COMPLEX LIFE-HISTORIES AND BIOGEOCHEMICAL CYCLES; INTERACTIONS BETWEEN AMPHIBIAN LIFE-HISTORY STRATEGIES AND ELEMENTAL CYCLING

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by

THOMAS MARSHALL LUHRING

Dr. Raymond D. Semlitsch, Dissertation Advisor

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled:

COMPLEX LIFE-HISTORIES AND BIOGEOCHEMICAL CYCLES;
INTERACTIONS BETWEEN AMPHIBIAN LIFE-HISTORY STRATEGIES AND ELEMENTAL CYCLING

Presented by Thomas M. Luhring,

a candidate for the degree of doctor of philosophy,

and hereby certify that, in their opinion, it is worthy of acceptance.

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Professor Raymond D. Semlitsch

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Professor Reginald B. Cocroft

_____

Professor H. Carl Gerhardt, Jr.

_____

Professor Dylan C. Kesler

Dedicated to all the kind souls with whom I have had the fortune to share fleeting moments of life. Their impacts greatly exceed the duration of our shared interactions.

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ABSTRACT

All life must balance two key currencies: energy and matter. My dissertation focuses on the struggle that animals face in balancing various permutations of the latter currency: elements. The availability of essential elements governs life processes from cellular to landscape scales. Likewise, biogeochemical cycles are intimately tied to and affected by biotic processes.

At the ecosystem level, exchanges of materials and energy across system boundaries (e.g., between terrestrial and aquatic) vary in their spatial and temporal voracity, but are often essential to the functioning of recipient systems. Animals with complex life-histories (e.g., pond-breeding amphibians, diadromous fishes, holometabolous insects) use multiple habitats at various stages of their lives. In doing so, they translocate energy and matter between disparate systems as well as serving as within-system cyclers. We use amphibians to test various interactions of animals with biogeochemical cycles and their role in shaping spatial subsidies.

CHAPTER 1

INTERACTIONS OF BIOGEOCHEMICAL CYCLES AND LIFE-HISTORY STRATEGIES

Thomas M. Luhring

INTRODUCTION

The interactions between organisms and the environment form the underpinnings of ecology. Animals, in particular, shape ecosystem processes through a combination of their direct effects (e.g., ingestion; Vanni 2002) and movement capacity (sensu lato; Nathan et al., 2008). By shifting biomass and nutrients between ecosystems, animals engage in the active translocation of spatial subsidies (Polis et al., 1997). The chemical form and quantity of elements being translocated depends on the process that causes its movement. In many aquatic-terrestrial interfaces the focus on biomass movement is either through insect-mediated biomass transfers, excretions (e.g., seabird guano; Anderson and Polis 1999), or through third-party interactions (e.g., bears moving salmon into woods; Schindler et al., 2003).

The complex life-histories of amphibians enable us to investigate aquatic-terrestrial links (Regester et al., 2006). While we know that amphibians move biomass

between aquatic and terrestrial habitats, the drivers of subsidy quantity and quality (elemental composition, stoichiometry) remain ambiguous. In amphibian systems, obligatory life-history ties to multiple habitats result in the transfer of vertebrate biomass between them. However, the quantity and quality of each subsidy is not a random conglomeration. Terrestrially-derived biomass arrives in aquatic habitats in the form of ova and aquatically-derived biomass arrives in terrestrial habitats in the form of recently-metamorphosed juveniles. What determines the quantity (biomass) and quality (stoichiometry) of these subsidies?

The first manuscript chapter (chapter 2) targets two main questions: 1) what effects do amphibians have on within-system nutrient cycling and community processes? 2) how do bottom-up and top-down pressures affect the quantity of biomass and nutrient export from pond systems? This is followed by a chapter (chapter 3) that investigates how amphibian stoichiometry changes across ontogeny (fig. 1). Most of this dissertation focusses on the aquatic ontogeny (slope A in fig. 1) of amphibians, however, the terrestrial ontogeny (slope B in fig. 2) of maturing animals or wholly-terrestrial animals deserves future consideration. Chapter 4 combines data from chapter 2 on bottom-up and top-down effects and data from chapter 3 on ontogenetic changes in stoichiometry to create element-specific scenarios of translocation based on per-capita effects of amphibian life cycles.

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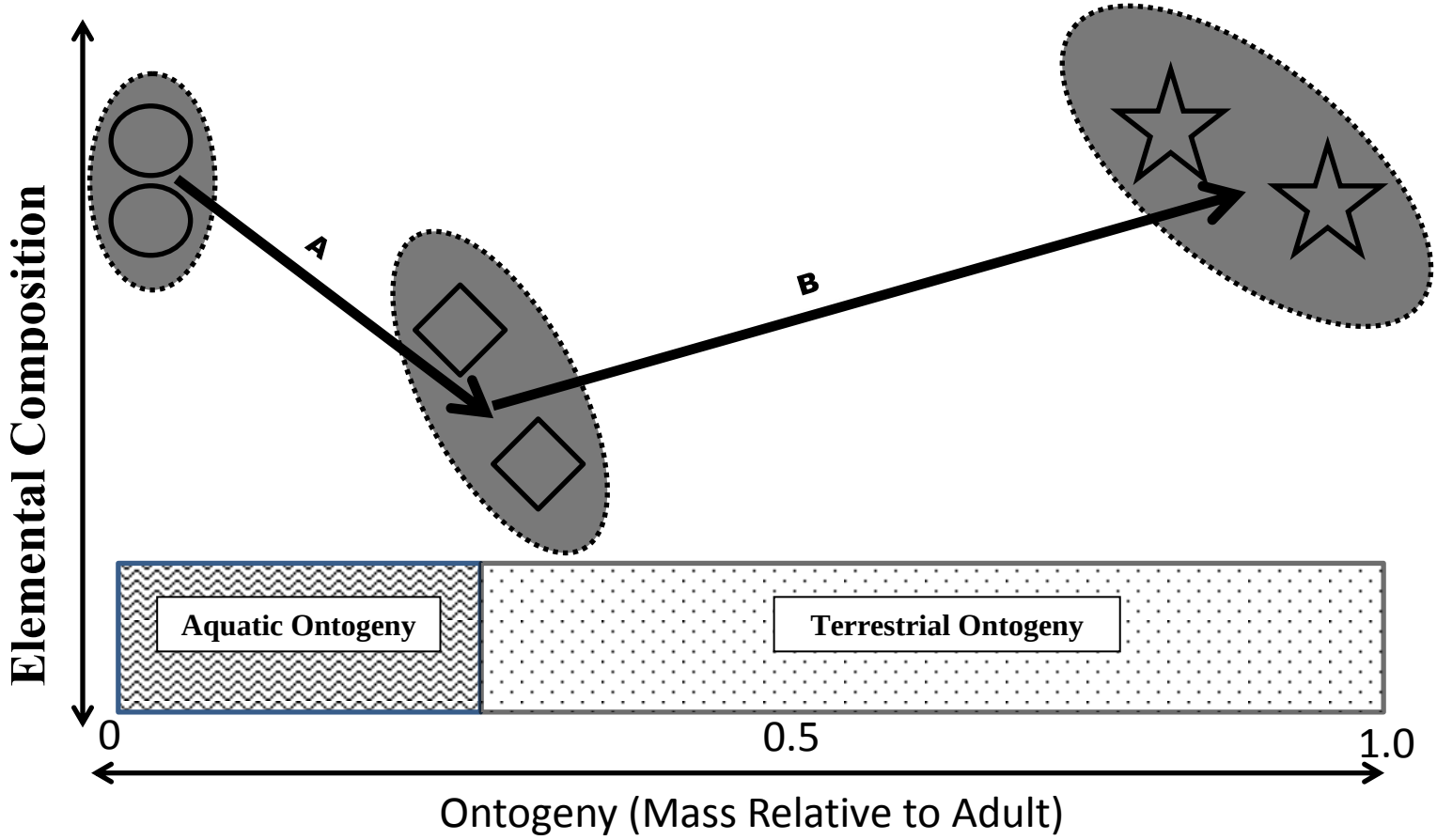
Figure 1. Ontogenetic stoichiometry of amphibians with between-stage shifts (arrows) and within-stage (shaded clouds) variation across ontogeny from birth (ova; o) to maturity (☆) with a transition stage (metamorphosis; ◊).



CHAPTER 2

Bottom-up and top-down effects in aquatic amphibian communities impact nitrogen and phosphorus cycling and community-level productivity

Thomas M. Luhring, Julia E. Earl, and Raymond D. Semlitsch

ABSTRACT

Top-down and bottom-up forces shape the ecological roles of both producers and consumers. In many aquatic systems, amphibians with complex life-histories tie together terrestrial and aquatic systems. Studies of aquatic amphibian larvae generally investigate the effects of the larval habitat on survival and fitness of the amphibians themselves. Fewer studies yet investigate or document the effects of amphibians on their aquatic habitats. We use a series of manipulative mesocosm treatments varying in bottom-up and top-down pressures to investigate the effects of amphibian larvae on aquatic productivity and water nutrients. Decreasing the strength of bottom-up effects through increased shading resulted in not only reduced productivity, but switches in the effects of amphibians on their aquatic environments. Top-down effects of predation generally increased productivity through a reduction of primary consumer biomass. Predation prevented the drawdown of phytoplankton in high light tanks and thus maintained total water P in phytoplankton biomass, whereas high light tanks with large primary consumers were most likely supplemented by the translocation of benthic P to the water column as was evident from an increase in dissolved P.

Key Words: Nutrient Recycling, Tri-trophic Effects, Community, Phosphorus, Nitrogen, Depletion, Competition, Predation, Primary Productivity

Introduction

Several nutrients in natural systems can be co-limiting or vary in degree of limitation across time and space (Townsend et al., 2011). In aquatic habitats, nitrogen and phosphorus availability often limit primary production and the growth of consumers (Elser et al., 2000). Interactions between element and organism is bi-directional, with animals impacting nutrient cycles through direct (e.g., consumption) and indirect (e.g., changes in community structure) mechanisms (Vanni 2002).

Animals shape the biogeochemical cycling of elements in various lentic and lotic waters. In stream systems, consumers can change the stoichiometry of primary producers (Rosemond et al., 1993) and nutrient recycling (Vanni et al., 2002). Animals are important nutrient sinks and cyclers in a variety of aquatic and terrestrial systems (e.g., Kitchell et al., 1979, Pastor et al., 1993, Layman et al., 2011). In many temperate (e.g., Burton and Likens 1975, Seale 1980, Regester et al., 2006) and tropical systems (e.g., Whiles et al., 2006), amphibians are thought to be important agents of nutrient cycling, however, their role in such processes and how bottom-up and top-down pressures affect those roles remains understudied.

Amphibians with complex life-histories require both aquatic and terrestrial ecosystems to complete their life cycles (Wilbur 1980). Community composition of

larval amphibians during the aquatic phase of their life cycles are shaped by a variety of top-down and bottom-up pressures including invertebrate (e.g., odonates; McCollum et al., 1997) and vertebrate (e.g., caudates; Morin 1983) predators, hydroperiod (Babbitt et al., 2003) and canopy cover (Skelly et al., 2005). While several studies have investigated the effects of light, life-histories, and predators on amphibian survivorship and growth in aquatic habitats (e.g., Morin 1983; Earl et al., 2011), none have experimentally manipulated the impacts of these top-down and bottom-up forces to study amphibian mediated biogeochemical cycling in lentic waters.

We used experimental aquatic mesocosms to raise anuran species with varying life-histories to test the effects of bottom up (light level) and top-down (predator) pressures on amphibian larvae and their effects on water N and P. Although this study was primarily interested in the general effects of amphibians (anuran larva and predatory adult caudates) on water nutrients (N and P) in aquatic ecosystems, we also tracked biotic indicators (e.g., gross primary productivity, community respiration and net primary productivity) to approximate possible mechanisms through which amphibians affect nutrient cycling.

Methods and Materials

Study Organisms

Three anuran species were chosen to represent a continuum of life-history characteristics (namely time to and size at metamorphosis). *Anaxyrus americanus,* American Toad, has a larval period of 50-60 days and metamorphoses at 7-12mm snout-vent length (SVL; Wright and Wright 1949). *Hyla versicolor,* Gray Treefrog, has a larval period of 45-65 days and metamorphoses at 16mm SVL (Wright and Wright 1949). *Lithobates*

sphenocephalus, Southern Leopard Frog, has a larval period of ~90 days (Ashton and Ashton 1988) and metamorphoses at 20-33mm (Wright 1932). All three species are widely distributed and commonly share larval habitats. *Notophthalmus viridescens,* Eastern Newt, is a wide-ranging caudate species with a predatory aquatic adult stage that can have major impacts on structuring larval assemblages (Morin 1983).

Mesocosms

A total of 72 1000-L cattletank mesocosms (Semlitsch and Boone 2009) were initiated in early March 2010. Mesocosms were initiated in the same general fashion as previous studies (Earl et al., 2011) with a substrate of 1kg of mixed oak deciduous litter. A total of seven community treatments: 1) control (CONT; no amphibians) 2) *Anaxyrus americanus* (ANAM; American Toad) 3) *Hyla versicolor* (HYVE; Gray Treefrog) 4) *Lithobates sphenocephalus* (LISP; Southern Leopard Frog) 5) *Notophthalmus viridescens* (NOVI; Eastern Newt) 6) Competition (COMP; all three frog species) 7) Competition and Predation (PRED; all three frog species and adult newts) were crossed with two light level treatments (high; 77% ambient, low; 27% ambient). Light levels were manipulated with high-density polyethylene PAK knit shade cloth of the appropriate level with 1mm (high shade) or 2mm (low shade) gauge mesh (Hummert International, St. Louis, Missouri), which also prevented colonization and oviposition of predators (e.g., odonates) and competitors (e.g., hylid treefrogs).

A random numbers table was used to assign tank community treatments and then light level treatments. Plankton were collected from wetlands at Thomas Baskett Research Area (TBRA; Boone County, Missouri, USA) and added to each tank on 27 April 2010. On 1 May 2010 we collected adult *Notophthalmus viridescens* (Eastern

Newt) from TBRA. On 2 May 2010, 2 male and 2 female *N. viridescens* were added to appropriate tanks. On 18 May 2010, *Hyla versicolor* (Gray Treefrog) tadpoles were redistributed from one tank where a female had oviposited to other appropriate tanks (n=45 each). *Lithobates sphenocephala* (Leopard Frog) eggs were collected from TBRA on 20 May 2010 and hatched in captivity. *Anaxyrus americanus* (American Toad) tadpoles were collected from Forum Nature Area (Boone County, Missouri, USA) on 25 May 2010. On 25 May 2010, *L. sphenocephala* (n=30) and *B. americanus* (n=45) were added to appropriate tanks. Day 1 of the experiment was considered to be 26 May 2010 as that was the first full day in which all of the experimental treatments were in place. Larvae were generally added to tanks immediately after they were able to swim on their own and had absorbed external gills (Gosner stage 25; Gosner 1960). The exception being field-collected *A. americanus* tadpoles, which were generally between Gosner stages 25 and 30.

Biotic Measurements

We used the diel oxygen method to estimate gross primary productivity (GPP) and community respiration (CR_{24}; Wetzel and Likens 2000). Dusk and dawn dissolved oxygen (DO; to 0.01 mg L^{-1}) and temperature (within 0.1 ^{o}C) were recorded using a YSI 55 handheld meter (Yellow Springs Instruments, Yellow Springs, OH, USA) from 3 June (Day 9) to 29 September 2010 (Day 127). Measurements were taken within an hour of sunset or sunrise (Fontaine and Ewel 1981) when the sampling window (three total consecutive sunrise or sunsets) was free of precipitation and had little to no cloud cover or wind (<8 mph). This minimal disturbance to the water-air interface allowed us to use a 0.05 diffusion coefficient (k; Wetzel and Likens 2000). Previous studies on similar

mesocosms indicate that oxygen concentrations an hour prior to sunset were indistinguishable from afternoon readings (Williams et al., unpublished data; Williams et al., 2008), indicating that sunset measurements also serve as a measure of peak oxygen concentration. Dawn-dusk DO readings were used to calculate net respiration (NR = dusk DO – dawn DO – reaeration), community respiration (CR = hourly NR * 24 hours), net primary productivity (NPP = dawn DO – dusk DO – reaeration) and gross primary productivity (GPP = NPP + amount of respiration occurring over period NPP measured; Wetzel and Likens 2000).

Nutrients

The timing of the water samples were targeted to broadly correspond with the metamorphosis of amphibian juveniles. Water samples for analysis of total nitrogen (TN), total phosphorus (TP), dissolved nitrogen (DN), and dissolved phosphorus (DP) were taken from each tank on day 11 (5 June 2010) and day 61 (25 July 2010). One raw and one filtered sample (60 mL each) were collected from each tank and frozen until they were analysed on a Technicon Flow Injection Auto-Analyzer II (Technicon Systems, Oakland, CA, USA). Samples used for DN and SRP analysis were filtered through glass fiber filters with a 0.7 μm pore size (AP40 filters, Millipore, Billerca, MA, USA). Raw water samples were run through a sulfuric acid-nitric acid digestion to measure total nitrate and total SRP (Clesceri et al. 1989). Nitrate (to 0.01 mg L^{-1}) and SRP (to 1 μg L^{-1}) were analyzed using the cadmium reduction and molybdenum blue methods, respectively (Clesceri et al. 1989).

Statistical Analyses

Comparisons – We used two-way ANOVA's with an interaction between amphibian treatment and light level to test for whole treatment and light effects. Light effects were generally predictable and strong. Our primary interests were the effects of amphibians on nutrient levels and community biotics (hereafter, "amphibian effects"). To test for amphibian effects while minimizing multiple comparisons, we used an *a priori* contrast similar to a Dunnett's test (Dunnett 1955) in which we compare amphibian treatments to control (no amphibian) tanks within the same light treatment. These contrasts were set up within a single test (i.e., using 12 d.f. for 12 comparisons of treatment by light group to corresponding control by light group). A second contrast compared COMP to PRED treatments within each light treatment to test for the effects of predators (hereafter, "predation effects").

Transformation – Nutrient data were log-transformed to meet assumptions of normality and homoscedasticity. Biotic measurements (e.g., CR, GPP) were not transformed as they generally followed a normal distribution. All statistical analyses were run in JMP (JMP®, Version 10.0.0. SAS Institute Inc., Cary, NC, 1989-2007) by running a two-way ANOVA with an interaction effect (Light X Amphibian Treatment) and then manually setting up our contrasts of interest through the LSMeans contrast option. Because of the noisy nature of complex systems and the relatively low number of tanks (4-6) within each treatment by light level combination, we also include "marginally" significant effects ($p<0.1$ and $p>0.05$) in the results section.

Results

Dissolved Nitrogen – Dissolved nitrogen (DN ± standard deviation) levels decreased from day 11 (0.0069 ±0.0048mg/L) to 61 (0.0015 ±0.0012mg/L). No significant

treatment effects were observed for day 11 and most readings of DN by day 61 were at the lower threshold of detectability (~0.001 mg/L) and not analyzed statistically (Table 1). There were no observed predator effects on DN at either sampling period (Table 2).

Dissolved Phosphorus – Dissolved phosphorus (DP) levels decreased from day 11 (16.31 ±10.17µg/L) to 61 (5.34 ±2.28µg/L). By day 11, high light ANAM and LISP tanks decreased DP in the water column relative to their respective controls (Table 1). High light LISP tanks elevated DP relative to high light control tanks on day 61. There were no observed predator effects on DP (Table 2).

Total Nitrogen – Total nitrogen was depressed in low light ANAM tanks on day 11 (Table 1). By day 61, low light LISP and low and high light COMP tanks depressed TN. High light NOVI tanks had elevated TN in the water column relative to the control at day 11. Predation effects (COMP vs. PRED) elevated TN in both high and low light treatments at day 61 (Table 2).

Total Phosphorus – Under low light conditions, total phosphorus was depressed on day 11 in ANAM tanks and by day 61 in HYVE, LISP, NOVI, and COMP tanks (Table 1). Total phosphorus was elevated by day 61 in both LISP and PRED tanks relative to the control under high light. Predation effects decreased TP at day 11 in the low light tanks, but elevated TP in the high light treatment at day 61 (Table 2).

Tank Stoichiometry – The molar ratio of dissolved N:P on day 11 was not affected by light or overall treatment effects. However, within high light tanks, ANAM and COMP both elevated water dissolved N:P ratios. Total water N:P was likewise unaffected by light or treatment on day 11. Two marginal amphibian effects were seen on day 11 total water nutrient stoichiometry: high light ANAM elevated N:P ($p=0.08$) and low light

COMP depressed N:P ($p=0.06$; Table 1). For low light treatments, predation prevented the depression of total water N:P seen in COMP tanks on day 11 (Table 2).

Dissolved nitrogen was at or below the detection limit in most tanks by day 61. Because of this, only the stoichiometry of total water N:P was statistically tested. Total water N:P on day 61 was greater in high light tanks ($p=0.0002$). Control and ANAM tanks had the lowest N:P in low light tanks and the highest N:P in high light tanks. Amphibian effects on total water N:P thus varied according to light treatment. In high light tanks, LISP and PRED lowered total water N:P (Table 1). In low light tanks, HYVE and NOVI raised total water N:P (Table 1). Total water N:P increased from day 11 to 61 in both light treatments, but increased more in high light tanks ($p=0.016$). In high light tanks, although a moderate increase in total water N:P was seen over time, LISP prevented the degree of increase seen in CONT tanks (Table 1). A similar but marginal effect was seen in PRED tanks ($p=0.08$).

Community Respiration – Community respiration (CR) was higher in high light tanks across all five sampling periods ($p<0.0001$ in all but day 127, where $p=0.005$). Amphibian effects contrasts revealed that COMP high light tanks had higher respiration rates than that of CONT high light tanks (Table 3). By day 28, treatment effects remained mildly significant ($p=0.07$) and were driven by the greater than two-fold difference between CR in LISP (3.69) and HYVE (1.50). Amphibian effects contrasts likewise revealed only mildly significant differences between treatment x light combinations with high light LISP ($p=0.05$) and NOVI ($p=0.06$) showing elevated CR's.

Day 45 of the experiment showed the strongest treatment effects ($p=0.0002$) with NOVI and ANAM showing higher CR than COMP and LISP. Community respiration

values for CONT tanks were only 0.4 lower than ANAM and nearly fell out with ANAM and NOVI at this time period. Four high light amphibian treatments, HYVE, LISP, COMP, and PRED showed depressed CR relative to high light controls (Table 3). Predation elevated CR in high light tanks (Table 4).

By day 86, no treatment effects remained ($p=0.13$) and only a borderline amphibian effect was detected (COMP low light depressed CR $p=0.08$). Day 127 saw the lowest overall CR values (-1.40) and light effects ($p=0.005$). At this time, a borderline treatment effect ($p=0.07$) was driven by low values for ANAM (-2.11) and COMP (-1.98) tanks relative to CONT (-0.85). Amphibian effects were detected for low light COMP and high light ANAM, which both depressed CR relative to controls (Table 3).

Net Primary Production – As expected, hourly net primary productivity (NPP) was higher in high light tanks (all five intervals $p<0.001$). No treatment, amphibian or predator effects on NPP were evident at day 9. By day 28, a treatment effect ($p=0.017$) was driven by the divergence of ANAM (depressed) and COMP treatments (elevated). The only amphibian effect at day 28 was the elevation of NPP in high light COMP tanks (Table 3). On day 45 no predator effects were observed, and NOVI and PRED only marginally depressed NPP in high light treatments (both $p=0.09$).

Day 86 had the strongest overall amphibian effects on NPP. Four high light treatments, ANAM, LISP, NOVI, and PRED demonstrated reduced NPP relative to the high light control (Table 3). Low light COMP tanks also depressed NPP relative to the low light control. Predation marginally depressed NPP in low light ($p=0.1$) and elevated NPP in high light tanks (Table 4). By day 127, no predation effects were evident and

NPP was depressed in all three high light amphibian treatments containing *L. sphenocephalus*, LISP, COMP, PRED (p=0.09). Within high light tanks, total emergent anuran wet mass was negatively correlated with NPP on day 127 (NPP/hr = 0.16 – 0.0013*Total Anuran Mass; $F_{1,34}$ = 13.09, p=0.001, adj. R^2=0.26). There was a negative but non-significant trend in low light tanks (p=0.16).

Gross Primary Productivity – As expected, gross primary productivity (GPP) was higher in high light tanks (all five intervals p<0.001). Treatment effects (p=0.03) were evident by day 9 with ANAM showing decreased GPP relative to COMP tanks. The only amphibian effect detected at day 9 was a mild elevation of GPP in high light COMP tanks (p=0.09). Treatment effects at day 28 were driven by differences in mean ANAM (5.04) and COMP (2.7) GPP. In high light treatments, COMP treatments marginally elevated GPP (p=0.056).

Day 45 had the highest number of and strongest effects of treatments on GPP. An overall treatment effect (p=0.004) was driven by lower GPP in both COMP and LISP treatments relative to NOVI. In the high light treatment, three amphibian effects resulted in reduced GPP relative to the control: HYVE (p=0.07), LISP, COMP (Table 3). By day 45, predation increased GPP (Table 4). No treatment, amphibian or predation effects were significant on day 86. By day 127, only a weak signature of a treatment effect (p=0.09) was caused by a difference between COMP (-0.10) and CONT (1.08) GPP. Three amphibian treatments reduced GPP: low light COMP (p=0.07), high light COMP, and high light ANAM (p=0.09).

Discussion

Consumers directly affected water chemistry and community biotic measurements, with bottom-up and top-down processes shaping their influence (Tables 1-4). As expected, light levels (bottom-up driver) had pervasive and consistent effects across time, mainly through increased primary productivity. Predation (top-down driver) had temporally varying effects on mesocosm productivity, and water chemistry.

Amphibians with complex life-histories are transient consumers that directly impact aquatic systems for a finite portion of time between hatching and metamorphosis. All single-species treatments affected water chemistry at one point during the course of the experiment with the exception of high light HYVE tanks (Table 1). The majority of water nutrient effects coincided temporally with the presence of amphibians in tanks (8 of 10 effects seen in single-species anuran tanks; Table 1). The exceptions were low light HYVE, which decreased TP (and thus increased molar TN:TP) approximately 46 days after their mean day of emergence (15.3; Table 1). Top-down effects on nutrient levels interacted with time and bottom-up pressures. Total phosphorus was depressed in low light predation tanks by day 11 (resulting in a 76% increase in total water molar N:P), whereas 50 days later, only high light treatments demonstrated significant effects on total water nutrients corresponding to increases of TN and TP (marginal increase of TN in low light; Table 2).

