flows of organic matter across habitat and ecosystem boundaries, or spatial subsidies, have the potential to alter ecosystem processes as they can represent an ecologically important flux of energy and nutrients across landscapes (Polis, Anderson & Holt, 1997). These subsidies can influence abundance and interactions of species in recipient habitats (Nakano & Murakami, 2001; Baxter, Fausch & Saunders, 2005; Rubbo, Cole & Kiesecker, 2006). Consumers with biphasic life cycles can provide spatial subsidies by transporting and transforming energy and nutrients across system boundaries (Regeer, Whiles & Lips, 2008).

Investigations of cross-habitat flows have often described the movement of energy and nutrients from terrestrial to aquatic habitats as aquatic systems are predicted to receive higher amounts of materials from terrestrial environments than vice versa (Lindeman, 1942; Bartels et al., 2012). However, the quality of these subsidies can differ, since the stoichiometric composition of spatial subsidies is highly variable across ecosystems and types of subsidies (Earl & Semlitsch, 2012). Materials moving from terrestrial to aquatic habitats are commonly dominated by detritus (Bartels et al., 2012). Leaf litter and other forms of detritus can have high carbon (C) to nitrogen (N) or phosphorus (P) ratios (C:N:P) and are low-quality subsidies. Conversely, subsidies moving from aquatic to terrestrial environments are predominantly created by the movement of living organisms (Bartels et al., 2012) and typically have much lower C:N:P ratios, forming higher-quality subsidies. For instance, in lotic

systems, migrating salmon can generate large fluxes of elements across habitat boundaries and represent important components of food webs in recipient ecosystems (Tiegs et al., 2009, 2011).

Examinations of the flux of energy and nutrients across freshwater–terrestrial boundaries have primarily been conducted in lotic systems (Bartels et al., 2012), yet spatial subsidies can be important fluxes of elements into and out of other kinds of freshwater habitats (Regerter, Lips & Whiles, 2006; Earl & Semlitsch, 2012; Reinhardt et al., 2013). Seasonal wetlands occur globally and often support high densities of organisms adapted to breeding in ephemeral habitats (Leibowitz, 2003). Examples of seasonal wetlands include playas, prairie potholes, Carolina bays and vernal pools – the subject of this study. Vernal pools occur in a diversity of landscape settings from isolated upland depressions to larger wetland complexes (Calhoun & Demaynadier, 2008). Because of their ephemeral nature, vernal pools are free of fish and provide critical breeding habitat for organisms sensitive to predation by fishes and other predators that require permanently inundated habitats. Pool-associated species include invertebrates such as fairy shrimp (*Eubranchipus* spp.) and amphibians such as mole salamanders (*Ambystoma* spp.) and wood frogs (*Lithobates sylvaticus*) (Semlitsch, 2000).

Animals using both aquatic and terrestrial habitats as part of their life cycle transport elements across habitat boundaries (Nakano & Murakami, 2001; Regerter et al., 2008; Kraus & Vonesh, 2012). An important example is organisms with biphasic life

cycles that use aquatic habitats for larval development and emerge as juveniles or adults into terrestrial environments (Regester et al., 2008; Hoekman et al., 2011; Kraus & Vonesh, 2012). Pool-breeding amphibians depend upon aquatic habitats for reproduction and require adjacent upland habitat for dispersal, foraging and hibernation (Semlitsch, 2002; Faccio, 2003).

Movement of amphibians between aquatic and terrestrial habitats in the forest-wetland matrix may generate a substantial flow of nutrients and energy between breeding pools and adjacent terrestrial ecosystems (Regester & Whiles, 2006; Earl et al., 2011; Earl & Semlitsch, 2012). However, the flow of energy via dispersing amphibians has been quantified only for a few species (e.g. Burton & Likens, 1975; Regester et al., 2006), and generalised patterns in the variability, magnitude and direction of the flux of amphibian-derived elements are poorly understood. Moreover, most investigations have been limited to one- to three-year study periods (e.g. Seale, 1980; Regester et al., 2006; Reinhardt et al., 2013). Often, pool-breeding amphibians are characterised by boom-and-bust population cycles that are driven by variability in the numbers of breeding adults, the extent of successful larval development and the recruitment of emergent juveniles (Berven, 1990, 1995; Whiteman & Wissinger, 2005). However, long-term studies are needed to understand variability in the quality and quantity of spatial subsidies produced by populations of organisms with biphasic life cycles.

The purpose of this study was to model the influence of pool-breeding wood frogs on

nutrient transport and transformation through time. We coupled long-term population monitoring data (1985–2005) of a single wood frog population with data on the body elemental composition of wood frog egg masses and emergent juveniles to estimate the magnitude of elemental flux between terrestrial and aquatic habitats. We predicted that wood frogs would be a substantial, yet highly variable, flux of C, N and P between terrestrial and aquatic habitats. We hypothesised this effect would vary among elements and depend upon life-stage stoichiometry.