Community biotics were consistently affected in high light tanks (at least one effect per sample day (Table 3). In low light tanks CR, GPP, and NPP were all depressed relative to tanks with high light input. This low relative productivity may have resulted in a reduced probability of amphibian effects to be detected due to an already low

background rate of production relative to background variance. Only the largest treatment of consumers (by emergent biomass: COMP) had any measurable effects in low light treatments. Interestingly, these effects were only significant after the vast majority of anurans had metamorphosed (days 86 and 127).

Under high light conditions nearly all of biotic measurements through day 45 corresponded temporally with the presence of anuran larvae (8 of 10 treatment effects), with two exceptions, delayed effects in high light HYVE tanks on CR and GPP on day 45. For the two sampling periods (day 86 and 127) following day 45 few anuran larvae remained in tanks and none metamorphosed after day 80. However, when including the day 45 effects in HYVE tanks, delayed production effects in high light tanks were present in every treatment (figures 1-6).

Top-down effects on biotic measures were multi-trophic and generally resulted from the effects of predators on the largest primary consumer in the community (*L. sphenocephalus*). Top-down effects on high light community productivity were strongest at day 45 when *L. sphenocephalus* emergent biomass production was peaking (figures 3,5; Table 3). In high light tanks without predators, anuran communities (COMP) decreased both CR and GPP by 50 and 24%, respectively, in comparison to control tanks. The addition of predators to otherwise identical tanks reduced the drawdown of CR and GPP seen in high light COMP tanks (Table 4) to levels commensurate with the no-amphibian control tanks (Table 3).

While the majority of effects on nutrients and tank biotics were associated with the presence of *L. sphenocephlus*, even the smallest consumer (*A. americanus*) had measureable effects. The timing of ontogenetic development and rate of change in

whole-body stoichiometry during the transition from hatchling to metamorph may explain differences in the early effects of *L. sphenocephalus* and the smaller and faster developing *A. americanus.* Both species decreased DP in high light tanks indicating a selective retention of P by tank biota (e.g., reduced P excretion). However, ANAM tanks increased N:P molar ratios in the dissolved pool by 143% compared to a 30% elevation in LISP tanks. The increase in ANAM tank dissolved N:P was bolstered by a moderate, but not statistically significant increase (+27%) in DN compared to a decrease of the same magnitude (-25%) in LISP DN. Phosphorus is associated with bone formation and relative growth rate (Calder 1984; Elser et al., 1996) and dissolved phosphorus in the water column may be more strongly affected through selective retention by animals that are growing fast and developing bone (i.e., anurans preparing for metamorphosis). Nitrogen, which was depressed in its dissolved form in LISP tanks, is associated with protein creation and could have been affected more strongly by animals that were incorporating proportionally more "soft" biomass than structural investments (e.g., muscle and soft tissue versus bone).

Anuran larval communities demonstrate priority effects in which the timing of hatching (e.g., beginning of larval period) influences the strength of competition between two or more species (Wilbur and Alford 1985, Alford and Wilbur 1985, Lawler and Morin 1993). The currency through which these effects occur is usually attributed to resource availability. Phytoplankton and algae are generally assumed to be that currency in pond systems and are reduced in abundance through top-down effects of anuran larvae (Seale 1980). This reduction of phytoplankton, in turn, reduces primary productivity and the standing stock of particulate N (Seale 1980). While a myriad of studies on amphibian

competition have demonstrated the effects of anuran larvae on each other, their effects on nutrient cycling and primary production dynamics in lentic systems are only known from this one pond.

In our systems, the relative strength of bottom-up effects determined the net effect of amphibian composition on post-metamorphosis water nutrients, stoichiometry, and whole-tank productivity. Although initially similar at day 11, reduction of bottom-up forces through lowered solar input, resulted in higher TP in low light controls than high light controls by day 61 (22.39 versus 11.63 ug/L). While controls differed in their total water column P, so did the relative effects of anurans. In the P-enriched low light tanks, anurans generally drew down whole-tank TP by day 61, whereas in P-depleted tanks, *L. sphenocephalus* increased dissolved and total water P (Table 1). Total N in each control was similar and anurans drew down total N in each light treatment. Amphibians as a whole, including primary and secondary consumers, tended to moderate the N:P balance of whole-pond systems. When compared to low light controls with a mean molar TN:TP of 12.54 amphibians tended to increase TN:TP. Conversely, when compared to high light controls with a higher mean molar TN:TP of 29.12, amphibians decreased whole tank TN:TP (Table 1).

While N and P generally decreased in whole-tank abundance across the course of the experiment, decrease in dissolved and total P was more dramatic. This may in part explain the pattern of increased dissolved and total P in LISP tanks. Tadpoles respond to reduced phytoplankton by switching to foraging strategies to detrivory (Seale 1980). Detrivorous fish can increase dissolved P in the water column by eating benthic sediments and excreting nutrients in the water column (Vanni 2002). This differs from

the increased TP seen in the predator treatment at day 61 which was not caused by an increase in DP. Thus it appears that the increased NPP at day 86 in high light tanks was caused by both top-down effects of newts (NOVI and PRED tanks) and bottom-up P supplementation of the water column (LISP tanks).

These manipulative studies of amphibian effects on aquatic systems are useful for understanding potential mechanisms of how amphibians interact with and affect biotic functioning of aquatic systems and biogeochemical cycling. It also highlights the sensitivity of aquatic systems to the interactions of amphibian-mediated top-down and bottom-up forces. Anuran larvae exist in a wide range of densities, however, our experiment used a highly conservative density in relation to natural systems, meaning that our effects may likewise be highly conservative in regards to natural systems. Future studies varying densities and bottom-up resource supplementation would be a welcome addition to our understanding of how amphibian larvae shape both productivity of and nutrient cycling within natural systems.

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Table 1. Amphibian treatment effects (in percent difference relative to control) on water nutrients. Control mean phosphorus values in µg/l, nitrogen values are in mg/L. Significant effects denoted at $p<0.05$ (*), or at $p<0.10$ (.).

	Day 11						Day 61					Change
	Phosphorus		Nitrogen		Molar	Molar	Phosphorus		Nitrogen		Molar	Molar
	DP	TP	DN	TN	DN:DP	TN:TP	DP	TP	DN	TN	TN:TP	TN:TP
Low Light												
Control $\bar{x}$	13.65	40.37	0.008	0.83	0.24	9.46	5.61	22.39	0.001	0.61	12.54	3.08
ANAM	0.09	-0.45*	-0.10	-0.33*	-0.08	0.31	-0.27	-0.20	-	-0.12	0.10	-0.54
HYVE	-0.11	-0.05	-0.22	0.02	0.04	0.24	-0.05	-0.51*	-	-0.23	0.66*	1.94
LISP	0.26	-0.18	-0.28	-0.13	-0.17	-0.14	-0.03	-0.39*	-	-0.29*	0.24	1.42
NOVI	0.54	-0.19	0.05	-0.08	-0.33	0.25	-0.17	-0.41*	-	-0.22	0.74*	2.24
COMP	0.41	0.38	0.28	-0.03	0.96	-0.28.	-0.12	-0.52*	-	-0.36*	0.35	2.28
PRED	-0.01	-0.07	-0.17	0.09	0.26	0.27	0.07	-0.28	-	-0.14	0.25	0.21
High Light												
Control $\bar{x}$	22.94	33.70	0.005	0.68	0.13	9.59	4.70	11.63	0.002	0.63	29.12	19.52
ANAM	-0.54*	-0.26	0.27	0.01	1.43*	0.39.	0.57	0.02	-	0.03	-0.07	-0.30
HYVE	-0.09	0.14	0.64	0.19	0.49	0.06	-0.04	0.14	-	-0.08	-0.23	-0.38
LISP	-0.52*	-0.13	-0.25	0.05	0.31	0.28	0.65*	0.68*	-	-0.01	-0.42*	-0.77*
NOVI	-0.05	0.51	-0.06	0.68*	0.06	0.12	0.09	0.39	-	0.29	-0.18	-0.32
COMP	-0.40	0.12	0.80	0.29	1.59*	0.19	-0.25	-0.17	-	-0.25*	-0.11	-0.26
PRED	-0.36	0.07	0.09	0.27	0.58	0.20	0.23	0.53*	-	0.11	-0.33.	-0.59.

Table 2. Predation effects (in percent difference relative to competition tanks) on water nutrients. Competition treatment phosphorus values in µg/l, nitrogen values are in mg/L. Significant effects denoted at $p<0.05$ (*), or at $p<0.10$ (.). Light level denoted with treatment (L=low; H=high).

	Day 11						Day 61				Change
	Phosphorus		Nitrogen		Molar		Phosphorus		Nitrogen	Molar	Molar
	DP	TP	DN	TN	DN:DP	TN:TP	DP	TP	TN	TN:TP	TN:TP
L COMP $\bar{x}$	19.29	55.68	0.01	0.81	0.48	6.82	4.94	10.73	0.39	16.91	10.09
L PRED	-0.30	-0.33.	-0.35	0.13	-0.36	0.76*	0.21	0.51	0.34.	-0.07	-0.63
H COMP $\bar{x}$	13.70	37.72	0.01	0.87	0.33	11.37	3.53	9.61	0.47	25.88	14.52
H PRED	0.06	-0.04	-0.39	-0.01	-0.39	0.01	0.64.	0.85*	0.48*	-0.24	-0.44

Table 3. Amphibian treatment effects (percent difference from control) on biotic measurements. Control values shown for NPP are hourly rates, whereas CR and GPP are averages for 24-hour time periods. Significant effects denoted at $p<0.05$ (*), or at $p<0.10$ (.).

	Day 9			Day 28			Day 45			Day 86			Day 127		
	CR	GPP	NPP	CR	GPP	NPP	CR	GPP	NPP	CR	GPP	NPP	CR	GPP	NPP
							Low Light								
Control $\bar{x}$	-1.50	-0.73	0.01	0.90	2.43	0.13	4.10	6.25	0.26	3.99	7.13	0.35	-1.10	0.45	0.08
ANAM	0.56	1.21	-1.84	0.64	0.23	0.12	0.24	0.15	0.10	0.18	0.07	0.01	0.71	-0.97	-0.05
HYVE	0.45	0.74	-0.62	-0.88	-0.26	-0.08	0.09	0.06	0.04	0.06	0.07	0.07	0.44	-0.51	0.01
LISP	0.47	0.76	-0.62	0.68	-0.03	-0.24	-0.24	-0.08	0.03	-0.45	-0.18	-0.05	0.68	-1.28	-0.20
NOVI	0.16	0.47	-0.99	-0.24	0.01	0.08	0.41	0.14	-0.04	-0.28	-0.10	-0.01	0.37	-0.82	-0.17
COMP	-0.59	-1.30	1.99	-0.31	-0.17	-0.13	-0.04	0.05	0.11	-0.55.	-0.07	0.16*	1.31*	-2.35.	-0.34
PRED	0.18	0.15	0.29	0.62	0.03	-0.14	0.27	0.14	0.05	-0.06	0.02	0.06	0.91	-1.51	-0.18
							High Light								
Control $\bar{x}$	0.86	1.98	0.10	3.71	5.51	0.23	9.06	9.98	0.31	8.26	10.70	0.43	-0.60	1.73	0.17
ANAM	-1.21	-0.57	-0.33	0.35	0.29	0.24	-0.02	0.01	0.05	0.05	0.08	0.11.	2.91*	-0.58.	-0.07
HYVE	0.38	-0.21	-0.43	-0.22	-0.20	-0.19	-0.27*	-0.15*	0.00	-0.05	0.01	0.05	-0.77	-0.11	-0.20
LISP	0.98	0.32	0.08	0.58.	0.17	-0.10	-0.42*	-0.19*	0.08	-0.18	-0.02	0.11*	1.00	-0.52	-0.29*
NOVI	0.69	0.36	0.24	0.57.	0.23	-0.01	0.03	0.07	0.12.	0.01	0.06	0.10*	-0.06	-0.11	-0.11
COMP	1.70*	0.67.	0.30	-0.29	-0.37.	-0.42*	-0.50*	-0.24*	0.09	-0.12	-0.04	0.03	1.38	-0.77*	-0.45*
PRED	0.84	0.25	0.04	0.00	-0.14	-0.23	-0.22.	-0.07	0.11.	-0.18	-0.01	0.13*	0.56	-0.36	-0.23.

Table 4. Predation effects (in percent difference relative to competition tanks) on biotic measurements. Competition treatment values shown for NPP are hourly rates, whereas CR and GPP are averages for 24-hour time periods. Significant effects denoted at $p<0.05$ (*), or at $p<0.10$ (.).

	Day 9			Day 28			Day 45			Day 86			Day 127		
	CR	GPP	NPP	CR	GPP	NPP	CR	GPP	NPP	CR	GPP	NPP	CR	GPP	NPP
L COMP $\bar{x}$	-0.61	0.22	0.04	0.62	2.01	0.11	3.91	6.54	0.29	1.82	6.62	0.40	-2.54	-0.61	0.06
L PRED	1.90.	-4.87	-0.57	1.36	0.25	-0.01	0.33	0.09	-0.05	1.07	0.10	-0.09.	-0.18	-0.62	0.24
H COMP $\bar{x}$	2.33	3.31	0.13	2.63	3.47	0.13	4.49	7.62	0.34	7.29	10.31	0.44	-1.43	0.40	0.09
H PRED	-0.32	-0.25	-0.20	0.41	0.37	0.34	0.57*	0.22*	0.02	-0.07	0.03	0.10*	-0.34	1.73	0.41

Figure 1. *Anaxyrus americanus* treatment distribution of metamorphosis time (A,B), biomass production (C,D), and effect sizes (percent difference from control) of nutrient (E,F), and biotic measurements (G,H).

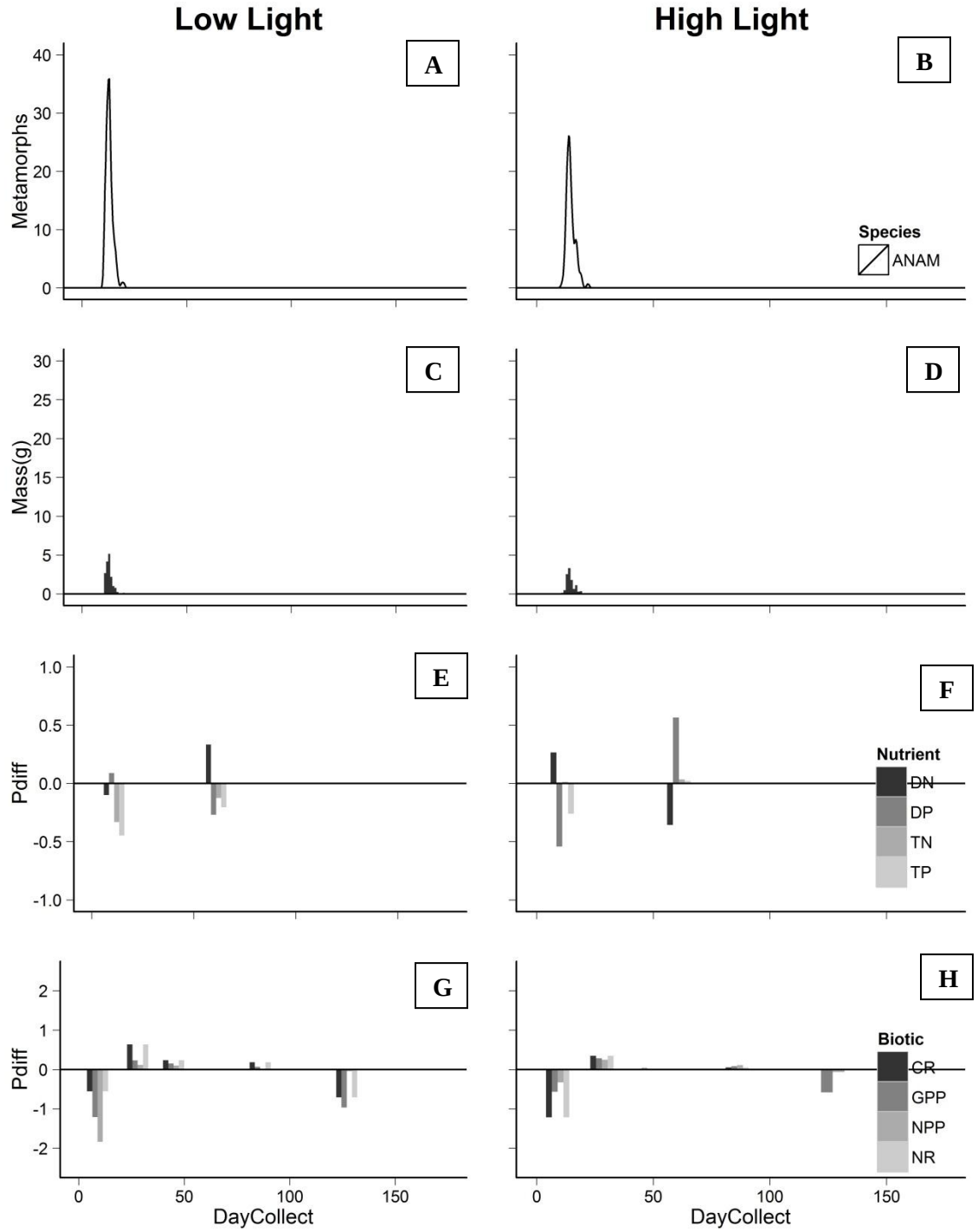


Figure 2. *Hyla versicolor* treatment distribution of metamorphosis time (A,B), biomass production (C,D), and effect sizes (percent difference from control) of nutrient (E,F), and biotic measurements (G,H).

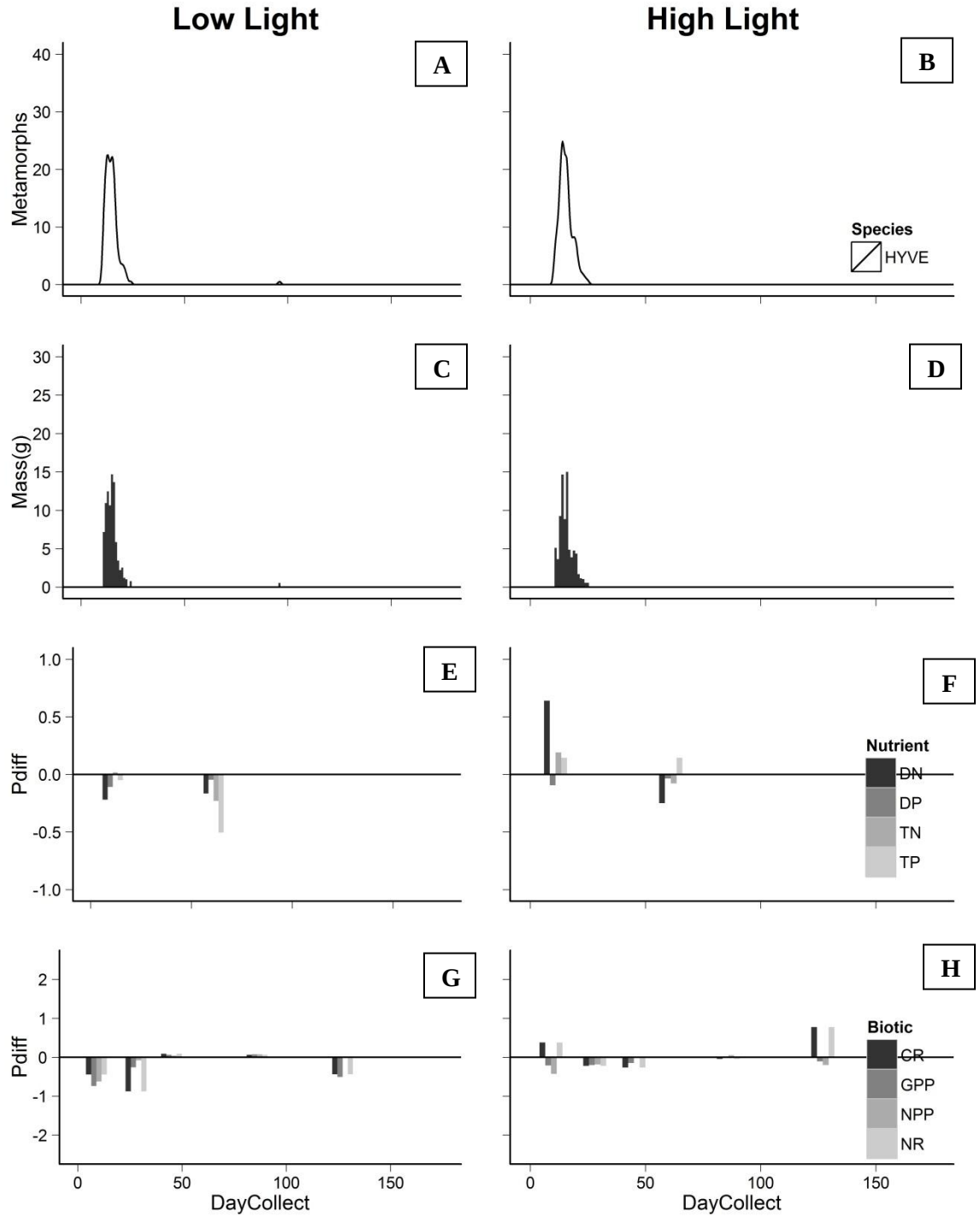


Figure 3. *Lithobates sphenocephalus* treatment distribution of metamorphosis time (A,B), biomass production (C,D), and effect sizes (percent difference from control) of nutrient (E,F), and biotic measurements (G,H).

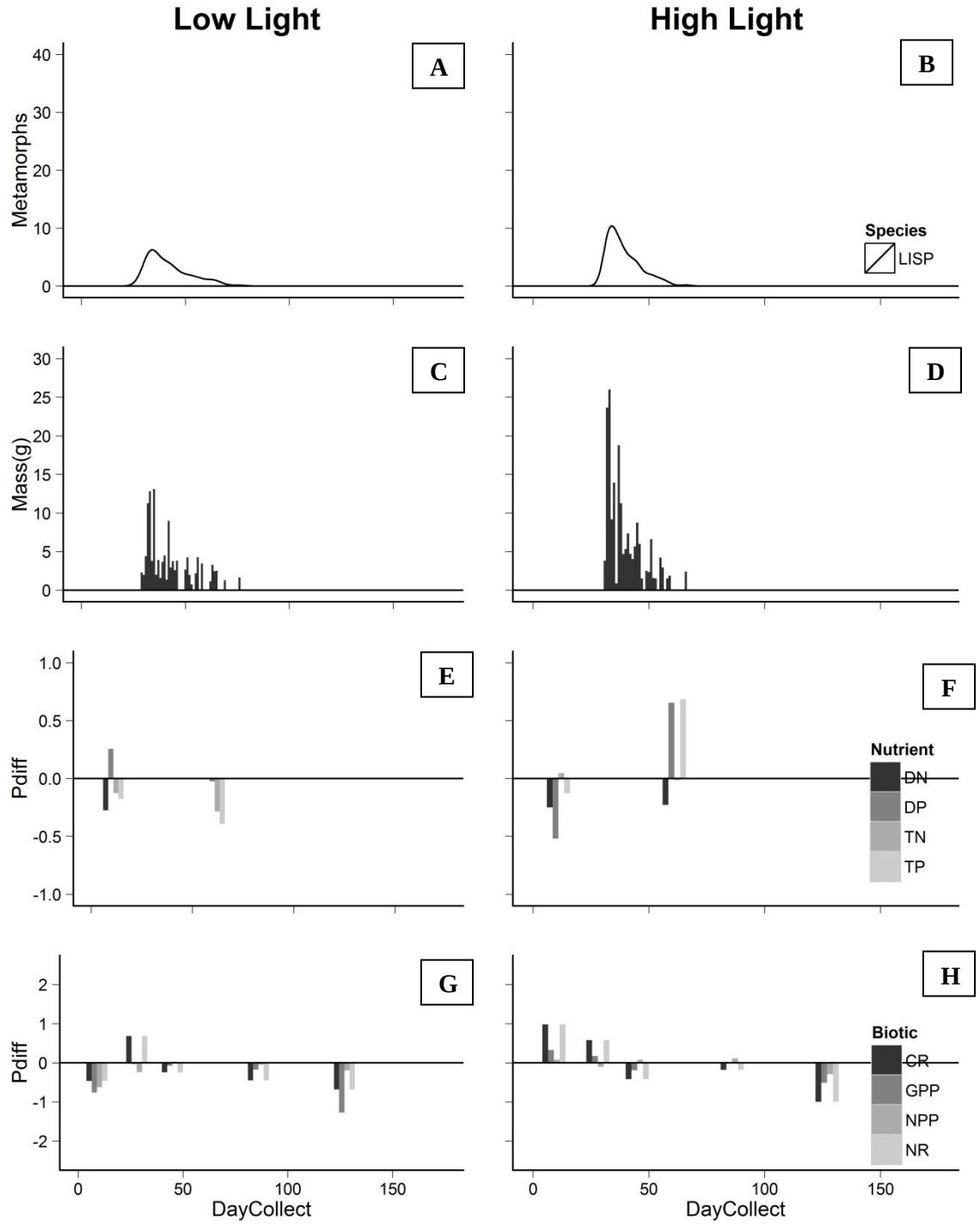


Figure 4. *Notophthalmus viridescens* treatment distribution of metamorphosis time (A,B), biomass production (C,D), and effect sizes (percent difference from control) of nutrient (E,F), and biotic measurements (G,H).

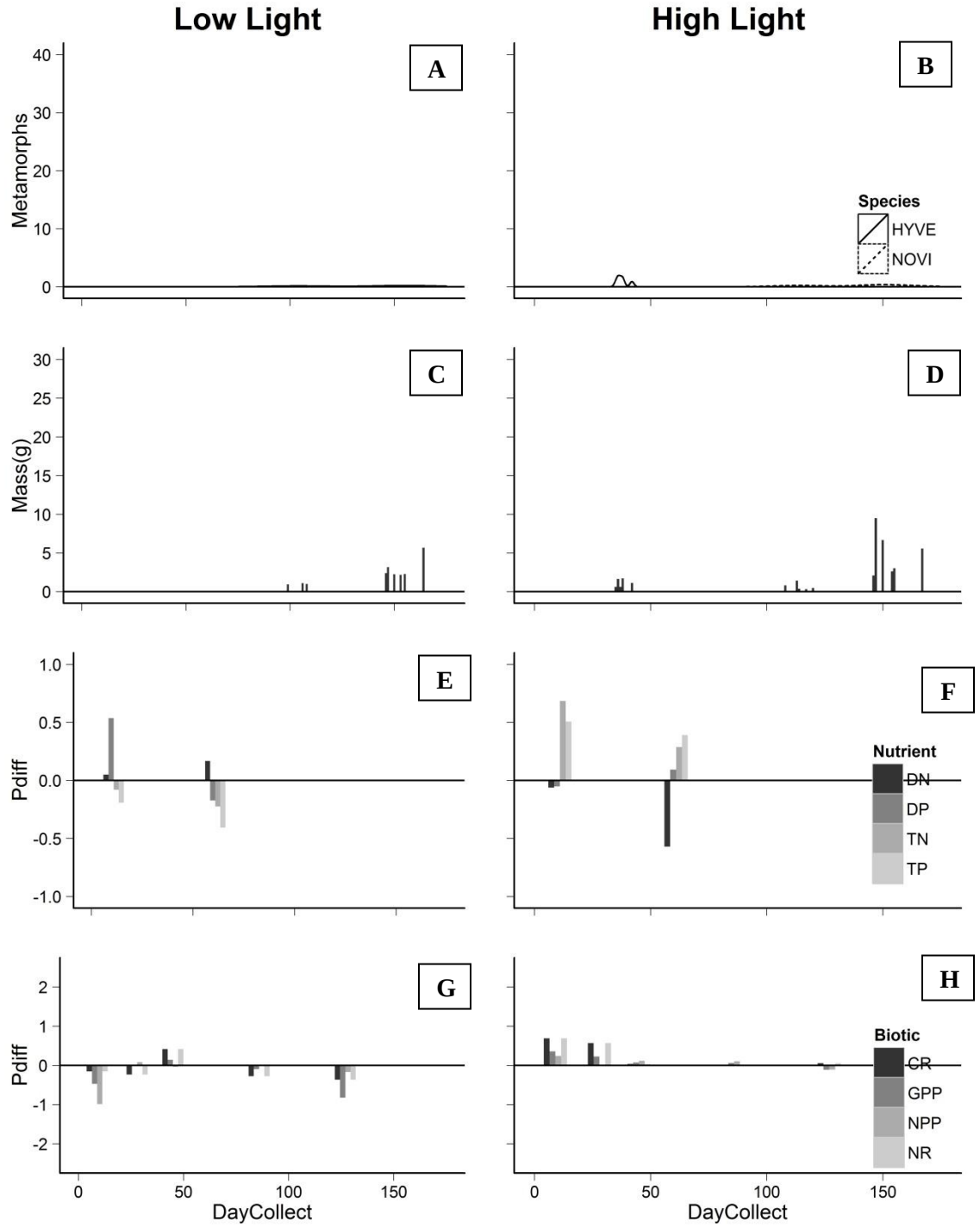


Figure 5. Competition treatment distribution of metamorphosis time (A,B), biomass production (C,D), and effect sizes (percent difference from control) of nutrient (E,F), and biotic measurements (G,H).

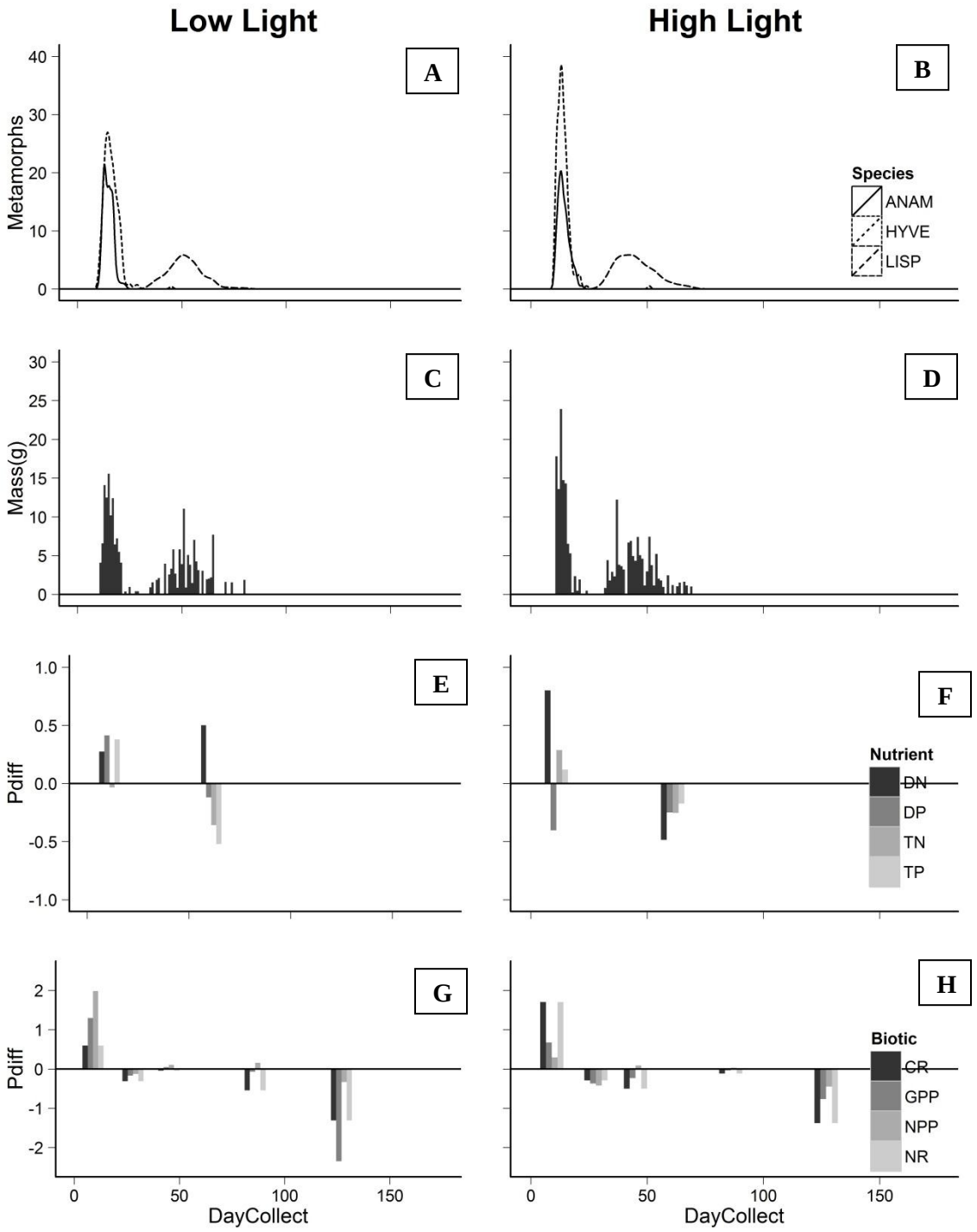
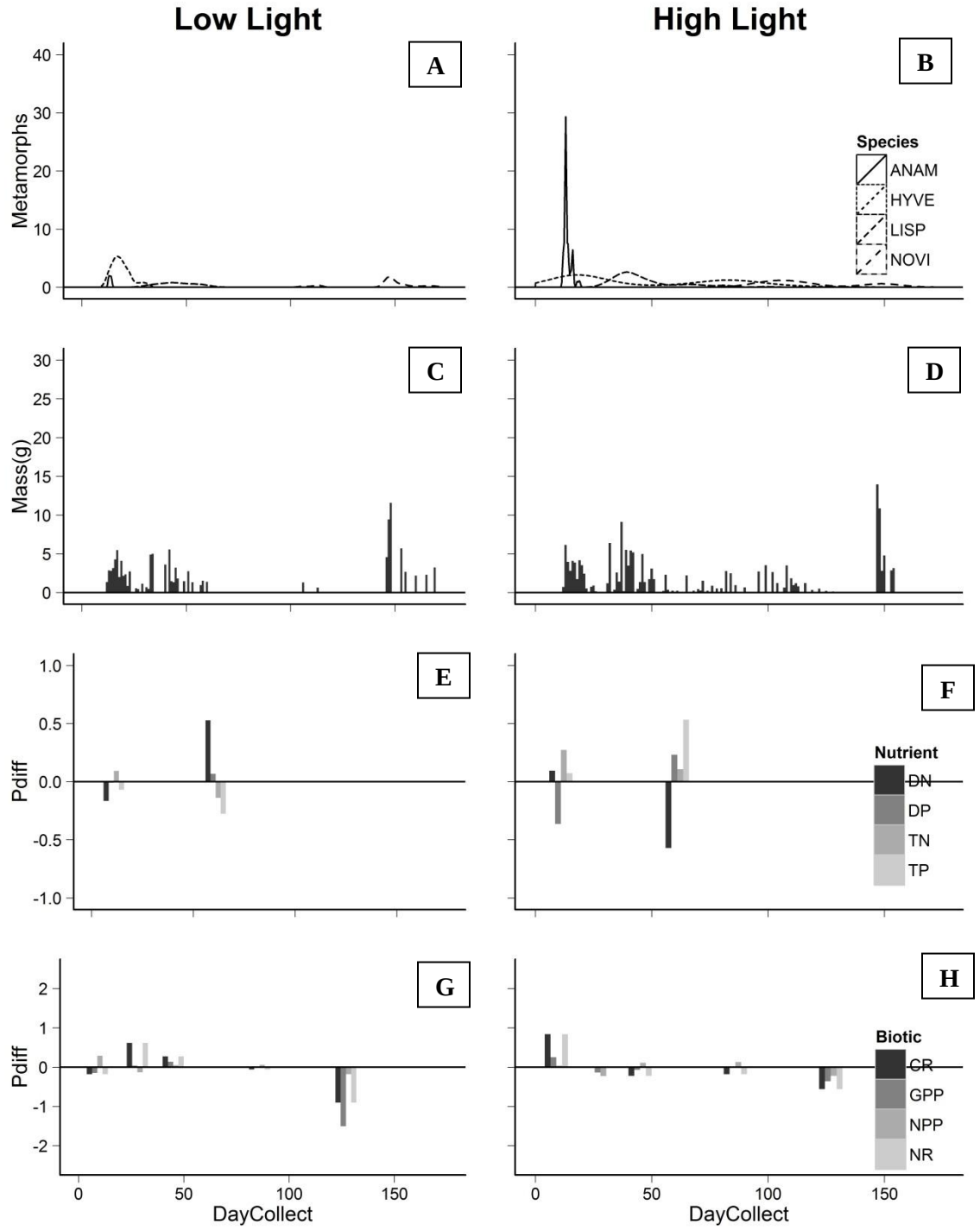


Figure 6. Competition + Predation treatment distribution of metamorphosis time (A,B), biomass production (C,D), and effect sizes (percent difference from control) of nutrient (E,F), and biotic measurements (G,H).



CHAPTER 3

Time to face the strain: Changes in vertebrate stoichiometry across ontogeny when complex life-histories present stage-specific demands.

Thomas M. Luhring,[1,2,*] Diana L. Soteropoulos,[2] David E. Scott,[2] Gary M. Mills,[2] Raymond D. Semlitsch[1]

1. Division of Biological Sciences, University of Missouri, Columbia, MO, USA 65211

2. Savannah River Ecology Laboratory, Drawer E, Aiken, SC, USA 29802

Abstract

The availability of essential elements governs life processes from cellular to landscape scales. Elements change in their relative importance to organisms as a function of changes in growth rate and body structure. Whereas coping with the elemental demands of living within one type of habitat can be in itself a challenge, several groups of organisms change habitats during the course of their own developmental progression (ontogeny). We used vertebrates (pond-breeding amphibians) with complex life-histories to investigate the influences of life-stage-specific demands on body stoichiometry during a major life transition (metamorphosis). We targeted three stages encompassing the life-cycles of amphibians; ova, recently metamorphosed juveniles, and adults. Nearly all of the elements examined (15 of 17) varied in their relative abundances

between life stages. Patterns of change across ontogeny varied from a monotonic increase or decrease to stage-specific spikes in the relative amount of an element present. Larval development strategy influenced among-species differences in stoichiometry of both ova and recently-metamorphosed juveniles. For mature animals, body length (SVL) and the presence or absence of a metamorphosis into a terrestrial form explained most among-species differences. For animals navigating stage-specific changes in ontogeny, major transition points such as metamorphosis have specific elemental requirements and potentially operate as stoichiometric bottlenecks.

Introduction

The availability of elements modulates ecosystem function (Vitousek 2004) and the ecologies of organisms across several scales of organization. From cellular growth and replication (Elser et al., 1996) to the landscape distribution of wildebeest during migration in the Serengeti (Holdo et al., 2009), elements are tightly coupled to the behavior, ecology and morphology of all life. Every organism endures a life-long struggle to maintain specific combinations of elements in their bodies that differ substantially from that of their surrounding environment (Lotka 1925). The vast majority of studies that document this requisite process generally focus on the last stage – when organisms arrive at a state of relative homeostasis (but see Villar-Argaiz et al., 2002; Pilati and Vanni 2007).

The nutritional needs and body composition of organisms are dynamic and change throughout ontogeny with body size, growth rate, and morphology (Elser et al., 1996). In organisms without major structural investments (i.e., bone, chitin), percent body phosphorus increases with relative growth rate as a function of increased body mass allocation to RNA (~9% P by mass) and ribosomal activity associated with growth (Sterner and Elser 2002). Increases in body size are associated with a decreased growth rate, relative contribution of biosynthetic

machinery (e.g., ribosomes and RNA) to whole body mass, and percent body phosphorus. For organisms with higher contributions to structural components (e.g., bone), other factors such as body composition weigh more heavily on their elemental composition as body size increases.

Vertebrate body composition and stoichiometry change concomitantly with body size (Calder 1984). For vertebrates, increase in size is generally accompanied by a disproportionate increase in bone. Bone is richer in several elements relative to other somatic tissues (e.g., phosphorus, calcium), which results in those elements being of higher relative abundance in larger vertebrates (mammals; Prange et al., 1979, teleost fishes; Cassadevall et al., 1990). While elements should generally increase or decrease in abundance across ontogeny solely as a function of changing body composition, some elements serve multiple dynamic roles within the developing body, which change throughout development (e.g., phosphorus for growth and bone). While life within a single habitat requires a successful balancing of several elements, animals that are tied to multiple habitats as obligate parts of their life cycle must navigate the elemental requirements and limitations of each system.

Ontogenetic changes in stoichiometry should be especially pronounced in organisms that have complex life-histories. The copepod *Mixodiaptomus laciniatus,* varies in P content across ontogenetic stages with relative P content reaching a peak during intermediate life-stages (Carillo et al., 2001). We investigate changes in elemental composition across ontogeny and taxonomy for a group of vertebrates with complex life histories and differing life-history strategies. Many amphibians experience a major developmental transition from aquatic larvae to terrestrial juvenile and adult. This transition stage (metamorphosis) should be accompanied by an increase in body mass dedicated to structural support. Because animals experience differing pressures at

each life stage, we investigated a suite of elements that are potentially affected by changes in life-history, physiology, and morphology.

Several groups of amphibians require both terrestrial and aquatic habitats to fulfill stage-specific needs of their life-cycle. Because we are interested in stoichiometric changes across ontogeny in each habitat, we selected three stages that encapsulate a beginning and endpoint for the aquatic (ova to metamorphosis) and terrestrial (metamorphosis to adult) segments of amphibian life-cycles. While there are continuous and possibly dynamic changes in the developmental time between these discrete stages, we selected these three points in ontogeny to provide beginning and end-points for each phase (aquatic and terrestrial) that are standardized and easily comparable between different taxa. These beginning and endpoints provide insight into the minimal elemental requirements to progress through ontogeny for animals with complex life-histories.

Conceptual ontogenetic stoichiometry models are developed using these stages to provide a common framework for studies investigating changes in body composition across ontogeny (Fig. 1). These models scale ontogeny in relation to the adult size across the horizontal axis (independent variable) and elemental composition on the vertical axis (dependent variable). Steep positive slopes between stages indicate potential elemental constraints on development as a function of accelerated disproportionate incorporation of that element into body mass. Neutral slopes indicate that an element is being incorporated at levels consistent with the previous stage, whereas negative slopes indicate that an element is being incorporated at levels lower than that of the previous stage. The system mass (e.g., whole body) of a particular element can still increase with a negative slope (i.e., a negative slope only indicates a decreasing relative rate of

element-specific incorporation into biomass not a net loss from system mass) because overall body mass is increasing.

We use a range of species with a variety of life-histories, including size at metamorphosis (horizontal position) to investigate factors influencing stoichiometry of animals within each stage (vertical position) and the changes in stoichiometry between stages (slope). We test two general hypotheses for each element:

1) Elemental composition changes across ontogeny.
2) Elemental composition varies among individuals within each stage with respect to life-history, taxon, and size.

Support for either hypothesis then leads to further hypotheses associated with various stoichiometric theories revolving around specific elements (e.g., phosphorus and the growth-rate hypothesis; Elser et al., 1996).

Methods

Specimen collection

Adults, recently-metamorphosed juveniles (hereafter referred to as metamorphs), and ova were collected from four families of amphibians including two species of caudates and four species of anurans. We used ova in gravid females (hereafter referred to as ova) rather than freshly oviposited eggs (hereafter referred to as eggs) as the initial stage to avoid issues of external contamination. Additionally, the composition of ova gave us a direct measure of maternal elemental investment. Additional adults, tadpoles and eggs were collected for additional comparisons across taxa (a total of eight families and eleven species). Most specimens were salvaged animals that died during various trapping efforts at locations across the Savannah River

Site (Aiken and Barnwell Counties, South Carolina, USA; most recent SC DNR Collecting Permit 23-1012). All salvaged animals were intact and frozen prior to sample preparation. Live animals were captured and physically euthanized to avoid chemical contamination that might affect stoichiometric analyses (MU ACUC 6144, 7403).

Specimen preparation

Ova, eggs, and metamorphs were generally too small to be used individually for analyses and were combined to provide a composite sample. Snout-vent length (SVL) and mass were collected for metamorphs and adults. When possible, two composite samples of metamorphs were created from each end of the size continuum for a species (i.e., one large and one small composite sample). Ova and eggs were enumerated and weighed to provide per capita mass (dry and wet). Because we were interested in whole-body elemental content, we included all organs in samples (minus ova for gravid females). Adults, large metamorphs, and juveniles were dissected and checked for food items (which were then removed and G.I. tracts rinsed if present). All specimens were vacuum dried in a Labconco Freezone vacuum dryer prior to being homogenized.

Stoichiometric Analyses

Dried and homogenized samples were then subsampled, packed in glass vials, and sent to the MBL Stable Isotope Laboratory, Woods Hole, MA for carbon and nitrogen content analysis. Carbon and nitrogen content was determined using a Europa ANCA-SL elemental analyzer – gas chromatograph preparation system attached to a continuous-flow Europa 20-20 gas source stable isotope ratio mass spectrometer. Subsamples taken for the other elements were analyzed at the University of Georgia's Soil, Plant, and Water Laboratory through microwave assisted digestion (EPA method 3052) followed by axially viewed ICP-AES (EPA method 6010b).

STATISTICAL ANALYSES

Ontogenetic Stoichiometry – We analyzed elemental concentrations in six species (Marbled Salamander; *Ambystoma opacum,* Mole Salamander; *A. talpoideum,* Southern Toad; *Anaxyrus terrestris,* Bullfrog; *Lithobates catesbeianus,* Southern Leopard Frog; *L. sphenocephalus,* Eastern Spadefoot Toad; *Scaphiopus holbrookii*) at each ontogenetic stage (ova, metamorph, adult). To test for ontogenetic changes in the relative contribution of individual elements to biomass, we ran generalized linear models with an interaction term between order (Caudata, Anura) and ontogenetic stage (ova, metamorph, adult) followed by a Tukey's HSD test if there was a significant effect. Replicates for ova and metamorphs were generally composited samples of several individuals whereas adult replicates represent individuals (Luhring, unpublished dissertation). When taxonomic order effects were significant, we conducted an additional test on species-ontogeny interactions within each order. All analyses were run with the GLM function in program R (version 2.15.1; R Development Core Team 2005) within the RStudio environment (version 0.96.331; RStudio 2012). The GLMs assumed a Gamma error distribution as it best reflected the distribution of most datasets. Although the elemental data are in fact proportions (ppm), they never approach 1 and behave as positive continuous data.

Across Taxa Stoichiometry – While the aforementioned GLM's tested for changes between stages, we still wanted to understand the factors influencing the composition of different individuals within each stage. In addition to the data used for the between-stage analyses, samples from additional species were added to the ova (Pinewoods Treefrog; *Hyla femoralis*) and adult (Two-toed Amphiuma; *Amphiuma means,* Eastern Narrow-mouthed Toad; *Gastrophryne carolinensis,* Green Treefrog; *Hyla cinerea, H. femoralis,* Greater Siren; *Siren lacertina*) datasets. Because these data include species that are not biphasic (*Siren* and

Amphiuma remain aquatic throughout life), it captures a wider scope of amphibian life history strategies. For each developmental stage (ova, metamorph, adult), we used an information-theoretic approach to compare competing models to explain elemental composition (Burnham and Anderson 2002). Candidate models included factors related to body composition and size (individual dry mass or snout-vent length [SVL]), taxa (order or family), and life-history characteristics related to growth and development at each stage (average larval period for ova and metamorphs; presence or absence of a terrestrial form for adults). We included multiple metrics of each category (e.g., dry mass and SVL for size) in the initial suite of models as we did not have *a priori* expectations of which would perform better for each element and ontogenetic stage. While two metrics of taxa and size were included in initial model tests, we limited the final model comparisons to one measure of each (i.e., eliminated either SVL or dry mass, eliminated family or order). Candidate models included each parameter as a sole predictor as well as every combination of size metrics and a life-history parameter (larval period for ova and metamorphs, habitat for adults). One size metric was used in combination with the life-history parameter for the models (e.g., intercept, size, life history, size + life history) used to determine model weights. Interaction effects between model parameters were left out due to small sample sizes. Model comparisons were conducted using the bbmle package in program R (version 2.15.1; R Development Core Team 2005).

Results

Ontogenetic Stoichiometry

Elemental composition for six species of amphibians were largely explained by differences between ontogenetic stages (13 of 17 elements), although taxon (order, species), and/or a taxon by ontogenetic stage interaction were also significant in some cases (Tables 1-2, A1-A2).

Ontogenetic stoichiometry trajectories varied considerably among species and elements (Figs. A1-6; Luhring, Unpublished dissertation). However, some elements demonstrated consistent positive (e.g., Ca; Fig. A4) or negative trends (e.g., C; Fig A1) through ontogeny. In other cases, individual stages were elevated in a particular nutrient (e.g., Fe in metamorphs; Fig A5, Zn in ova; Luhring unpublished dissertation).

Start of the Aquatic Phase: Ova

For 9 of the 17 elements examined, ova elemental content was better explained by one or more components of life-history, taxon, or size than by an intercept (null) model (Table A3). Only 5 of these elements (Mg, N, S, P, Zn) demonstrated strong patterns ($w_i \geq 0.8$ and a significant GLM for any component).

The concentrations of four elements (Mg, N, P, S) were explained by larval period (Table A3). Maternal investment of per-ovum P and Mg in anurans was higher in species that typically develop faster (shorter larval period; Fig. 2), whereas N content was positively correlated with larval period in all amphibians. The relationship between S and larval period was driven by the high S content of *L. catesbeianus* and is better explained by taxonomic differences (see below). Further evidence of selective stoichiometric provisioning was seen in the disproportionately high amount of Ca observed in *S. holbrookii* ova (Fig. 3).

Nitrogen was the only element that demonstrated a relationship with dry mass (included in a model with larval period). This pattern was more evident in the anuran subset of data than with caudates (Fig. 4), which maintained a relatively constant level of N in their ova (10.36%). While the proportion of N in anuran ova decreased with increased ova dry mass, the total quantity of N still increased. In other words, as anurans increased the size of their ova, they incorporated N at a slightly lower rate.

The ovum content of three elements (P, S, Zn) was explained by order. Because we sampled from animals within the same landscape, we were limited to one family of biphasic caudate (Ambystomatidae). This is in contrast to multiple families of anurans with highly divergent life-histories. Because of these limitations, we are cautious in ascribing the differences entirely to taxonomic differences between caudates and anurans.

Variation in the S content of ova and other stages was generally explained better by differences between caudates and anurans than by other factors. In the case of ova, caudates demonstrated lower levels of S (5624 ± 244 ppm) than anurans (6492 ± 387 ppm; Table A3). Caudates showed higher levels of P investment in their ova (12512 ± 647 ppm) than anurans (11330 ± 1361 ppm; Table A3). However, anuran ova P covered a wider spectrum from 8811-12988 ppm (as compared to caudates; 11834-13229 ppm) which corresponded to vast differences in larval period (Fig. 2). Anuran ova were elevated in Zn content (202.4 ± 77.6 ppm) relative to caudates (89 ± 17.0 ppm; Table A3).

We purposely collected ova from females in reproductive condition to avoid elemental contamination from the water and male ejaculate. However, each of these could potentially play a role in the early nutrition and development of embryos. Water in recently-filled wetlands is rich in several elements (Schalles et al., 1989) that are necessary for metabolic processes. Preliminary comparisons of ova and freshly-oviposited egg stoichiometry reveal differences in a number of elements that are consistent between both species examined (*A. terrestris, H. femoralis*). The largest relative increases (11 to 285-fold) from ova to egg (for *A. terrestris* [ova n=2, egg n=1] and *H. femoralis* [ova n=1, egg n=1]) were seen in the 2nd, 3rd, and 4th most common elements in the Earth's crust (Si, Al, Fe, respectively; Lotka 1925) and may reflect the stoichiometry of the water and suspended sediments in which they were laid (both sets of eggs

were collected from recently-filled pools that were fairly turbid) or a degree of selective membrane permeability. Two elements demonstrated large decreases that likely reflect a change from the maternal to external environment (e.g., K and Na in eggs were around 20-30% of that in ova). The elemental changes that ova experience in the transition from the maternal to external environment are demonstrably massive (*A. terrestris* eggs had 2859 ppm Al whereas ova in a female were below detection limits; <10 ppm) and deserve further attention. Other elements showed moderate increases (1.5 to 3-fold; Ca, Cr, Cu, Mn, Ni, TML unpublished data) and may likewise reflect the stoichiometry of the water or disturbed sediments in recently refilled wetlands.