METHODS

Study site and field methods

We collected population data from a single population of wood frogs in University of Michigan's Saginaw Forest Preserve (42°40'N, 83°13'W) between 1985 and 2005. The forest preserve is ~32 hectares and is surrounded by a matrix of woodland, pasture and commercial and residential developments. Wood frog breeding in this forest occurs predominantly in an ephemeral pond (~2912 m² area; 1.5 m maximum depth) that was the subject of our study (Berven & Boltz, 2001; Berven, 2009). All adults entering the pond and all juveniles leaving the pond were collected in a pit-fall array created using aluminum window screening (height: 75 cm) and wooden stakes. The fence completely encircled the pond and was buried to a depth of 20 cm. Pitfall traps were left open and monitored daily during the breeding season (1 March–30 April) and during the period of juvenile emergence (1 June–28 July); however, the

traps were closed during the rest of the year to permit organisms to freely enter and exit the pool (Berven, 2009).

We measured the wet masses each year of a sub-sample of the breeding adults (~140 individuals of each sex) in the laboratory and emergent juveniles (~100) in the field using an electronic balance. To estimate dry mass from wet mass, a subset of eggs and juveniles collected in 2012 were oven-dried to a constant mass, cooled in a desiccator and weighed to the nearest 1 mg. We used the wet mass/dry mass relationship to convert wet mass to dry mass for each sample year. Total egg mass number was estimated as the total number of females returning to the pool each year, as all females entering the pool were gravid (Berven, 1981). We determined clutch size by pairing females (average of 40 females each year; range 13–79) with a male in a pan of water and allowing them to deposit their eggs. After egg deposition, we counted the number of eggs in each clutch. We then used the relationship between female wet mass and egg number to determine the average clutch size for the average-size female breeding each year. Wet mass of the egg masses was estimated using the relationship between the total egg mass (difference between mass of females before and after egg deposition) and wet mass of fully expanded egg masses ($y = 16.14x + 17.63; r^2 = 0.79$). We estimated the dry mass of egg masses using the relationship between wet and dry mass ($y = 0.0174x + 0.393; r^2 = 0.92$).

Individual adult remineralisation rates were estimated using five male frogs collected in 2013 from the Michigan population using methods modified from Vanni et al.

(2002). Briefly, 10 plastic aquaria were filled with 1 L of unfiltered pond water, and a single adult frog was added to five of these. The five remaining aquaria served as controls. The frogs were incubated for 24 hr. Water from each aquaria was then filtered (Whatman GF/F), frozen and subsequently analysed for NH_4^+ -N and SRP-P using methods outlined below. We did not measure the contribution of adult remineralisation to C flux into the pools. Remineralisation estimates were made by subtracting the change in ambient chemistry in control chambers (no frog) from the change in ambient chemistry in treatment chambers (frog) during the incubation period. Total contribution of adult remineralisation to the flux of elements into the study pool was made using the following formula: $\text{RF}_i = [(M \times R_x \times B_M) + (F \times R_x \times B_F)]/1,000,000$ where RF = element flux due to adult remineralisation (g); $i = \text{NH}_4^+$ -N or SRP-P; M = average mass of males (g); R = element remineralisation rate of adults ($\mu\text{g element} \times \text{g wet mass adult}^{-1} \times \text{d}^{-1}$); B_M = annual breeding period for males (days); F = average mass of females; and B_F = annual breeding period for females. Using previously published work on breeding behavior of wood frogs (Berven, 1981), we assumed females remained in the pool for 24 h (therefore B_F is always equal to 1) and that males remained in the pool for the entire annual breeding period (3–30 days).

Analytical methods

To estimate the C, N and P content of egg masses and emergent juveniles, we collected

five replicate samples of each life stage for analysis. Egg masses were harvested from a wood frog population in the University of Maine Dewitt Forest Preserve in Old Town, Maine (44°55' N, 68°41' W), in 2013, as data from the Michigan population were not available. Ten egg masses were brought back to the laboratory, and five were immediately dried and processed. The remaining five masses were hatched in the laboratory, and tadpoles were fed unlimited amounts of blanched kale throughout their development to minimise differences in stoichiometry due to diet. Juveniles were haphazardly harvested from this laboratory-reared population. Because of their small mass, each replicate of egg masses consisted of an entire egg mass ($n = 5$ replicates) and each replicate of juveniles consisted of 10 individuals ($n = 5$ replicates).