Aquatic Ontogeny: From Ova to Metamorph

Seven elements increased in their relative abundance from ova to metamorphosis (Al, Ca, Fe, K, Mn, Na, Si) while C decreased (Table 1). Iron concentrations were higher at metamorphosis (490.6 ± 97.4 ppm) than in ova (148.4 ± 50.5 ppm) or adults (206.0 ± 40.4; Fig. A5, Table 1). Two elements associated with electrochemical processes and bodily fluid balance, Na and K, increased from ova to metamorphosis. Available egg samples indicate that most of the Na and K seen in ova is not retained in deposited eggs and suggests that the slope for this transition would be even steeper than appears from ova stoichiometry. Further data on the stoichiometry of oviposited eggs would help to tease out some of the potential biases of sampling ova (e.g., maternal body fluid concentrations of Na and K).

End of the Aquatic Phase and Beginning of Life on Land: Metamorphosis

For 4 of the 17 elements examined, metamorph elemental content was better explained by one or more components of life-history, taxa, or size than by the intercept (null) model (Table A4). Three of these elements (Mg, P, S) had model weights above 0.8 and significant effect

sizes. Although C content was not explained well by candidate models, there was an increase with larval period that would have been significant if not for an apparent outlier (*S. holbrookii*) at the extreme short end of larval period development.

As was the case in ova, S content was predicted largely by differences between caudates and anurans. However, contrary to the pattern seen with ova, caudate metamorphs were comprised of a higher proportion of S (6724 ± 319ppm) than anuran metamorphs (5480 ± 171 ppm). This represented an overall average decrease in relative S from ova to metamorph for anurans and an increase for caudates.

Both Mg and P were explained best by a model that used larval period and snout-vent length (SVL) and were moderately correlated with one another (Mg=0.04*P + 402.70; p=0.01 adj R^2=0.57). Snout-vent length was the strongest predictor of Mg content and was more apparent when comparing large and small metamorphs of the same species (Fig. 5). Both Mg and P decreased in relative content with an increase in body length (SVL) within a species. However, both appeared to increase with length between species (especially if we ignore the data point from *S. holbrookii* on the smallest end of the spectrum; Fig 5). Larval period was positively correlated with P content in metamorphs.

Terrestrial Ontogeny: From Metamorph to Adult

Seven elements (Al, B, C, Ca, Fe, Mg, P) changed in their relative proportion from the metamorph to adult stage (Table 1.). Aluminum, B, C, and Fe decreased from metamorphosis to adulthood, whereas Ca, Mg, and P (all three of which are components of bone) increased (Table 1). In anurans, S increased from metamorphosis to adulthood and regained levels near that seen in ova (Table A1).

End of Ontogeny: Adult

For adults, eight elements were better explained by one or more components of life-history (in this case, terrestrial or aquatic form), taxa (family), or size than by the intercept (null) model (Table A5). None of the individual models had weights above 0.8, however, five elements (C, K, Mn, N, Na) had top models that combined for at least 0.7 and significant effect sizes.

The differences between terrestrial and aquatic adults drove the variation seen in four (C, K, N, Na) elements. Carbon, K, and N were all proportionally higher in aquatic adults, and Na was higher in terrestrial adults (Table A5). These differences are likely driven by the proportionately higher investment of body mass in bone seen in terrestrial forms (Fig. 17).

Because of the highly divergent nature of aquatic adults, we re-ran the adult analyses with terrestrial species only (i.e., excluded sirenids and amphiumids). In the terrestrial adult subset, stoichiometry for six elements was better explained by one or more component of taxa (Order) or size (SVL or mass) than by the intercept (null) model (Table A6).

No individual model had a weight above 0.8. However, P content was better explained by three models which received higher weights than the intercept and combined for a model weight of 0.85. Of these three models, only dry mass had a significant effect size, but taxa was marginally significant ($p=0.1$ and 0.08). Phosphorus content was generally driven by dry mass, although there is considerable spread in the data along that trend. Taxonomic differences may be real, however, data for anurans covered a wider spectrum of body sizes than that of caudates. When combined with the fact that body P generally increased with body size, the apparent elevation of P in anurans may be an artifact of the sampling regime including larger (and thus P-enriched) adults. Calcium demonstrated a similar pattern to P, however, only size (dry mass) had a significant effect size. Magnesium was the only element that demonstrated a solely taxonomic

effect with caudates demonstrating a lower concentration than that of anurans. The difference in Mg content does not appear to be an artifact of differences in the body sizes of the samples as anurans larger and smaller than the caudate samples were higher in Mg content than caudates (only 3 anuran samples had Mg lower than the highest caudate).

Discussion

Body stoichiometry changes as a function of growth rate, structural composition, and diet throughout ontogeny (Sterner and Elser 2002, Pilati and Vanni 2007). While some ontogenetic trajectories feature only minor incremental changes in body composition, others are marked by more abrupt transitions between different body plans (i.e., metamorphosis in anurans). Any major stoichiometric differences between the transitional stage and the initial or terminal stage indicate potential elemental bottlenecks that occur at a critical point in ontogeny prior to relative homeostasis as an adult (Fig. 1). These differences should be especially strong as organisms change body plans, diets, or habitat throughout ontogeny. Environmental constraints on resource quality, quantity, and ephemerality during these transition periods are particularly important. Many species of amphibians demonstrate varying degrees of these shifts (tadpole to frog, herbivore to carnivore, aquatic to terrestrial) and face a continuum of resource windows (i.e., hydroperiods of larval wetland habitats) in which to make those transitions. Based on our results, we follow the ontogenetic trajectory of amphibians from ova to metamorphosis to maturity and outline the selective pressures that shape the stoichiometry of each stage and the transitions between them.

Ova – Larval period is implicated with ova stoichiometry for a variety of elements (Table A3) and indicates that female anurans contribute to the stoichiometric configuration of their ova, perhaps in response to the selective pressures of the larval environment. While the exact

disposition of additional P in ova with a shorter larval period is not known, it is consistent with the increased P demand of an increased relative growth rate (Elser et al., 1996). The apparent exponential decay of Mg content in ova with increase in larval period suggests that some process may have to be expedited for metamorphosis within a short period of time. Animals use Mg for a variety of functions including structural (e.g., bone), electrochemical and mechanical (Sterner and Elser 2002, Fraústo da Silva and Williams 1991). Magnesium concentrations in cells are especially vital for the formation and stabilization of ribosomal subunits and rates of translational activity that produce proteins (Sperrazza and Spremulli 1983; Cate et al., 1997; Shenvi et al., 2005). The higher amount of P and Mg in faster-developing ova suggests that larval environments select for element-specific investments that facilitate fast biosynthesis of a metamorph from an ova.

On a per-ovum basis, *S. holbrookii* ova contain as much calcium as *A. opacum* ova despite being nearly three times smaller. The extreme environmental pressures of highly ephemeral aquatic larval habitats and stoichiometric limits on extremely short *Scaphiopus* larval development (relative to other anurans) appear to play an important role in their ova stoichiometry. However, the degree to which physiology and/or phylogeny determine element-specific provisioning in ova is unknown and presents an interesting topic for further study.

Aquatic Phase: Transition from Water to Land – While several elements have multiple roles in the biochemical physiology of vertebrates, some patterns are generally attributable to well-known processes that are taking place within the developing body of amphibians in preparation for a terrestrial existence. Ossification of bone in preparation for bearing the weight of a terrestrial body is likely responsible for the increase of elements related to the composition (Ca, nearly significant $p=0.059$, increase of P; Fig. 5) and development of bone (Mn; required for

bone and cartilage development; Neilson 2006). Other physiological changes such as preparations for breathing air reach their zenith during or just prior to metamorphosis, and their signature is apparent in the stoichiometry of metamorphs (changes in blood and liver content of Fe; Osaki et al., 1974). Indeed, two of the highest values of Fe concentrations were observed in *L. catesbeianus* tadpoles undergoing metamorphosis (Gosner stage 40 = 1600ppm, Gosner stage 42 = 621ppm; Gosner 1960).

The elevation of Al and Si in metamorphs relative to ova may be a signature of the aquatic environment as Al and Si are at their highest concentrations in eggs, tadpoles, and metamorphs. Indeed, Al and Si both increased in their abundance from ova to egg (Supplementary Electronic Raw Data Table). Amphibians have been shown to accumulate and transport several trace elements throughout the course of their life-histories (Roe et al., 2005; Hopkins et al., 2006). Contaminants such as Se and Sr (common byproducts of coal combustion waste), which function as biochemical analogs for S and Ca, respectively, accumulate in amphibian body tissue and remain through adulthood (Roe et al., 2005; Metts et al., 2012). Elements such as Al, which is not used in biochemical reactions, would be expected to decrease. It would be useful to know whether a stoichiometric framework of ontogeny and taxonomy can predict the uptake and turnover of different elemental contaminants (especially those that are chemical analogs to vital nutrients). The lethality of these substitutions has potential to affect not only amphibian population viability, but the distribution of contaminants within a landscape.

Of the seven elements that increased during aquatic ontogeny ("A_3"; Figure 1), six (Si, Al, Fe, Ca, Na and K) are the 2^{nd}-7^{th} most common elements in the Earth's crust. Of these, only Al is not a major contributor to biotic metabolism. If these elements are more prevalent in recently-refilled wetlands (e.g., Schalles et al., 1989) and if amphibians are metabolizing these

elements during their aquatic stage, then recently refilled wetlands may represent transient havens with reduced predation and increased quality of resources (from a stoichiometric perspective).

Metamorphs – Recently-metamorphosed juveniles (metamorphs) are the first terrestrial stage for many species of amphibians. As such, the stoichiometries of metamorphs that span a variety of life-history strategies provide us with a spectrum of successful body types and the elemental requirements of creating them. In contrast to ova P content, which was negatively correlated with larval period (Fig. 2), larval periods in metamorphs were positively correlated with P content (P~47.83*Larval Period + 12268.5; p=0.05, adj. R^2=0.36). The positive relationship between larval period and P in metamorphs is likely caused by the increased body size (and resulting increased structural investment) associated with a longer larval period (SVL and larval period are positively correlated for the species examined; p=0.006, adj R^2=0.64). The switch from a negative correlation of ova P with larval period to a positive correlation of metamorph P with larval period thus embodies the shift in the relative importance of P in a vertebrate from growth and cell replication (ova) to that of an investment in structural support (metamorph). Larger metamorphs within a species had higher proportions of C content than smaller metamorphs (large vs. small metamorphs % of dry mass as C of four species, *A. opacum*; 47.40% vs. 44.19%, *A. talpoideum*; 45.40% vs. 41.33%, *L. catesbeianus*; 47.02% vs. 44.49%, *L. sphenocephalus*; 42.34 vs. 41.43%).

What these general patterns suggest is that a larger size at metamorphosis (among species) requires a higher investment of P and Mg (and potentially other elements associated with bone formation) relative to C (i.e., low C:nutrient ratios), making them potentially more limiting for larger species. However, additional growth around species-specific sizes at

metamorphosis is higher in C and potentially less limited by nutrients. In other words, nutrient availability of P and Mg (e.g., resource quality) could be more important for determining whether or not an amphibian could metamorphose, whereas variation around the species-specific size at metamorphosis would be driven more so by availability of carbon sources (e.g., resource quantity). Because of the broad survey nature of our sampling regime, more resolution within a stage or species is still needed to tease out more detailed mechanisms.

The Terrestrial Phase: From Metamorphosis to Maturity – The stoichiometric transition from metamorph to adult in amphibians with complex life-histories is essentially the increase in size of a terrestrial vertebrate. Elements that appear to be carry-overs from the aquatic environment in metamorphs (e.g., Al, B) decrease in their prevalence in adults, whereas elements associated with bone formation (Ca, Mg, P) all increase from metamorphosis to maturation (Table 1). Carbon continues to decrease (Fig. A1) in relative proportion of biomass as metamorphs grow and allocate increasingly higher amounts of biomass to structural support (bone; Fig. 6). The decrease in proportion of mass in Fe from metamorph to adult follows the peak of body Fe composition at metamorphosis (Fig. A5) and may reflect a lowered relative body demand for O_2 transport concomitant with an increased body size.

Adults (Aquatic and Terrestrial) – Our dataset with terrestrial and aquatic adults represents the most taxonomically-diverse sample of our stages. Adults are the ontogenetic stage most often used for taxonomic comparisons, although, as the data from this study suggests, there can be several important changes in an individual's stoichiometry prior to maturation. Because of the conflicting patterns seen in aquatic species, we analyzed terrestrial species separately from aquatic species.

Aquatic Adults – While *S. lacertina* demonstrates several stoichiometric properties that we expected in an aquatic species (i.e., proportionately less Ca, and P resulting from lowered bone investment), *A. means* appears to be more stoichiometrically similar to terrestrial adults in terms of body Ca and P. This is potentially because *A. means* demonstrates a few more "terrestrial" tendencies (e.g., brooding; Gunzberger 2003) and a more recent terrestrial ancestor (Zhang and Wake 2009). Although SVL was an important contributor to stoichiometric differences in the combined aquatic and terrestrial adult dataset, it was generally confounded with habitat type (the largest adults were aquatic).

Terrestrial Adults – Bone mass and body size appeared to drive stoichiometric differences in terrestrial adults. Terrestrial adults differed from each other primarily in three elements that are incorporated into bone (Ca, Mg, and P; Table A6). While Mg differed between anurans and caudates, P and Ca (two of the major elements that make up the majority of bone mass) content was related to body mass. Dry mass apparently drove P and Ca content; however, this was heavily influenced by the largest animal, a female *L. catesbeianus*. There was considerable variation among the remaining animals in both Ca and P content that was not apparently related to size alone (Luhring unpublished dissertation). Amphibians store varying amounts of Ca and P under the epithelium of their skin as apatite (Zadunaisky and Lande 1972). Although the exact purpose of these deposits are not known, it is possibly related to water regulation (terrestrial species tend to have higher amounts than aquatic species) and as a potential reservoir for calcification (Zadunaisky and Lande 1972). Further studies that parse out the compartments of P and Ca would be beneficial in understanding where the elements are going in each of these species (e.g., bone versus skin) and how they differ across ontogeny.

Ontogenetic Changes in Stoichiometry

In the case of amphibians with varying life-history strategies, few patterns in ontogenetic stoichiometry were universal. The strongest and most consistent changes in body composition were a decrease in C and an increase in Ca from ova to metamorphosis (Figs. A1, A4). An increase in Ca from ova to metamorphosis would be consistent with increased ossification in a developing body preparing for the rigors of a terrestrial environment. The source of this Ca, however, likely differs between the larvae of carnivorous caudates and omnivorous anurans.

Non-carnivores often face stoichiometric imbalances in several elements that are scarce in their diets (e.g., Na and P in large free-ranging herbivores; WallisDeVries 1998). Vertebrates, in particular, can face substantial hurdles in acquiring enough Ca through their diet (e.g., Barclay 1994). Although data for a variety of species is lacking, some anuran larvae have evolved various non-dietary mechanisms for Ca metabolism including absorption through gills and skin (Stiffler 1993). Physiological or environmental limitations on Ca^{2+} absorption rates through non-dietary pathways may play a major role in breeding phenology, dietary specialization, and parental investment.

A few species demonstrated relatively steep slopes in Ca content for the transition from ova to metamorph that result from a change in stoichiometry combined with their size at metamorphosis being relatively small compared to that of the full-sized adult. The implications for each of these species differ as a consequence of their life-histories. In the case of *L. catesbeianus*, which usually deposit eggs in permanent wetlands, the time between ova deposition and metamorphosis (larval period) can be more than a year, thus relaxing the timing constraints on a steep stoichiometric transition. In contrast to *L. catesbeianus,* various *Scaphiopus* species oviposit in bodies of water that are highly ephemeral (short in duration).

Because of this potential constraint on development time, *Scaphiopus* tadpoles have to expedite Ca uptake rates through dietary or other pathways.

One potential mechanism for avoiding Ca limitation during a short larval period is through maternal investment. *Scaphiopus holbrookii* ova show a disproportionately higher per-ova investment in Ca than other amphibians examined (Figure 3). Additionally, *Scaphiopus* often demonstrate alternative larval phenotypes that appear to be related to tradeoffs associated with resource availability and pool ephemerality (Pfenning 1992). Briefly, carnivorous phenotypes are prevalent in short-duration pools where fairy shrimp are often abundant, and omnivorous phenotypes are prevalent in longer-duration pools where fairy shrimp are less abundant and detritus serves as a more abundant food resource. While alluded to as more nutritious (Pfenning 1992), the carnivorous diet of fairy shrimp would be proportionately higher in P (important for cell growth and replication; Elser et al., 1996) and Ca (important for ossification) than the omnivorous diet on detritus. While growth rate and development rate in tadpoles are often confounded, understanding the stoichiometric needs of each (e.g., lower Ca demand for growth than development) would provide a proximate mechanistic driver of alternative larval strategies.

Phosphorus: Growth Rate and Bone

Body content of P is positively correlated with growth rate and bone content. This generally produces contradictory patterns of body content of P between groups with and without bone. In groups without bone, body content of P decreases with body size as a function of decreased relative growth rate (e.g., insects; Woods et al., 2004). Amphibians demonstrated two patterns consistent with theoretical predictions of vertebrate body P relative to body mass.

The prediction that body P increases in vertebrates as a result of additional content of bone was supported by our data (Fig. 6, A7, A8). Phosphorus and Ca are major contributors to bone in vertebrates (hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, comprises ~60%70 of bone; Simkiss and Wilbur 1989). Phosphorus content of all metamorphs, juveniles and adults (including aquatic species) was positively correlated to Ca content with a slope (2.54) that is nearly identical to the mass ratio of a 2:1 ratio of Ca to P atoms (2.59; Fig. 7) indicating that bone was a major contributor to body P as is consistent with other vertebrates (gizzard shad; Pilati and Vanni 2007).

The second theory, that whole body P (outside of bone) content decreases with body size, was also supported by our data. After accounting for P in bone (through Ca content), amphibians showed an overall negative trend in body P with increased body size (Fig. 6). This is consistent with a decreased relative growth rate and thus a decreased need for P-rich cellular machinery dedicated to cell growth and replication (Elser et al., 1996). The interaction of body P with body size and amount of ossification was further supported by large animals with low skeletal investment (the aquatic salamander, *S. lacertina*) following a pattern of decreased P and Ca with increased size (Fig. A7, A8).

Amphibians shift their nutritional constraints when transitioning from an aquatic larva to a terrestrial juvenile/adult. The obligate formation of bone for a terrestrial vertebrate existence has major implications for larval amphibian nutritional ecology and nutrient provisioning in ova. While transitioning from an aquatic to terrestrial existence has major nutritional demands, maintaining an aquatic adult form can relax potential stoichiometric limits on growth through increased C:nutrient ratios. The reduction of body mass allocated to skeletal investment in *S. lacertina* results in C:P and C:Ca ratios that terrestrial species only approach as ova and

metamorphs (Fig. 8). A fast growth rate and large body size are adaptations that enable this species to persist in drought-prone wetland habitat (T.M. Luhring and R.M. Holdo, unpublished manuscript). The low C:P and C:Ca of *S. lacertina* may be a stoichiometric adaptation that enables it to maximize growth rate through allocation of P to growth (instead of ossification) and decreased nutrient demands.

Interactions of Amphibians with Biogeochemical Cycles

Mechanisms that facilitate the availability of elements for biotic synthesis directly affect the functioning of ecosystems. In aquatic systems, water-logged soils are anaerobic and retain several elements in forms that are not biologically available. In more permanent waters, fishes (e.g., gizzard shad) increase nutrient availability by eating benthic sediments and excreting them into the water column (Vanni 2002). In ephemeral wetlands, the process of drying and refilling may serve a similar purpose for releasing bound elements. In a drying wetland, previously anoxic sediments are oxidized and some nutrients become soluble. When the wetland refills, these elements are released into the water column and are biologically available for a finite period of time (Schalles et al., 1989).

Many species of amphibians specialize in breeding in ephemeral wetlands, which is often attributed to the benefits of avoiding top-down effects of aquatic predators or competition (e.g., Resetarits and Wilbur 1989). While top-down effects of aquatic predators on amphibian larvae are undeniable, our understanding of the bottom-up effects of nutrient pulses from re-filling wetlands on amphibians is relatively poor. Several elements present as cations in refilled wetlands are essential for the formation of bone (e.g., Ca, Mg, Mn, Na) and are a potential resource for amphibian larvae to capitalize prior to their transition to a terrestrial existence. Many studies chronicle the particular concentrations of various elements needed to successfully

raise amphibian larvae in laboratory settings (e.g., Mg; Brown 1961). However, none to our knowledge tie these requirements back to the natural variation within and among wetlands across time and space. The role of seasonal pulses of nutrients in wetlands are poorly understood and are another potential limiting "resource" that remains underappreciated in amphibian ecology and the structuring of transient aquatic communities.

There can be a high degree of variability in cation concentrations seasonally and among wetlands with otherwise seemingly similar physical and biological features (Schalles 1989; Schalles et al., 1989). Non-dietary absorption of cations for biological syntheses would provide amphibian larvae with an alternative nutrient source that is scarcely considered in the amphibian literature. If cation concentrations are drawn down by active ion pumping in amphibians, then there is potential for aquatic larvae to compete with each other in a manner similar to plants for limiting elements in soil (i.e., Tilman 1982). The degree to which this factors into aquatic community structure is wholly unknown. However, a shift from one terrestrial salamander species to another along a soil Ca gradient (Beier et al., 2012) supports the idea that elemental availability potentially shapes amphibian communities.

The movement of amphibian biomass during seasonal migrations is potentially massive. In pond-breeding amphibians, more than a ton of amphibian biomass can be produced (e.g., juveniles) from a single wetland within a season (Gibbons et al., 2006). Forested ponds with ambystomatid salamander assemblages and high natal mortality demonstrate net biomass import into ponds as a result of amphibian breeding activities (Regester et al., 2006). However, the stages that move into (eggs) and out of (metamorphs) ponds differ substantially in their stoichiometries. In addition to the multitude of factors that affect the amount of amphibian biomass moving between systems (e.g., Earl et al., 2011), stoichiometric asymmetries among

life-stages and species may result in patterns of net elemental movement that cannot be explained by biomass alone (Luhring unpublished dissertation).

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Table 1

ONTOGENETIC CHANGES IN 17 ELEMENTS FOR TWO ORDERS (SIX SPECIES) OF AMPHIBIANS.

Element	Effects	Differences (With Tukey HSD)	F-statistic p-value
Aluminum (Al)	Stage	Ova, Adult < Meta	$F_{2,33}$=5.93 p=0.007
Boron (B)	Stage	Adult<Ova,Meta	$F_{2,33}$=3.99 p=0.03
	Stage*Order	Meta is highest stage for Anura, lowest for Caudata	$F_{2,33}$=4.48 p=0.02
Carbon (C)	Stage	Adult<Meta<Ova	$F_{2,34}$=32.4 p=4.0e-08
Calcium (Ca)	Stage	Ova<Meta<Adult	$F_{2,33}$=47.11 p=1.1e-09
Chromium (Cr)	None	None when outlier removed	N/A
Copper (Cu)	Order	Caudata<Anura	$F_{1,33}$=5.78 p=0.02
Iron (Fe)	Stage	Ova, Adult<Meta	$F_{2,33}$=6.47 p=0.005
Potassium (K)	Stage	Ova<Meta,Adult	$F_{2,33}$=37.72 p=1.1e-08
Magnesium (Mg)	Stage	Meta<Adult (p=0.05)	$F_{2,33}$=3.51 p=0.04
	Order	Caudata<Anura	$F_{1,33}$=4.58 p=0.04
Manganese (Mn)	Stage	Ova<Meta	$F_{2,33}$=5.40 p=0.01
Molybdenum (Mo)	*Stage	*Only Adults and Large Meta above detection limit	Not run
Nitrogen (N)	None	N/A	N/A
Sodium (Na)	Stage	Ova<Meta,Adult	$F_{2,33}$=23.67 p=9.6e-07
Phosphorus (P)	Stage	Ova<*Meta<Adult *p=0.059	$F_{2,33}$=25.15 p=5.6e-07
	Stage*Order	Lower P in Adult Caudata, other stages similar	$F_{2,33}$=3.62 p=0.04
Sulfur (S)	Stage*Order	Ova highest stage in Anura, lowest in Caudata. Meta lowest stage in Anura, highest in Caudata	$F_{2,33}$=12.14 p=0.0002
Silicon (Si)	Stage	Ova<Meta	$F_{2,33}$=3.83 p=0.03
Zinc (Zn)	Stage	Adult*,Meta**<Ova *p=0.05, **p=0.07	$F_{2,33}$=3.91 p=0.03
	Order	Caudata<Anura	$F_{1,33}$=7.56 p=0.01

Note – Analyzed as Generalized Linear Models (Nutrient~Stage*Order) with gamma error distributions. Recently metamorphosed juveniles are abbreviated as "Meta." Commas between groups indicate no differences whereas "<" denotes a significant difference between each group on either side of the sign (e.g., A,B<C means that A is not different from B, but A and B are less than C). All between group differences are significant at the $p<0.05$ level unless denoted with an asterisk.

Table 2

SUMMARY OF ELEMENTAL CONTENT (PPM ± STD. ERR.) FOR THREE ONTOGENETIC STAGES.

Element (detection limit)	Ova	Metamorph	Adult
Aluminum (10)	12.5 ± 3.0	138.2 ± 52.4	40.5 ± 7.5
Boron (1)	6.8 ± 1.3	9.9 ± 2.1	2.4 ± 0.6
Carbon	506569.3 ± 2213.6	438070.1 ± 12043.8	418290.6 ± 9670.3
Calcium	781.4 ± 127.1	26680 ± 1467.7	42767.1 ± 3779.7
Chromium (1)	0.75 ± 0.14	1.9 ± 0.3	3.6 ± 1.7
Copper (5)	6.4 ± 1.2	8.1 ± 2.2	91.4 ± 63.9
Iron	145.5 ± 45.0	490.6 ± 97.4	206.0 ± 29.8
Potassium	4062.1 ± 170.3	9612.4 ± 819.3	9457.1 ± 326.8
Magnesium	1108.7 ± 48.8	1074.3 ± 47.3	1295.8 ± 43.7
Manganese (5)	19.8 ± 7.1	93.2 ± 25.1	69.3 ± 11.0
Molybdenum (1)	below detection	0.58 ± 0.08	1.1 ± 0.06
Nickel (1)	0.76 ± 0.21	1.2 ± 0.35	1.8 ± 0.7
Nitrogen	103244.6 ± 1504.4	99163.2 ± 2130.1	107059.5 ± 2372.4
Sodium	1749.4 ± 180.8	5384.4 ± 712.9	4521.6 ± 259.7
Phosphorus	11705.7 ± 331.2	15856.0 ± 881.8	22625.5 ± 1383.0
Sulfur	6182.8 ± 144.4	5895.1 ± 252.2	6277.3 ± 83.4
Silicon (50)	36.1 ± 8.2	78.2 ± 18.5	50.0 ± 9.6
Zinc	179.1 ± 24.5	109.8 ± 20.0	149.6 ± 32.5

Note – Metamorph data is based on 9 samples, adults on 22, and ova on 10 (with the exception of C and N having 9 samples). Cadmium (Cd) was not detected in any samples (detection threshold at 1 ppm). Samples below detection were given values equal to the midpoint between 0 and the detection threshold.