Juveniles were euthanised using an overdose of MS- 222. Samples for elemental analysis were dried in a convection oven to a constant mass at 45 °C and ground to a fine powder. All samples were analysed at the Maine Agricultural and Forestry Experiment Station Soils Lab. For C and N, dried material was analysed using a Leco TruMac Series Macro CN-Analyzer. For particulate P analysis, subsamples of material were combusted at 500 °C and digested with 1 N HCl for 2 h and the digested solution was analysed using an iCap 6000 Series ICP-OES. Water chemistry from the remineralisation estimates were measured using standard analytical techniques at the Environmental Analysis Laboratory at Lake Superior State University. Net flux into the terrestrial habitat (NFT) was estimated each year for each element using the following equation: $NFT_j = [A_j \times J] - [(A_j \times EM) + RF_i]$ where $j = C,$

N or P; A = % element; J = the annual estimate of the dry mass (g) of all of the juveniles emerging; EM = the annual estimate of the dry mass (g) of all of the deposited egg masses; RF = element flux due to adult remineralisation (g); and $x = \text{NH}_4^+ \text{-N}$ or SRP-P (RF is 0 for estimating flux of carbon). Positive values of NFT indicate greater amounts of the element were moved from the aquatic to the terrestrial habitat in emergent juveniles than were transferred from the terrestrial to aquatic habitat by breeding adults. To make areal flux estimates (g m^{-2}), we assumed a constant pool area of 2900 m^{-2} , the maximum potential fill area of the pool. To determine differences in body nutrient concentration and stoichiometry between egg masses and juveniles, we used Student's t-test. All data were \log_{10} -transformed to address non-uniform variance. All analyses were conducted using JMP 10 statistical software (SAS Institute, 2012).

RESULTS

Over the 21-year study period, the number of breeding adults returning to the pond and the number of juveniles emerging from it varied by orders of magnitude [adults: 2056 (mean); 900–9840 (range); juveniles: 29 274 (mean); 0–113 686 (range)]. Over the entire study period, 86 359 adults returned to breed in the ephemeral wetland and produced 20 481 egg masses (range: 122–4427; Fig. 1a) with an average of ~600 eggs per mass (Fig. 1b). Average excretion rates for adults were $49.02 \mu\text{g N}$ and $9.21 \mu\text{g P}$ per g wet mass $\text{frog}^{-1} \text{ day}^{-1}$. The average dry mass of the egg masses ranged from 0.670

to 1.207 g per mass (Fig. 1c). During the study period, a total of 614 755 juveniles were exported to the surrounding forest habitat, 18.5% of which were produced in a single year (1997). No juvenile recruitment was observed in 4 of the 21 years. Average dry mass of individual juveniles varied greatly among years and ranged from 0.031 to 1.27 g.

Nutrient content and stoichiometry differed between eggs and juveniles. Although egg masses had significantly greater C content than emergent juveniles [egg mass (mean % mass \pm SD): $46.1 \pm 2.36\%$; juvenile: $40.8 \pm 2.41\%$; $P = 0.008$], juveniles were significantly richer in N (egg mass: $9.2 \pm 0.61\%$; juvenile: $10.4 \pm 0.84\%$; $P = 0.042$) and P (egg mass: $0.86 \pm 0.21\%$; juvenile: $2.5 \pm 0.86\%$; $P = 0.003$). These differences also produced significant differences in stoichiometry between egg masses and juveniles {mass C:N [egg mass (mean \pm SD): 5.0 ± 0.20 ; juvenile: 3.8 ± 0.47 ; $P = 0.003$], C:P (egg mass: 57.6 ± 17.03 ; juvenile: 18.2 ± 8.14 ; $P = 0.004$) and N:P (egg mass: 11.6 ± 3.10 ; juvenile: 4.4 ± 2.03 ; $P = 0.005$).

In years with juvenile recruitment (all years except 1991, 1992, 2000, 2004), the dry mass exported from the aquatic habitat to the terrestrial environment was greater than the dry mass imported through egg deposition and remineralisation (NFT >0) in all but two years (Fig. 2a). On average, dry mass export to terrestrial habitat was ~450% greater than dry mass imported to the aquatic habitat through egg masses. During the study period, a total of 19.3 kg of egg masses and 50.3 kg of juveniles (dry mass) moved into and out of the vernal pool (Fig. 2a), a pattern reflected in the import and export of

N and P (Fig. 2b). Relative to N and P flux in the form of deposited egg masses, estimated adult N and P remineralisation was on average 15% of the N (min: 3%; max: 52%) and 25% of the P (min: 7%; max: 69%) imported into the system. However, these values were highly variable and in two of the 21 years (1994 and 2000), excretion contributed more than 35% of the N and 55% of the P transferred to the pool via wood frogs (Fig. 2b).

Similar to patterns in biomass export, the total elemental flux (Fig. 3a) and the average elemental flux (Fig. 3b) of juvenile export to the terrestrial habitat were Greater than the elemental flux of nutrients into the aquatic habitat via egg masses (C, N and P) and remineralisation (N and P only). Because the export of juveniles was the dominant flux of nutrients in most years, the average stoichiometry of the flux more closely resembled body stoichiometry of juveniles than that of egg masses (Table 1; Fig. 3c). Average fluxes of C, N and P from the forest into the pond were 0.15 g m^{-2} , 0.03 g m^{-2} and 0.003 g m^{-2} , respectively. Average fluxes of C, N and P from the pond into the forest were 0.35 g m^{-2} , 0.09 g m^{-2} and 0.02 g m^{-2} , respectively. Net flux of C, N and P into the terrestrial environment was positive in most years of the study and was strongly related to the flux of biomass into and out of the study pond (Fig. 4a); however, these effects were element dependent (Table 1; Fig. 4a,b). Differences in life-stage stoichiometry were driven by P content and had a pronounced effect on the amount of P imported into the vernal pool through egg masses (Fig. 4b). Thus, years with low biomass export relative to import, such as 1998 and 2003, had the potential to

produce negative NFT values for one element and positive NFT values for other elements (Table 1; Fig. 4). This was especially true for the flux of P, which was strongly related to juvenile recruitment (Fig. 4b).