Figure 1. Generic model of ontogenetic stoichiometry. The slope of transition lines (A and B) are driven by factors influencing the position of points on the horizontal (relative size) and vertical (elemental composition) axes. In this case, ova (o), metamorphs (◊), and adults (☆) represent initial, transitional, and terminal stages of ontogeny. Three possible scenarios (subscripts 1-3) exist for each slope leading to (A) or away (B) from the transition stage. Scenario 1: the transition stage has a lower concentration of an element than the initial (A_1) or terminal (B_1) stage. Scenario 2: the transition stage does not differ from the initial (A_2) or terminal (B_2) stage. Scenario 3: the transition stage is elevated in an element relative to the initial (A_3) or terminal (B_3) stage.

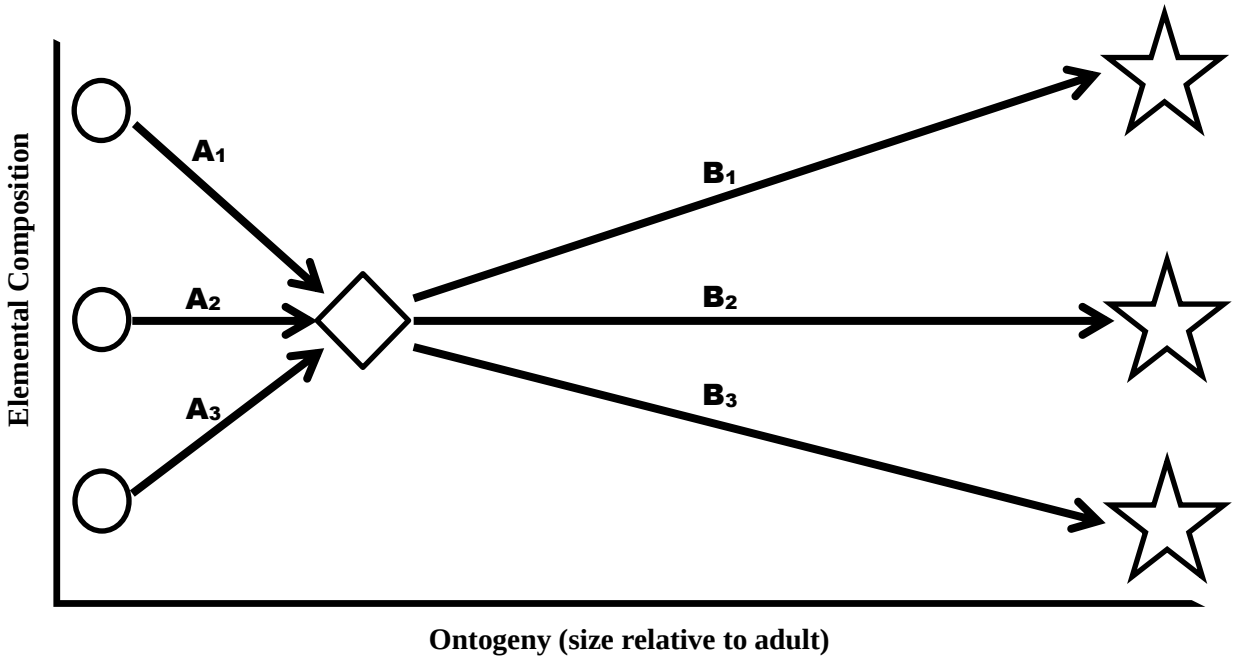


Figure 2. P (A) and Mg (B) content of ova in relation to larval period for anurans (■) and caudates (□).

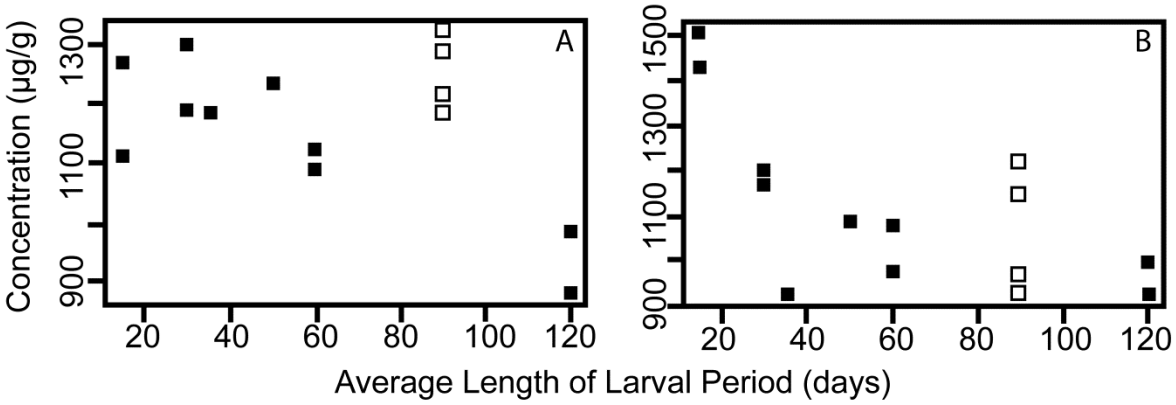


Figure 3. Per-Ovum investment of Ca vs. ova size for anurans (●) and caudates (o).

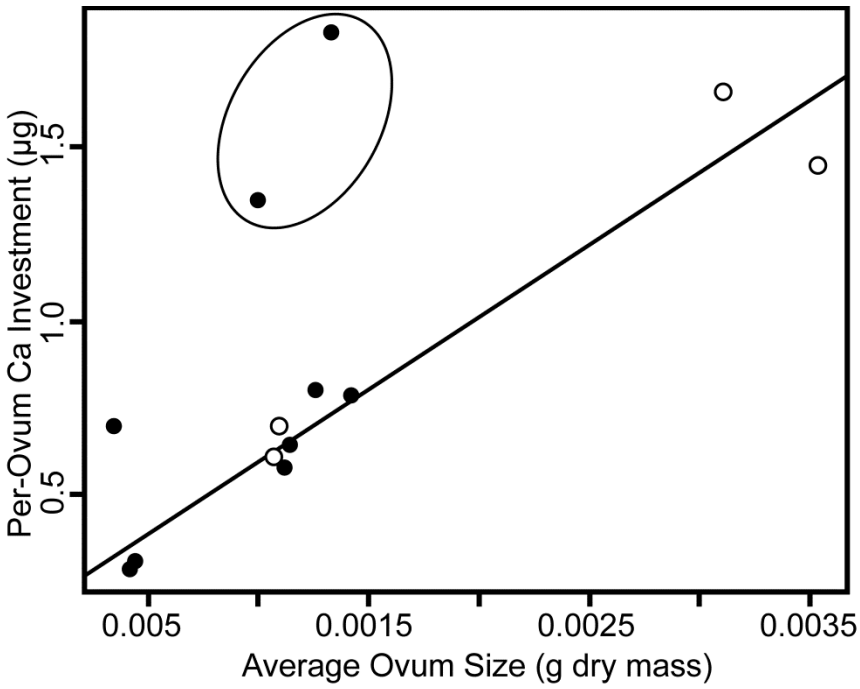


Figure 4. Per-Ovum concentration of N vs. ova size for anurans (●) and caudates (o).

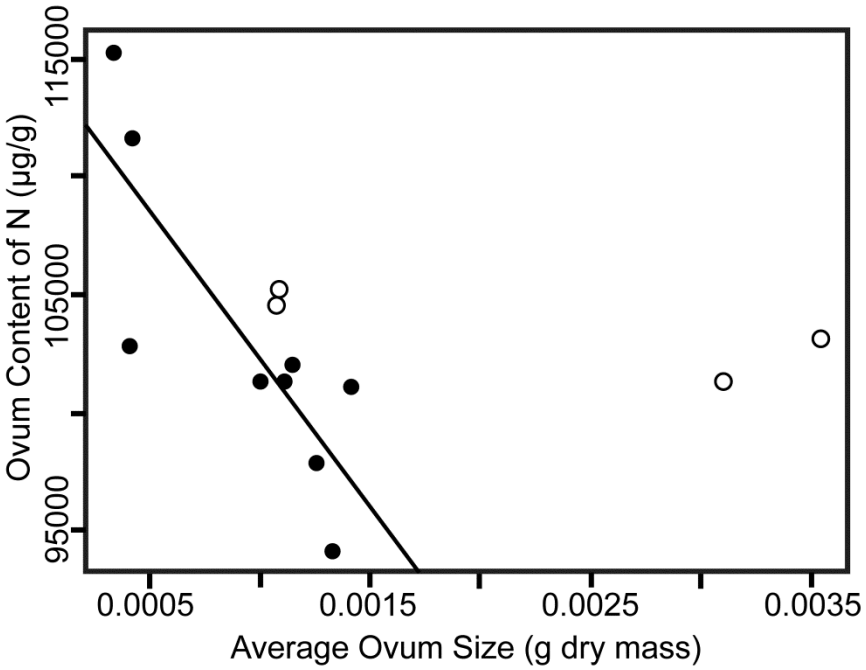


Figure 5. Intra- and inter-species differences in metamorphosed juvenile whole body P (A) and Mg (B) in relation to body size (mm SVL). Samples of *A. opacum* (◊ with solid line), *L. catesbeianus* (♦with dashed line), *L. sphenocephalus* (♦ with solid line), with differing average SVL's show a within-species decrease in Mg and P content with increase in metamorph size. Individual points indicate *A. terrestris* (+), *S. holbrookii* (▼), and *A. talpoideum* (Δ).

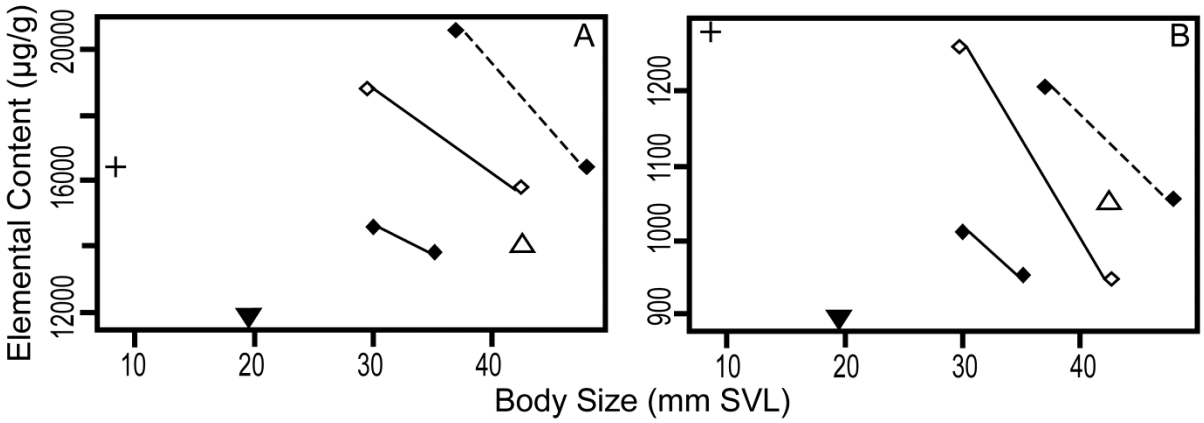


Figure 6. Interaction between body size (g dry mass), whole body P and whole body Ca for amphibians. The circle represents a theoretical stoichiometric position of an ovum. Dashed lines trace the stoichiometric resistance encountered along a terrestrial (☆) and aquatic (Δ) ontogeny.

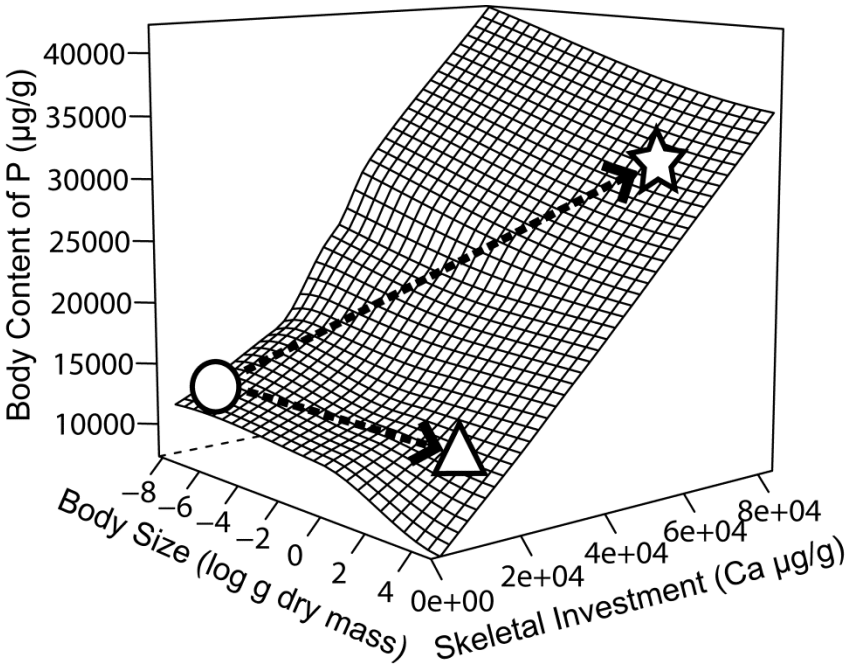


Figure 7. Relationship between body Ca and P for non-ova stages of amphibians (solid line) compared to that of gizzard shad (dashed line; Pilati and Vanni 2007). Amphibian ova and eggs (o) show an initial investment in P, but not Ca. Aquatic phases (Δ) and terrestrial phases (+) generally follow the same pattern with tadpoles (two upper left Δ's) differing from aquatic species (siren and amphiuma represent four remaining Δ's).

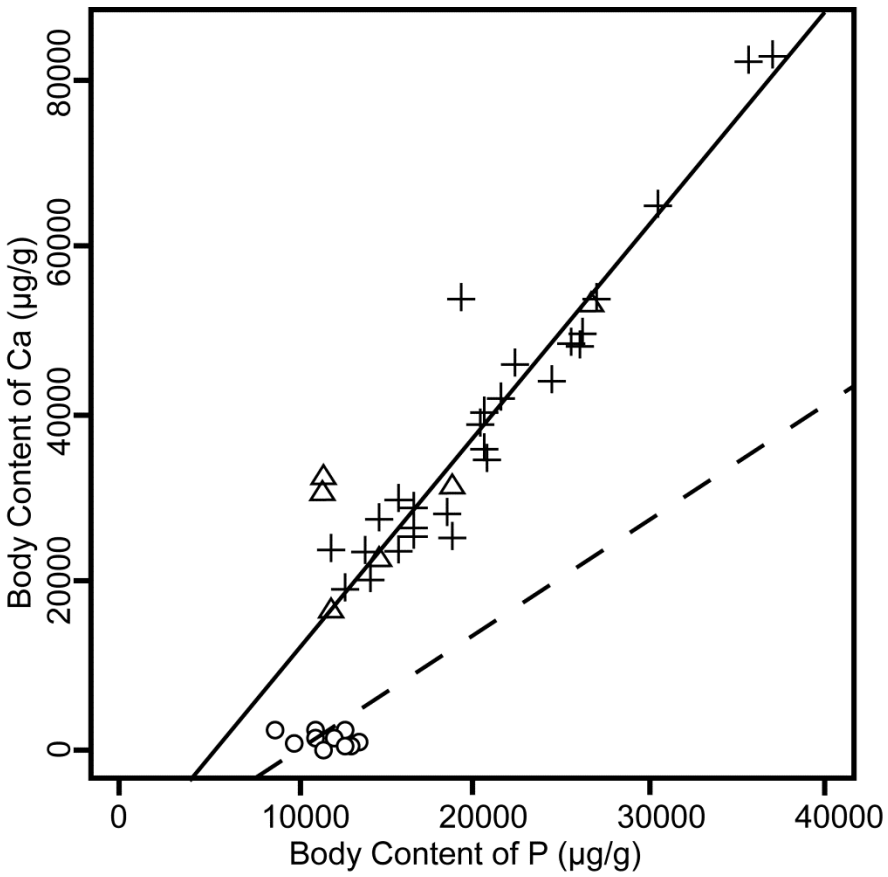
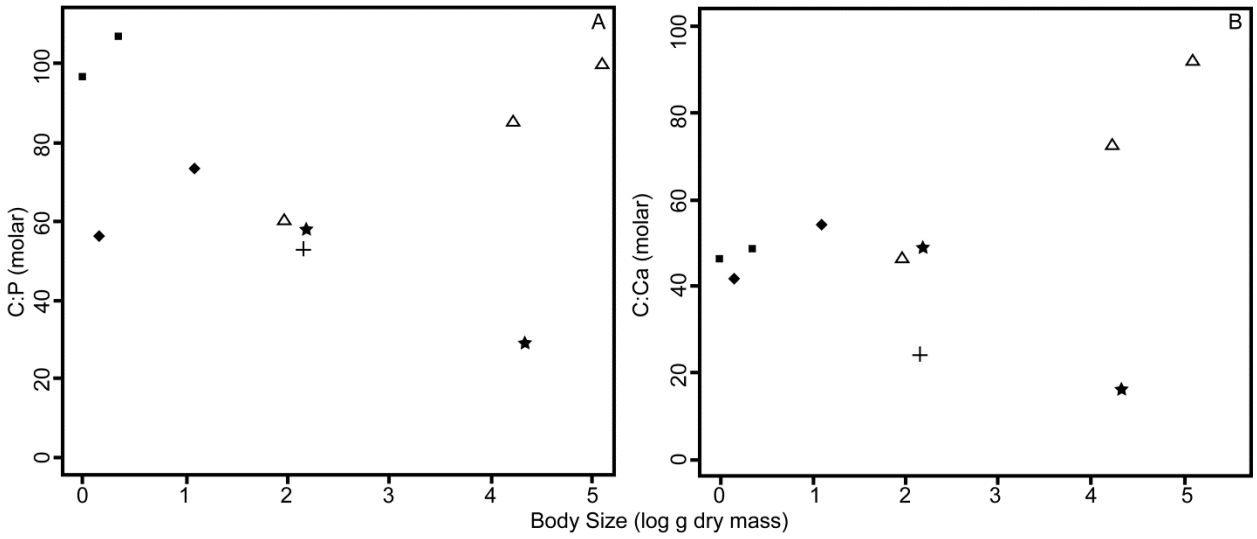


Figure 8. Differences in C:P (A) and C:Ca (B) ontogeny between a large terrestrial (*L. catestbeianus*; closed symbols and +) and large aquatic (*S. lacertina*; Δ) amphibian. For scaling purposes, ontogeny restricted to tadpoles (■), metamorphs (♦), juveniles (+), and adults (★, Δ).



CHAPTER 4

What governs the quantity and quality of active subsidies resulting from complex life-histories?

Thomas M. Luhring and Raymond D. Semlitsch

Abstract

Ecosystems are tied together through the transfer of materials and energy. Many cross-systems transfers are the direct result of organisms that utilize multiple habitats for the completion of their life-histories (e.g., diadromous fishes, holometabolous insects, amphibians). We use amphibians with aquatic larval and terrestrial adult phases to develop theoretical expectations of biomass subsidy movements that result from obligatory succession of life-history stages. Although wetland ecosystems can be exceedingly complex in both shape and structure, it is relatively simple to build a conceptual model of the movement of biomass from terrestrial systems to aquatic systems (in the form of eggs and adult mortality) and from aquatic to terrestrial systems (in the form of recently metamorphosed juveniles). The biomass of each directional pulse (into and out of the wetland) is further parsed into specific elements according to taxonomic differences among its constituent parts. We then inform theoretical models with data from experimental mesocosms and stoichiometric data to simulate the effects of top-down and bottom-up pressures on subsidy quantity (biomass) and quality (stoichiometry). Anuran

biomass exported from wetlands was more strongly depressed by predation when the species in question had a smaller size at metamorphosis and thus did not increase in average size as a function of predatory release from competition. Changes in taxonomic composition of wetland biomass export from anuran to caudate-dominated increased the likelihood of net sulfur export because of the relatively higher concentration of S in caudate metamorphs relative to their ova (1.20-fold higher). Overall, two major factors influenced the magnitude of asymmetrical per-unit of biomass transfers between aquatic and terrestrial systems: 1) relative growth and 2) stoichiometric differences between the subsidy life stages involved in biomass translocation.

Introduction

Few if any ecosystems lack movement of materials and energy across their boundaries (Polis et al., 1997). Linkages between terrestrial and aquatic ecosystems are well-established and serve as a somewhat easier to define boundary between two interacting but disparate systems. Sea birds support the productivity of isolated oceanic islands through translocation of nutrients from fish in the ocean to land via guano (Maron et al., 2006). A variety of mechanisms move nutrients and biomass of spawning salmon (carrying oceanic nutrients) from streams to riparian forest where trees and other organisms benefit from the spatial subsidy (Schindler et al., 2003). Not all spatial subsidies, however, need to take place over large geographic scales to be of importance. Within pond nutrient translocation by fishes from benthic sediments to the water column are capable of supporting and enhancing primary productivity (Vanni 2002).

In addition to examples of nutrient recycling and translocation that involve excretion and elimination of ingested material (see Vanni 2002), some systems experience a transfer of elements bound within living biomass. Active subsidies such as diadromous fishes, holometabolous insects, and amphibians translocate biomass among aquatic and terrestrial

ecosystems through ontogenetic shifts in habitat usage. The transfer of this living biomass can subsequently enter food chains in a spatially and temporally finite scale (e.g., breeding adult salmon senescence; Helfield and Naiman 2001) that have massive and immediate effects on recipient food chains. While several studies have demonstrated the importance of active subsidies for recipient systems, we still lack a general theoretical approach to understanding how complete life-cycles of organisms influence biomass and nutrient transfer among systems.

Amphibians subsidize aquatic ecosystems with terrestrially-derived biomass through oviposition of their eggs in wetland habitats (Regester et al., 2006). Likewise, amphibians subsidize terrestrial systems with aquatically-derived biomass through the emergence of their recently-metamorphosed juveniles from wetlands. With the exception of Antarctica, amphibians are found on every continent on Earth (Zug et al., 2001). As ectotherms, amphibians are efficient transformers of energy into biomass and often reach higher densities than other vertebrates in the same systems (Burton and Likens 1975; Gehlbach and Kennedy 1978; Petranka and Murray 2001). Whereas there are several species of amphibians that remain wholly aquatic (e.g., sirenids, cryptobranchids) or terrestrial (e.g., several plethodontids) throughout their ontogeny (simple life cycle), we use "amphibians" in this paper to refer to the subset of anurans and caudates characterized by obligate ties to aquatic (for breeding and larval development) and terrestrial (for juvenile or adult growth) environments (complex or bi-phasic life cycle; Wilbur 1980).

When combined with the potentially high biomass productivity of amphibians (Peterman et al., 2008, Sorensen 2004), finite temporal and spatial scales of life-history events (i.e., breeding and metamorphosis) can lead to large local material fluxes (e.g., 1,400kg of recently-metamorphosed juveniles emigrating from a 10-ha wetland in one year; Gibbons et al.,

2006). While several factors can influence their magnitude (e.g., predation and competition; Morin 1986, canopy cover; Earl et al., 2011) the actual effects of these subsidy modifiers on the bi-directional translocation of specific quantities and ratios (stoichiometry) of elements remains unknown. One of the key limitations in understanding the effects of amphibian-mediated subsidies on biogeochemical cycles is the lack of data on the elemental composition of the subsidies themselves. Although it may be apparent that an amphibian ova would have a vastly different stoichiometry than that of a metamorph, the actual differences for several elements were, until recently (Luhring, unpublished dissertation), unavailable.

The species composition of amphibian communities is affected by several, often interacting, factors (Morin 1983; Wells 2007). Changes in community composition could likewise change the quantity and quality of subsidies between aquatic and terrestrial systems. Species differ substantially in the per-capita sizes of their eggs (subsidy to aquatic) and metamorphs (subsidy to terrestrial), changes in either would directly affect the quantity of material moving between the two habitats. Likewise, differences between species stoichiometries of either eggs or metamorphs would change the quality (stoichiometry) of subsidies. Within-species differences of mass at metamorphosis or stoichiometry of either subsidy life-stage would also shape subsidy quantity and quality.

Although this study uses the two-habitat life-cycle of amphibians to derive simplified conceptual and quantitative models, the findings and mass-balance approach are transferable to any system with organisms that use multiple habitats throughout the course of their life history. We meld recently developed stoichiometry datasets and experimental tests of bottom-up and top-down forces on subsidy magnitude to create element-specific predictions of subsidy movement resulting from complex life-histories. Three general factors in our simplified conceptual model

(transition probability, asymmetry in size, asymmetry in stoichiometry) are parameterized from experimental and *in situ* data and then simulated to explore factors influencing 1) subsidy quantity, and 2) subsidy quality.

Methods and Materials

Conceptual Biomass Model – In a simplified biomass subsidy model, amphibians move terrestrially-derived biomass into aquatic habitats in the form of eggs and move aquatically-derived biomass out of aquatic habitats and into terrestrial habitats in the form of recently metamorphosed juveniles. To further understand the two-way dynamics of biomass movement between these systems, we can use a mass balance equation (i.e., Total Aquatic Subsidy = Total Terrestrial Subsidy). We can then define the subsidy that the aquatic system receives as equal to the product of the number of eggs and their per capita mass. Likewise, the subsidy that the terrestrial system receives is equal to the product of the number of recently metamorphosed juveniles (metamorphs, hereafter) emigrating into the terrestrial habitat and their per capita mass (eq.1). The numerical component of the right side of the equation (eq.1) can then be modified by defining the number of metamorphs exiting a system as being the product of the number of eggs being deposited in the aquatic environment and their chance of surviving to metamorphosis (eq. 2).

$$N_{Egg} * Mass_{Egg} = N_{Meta} * Mass_{Meta} \qquad \text{Eq. 1}$$

$$N_{Meta} = N_{Egg} * P_{Larval} \qquad \text{Eq. 2}$$

Substituting Eq. 2 into Eq. 1 results in total number of eggs (N_{Egg}) cancelling from each side (eq. 3).

$$Mass_{Egg} = P_{Larval} * Mass_{Meta} \quad \text{Eq. 3}$$

We can then divide both sides by metamorph mass ($Mass_{Meta}$) to put both units of mass on the same side. This creates a ratio between the mass of a single egg ($Mass_{Egg}$) and the mass of a single metamorph ($Mass_{Meta}$) and balances it with the likelihood (P_{Larval}) that an egg will become a metamorph (Eq.4).

$$Mass_{Egg} * Mass_{Meta}^{-1} = P_{Larval} \quad \text{Eq. 4}$$

This equation can then be solved for values where both sides are equal to each other and used as a reference isocline to graphically depict expected patterns of biomass transport given aquatic survival and the relative size of an egg versus metamorph (Figure 1).

Element-Specific Models

To specify the relative amounts of specific elements moving in each direction, we can further adjust our biomass isocline equation (Eq. 4) to include element-specific modifiers (Eq. 5). The modifiers $PPM(E)_{Egg}$ and $PPM(E)_{Meta}$ are the proportion (ppm) of the egg or metamorph that is made of a certain element (E).

$$Mass_{Egg} * PPM(E)_{Egg} * Mass_{Meta}^{-1} * PPM(E)_{Meta}^{-1} = P_{Larval} \quad \text{Eq. 5}$$

This equation is further simplified by taking the inverse of each side (Eq. 6) and making ratios of the transitions from egg to metamorph. These ratios represent the relative amount of mass gained from egg to metamorphosis (relative growth, RG; Eq. 7) and the shift in stoichiometry between ontogenetic stages (ontogenetic stoichiometry, OS; Eq. 8).

$$Mass_{Egg}^{-1} * PPM(E)_{Egg}^{-1} * Mass_{Meta} * PPM(E)_{Meta} = P_{Larval}^{-1} \quad \text{Eq. 6}$$

$$Mass_{Meta} / Mass_{Egg} = \text{Relative Growth} \quad \text{Eq. 7}$$

$$PPM(E)_{Meta} / PPM(E)_{Egg} = \text{Ontogenetic Stoichiometry} \quad \text{Eq. 8}$$

These ratios (Eqs. 7-8) substitute back into the element-specific mass balance equation (Eq. 6) to produce a more conceptually appealing and simplified zero-net transfer of specific elements (Eq. 9).

$$RG * OS = P_{Larval}^{-1} \quad \text{Eq. 9}$$

Simulation Models

Mass and Relative Growth – To calculate a range of amphibian RG's, we collected dry mass data from eight amphibian species in South Carolina (Table 1), three species of anurans from manipulative mesocosm experiments in Missouri (Luhring, unpublished dissertation), and three species of ambystomatid salamanders from Illinois (Regester et al., 2006). Metamorphs collected for the South Carolina data set were intentionally biased toward smaller and larger individuals for stoichiometric analyses. Because of the inherent bias of calculating an average body mass from a non-representative sample, we used the midpoint between the smallest and largest individual as the "mean" mass for each species. Variation of ova and metamorph mass were simulated 1000 times by drawing from a random normal distribution with standard deviations that were a $1/6^{th}$ of the total range between the minimum and maximum range for each species (i.e., the minimum and maximum were considered to enclose approximately 99.8% of the species variance). In instances where max and min sizes were not obtained (e.g., *Schaphiopus holbrookii* metamorphs, ambystomatids from Regester et al., 2006), we used target standard deviations (as a percent of the mean) that were similar to other species within their taxonomic order (caudates; 0.22-0.23, anurans; 0.14-0.16 typical with a max of 0.21).

Elements and Ontogenetic Stoichiometry – We created OS's from data on ontogenetic stoichiometry in amphibians (Luhring, unpublished dissertation). This dataset was limited to eight species for which metamorph and ova stoichiometry data exist. We created OS standard deviations in a similar manner to that of RG's with simulations of stoichiometric values based on observed variation in amphibians. Random values were drawn from distributions that best reflected available data (either normal or gamma). Simulating elemental values for ova, which often had smaller amounts of elements and large variances, often resulted in values drawn that were nearly zero. Because ova values were used as the denominator in calculating OS's, this would result in inflated estimations and long right-handed tails to OS distributions. To account for this, we added a qualifier in the simulation that caused values drawn below 10% of the minimum observed value in real samples to be redrawn from the distribution. After simulating OS's for some elements, a long right-hand tail would still be produced, in which case we removed the upper 5% of simulated values.

Elemental-specific Subsidy Simulations – We used RG and OS values to calculate the element-specific rate of larval survival needed to have a zero net subsidy (Eq. 9). Standard deviations around each values were simulated in the same manner as RG's and OS's.

Top-down and Bottom-up Influences on Biomass Subsidies – We used survival and body size data from experimental mesocosms (Luhring, unpublished dissertation chapter) to simulate the influences of top-down (predation) and bottom-up (canopy cover) forces on subsidy magnitude. Briefly, the mesocosm experiment used to parameterize this set of simulations used a combination of three species of anurans with divergent life-history strategies (namely body size at metamorphosis). Each species was placed in a mesocosm with only intra-specific competition, competition with the other two anuran species, or competition with the other two

anurans and predators (eastern newts, *Notophthalmus viridescens*). Each of these trophic treatments was further nested within light treatments (high: 67% ambient light, and low: 27% ambient light) with each trophic by light combination replicated 4-6 times (see Luhring unpublished dissertation).

Ova dry masses for each species were calculated based on field-collected samples (Table 1). Subsamples of each species of metamorphs were used to estimate a species-specific wet to dry mass conversion, which was used to convert each mesocosm's species-specific total wetmass into drymass. The RG for each tank was then calculated (Eq. 7) and paired with the survival data for that tank. The survival and RG for each species in each mesocosm were then resampled with replacement in a bootstrapping procedure to produce 2-dimensional confidence intervals of biomass subsidy magnitudes.

Biomass Export into Terrestrial Systems – We use published aquatic productivity values of emergent metamorphosing amphibian biomass from whole-wetland studies on large open (Gibbons et al., 2006) and smaller closed-canopy wetlands (Regester et al., 2006) to simulate biomass and elemental export into the terrestrial environment as a function of emigrating metamorphs. This export was then given a uniform distribution around the wetland at varying distances to quantify the concentration of elements exported in a single year in the form of emergent amphibian metamorphs (Fig. 2). All simulation models were created and run with the MATLAB platform (R2009a, The MathWorks, Natick, MA).

Results

Relative Growth – Species varied considerably in the amount of dry mass gained from ova to metamorphosis with orders of magnitude separating the smallest (*A. terrestris*; RG of 11) from the largest (*L. catesbeianus*; RG of 5146) relative gainers (Table 2). For caudate data, a similar

spread in RG values was seen between the smallest (*A. opacum* from Regester et al., 2006; RG of 12) and the largest (*A. talpoideum*; RG of 1640.9) relative gainers.

Ontogenetic Stoichiometry – Carbon was the only universally imported element among amphibians on a per-mass basis (Table 3). While nearly all of the remaining elements were exported on a per-mass basis (OS > 1), Ca demonstrated the largest relative increase from ova to metamorph (~34-fold increase). On a per-mass basis, anurans imported S at nearly the same magnitude (0.86 export = 1.16-fold import) as it was exported by caudates (1.20-fold export). Simulating OS values of elements with non-normal distributions resulted in simulated averages of OS's that were divergent from that of the OS calculated from the midpoints of field data (evident from differences in mean versus median values in Table 3).

Elemental-specific Subsidy Simulations – When combined, the effects of change in size and stoichiometry between the two subsidy stages revealed large differences in specific elemental fluxes (Table 4). The most drastic differences occurred between C and Ca fluxes. Carbon content in ova is higher than that of metamorphs and thus dampens total C export caused by RG, whereas Ca is disproportionately higher in metamorphs relative to all other elements examined and amplifies Ca export caused by RG. For the species with the smallest RG, *A. terrestris*, 1 out of 9.4 ova have to reach metamorphosis (P_{Larval} of 0.11) for there to be zero net transfer of C between systems within a year. However, for the same species, only 1 in 478.7 ova have to reach metamorphosis for there to be a zero net transfer of Ca between aquatic and terrestrial systems (P_{Larval} of 0.002). The highest threshold P_{Larval} for zero net transfer was for S in *A. terrestris* (1 in 9.2; 0.11). The lowest threshold P_{Larval} for zero net transfer was for Ca in the species with the largest RG, *L. catesbeianus*, which only required 1 in 2.3×10^5 to have zero net transfer of Ca between systems.

Top-down and Bottom-up Influences on Biomass Subsidies – Top-down effects of predators on net anuran biomass export depended largely on potential size at metamorphosis and the gape-limited nature of our predators (*Notophthalmus viridescens*). The anuran with the smallest size at metamorphosis (*A. americanus*) metamorphosed with an estimated RG of 10 (Table 2) which would require a P_{Larval} of 0.10 to meet the zero net transfer isocline. When raised alone or with competitors, all 20 mesocosms had survival rates high enough to export *A. americanus* biomass. With the addition of predators only 2 out of 12 tanks (17%) had survival rates high enough to export *A. americanus* biomass.

Because of their larger RG values, the other two species used for this study only needed one metamorph to emerge to cause a net biomass export (based on the 30-45 individuals placed in each mesocosm). While this 1 per tank emergence threshold probably overestimates the likelihood that a species would be an exporter, the overall effects of predation on each population's transition probability and size at metamorphosis demonstrate meaningful patterns. For the second largest species, *H. versicolor*, only 2 out of 12 predator mesocosms failed to recruit metamorphs and another 2 had only one recruit. For the largest species, *L. sphenocephalus,* three (25%) predator tanks failed to recruit metamorphs.

Predation generally increased the chance that aquatic habitats would receive a net input (Figs. 1B-D). However, *L. sphenocephalus* (and to a lesser degree *H. versicolor*) larvae demonstrated an increase in per capita size in mesocosms with predators (Fig. 3). While fewer individuals were surviving as a result of predation, the size of each individual (and thus their RG) was greater than tanks with higher overall survival. By reducing competition, predators appeared to release the larger species from size constraints caused by density dependence. After a certain size was attained, the larger species would also be immune from predation of gape-

limited predation (in effect becoming mega-herbivores). While survival and total biomass exported from predator tanks was lower than tanks without predators, the larger species' increased size in response to reduced competition caused the decline in overall export to be more gradual (Figs. 1B-D).

Bottom-up effects on subsidy production yielded contrasting results. As intended, primary productivity was higher on average in mesocosms with higher light levels (Luhring, unpublished dissertation). Anuran biomass production, however, demonstrated species-specific patterns. Low light mesocosms were the most productive in terms of total biomass for both *A. americanus* and *H. versicolor* whereas *L. sphenocephalus* produced more total emergent biomass in mesocosms with high light levels (Figs. 1B-D). In combined mesocosms with competition or competition and predation, the increased productivity of *L. sphenocephalus* in high light treatments resulted in an overall higher amount of subsidy production (Luhring unpublished dissertation).

Discussion

Although amphibians are used here as an example, conclusions drawn from this exercise are applicable to any biomass subsidies driven by organismal life-histories. Ova and metamorphs are the currencies exchanged between aquatic and terrestrial systems during the complex life-cycle of amphibians. The direct dependence of one currency (metamorphs) on another (ova) yields a simplified mass balance equation of a transition probability (P_{Larval}) and modifiers on quantity (RG) and quality (OS) transitions between subsidy type (i.e., life stages involved in the transfer of material between systems). Transition probability and asymmetries in subsidy quantity (biomass) and quality (stoichiometry) determine the specific amount of elements moving in each direction. While several factors contribute to the dynamic nature of

biomass subsidy magnitude, biologically-fixed asymmetries in elemental composition ameliorate or exacerbate the net translocation of specific elements. Using a simplified framework, three readily estimated factors can be used to simulate and test hypotheses about subsidy movement by animals with complex life-histories: transition probability between stages (P_{Larval}), asymmetry in per capita size (RG), and asymmetry in stoichiometry (OS).

While survival estimates vary from one study to another, estimates of survival from oviposition to metamorphosis in anurans (0.04 P_{Larval} *L. sylvaticus*; Herreid and Kinney 1966, 0.05 P_{Larval} *L. aurora*; Calef 1973) and caudates (0.01-0.1 P_{Larval} *A. maculatum*; Shoop 1974, 0.0001-0.04 P_{Larval} *A. talpoideum*; Semlitsch 1987, 0.002-0.008 P_{Larval} *A. maculatum*, 0.08-0.13 P_{Larval} *A. tigrinum*; Regester et al., 2006) *in situ* are generally low. Additionally, in natural populations, stochastic environmental perturbations such as drought or flood can result in catastrophic breeding failure of entire wetland complexes, resulting in a total import without export for all or part of that breeding season (Semlitsch et al., 1996). For years with high larval survival (above 0.1), all of the species we examined would cause net exports of C, Ca, Fe, K, Na, P and S except for the species with the smallest RG (*A. terrestris*) showing a marginal import of C and S. As P_{Larval} decreases, several elements in species with smaller RG's begin to show net imports (Table 4). However, barring catastrophic breeding failure, calcium remains a net export for nearly all scenarios (minimum P_{Larval} for zero net transfer in two smallest RG species; 0.002 for *A. terrestris*, 0.0006 for *G. carolinensis*).

The stoichiometric asymmetries in subsidies moving in each direction can result in differential net displacement of elements between systems within a year. For example, in a population of *A. terrestris* P_{Larval} values 0.01 – 0.04 would result, on average in an import of C, K, P, S and an export of Ca, Fe, Na. Larval survival from 0.01 – 0.002 would result, on average

in an import of 6 of the 7 elements with Ca still being exported. Similar scenarios happen at varying larval survival values for each species (Table 4).

In our systems aquatic predation had the strongest effect on subsidy transition probability (Figs. 1 B-D). Predation in recipient systems reallocates incoming biomass subsidies into predator biomass and excreta. In the case of active subsidies that depend on the transformation of an incoming subsidy into outgoing subsidies (e.g., ova into metamorphs), a decrease of the transition probability between subsidies localizes the first subsidy and prevents the development and export of the second. In this case, the predator (*N. viridescens*) is also an agent for biomass transport because incoming biomass could be converted to ova production (if the predator is female) or leave with emigrating adults. However, energy and biomass transfer between trophic levels results in varying degrees of energy and mass loss. Additionally, any energy or biomass that is not directly transferred from the incoming subsidy (e.g., ova) to an outgoing subsidy (e.g., metamorphs, emigrating adults) would be an import into the recipient system (e.g., trophic transfer loss, egestion, excretion). Although our example predator demonstrates a multi-habitat life cycle, several predatory subsidy recipients do not use multiple habitats and would create an even stronger subsidy sink.

A reduction of transition probability through predation should reduce total export of biomass if RG remains constant. The reduction of survival in smaller species with little to no plasticity in mass at each subsidy stage (e.g., *A. americanus*) shows relatively no effect on RG (Fig. 1B), resulting in a decrease in total biomass that is directly proportional to a reduction in the number of individuals exporting biomass. This may not be the case for animals with larger and relatively more plastic subsidy stages as was demonstrated by larger species (*H. versicolor, L. sphenocephalus*; Figs. 1C-D). While predator depression of survival reduced the total number

of individuals emerging from our mesocosms, it also increased the size of metamorphs that did emerge through a reduction of density effects (e.g., Scott 1990; Fig. 3). This indicates that predator effects through density-dependent processes can create non-linear effects on total biomass export. Systems with gape-limited predators (e.g., *N. viridescens*) may be more likely to see such effects as larger prey species can reach a size refuge from predation (e.g., *L. sphenocephalus*).

The productivity of amphibians in aquatic habitats (i.e., the biomass of metamorphosing juveniles produced) is highly variable across space and time (e.g., Pechmann et al., 1989). However, we can make a few simplifying assumptions to approximate the amount of elements being moved in the course of one breeding season and their concentration in the recipient system. The 15.9 g/m^2 productivity rate seen in a notably productive year of amphibian metamorphs (Gibbons et al., 2006) combined with an average amphibian dry mass (20%) would see an export of 318,000g dry mass from a 100,000m^2 wetland. Converted to element-specific components this would result in an export of 139kg C, 31.5kg N, 5.1kg P, 8.6kg Ca, 1.9kg S, 3.2kg K, and 1.6kg Na. An even distribution of the dry mass within a 10m buffer around the edge of the pond (11,524m^2) would result in an average distribution of 12.08 g/m^2 C, 2.73 g/m^2 N, 0.44 g/m^2 P, 0.75 g/m^2 Ca, 0.17 g/m^2 S, 0.28 g/m^2 K, and 0.14 g/m^2 Na. Whereas a more conservative estimate of emergent biomass (5g wet mass is used to approximate 0.79 g/m^2 ash-free dry mass; Regester et al., 2006) produces a markedly lower estimate (Fig. 2).

Future Directions

Understanding the mechanisms driving biomass and nutrient transfer will not only help identify species that are important for ecosystem function, but also predict impacts of system perturbations on linked systems. Changes in subsidy quantity or quality have direct impacts on

recipient systems (Maron et al., 2006). Several linked ecosystems feature active subsidies that transport biomass and nutrients between them. The dependence of animals on multiple habitats makes them vulnerable to changes in any of their requisite habitats. Additionally, loss of animals with complex life-histories ultimately affects multiple ecosystems (e.g., Whiles et al., 2006). The pervasive declines in several groups of animals (Amphibians: Stuart et al., 2004, Fishes: Helfman et al., 1997; Schindler et al., 2003, Reptiles: Gibbons et al., 2000) involved in multiple-system subsidies have implications at the inter-ecosystem scale.

Other shifts in subsidy quality and/or quantity may be seen in the case of invasive species replacing native subsidies. In the great lakes region of North America, such a shift has already been underway for the last century with the introduction of non-native salmonids (Crawford 2001) and the onset of non-native sea lamprey invasions (Christie and Goddard 2003). Fish-driven subsidies between streams and lakes in the Great Lakes have undoubtedly changed in their species composition as a result of these perturbations. Has it likewise changed in quantity or quality? The shift from one subsidy to another may have influences on recipient systems that extend beyond the simple total biomass being moved between systems. The life cycle of amphibians indicates a strong calcium export into the terrestrial system. If an amphibian subsidy was replaced with an insect subsidy of the same biomass, the total calcium exported would be greatly diminished.

Differences in adult breeding mortality in these systems has a major impact on subsidy quantity and quality. Our conceptual model assumed no adult breeding mortality. Future models that include varying degrees of adult breeding mortality or stage-structured transition probabilities with spatially explicit deposition for mortality at all stages (even those intermediate to each subsidy stage) would be a welcome addition. In the case of semelparous animals, a

model with three subsidy stages would needed as the adult carcass subsidy is independent of that of the ova being deposited.

Future research into the temporal and spatial distribution of subsidies resulting from animal life-histories would be of great benefit to the field of ecology. Shifts in breeding or dispersal phenology associated with global climate change likewise shift the timing of subsidy movement (e.g., Todd et al., 2011). Additionally, any changes in the movement ability or landscape resistance facing dispersing animals will directly impact subsidy spatial distribution.

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Table 1. Summary of species-specific dry masses across ontogeny (rounded to nearest measured digit).

	Ova			Metamorphs			Adults		
Species	Mean	Sample Size (aggregates,*n*)	Range	Mean	Sample Size (aggregates,*n*)	Range	Mean	*n*	Range
Ambystoma opacum	0.0033	9, 695	0.0024 – 0.0040	0.1960	3, 15	0.1329 – 0.6944	1.3545	2	1.256 -1.453
Ambystoma talpoideum	0.0011	2, 2670	0.0011	1.0904	2, 5	0.614 – 2.996	2.492	2	2.126 – 2.858
Anaxyrus terrestris	0.0012	2, 3444	0.0011 - 0.0012	0.0125	9, 306	0.0067 – 0.0197	2.7131	3	1.5182 – 3.6115
A. terrestris (oviposited)	0.0007	10, 3919	0.0006 – 0.0010						
Gastrophryne carolinensis (oviposited)	0.0004	3, 1373	0.0003 – 0.0005	0.0167	25, 25	0.0091 – 0.0232	0.5065	9	0.3699 – 0.6186
Hyla cinerea	0.0006	6, 1925	0.0004 – 0.0008	0.139	19, 19	0.071 – 0.311	1.3417	7	0.9729 – 1.5205
Lithobates catesbeianus	0.0004	4, 31806	0.0003 – 0.0004	2.0585	2, 2	1.1529 – 2.964	72.9671	2	69.8327 – 76.1014
Lithobates sphenocephalus	0.0013	2, 4424	0.0011 – 0.0014	0.5845	3, 4	0.3539 – 0.9215	3.650	2	2.969 – 4.331
Schaphiopus holbrookii	0.0012	2, 2400	0.0010 – 0.0013	0.1038	1,13	0.1038	5.768	2	2.723 – 8.813

Table 2. Relative growths (RG) for the transition from ova to metamorphosis of species modeled in this study. Values are per capita dry mass (g) midpoints between minimum and maximum values, calculated from means of whole-pond composites* (AFDM data from Regester et al., 2006), or species estimates based on close relatives or conspecifics from another region (denoted with *). Values of larval survival as shown where the total biomass entering a wetland (ova) is equal to the biomass that is leaving (metamorphs).

Species	Ova Dry Mass	Metamorph Dry Mass	RG	Larval Survival	Data Source
Ambystoma opacum	0.0032	0.414	129.3	0.008	This study
Ambystoma talpoideum	0.0011	1.805	1640.9	6.1×10^{-4}	This study
Anaxyrus terrestris	0.0012	0.013	11.0	0.09	This study
Gastrophryne carolinensis	0.0004	0.016	40.4	0.02	This study
Hyla cinerea	0.0006	0.191	318.3	0.003	This study
Lithobates catesbeianus	0.0004	2.058	5146.1	1.9×10^{-4}	This study
Lithobates sphenocephalus	0.0013	0.638	490.5	0.002	This study
Schaphiopus holbrookii	0.0012	0.104	86.5	0.01	This study
Ambystoma opacum	0.0050	0.06	12.0	0.08	Regester et al., 2006
Ambystoma maculatum	0.0100	0.19	19.0	0.05	Regester et al., 2006
Ambystoma tigrinum	0.0100	1.79	179.0	0.006	Regester et al., 2006
Anaxyrus americanus	0.0012*	0.012	10.0	0.1	This study
Hyla versicolor	0.0006*	0.09	150.0	0.007	This study
Lithobates sphenocephalus	0.0013*	0.24	184.6	0.005	This study

Table 3. Ontogenetic stoichiometries (OS) for the transition from ova to metamorphosis of species modeled in this study. Values are means for each order or stage for which a previous study demonstrated stoichiometric differences (Chapter 3). Simulation probability density function (PDF) refers to the distribution from which random numbers were drawn.

Element	Significant Levels (n)	Mean PPM (Std Dev)	OS	Simulated OS* ± Std Dev*	Simulation PDF
C	Ova (13) Metamorph (9)	506569 (7981) 438070 (36131)	0.86	0.87 ± 0.07	Normal
Ca	Ova (14) Metamorph (9)	781 (476) 26680 (4403)	34.16	44.31 ± 26.96	Gamma
Fe	Ova (14) Metamorph (9)	146 (168) 490 (292)	3.36	7.39 ± 8.61	Gamma
K	Ova (14) Metamorph (9)	4062 (637) 9612 (2457)	2.37	2.44 ± 0.76	Normal
Mg	Anuran (34)	1211 (226)	NA		
	Caudate (16)	1108 (134)			
N	No differences (49)	103614 (9785)	NA		
Na	Ova (14) Metamorph (9)	1749 (676) 5384 (2139)	3.08	3.20 ± 1.73	Gamma
P	Anuran Ova (10) Anuran Metamorph (6)	11383 (1294) 15654 (2967)	1.38	1.39 ± 0.31	Normal
	Caudate Ova (4) Caudate Metamorph (3)	12512 (647) 16259 (2372)	1.30	1.31 ± 0.20	Normal
S	Anuran Ova (10) Anuran Metamorph (6)	6406 (455) 5481 (419)	0.86	0.86 ± 0.09	Normal
	Caudate Ova (4) Caudate Metamorph (3)	5624 (244) 6724 (552)	1.20	1.20 ± 0.11	Normal

Table 4. Simulated per-capita relative export of seven elements for eight species of amphibians.

	Per capita Relative Export (mean and median)						
Species	Carbon	Calcium	Iron	Potassium	Sodium	Phosphorus	Sulfur
Ambystoma	109.5	5.6×10^3	929.4	300.8	897.1	163.9	150.1
opacum	108.2	4.7×10^3	479.7	281.6	440.3	160.1	148.8
Ambystoma	1.4×10^3	7.3×10^4	1.2×10^4	4.0×10^3	1.2×10^4	2.1×10^3	1.9×10^3
talpoideum	1.4×10^3	6.1×10^4	6.1×10^3	3.7×10^3	6.1×10^3	2.1×10^3	1.9×10^3
Anaxyrus	9.4	478.7	82.6	26.3	82.2	15.0	9.2
terrestris	9.3	400.2	47.5	25.2	42.0	14.6	9.2
Gastrophryne	34.8	1.7×10^3	305.6	97.6	296.7	56.6	35.1
carolinensis	34.5	1.4×10^3	148.7	93.8	147.3	54.5	34.7
Hyla cinerea	281.6	1.4×10^4	2.3×10^3	788.0	2.4×10^3	442.5	278.2
	276.5	1.1×10^4	1.2×10^3	725.5	1.1×10^3	427.5	270.8
Lithobates	4.4×10^3	2.3×10^5	3.6×10^4	1.3×10^4	4.0×10^4	7.2×10^3	4.4×10^3
catesbeianus	4.4×10^3	1.9×10^5	1.8×10^4	1.2×10^4	2.0×10^4	6.9×10^3	4.4×10^3
Lihtobates	424.8	2.1×10^4	3.6×10^3	1.2×10^3	3.7×10^3	699.3	427.9
sphenocephalus	421.1	1.8×10^4	1.8×10^3	1.1×10^3	1.9×10^3	671.8	426.4
Schaphiopus	73.7	3.8×10^3	613.9	211.6	669.6	119.9	74.3
holbrookii	73.2	3.2×10^3	324.2	204.9	360.2	116.9	73.7

Figure 1. Graph of net import versus export. Plots of theoretical (A), and simulated exports from experimental mesocosms for (B) *Anaxyrus americanus,* (C) *Hyla versicolor,* (D) *Lithobates sphenocephalus.*

Figure 1.