DISCUSSION

Our study suggests that pool-breeding amphibians are an important, but highly variable flux of energy and nutrients between terrestrial and aquatic habitats in forested ecosystems. During the period of the investigation, the average net flux of C, N and P by wood frogs was from the aquatic to the terrestrial ecosystem in the form of juveniles, a flux of biomass with relatively low C:N:P. The magnitude and direction of the net flux of elements was element dependent; thus, the net flux of C, N and P did not always flow in the same direction. These findings highlight the necessity of using long-term population data sets to accurately model spatial subsidies generated by consumers, especially those with boom-bust reproductive cycles, such as wood frogs.

Variability in egg mass abundance and the number of emergent juveniles

Pond-breeding amphibian populations are strongly regulated by density-dependent processes in both aquatic and terrestrial stages of their life cycles (Berven, 1995, 2009; Skelly & Kiesecker, 2001). Larger numbers of emerging juveniles resulted in delayed maturation rates, reduced juvenile survival, smaller adult body size and reduced fecundity in the study population (Berven, 2009). Although most wood frogs within this population breed only once, in years with lower numbers of adults,

adults of both sexes live longer and reproduce more often (Berven, 2009). In our study, the number of egg masses deposited and the number of juveniles emerging from the pool ranged by orders of magnitude. Previous work with this population documented density-dependent factors that influence larval and juvenile success (Berven, 1990, 1995); conversely, the effect of changing environmental variables on egg mass number and juvenile recruitment are not well understood.

Most studies examining the role of amphibians in energy and nutrient transport have been based on data collected within a single breeding season (e.g. Burton & Likens, 1975; Regester et al., 2006; Reinhardt et al., 2013). Such data are time-consuming to collect; therefore, long-term population data sets of pool-breeding amphibians are rare. Had we based our flux estimates on time periods of 1–3 years, we may have produced very different projections about the direction, magnitude and stoichiometry of elemental transport and transformation by wood frogs. For example, if our study had been limited to either 1991–1992 or 2002–2005, years of relatively low (or no) juvenile recruitment, we would have predicted that there was no net flux of nutrients or that the net flux was into the aquatic system. This contrasts with a key finding of our study, which was that the net flux of nutrients over the 21 years was from the aquatic to the terrestrial habitat.

Climate variables including the precipitation and temperature regime also probably determine the abundance and condition of egg masses and juvenile emergents. Larger, older female wood frogs produce larger eggs in larger clutches (Berven, 2009). Thus,

overwinter conditions that favour wood frog survival may strongly, positively influence the flux of nutrients and energy into aquatic habitats from the terrestrial environment. Similarly, juvenile recruitment and the subsequent flux of elements from the aquatic to terrestrial environments can also be affected by climate. For example, cold spring temperatures may freeze egg masses and reduce larval survival. Additionally, hot, dry summers may also harm juvenile recruitment by causing the pool to dry prior to metamorphosis. Recent studies have begun to examine the influence of climate variables on the reproductive success of pool-breeding amphibians (e.g. Green et al., 2013; Hossack et al., 2013; Olker et al., 2013); as yet, few have employed long-term data sets of natural amphibian populations to understand these effects (but see, Scheele et al., 2012; O'regan, Palen & Anderson, 2014). Future investigations should integrate long-term amphibian population and long-term climate data to model the effects of climate on spatial subsidies generated by pool-breeding amphibians.

Implications of ontogenic shifts in stoichiometry on the transportation and transformation of nutrients across habitat boundaries

Like many other consumers (e.g. Pilati & Vanni, 2007; De Brabandere et al., 2009; Back & King, 2013), wood frogs experience ontogenic shifts in diet, body tissue composition and size. Each of these factors potentially contributes to the shifts in body nutrient concentration and tissue stoichiometry between eggs and juveniles that we documented in this study. Although changes in %C and %N were fairly small,

differences in P concentration between juvenile wood frogs and wood frog egg masses were pronounced. We documented steady increases in P concentration from egg to juveniles, and this significantly altered the C:P and N:P of body tissues. This pattern was expected since bone is the primary storage site of P invertebrates (Sterner & Elser, 2002) and wood frogs do not begin extensive skeletal development until later larval stages. Ontogenic shifts in tissue nutrient content and stoichiometry determine how organisms with biphasic life cycles transform and translocate elements across boundaries. In our system, net flux of C, N and P in wood frogs was from the aquatic to the terrestrial habitat in all but a few years (NFT > 0; Fig. 4). However, the amount and stoichiometry of the flux was highly variable through time (Figs 3 and 4; Table 1).