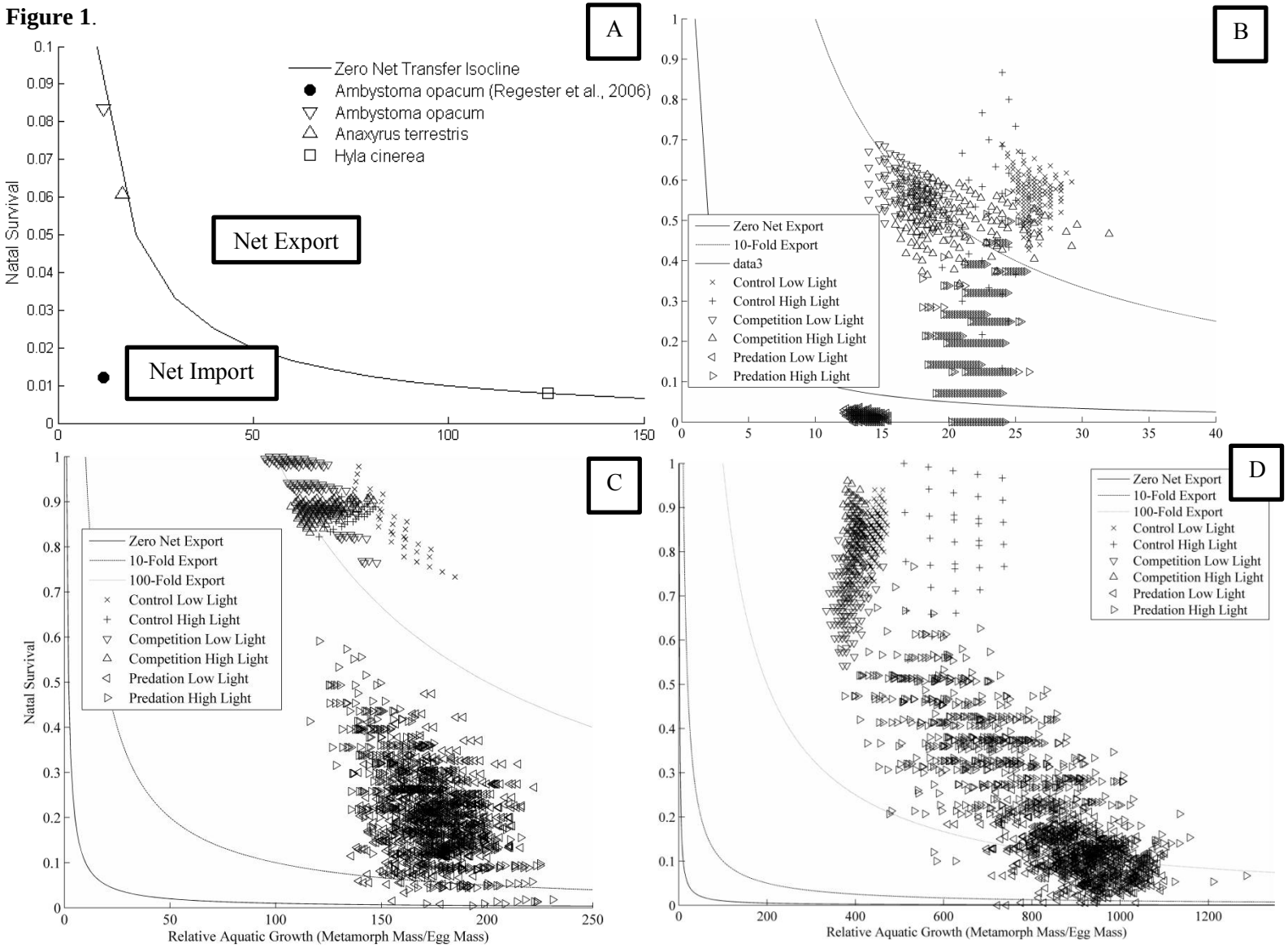
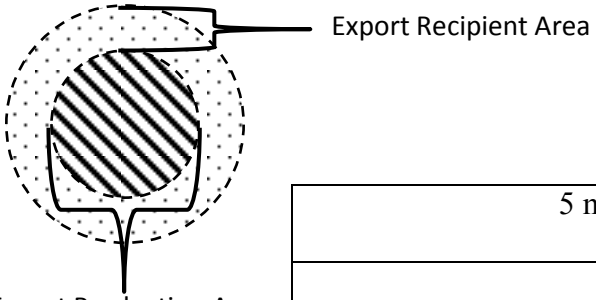
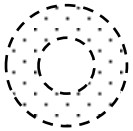

A
B
C
D
Zero Net Transfer Isocline
Ambystoma opacum (Regester et al., 2006)
Ambystoma opacum
Anaxyrus terrestris
Hyla cinerea
Net Export
Net Import
Natal Survival
Zero Net Export
10-Fold Export
100-Fold Export
data3
Control Low Light
Control High Light
Competition Low Light
Competition High Light
Predation Low Light
Predation High Light
Relative Aquatic Growth (Metamorph Mass/Egg Mass)

Figure 2. Elemental concentrations for subsidy export with varying donor and recipient system sizes. Modeled after donor (aquatic) and recipient (terrestrial) systems relating to metamorphosing amphibians. Average biomass production (ABD) is the amount of biomass (g wet mass) produced per season per m^2. Donor system denoted with total area whereas recipient system denoted with width of buffer (representing dispersal distance of subsidy). Values of elements less than 0.01 g drymass/m^2 denoted with <.

Figure 2.





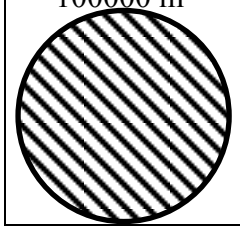
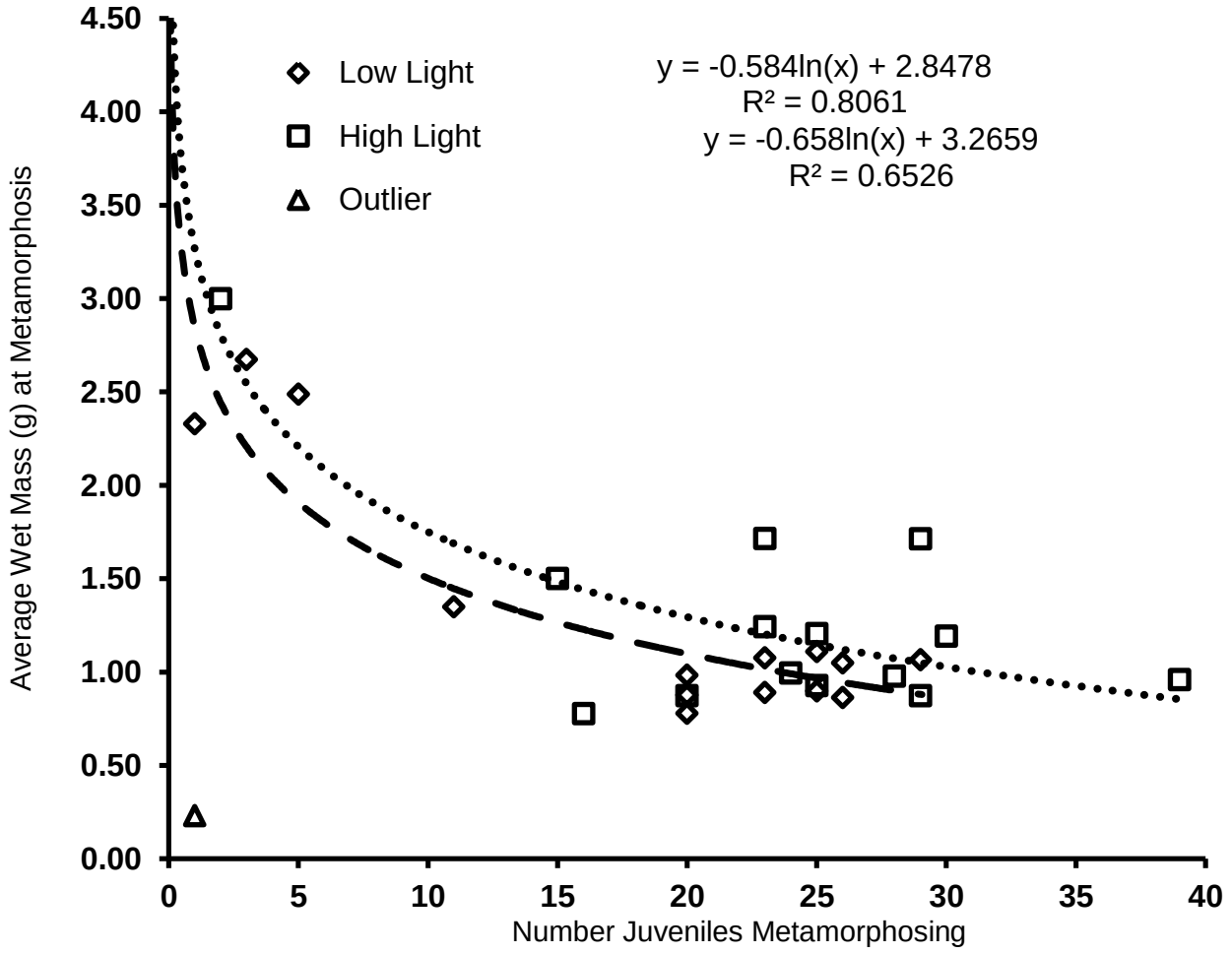
		5 m Dispersal							10 m Dispersal						
		Elemental Concentration (g/m^2)													
	ABP	C	N	Ca	P	K	S	Na	C	N	Ca	P	K	S	Na
100 m^2 	5	0.17	0.04	0.01	<	<	<	<	0.07	0.01	<	<	<	<	<
	15.9	0.54	0.12	0.03	0.02	0.01	<	<	0.21	0.05	0.01	<	<	<	<
1000 m^2 	5	0.69	0.15	0.04	0.03	0.02	0.01	0.01	0.31	0.07	0.02	0.01	0.01	<	<
	15.9	2.18	0.49	0.13	0.08	0.05	0.03	0.02	0.97	0.22	0.06	0.04	0.02	0.01	0.01
100000 m^2 	5	7.71	1.74	0.48	0.28	0.18	0.11	0.09	3.80	0.86	0.23	0.14	0.09	0.05	0.04
	15.9	24.51	5.54	1.51	0.90	0.56	0.34	0.28	12.09	2.73	0.75	0.44	0.28	0.17	0.14

Figure 3. Density dependent size regulation in *L. spenocephalus*. Average size at metamorphosis as a function of density. Each datapoint represents a single experimental mesocosm. The outlier is from a high light tank with a single metamorph.



CHAPTER 5

SUMMARY

The mobility of animals is what makes their interactions with biogeochemical cycles unique. Animals move nutrients in directions at odds with passive movement (e.g., against gravity), and in chemical forms not found in other organisms (e.g., bone, chitin). It is the movement and engineering of different chemical forms of matter throughout ontogeny that shapes the influence of animals on biogeochemical cycles.

This dissertation uses the obligatory movement of biomass and nutrients between terrestrial and aquatic ecosystems that result from amphibian life-cycles. What follows is a summary of each dissertation chapter's main findings.

Chapter 2 - Bottom-up and top-down effects in aquatic amphibian communities impact nitrogen and phosphorus cycling and community-level productivity

- Amphibians impact a variety of biotic and abiotic factors within aquatic ecosystems
- These impacts change with changes to bottom-up drivers (light)
- Top-down effects generally prevent water nutrient draw-down seen in systems with anuran larvae by preventing the consumption of primary producers

Chapter 3 - Time to face the strain: Changes in vertebrate stoichiometry across ontogeny when complex life-histories present stage-specific demands.

- Amphibian ontogeny is accompanied by significant changes in stoichiometry
- The change in relative calcium content between ova and metamorph is the largest relative increase between stages seen for any of the elements examined
- Carbon generally declined in relative contribution to overall amphibian biomass across ontogeny
- Life-history aspects such as larval period, size at metamorphosis and absence or presence of a terrestrial stage strongly influences amphibian stoichiometry

Chapter 4 - What governs the quantity and quality of active subsidies resulting from complex life-histories?

- Species that transfer nutrients between ecosystems as a predictable function of their life-histories are modeled for their net impacts on nutrient cycles.
- The net biomass and nutrient transfer that occurs as a result of sequential transfers that are a part of animal life-histories can be simplified into an equation that accounts for changes in biomass and stoichiometry and the transition probability from one stage to another.
- Species differences in body size and stoichiometry greatly shape the magnitude and likelihood of their nutrient translocations.
- Top-down pressures in one habitat can result in subsidy localization in the recipient habitat (e.g., a subsidy sink).

- Prey species that grow larger as they are released from competition via predatory thinning of their populations are less likely to show a strong reduction in net population biomass export from that system

Appendix A

Figures and Tables Related to Ontogenetic Stoichiometry

Table A1

ONTOGENETIC CHANGES IN SIX ELEMENTS FOR ANURANS (FOUR SPECIES).

Element	Effects	Differences (With Tukey HSD)	F-statistic p-value
Boron (B)	Stage	Adult<Ova<Meta	$F_{2,21}$=25.57 p=0.0001
	Species	ANTE,LICA<LISP,SCHO	$F_{3,21}$=14.73 p=0.0005
Copper (Cu)	Species*	LISP<SCHO	$F_{3,21}$=3.51 *p=0.06
Magnesium (Mg)	Stage	Meta<Adult Ova<Adult (p=0.06)	$F_{2,21}$=6.05 p=0.02
	Stage*Species*	SCHO Ova high	$F_{6,21}$=2.72 p=0.08
Phosphorus (P)	Stage	Ova,Meta<Adult	$F_{2,21}$=17.12 p=0.0006
Sulfur (S)	Stage	Meta<Ova,Adult	$F_{2,21}$=19.47 p=0.0004
Zinc (Zn)	Stage	Meta, Adult<Ova	$F_{2,21}$=6.01 p=0.02

Note – Analyzed as Generalized Linear Models (Nutrient~Stage*Species) with gamma error distributions. Recently metamorphosed juveniles are abbreviated as "Meta." Commas between groups indicate no differences whereas "<" denotes a significant difference between each group on either side of the sign (e.g., A,B<C means that A is not different from B, but A and B are less than C). All between group differences are significant at the $p<0.05$ level unless denoted with an asterisk.

Table A2

ONTOGENETIC CHANGES IN SIX ELEMENTS FOR CAUDATES (TWO SPECIES).

Element	Effects	Differences (With Tukey HSD)	F-statistic p-value
Boron (B)	None	None	N/A
Copper (Cu)	Stage*	Ova<Adult (p=0.06)	$F_{2,11}$=4.72 p=0.06*
Magnesium (Mg)	None	None	N/A
Phosphorus (P)	Stage	Ova <Adult	$F_{2,11}$=6.45 p=0.03
Sulfur (S)	Stage	Ova<Meta Ova<Adult* (p=0.09)	$F_{2,11}$=5.43 p=0.045
Zinc (Zn)	Species	AMTA<AMOP	$F_{1,11}$=18.57 p=0.005

Note – Analyzed as Generalized Linear Models (Nutrient~Stage*Species) with gamma error distributions. Recently metamorphosed juveniles are abbreviated as "Meta." Commas between groups indicate no differences whereas "<" denotes a significant difference between each group on either side of the sign (e.g., A,B<C means that A is not different from B, but A and B are less than C). All between group differences are significant at the $p<0.05$ level unless denoted with an asterisk.

Table A3

ELEMENTAL COMPOSITION OF OVA AS EXPLAINED BY AIC_C-RANKED CANDIDATE MODELS.

Element	Factors	Best Model (df)	AIC_c	ΔAIC_c	w_i	F-statistic p-value
Carbon (C)	Order	Order (3)	274.0	0.0	0.49	$F_{1,12}$=4.1 p=0.067
	Dry Mass	Intercept (2)	274.7	0.7	0.35	
Calcium (Ca)	Dry Mass	Dry Mass (3)	209.2	0.0	0.34	$F_{1,13}$=2.4 p=0.149
	Order	Intercept (2)	209.3	0.0	0.33	
Iron (Fe)	Larval	Larval Period (3)	164.2	0.0	0.73	$F_{1,13}$=2.37 p=0.15
	Order	Intercept (2)	169.1	4.9	0.13	
Magnesium (Mg)	Larval	Larval Period (3)	183.4	0.0	0.76	$F_{1,13}$=8.3 p=0.014
	Order	Intercept (2)	188.4	4.9	0.06	
Nitrogen (N)	Larval	Larval +	246.6	0.0	0.93	$F_{1,12}$=48.2 p=4.0e-05
	Dry Mass	Dry Mass (4)				$F_{1,12}$=11.6 p=0.007
		Intercept (2)	264.3	17.7	<0.001	
Sodium (Na)	Dry Mass	Dry Mass (3)	221.4	0.0	0.41	$F_{1,13}$=3.6 p=0.083
	Order	Order (3)	221.9	0.5	0.33	$F_{1,13}$=3.5 p=0.088
		Intercept (2)	223.4	1.9	0.16	
Phosphorus (P)	Larval	Larval +	233.2	0.0	0.99	$F_{1,13}$=22.4 p=6e-04
	Order	Order (4)				$F_{1,13}$=7.5 p=0.02
		Intercept	244.2	11.0	0.004	
Sulfur (S)	Order	Larval +	207.3	0.0	0.96	$F_{1,13}$=13.1 p=0.004
	Larval	Order (4)				$F_{1,13}$=20.7 p=8e-04
		Intercept	219.7	12.3	0.002	
Zinc (Zn)	Order	Order (3)	158.8	0.0	0.86	$F_{1,13}$=8.6 p=0.01
	Dry Mass	Intercept	162.7	8.7	0.01	

Note – Models tested aspects of life-history (larval period), taxa (order), and per capita size (dry mass). Final values shown for the best models with a ΔAIC_c <2.0 and higher support than the intercept model. Data for elements in which the intercept model was the best candidate are not shown. An ova sample for one species (*Hyla cinerea)* is absent from C and N data.

Table A4

ELEMENTAL COMPOSITION OF METAMORPHS AS EXPLAINED BY AIC-RANKED CANDIDATE MODELS.

Element	Factors	Best Model (df)	AIC_c	ΔAIC_c	w_i	F-statistic p-value
Iron (Fe)	Dry Mass	Dry Mass (3)	125.9	0.0	0.72	$F_{1,8}$=4.2 p=0.079
	Order	Intercept (2)	130.1	4.2	0.09	
Magnesium (Mg)	SVL	Larval +	111.0	0.0	0.98	$F_{1,8}$=3.8 p=0.098
	Larval	SVL (4)				$F_{1,8}$=56.1 p=3e-04
		Intercept (2)	119.4	8.4	0.01	
Phosphorus (P)	SVL	Larval +	157.5	0.0	0.998	$F_{1,8}$=56.7 p=3e-04
	Larval	SVL (4)				$F_{1,8}$=65.8 p=2e-04
		Intercept (2)	172.0	14.5	<0.001	
Sulfur (S)	Order	Order (3)	144.7	0.0	0.88	$F_{1,8}$=14.6 p=0.007
	Larval	Intercept (2)	149.6	4.8	0.08	

Note – Models tested aspects of life-history (larval period), taxa (order), and per capita size (dry mass or SVL). Final values shown for the best models within a ΔAIC_c <2.0. Data for elements in which the intercept model was the best candidate are not shown.

Table A5

ELEMENTAL COMPOSITION OF ADULTS AS EXPLAINED BY AIC-RANKED CANDIDATE MODELS.

Element	Factors	Best Models (df)	AIC_c	ΔAIC_c	w_i	F-statistic p-value
Carbon (C)	Habitat	Habitat (3)	537.0	0.0	0.41	$F_{1,21}$=4.7 p=0.042
	SVL	SVL (3)	537.6	0.6	0.30	$F_{1,21}$=4.0 p=0.059
		Intercept (2)	538.5	1.5	0.19	
Calcium (Ca)	Habitat	Habitat (3)	493.4	0.0	0.36	$F_{1,21}$=2.3 p=0.145
	SVL	Intercept (2)	493.6	0.2	0.32	
Iron (Fe)	Dry Mass	Dry Mass (3)	270.1	0.0	0.54	$F_{1,21}$=2.0 p=0.176
	Habitat	Intercept (2)	272.1	2.0	0.20	
Potassium (K)	Habitat	Habitat (3)	383.1	0.0	0.51	$F_{1,21}$=12.3 p=0.002
	SVL	SVL (3)	383.8	0.8	0.35	$F_{1,21}$=11.1 p=0.003
		Intercept (2)	389.2	6.2	0.02	
Manganese (Mn)	SVL	SVL (3)	221.7	0.0	0.72	$F_{1,21}$=7.2 p=0.01
	Habitat	Intercept (2)	232.8	11.1	0.003	
Nitrogen (N)	Habitat	Habitat (3)	462.4	0.0	0.79	$F_{1,21}$=23.7 p=9e-05
	Dry Mass	Intercept (2)	475.4	13.1	0.001	
Sodium (Na)	Habitat	Habitat (3)	370.9	0.0	0.57	$F_{1,21}$=8.3 p=0.009
	SVL	Habitat +	371.8	0.9	0.36	$F_{1,21}$=4.6 p=0.045
		SVL (4)				$F_{1,21}$=4.8 p=0.041
		Intercept (2)	377.7	6.8	0.02	
Phosphorus (P)	Habitat	Habitat (3)	450.8	0.0	0.38	$F_{1,21}$=2.7 p=0.12
	SVL	SVL (3)	452.3	1.5	0.18	$F_{1,21}$=1.4 p=0.26
		Intercept	451.2	0.4	0.31	

Note- Models tested aspects of life-history (aquatic or terrestrial habitat), taxa (Family), and per capita size (dry mass or SVL). Final values shown for the best models within a ΔAIC_c <2.0. Data for elements in which the intercept model was the best candidate are not shown.

Table A6

ELEMENTAL COMPOSITION OF TERRESTRIAL ADULTS AS EXPLAINED BY AIC-RANKED CANDIDATE MODELS.

Element	Factors	Best Model (df)	AIC_c	ΔAIC_c	w_i	F-statistic p-value
Calcium (Ca)	Dry Mass	Dry Mass (3)	402.6	0.0	0.41	$F_{1,17}$=6.4 p=0.02
	Order	Intercept (2)	403.8	1.2	0.22	
Iron (Fe)	Dry Mass	Dry Mass (3)	224.5	0.0	0.46	$F_{1,17}$=1.2 p=0.29
	Order	Intercept (2)	225.2	0.6	0.33	
Magnesium (Mg)	Order	Order (3)	243.0	0.0	0.74	$F_{1,17}$=9.4 p=0.007
	Dry Mass	Intercept (2)	249.0	6.0	0.04	
Manganese (Mn)	SVL	SVL (3)	193.3	0.0	0.47	$F_{1,17}$=2.79 p=0.11
	Order	Intercept (2)	193.9	0.6	0.34	
Phosphorus (P)	Order	Dry Mass +	366.7	0.0	0.30	$F_{1,17}$=5.8 p=0.03
	Dry Mass	Order				$F_{1,17}$=3.0 p=0.10
		Order	366.8	0.0	0.30	$F_{1,17}$=3.6 p=0.08
		Dry Mass (3)	367.2	0.4	0.25	$F_{1,17}$=5.12 p=0.04
		Intercept (2)	368.1	1.3	0.16	
Zinc (Zn)	SVL	SVL (3)	180.5	0.0	0.48	$F_{1,17}$=2.9 p=0.11
	Order	Intercept (2)	181.6	1.0	0.28	

Note – Models tested aspects of taxa (Order or Family), and per capita size (Dry Mass or SVL). Final values shown for the best models with a ΔAIC_c <2.0 and ranked higher than intercept model. Data for elements in which the intercept model was the best candidate are not shown.

Figures A1-A6. Ontogenetic stoichiometry plots of C, N, P, Ca, Fe, and S for A) *A. opacum* B) *A. talpoideum* C) *A. terrestris* D) *L. catesbeianus* E) *L. sphenocephalus* F) *S. holbrookii.* Symbols denote differences in taxonomy (caudates: open symbols, anurans: closed symbols) and ontogeny (ova: o, deposited ova: x, tadpoles: □, metamorphs: ◊, juveniles: +, adults: ✰).

Fig A1.