Although our estimate of C export by wood frogs (0.35 g m^{-2}) is comparable to the average of $0.27 \text{ g ash-free dry mass (AFDM) m}^{-2}$ exported by juvenile salamanders in Illinois (Reger et al., 2006), our NFT results contrast with their findings and those of Reinhardt et al. (2013). Contrary to Reger et al. (2006) and Reinhardt et al. (2013), the net flux of organic matter was from the aquatic habitats to the terrestrial habitats in our study. Most likely, the dissimilarity arose from differences in salamander and frog production and trophic ecology. On average, ambystomatid salamanders are less productive than wood frogs in a single year (Karraker & Gibbs, 2009); therefore, the potential annual juvenile recruitment per female wood frog would be much greater than that of salamanders. This pattern was documented by Seale (1980) who found that American toads (*Bufo americanus*) contributed almost 80% of the organic matter in amphibian egg masses compared with 11% contributed by three ambystomid species

in a pool in Missouri. Larval wood frogs are detritivorous grazers and feed on algae and microbes on the surfaces of decomposing leaf litter, food sources that are rarely limiting in vernal pools (Earl & Semlitsch, 2012). On the contrary, larval salamanders are carnivorous and may become food-limited as pools shrink and prey begin metamorphosing and leaving the pond (Davic & Welsh, 2004; Reinhardt et al., 2013). This difference in trophic ecology may be the driver of NFT values <0 in studies of ambystomid salamanders.

The biotic and abiotic factors regulating nutrient dynamics in amphibian species may be species specific; thus, different populations in the same pool may yield different NFT values. For example, Seale (1980) found that as a community, amphibians tended to produce positive NFT values for N as they exported more N in their juveniles than they deposited in their egg masses, but this pattern was not true for all species (e.g. *Bufo americanus*). However, Seale attributed this difference to lower juvenile recruitment due to predation of *Bufo* tadpoles by tiger salamander larvae (*Ambystoma tigrinum*) rather than differences in life-stage stoichiometry or the number of egg masses laid by adults (Seale, 1980). In her work, Seale (1980) documented stoichiometric differences in egg masses among amphibian species. Most likely, such differences are apparent throughout development. Future studies should examine changes in egg, larval, juvenile and adult stoichiometries in several amphibian species with varied trophic and reproductive ecologies, over a longer time period, and in response to changing environmental variables.

The physical form in which elements are transported across habitat boundaries (remineralisation, eggs, juveniles) by animals with biphasic lifestyles is also ontogenically dependent and potentially important to ecosystem function, especially in systems where low N or P availability limits primary productivity and/or respiration. For example, we examined the potential contribution of adult remineralisation relative to nutrients transported via fertilised eggs. This ratio was highly variable (Fig. 2b); in some years, adult remineralisation contributed a large portion of the nutrients transported by adults, and in some years, it was negligible. The effect was also element dependent, as egg masses have extremely low P concentrations. Although contribution through remineralisation may have been small in this study, in nutrient-limited systems, remineralisation may play a more important role in nutrient dynamics than indicated by the size of the flux. Water chemistry in isolated wetlands, such as vernal pools, can be highly variable (Whigham & Jordan, 2003), and they can have low ambient N and P concentrations ($<2 \mu\text{g L}^{-1}$ NO_3^- N, PO_4^- P) after the spring thaw (personal observation, K. Capps). Therefore, a source of labile nutrients may be important to initiate and maintain primary productivity and community respiration in these systems. Compared with excretion that releases N and P in forms that are immediately usable by algae and microbes, egg masses are relatively recalcitrant and need to be decomposed or consumed prior to entering the labile pool of nutrients. More work needs to be done to understand the pathways by which various forms of consumer-based nutrients are incorporated into biogeochemical cycling.

Landscape level effects of nutrient subsidies from pool-breeding amphibians

Compared with the quantity of nutrients transported by organisms such as fishes (e.g. Deegan, 1993; Brazner, Tanner & Morrice, 2001; Tiegs et al., 2011; Nelson et al., 2013), amphibians in forested landscapes may seem to provide a minor subsidy of elements crossing boundaries across larger spatial scales. However, the data presented in this study were collected from a single vernal pool. In glaciated areas in eastern United States with intact forests, vernal pools and wood frog populations can be the common features across the landscape (Calhoun & Demaynadier, 2008); thus, nutrients transported through amphibians may represent an important subsidy.

Vernal pools can be difficult to map across forested landscapes, and few municipalities have mapped their remaining pools. However, in the past decade, several towns in the north-eastern United States have mapped pools using aerial photographs and stereoscopes. For example, in the town of Orono in central Maine, there are approximately 9 ha (>100 vernal pools) of potential vernal pools (personal communication, A. Calhoun). Using data generated from the Michigan population of wood frogs, the average NFT of C, N and P into the forest from emergent wood frogs in the town of Orono would be 19 kg (range: -61 to 77 kg), 5.5 kg (range: -12 to 21 kg) and 1.6 kg (range: -1.1 to 5.1 kg), respectively. For context, N deposition in central Maine is estimated to be approximately $5 \text{ kg ha}^{-1} \text{ yr}^{-1}$ (Ollinger et al., 1993); therefore, our estimates suggest that net N flux from emergent wood frogs in vernal pools in Orono could be approximately 12% of the N inputs entering the pools from

N deposition. Notably, the NFT we projected for Orono was limited to wood frogs. Had we included other pool-breeding amphibian and macroinvertebrate species in our estimate, the NFT values may have been much greater. Our estimated NFT values for Orono were distributed among more than 100 vernal pools found throughout forested habitat. Therefore, vernal pools may function as subsidy hotspots across forested landscapes, transforming leaf litter, a low-quality food source (high C:N:P values), into emerging macroinvertebrates and amphibians, high-quality food source (lower C:N:P; Earl & Semlitsch, 2012).

Recent work has documented the importance of diet and ambient chemistry on consumer stoichiometry (El-Sabaawi et al., 2012a,b); thus, we would expect amphibian body chemistry to vary among years, across populations and among communities. Moreover, there are regional differences in population sizes, individual body size, clutch size and egg size in wood frog populations (e.g. Berven, 1990, 1995, 2009). In this study, we used frog body stoichiometry from a Maine population to model processes, including excretion, from Michigan, although the body and excretion stoichiometries from these two populations may be different. However, we argue that stoichiometric change we documented from egg masses to juveniles (higher N:P to lower N:P) would be consistent among populations as would be the relatively limited contribution of adult excretion to the flux of N and P to the ponds. Moreover, our use of a long-term data set to document the patterns of elemental flux across terrestrial/aquatic boundaries through time bolsters our argument that the Michigan population is representative of the boom-bust

population cycles of many populations of wood frogs.

Our NFT projections for the Michigan wood frog population were modelled using average dry mass (DM) estimates for egg mass import (0.32 g DM m^{-2}) that are smaller than published values from other pool systems ($1.1\text{--}9.0 \text{ g DM m}^{-2}$; Seale, 1980; Regester et al., 2006; Reinhardt et al., 2013). However, we collected population data in a relatively large vernal pool ($>2900 \text{ m}^{-2}$) that had an area that was at least an order of magnitude greater than pools from those studies ($3\text{--}900 \text{ m}^{-2}$; Seale, 1980; Regester et al., 2006; Reinhardt et al., 2013). Frequently, pool-breeding amphibians lay their eggs in edge habitats; therefore, our landscape-level estimates of nutrient transport by frogs may be small when expressed on an areal basis. Additionally, two earlier studies (Seale, 1980; Regester et al., 2006) collected data from a community of amphibian species rather than a single species. Vernal pools are important breeding areas for a suite of amphibians and macroinvertebrate species with biphasic life cycles (Calhoun & Demaynadier, 2008). Collectively, these factors provide additional evidence that the NFT results from this study underestimate the extent of the functional role vernal pool organisms may play in subsidising forest food webs.

Predicting nutrient and elemental flux by biphasic organisms in a changing environment

Environmental change in the form of habitat fragmentation, species invasions and the decline of native species can negatively influence the quantity and quality of spatial

subsidies across habitat boundaries (Tiegs et al., 2008; Stephens, Berven & Tiegs, 2013). To maintain ecosystem function in the wake of anthropogenic disturbance, conservation biologists and natural resource managers need to quantify spatial subsidies and understand the factors that determine their stoichiometric composition and size. Our study provides further evidence that organisms with biphasic life cycles can generate high-quality subsidies that cross freshwater–terrestrial boundaries. Our investigation also quantifies how ontogenic change can influence the stoichiometry, transformation and translocation of subsidies across system boundaries and that these subsidies are highly variable from year to year.

Future work should attempt to elucidate how elements transported via eggs, juveniles and adults are directly and indirectly incorporated into biogeochemical cycles in terrestrial and aquatic habitats. Predicting long-term trends in nutrient and energy flux through organisms with highly variable, biphasic life cycles should rely on long-term population data to account for this variability. This is especially true for organisms that are sensitive to long-term shifts in temperature and precipitation patterns, such as organisms breeding in ephemeral pools.

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Fig. 1 Long-term demography of the wood frog population. (a) Total number of egg masses laid in the pool each year; (b) average number of eggs per egg mass each year; (c) average dry mass of egg masses each year. Dotted lines indicate the average values for the entire study period.