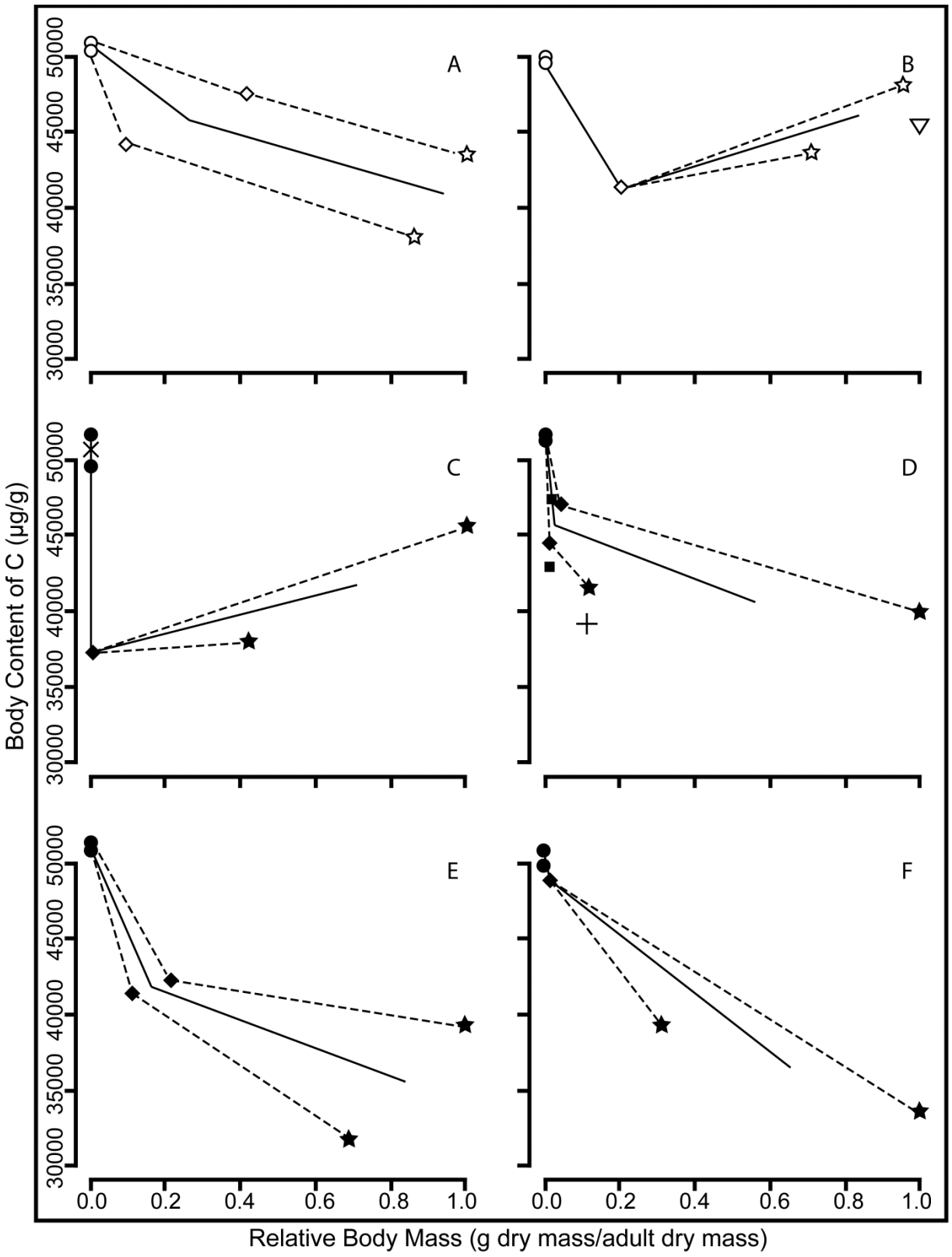

A
B
C
D
E
F
Body Content of C (µg/g)
30000
35000
40000
45000
50000
0.0
0.2
0.4
0.6
0.8
1.0
Relative Body Mass (g dry mass/adult dry mass)

Fig. A2.

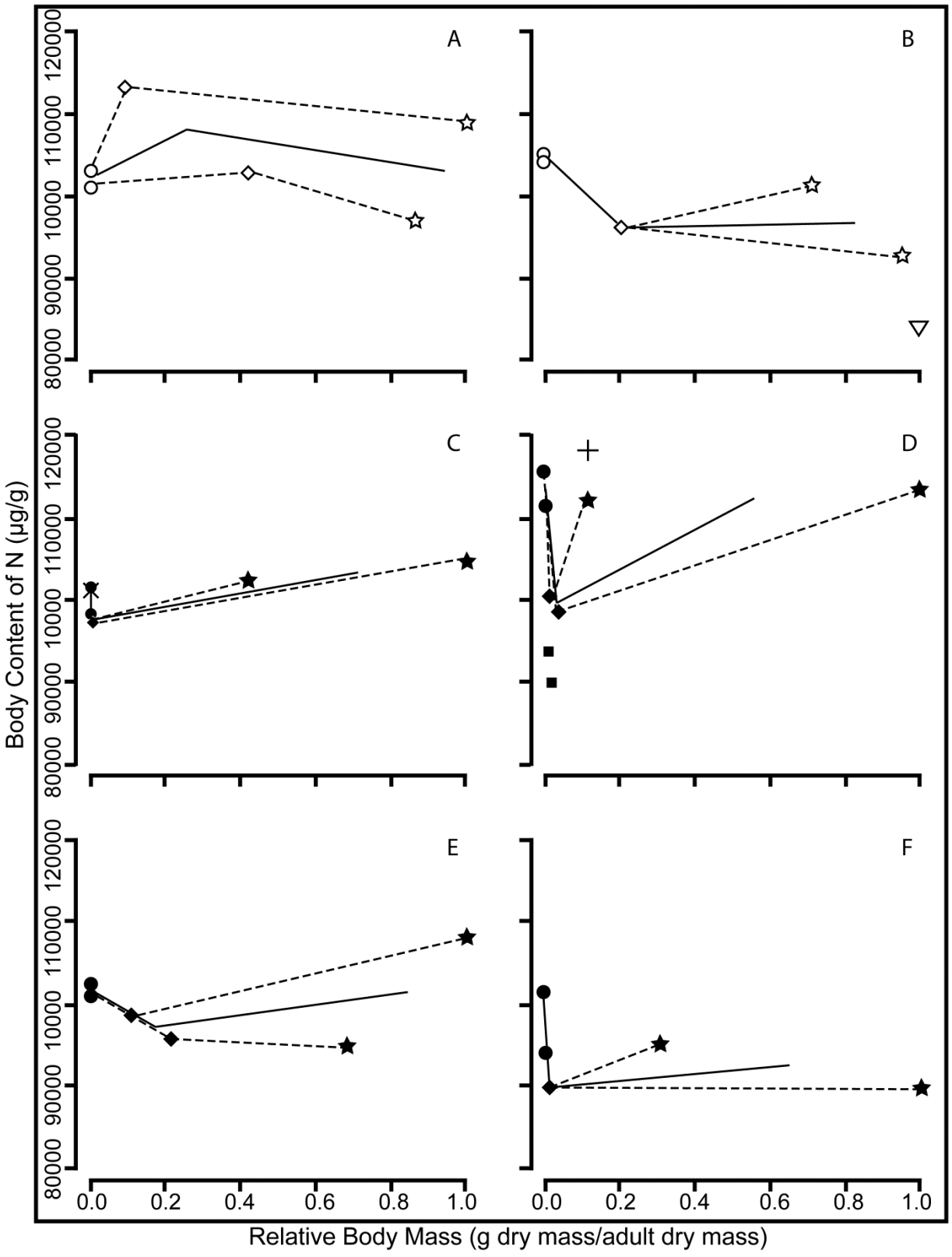

A
B
C
D
E
F
Body Content of N (µg/g)
80000
90000
10000
110000
120000
0.0
0.2
0.4
0.6
0.8
1.0
Relative Body Mass (g dry mass/adult dry mass)

Fig A3.

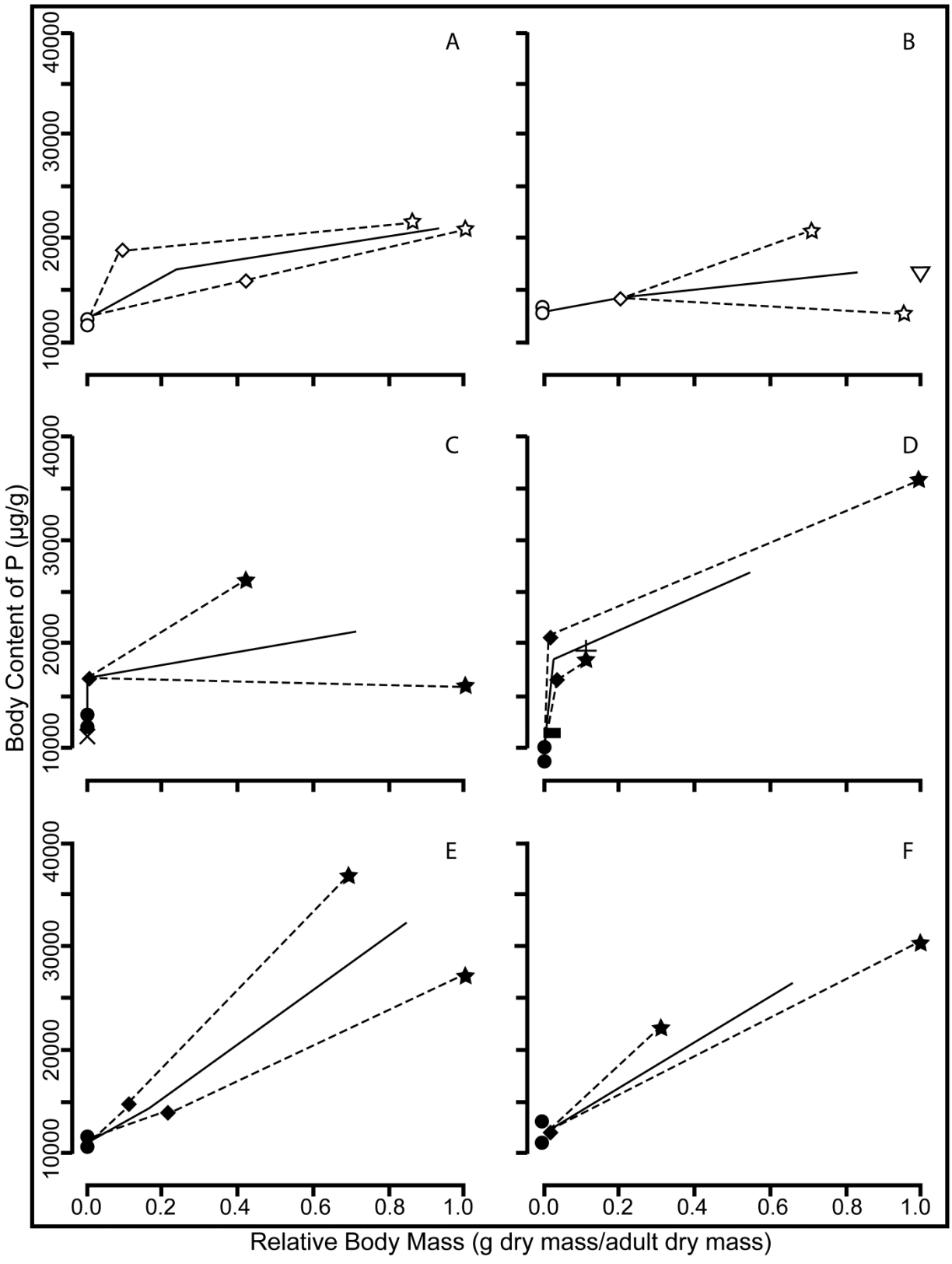

A
B
C
D
E
F
Body Content of P (µg/g)
10000
20000
30000
40000
0.0
0.2
0.4
0.6
0.8
1.0
Relative Body Mass (g dry mass/adult dry mass)

Fig. A4.

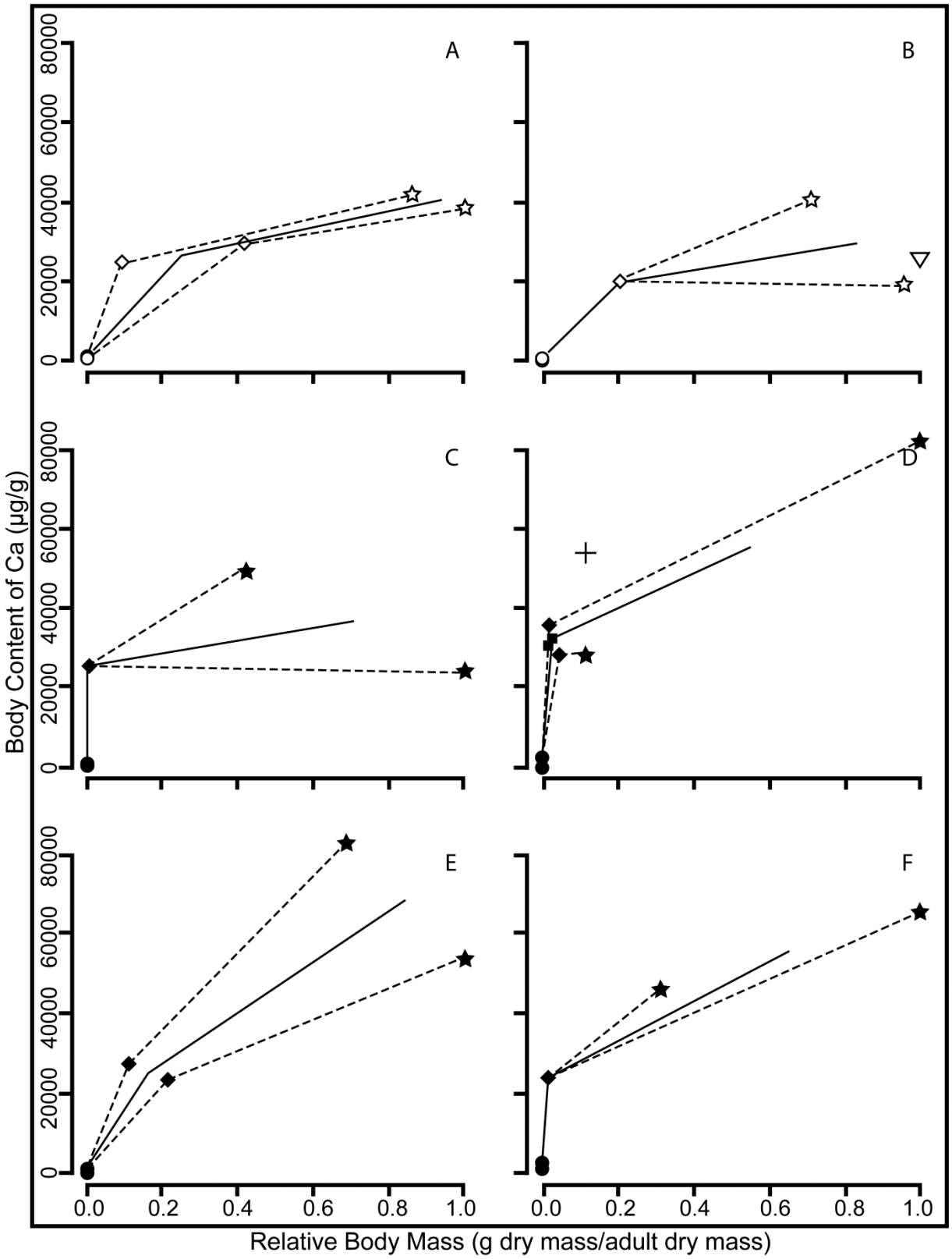

A
B
C
D
E
F
Body Content of Ca (µg/g)
0
20000
40000
60000
80000
0.0
0.2
0.4
0.6
0.8
1.0
Relative Body Mass (g dry mass/adult dry mass)

Fig. A5.

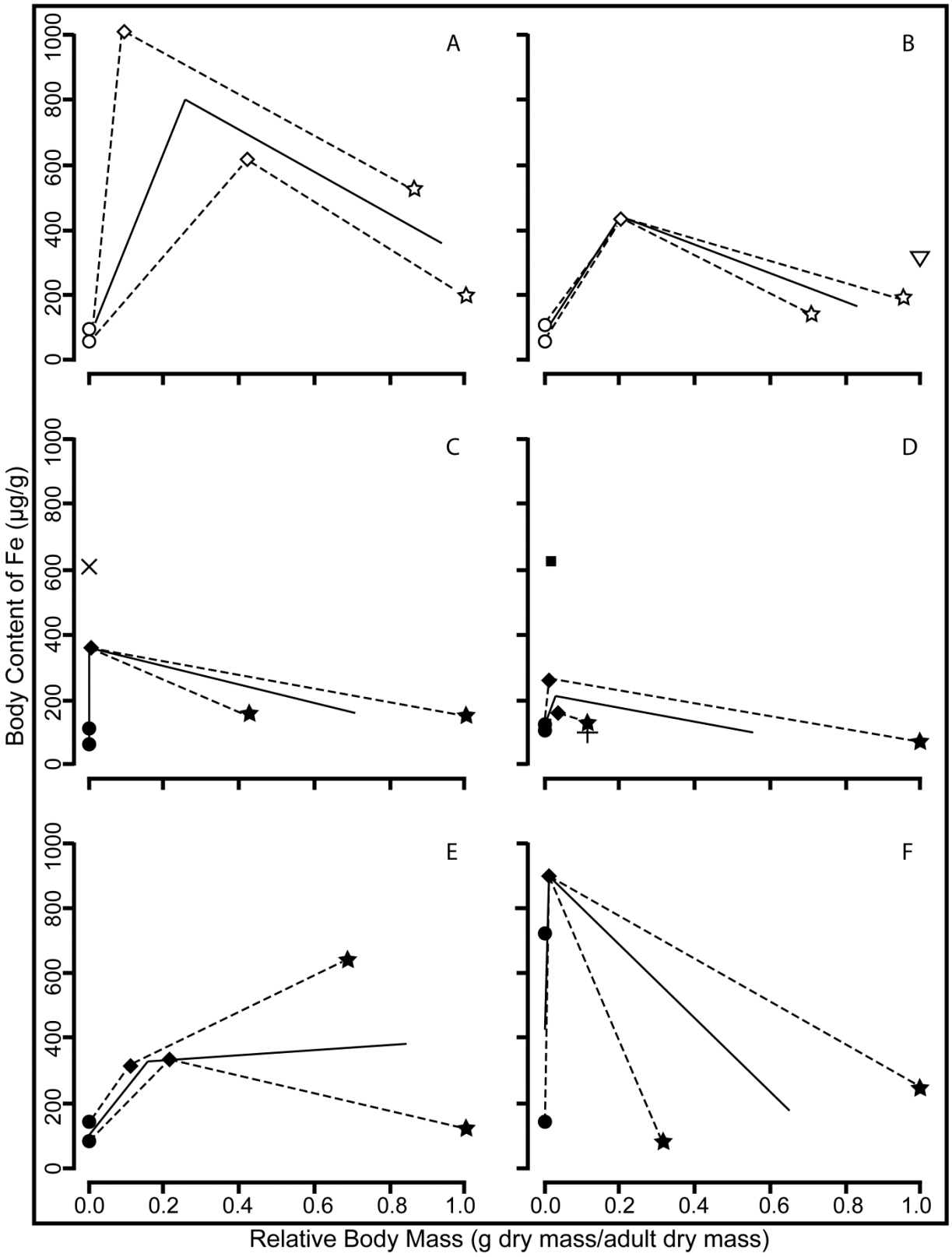

A
B
C
D
E
F
Body Content of Fe (µg/g)
0
200
400
600
800
1000
0.0
0.2
0.4
0.6
0.8
1.0
Relative Body Mass (g dry mass/adult dry mass)

Fig. A6.

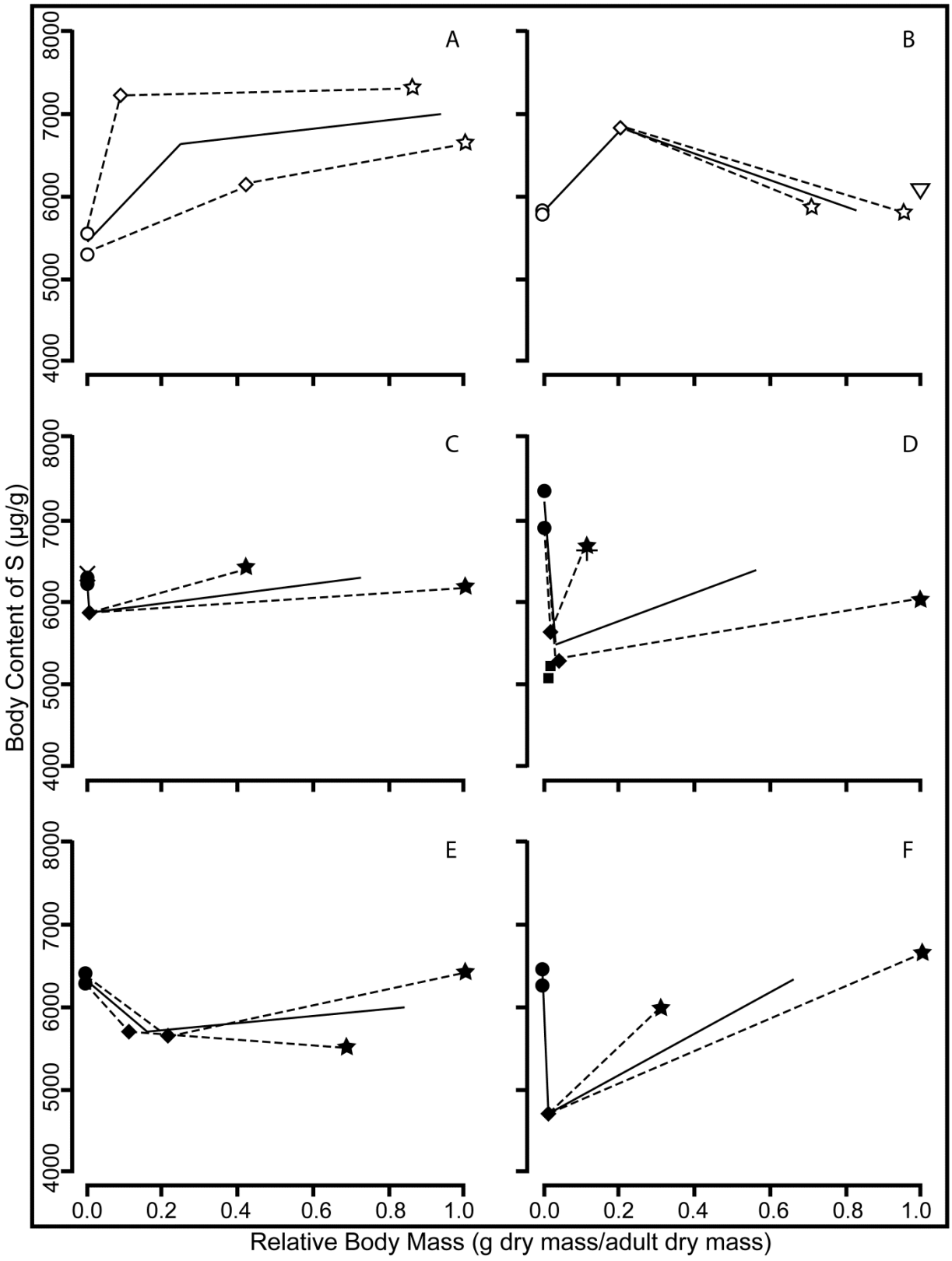
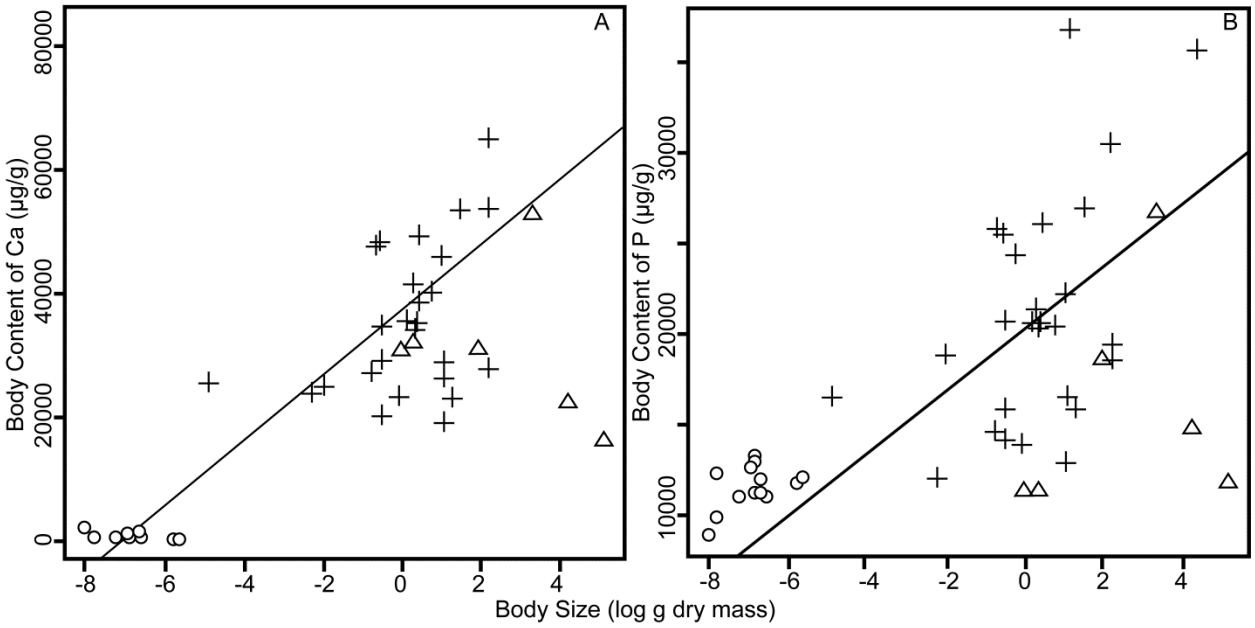

A
B
C
D
E
F
Body Content of S (µg/g)
4000
5000
6000
7000
8000
0.0
0.2
0.4
0.6
0.8
1.0
Relative Body Mass (g dry mass/adult dry mass)

Figure A7. Change in whole body Ca (A) and P (B) with size for terrestrial (+) and aquatic (Δ) stages of amphibians. Ova (o) show little to no initial investment of Ca. The positive relationships of body Ca and P against body mass for terrestrial forms (line) are significant (Ca: p=0.0035, P: p=0.013), but show considerable variation (Ca: adj $R^2 = 0.25$, P: adj $R^2 = 0.18$).



VITA

Thomas Marshall Luhring was born on January 10, 1982 in Midland Michigan. By the time he was in second grade, he had lived in Michigan, Delaware, Arizona, Pennsylvania, and Georgia. The variety of environments that he saw during the early years of his life only served to sharpen his curiosity of the natural world around him. As was the case with many budding naturalists, Tom spent countless hours conducting impromptu surveys of flora and fauna in creeks, ponds, swamps, and "excavation sites" in his parent's back yard.

During his grade school years, Tom spent most of his summers as a summer camp counselor at the local boy scout camp catching animals and teaching merit badge classes. As an undergraduate under Dr. Gary Barrett, Tom worked on a long-term small mammal project and learned to formalize and test questions related to natural processes. A herpetology class with Bob Reed and Cameron Young opened his eyes to graduate school and the possibility of a career working with animals with which he was fascinated.

In the summer of 2004, Tom was an REU student at the Savannah River Ecology Laboratory (SREL) under Betsie Rothermel and Whit Gibbons. He completely immersed himself in the experience and spent nearly every night road-cruising or photographing frogs (when he wasn't doing research). Tom graduated with a B.S. in biology and a B.S. in ecology from the University of Georgia in 2005. After graduation, Tom started his masters research under Whit Gibbons working on the ecology of giant salamanders. Graduating with a M.S. in Ecology in 2008, Tom entered the Ph.D. program at the University of Missouri to work with Ray Semlitsch. Upon finishing his Ph.D. in May 2013, he is starting a postdoctoral position at Michigan State University to work with Michael Wagner on alternative controls for invasive sea lamprey populations.