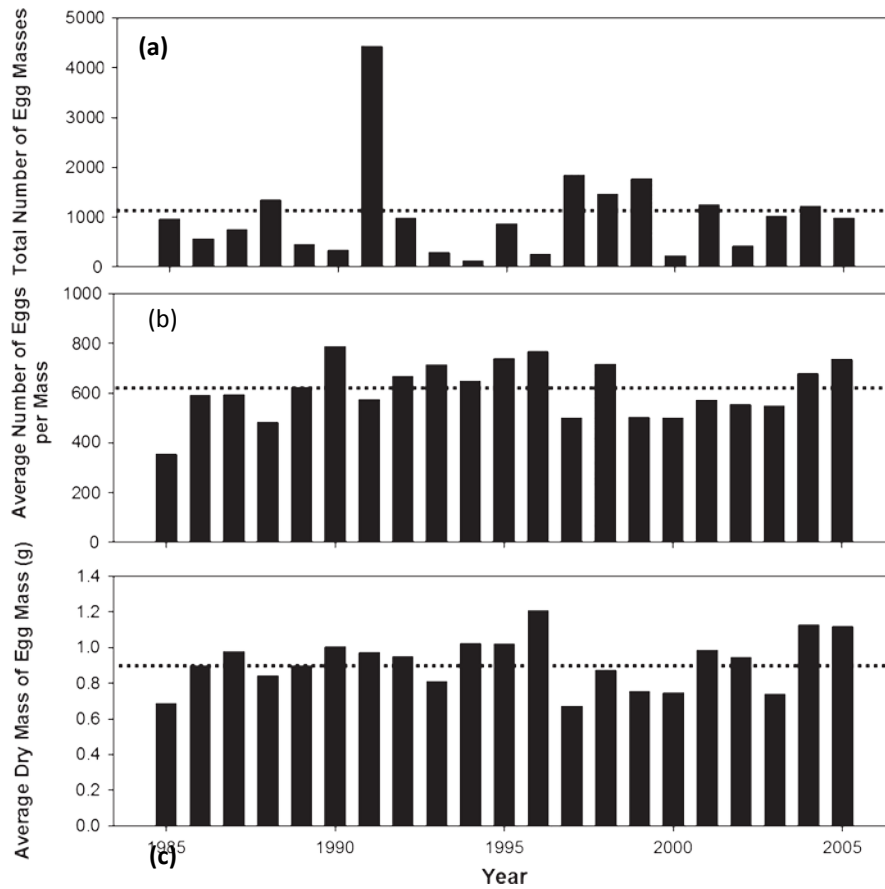


Fig. 2 Fluxes of materials carried by wood frogs between the vernal pool and the surrounding forest. (a) Biomass flux of wood frog eggs into aquatic habitat and emergent juveniles into terrestrial habitat. (b) Nitrogen and phosphorus flux of wood frog eggs and adult remineralisation into the aquatic habitat and emergent juveniles into the terrestrial habitat. Shaded area represents aquatic habitat.

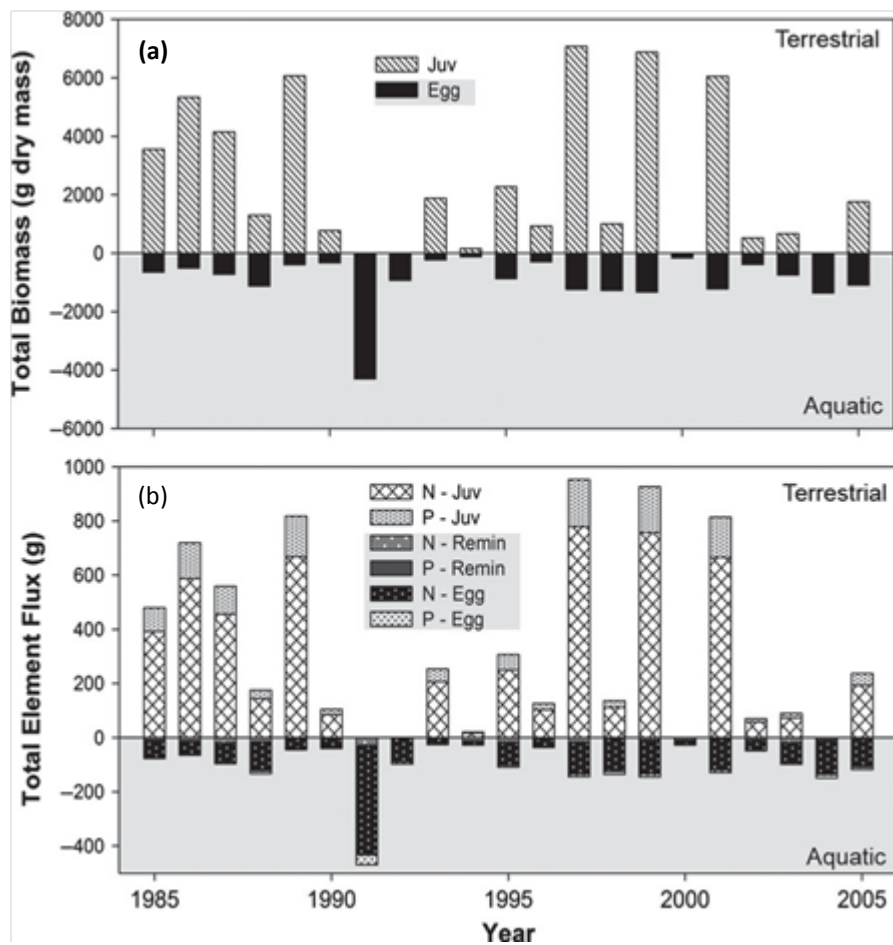


Table 1 Net flux of C, N and P between terrestrial and aquatic habitats in the study site.

Positive values indicate a net flux of elements into the terrestrial habitat (via emergent juveniles), and negative values indicate a net flux of elements into the aquatic habitat (via egg masses and adult remineralization for nitrogen and phosphorus). Stoichiometric ratios (mass) were calculated only for years when the net flux for all elements were in a single direction

Year	C flux (g)	N Flux (g)	P flux (g)	C:N	C:P	N:P	Recipient habitat
1985	1229	321	81	4	15	4	Terrestrial
1985	2064	529	126	4	16	4	Terrestrial
1987	1450	369	93	4	16	4	Terrestrial
1988	42	23	20	2	2	1	Terrestrial
1989	2426	627	146	4	17	4	Terrestrial
1990	182	49	15	4	12	3	Terrestrial
1991	-1977	-428	-41	5	48	10	Aquatic
1992	-426	-89	-8	5	51	11	Aquatic
1993	701	183	44	4	16	4	Terrestrial
1994	9	-7	0	-	-	-	Aquatic (N)- Terrestrial (C)
1995	575	152	46	4	13	3	Terrestrial
1996	264	70	20	4	13	4	Terrestrial
1997	2473	647	161	4	15	4	Terrestrial
1998	-153	-12	13	-	-	-	Aquatic (CN)- Terrestrial (P)
1999	2345	624	157	4	15	4	Terrestrial
2000	-76	-24	-3	3	25	8	Aquatic
2001	2037	547	138	4	15	4	Terrestrial
2002	42	13	8	3	5	2	Terrestrial
2003	-61	-16	6	-	-	-	Aquatic (CN)- Terrestrial (P)
2004	-627	-135	-13	5	48	10	Aquatic
2005	252	85	33	3	8	3	Terrestrial

Fig. 3 Stoichiometry and magnitude of elemental flux into terrestrial and aquatic habitats by wood frogs. (a) Total flux of C, N and P between habitats; (b) average net flux of elements into the terrestrial habitat (NFT). Positive values indicate a net flux of elements into the terrestrial habitat (via emergent juveniles), and negative values indicate a net flux of elements into the aquatic habitat [via egg masses and adult remineralisation (for N and P)]. Shaded area represents aquatic habitat; (c) stoichiometry (by mass) of the average flux of elements. This estimate was made using the 19 years of the study when the net flux of all of the elements was in a single direction (either terrestrial or aquatic; Table 1). Error bars are 1 SE.

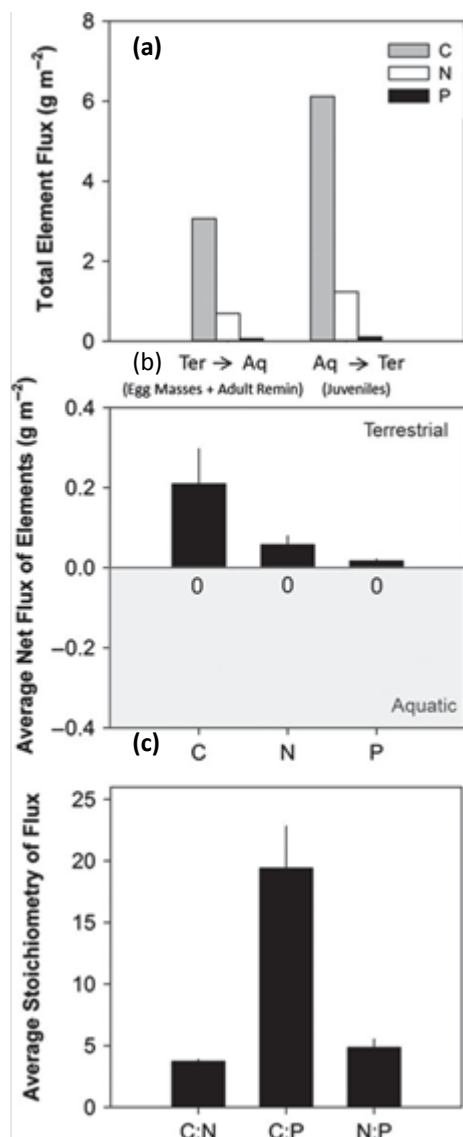


Fig. 4 Net flux of biomass and elements into terrestrial habitats (NFT) between 1985 and 2005. Positive values indicate a net flux of elements into the terrestrial habitat (via emergent juveniles), and negative values indicate a net flux of elements into the aquatic habitat [via egg masses and adult excretion (N and P only)]. Shaded areas represent aquatic habitat. (a) Biomass and C flux; (b) N and P flux.

