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Ontogenetic functional diversity: Size structure of a keystone predator drives functioning of a complex ecosystem

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Abstract. A central challenge in community ecology is to understand the connection between biodiversity and the functioning of ecosystems. While traditional approaches have largely focused on species-level diversity, increasing evidence indicates that there exists substantial ecological diversity among individuals within species. By far, the largest source of this intraspecific diversity stems from variation among individuals in ontogenetic stage and size. Although such ontogenetic shifts are ubiquitous in natural communities, whether and how they scale up to influence the structure and functioning of complex ecosystems is largely unknown. Here we take an experimental approach to examine the consequences of ontogenetic niche shifts for the structure of communities and ecosystem processes. In particular we experimentally manipulated the stage structure in a keystone predator, larvae of the dragonfly *Anax junius*, in complex experimental pond communities to test whether changes in the population stage or size structure of a keystone species scale up to alter community structure and ecosystem processes, and how functional differences scale with relative differences in size among stages.

We found that the functional role of *A. junius* was stage-specific. Altering what stages were present in a pond led to concurrent changes in community structure, primary producer biomass (periphyton and phytoplankton), and ultimately altered ecosystem processes (respiration and net primary productivity), indicating a strong, but stage-specific, trophic cascade. Interestingly, the stage-specific effects did not simply scale with size or biomass of the predator, but instead indicated clear ontogenetic niche shifts in ecological interactions. Thus, functional differences among stages within a keystone species scaled up to alter the functioning of entire ecosystems. Therefore, our results indicate that the classical approach of assuming an average functional role of a species can be misleading because functional roles are dynamic and will change with shifts in the stage structure of the species. In general this emphasizes the importance of accounting for functional diversity below the species level to predict how natural and anthropogenic changes alter the functioning of natural ecosystems.

Key words: Anax junius; *dragonfly larvae; ecosystem functioning; food web; functional diversity; ontogenetic niche shift; size structure.*

Introduction

Linking biodiversity to the structure and functioning of natural ecosystems has been a longstanding challenge in ecology. While much research has examined the impacts of species diversity on the functioning of ecosystems (McCann 2000, Loreau et al. 2001, Hooper et al. 2005, Duffy et al. 2007), variation below the species level is typically ignored in these studies (Reiss et al. 2009, Rudolf and Lafferty 2011). This reflects the classical approach to community ecology, which is based on the premise that we can predict the dynamics of communities by assuming that all individuals within a species are functionally identical. However, substantial evidence indicates that ecological variation among individuals within a species often exceeds variation among species (Polis 1984, Munoz and Ojeda 1998, Woodward and Hildrew 2002, Bolnick et al. 2003, Harmon et al. 2009). The unresolved question is whether this variation matters at the community and ecosystem scale in complex natural ecosystems.

By far the largest source of this intraspecific variation between individuals stems from differences in ontogenetic stage and size (Polis 1984, Werner and Gilliam 1984, Lomnicki 1988, Persson 1999, Benton et al. 2006). Individuals often experience substantial changes in their ecological role during their ontogeny. These changes are also called *ontogenetic niche shifts* and include shifts in habitat use, diet and resource use, predation risk, foraging rate, reproductive status, or other key life history traits (reviewed in Wilbur 1980, Werner and Gilliam 1984, Yang and Rudolf 2010). For instance, many predatory fish such as Eurasian perch forage in the littoral zone of lakes on zooplankton and invertebrates during the juvenile stage, but move to the pelagic zone to prey on other fish once they become mature (Mittelbach

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and Persson 1998). Ontogenetic niche shifts occur in >80% of animal taxa (Werner 1988) and a recent analysis indicates that the average resource overlap among stages of a species can range from 0% to 100% and be as low as 37% for entire ecological networks (Rudolf and Lafferty 2011). Plants experience similar changes in species interactions (e.g., due to changes in resistance to herbivory) during ontogeny (Boege and Marquis 2005, Yang and Rudolf 2010). Consequently, ontogenetic niche shifts are ubiquitous in natural communities (Rudolf and Lafferty 2011). While increasing evidence indicates the importance of ontogenetic niche shifts for the dynamics of species interactions (reviewed in Miller and Rudolf 2011), the consequences for the structure and functioning of complex ecosystems are largely unknown.

With ontogenetic niche shifts, stages within species often differ substantially in their ecological interactions and thus may also differ in their functional role. Given such functional differences across ontogenetic stages, any changes in the population size structure could strongly alter the functional role of a species and therefore its impact on the ecosystem. The size structure of populations often varies considerably within a season (Wissinger 1988, 1992, Urban 2007, Rudolf 2012), or over years and decades (Osenberg et al. 1992, Persson et al. 2003), or differs between populations in different habitats (Osenberg et al. 1992) due to changes (natural or anthropogenic) in environmental conditions. Thus, understanding the consequences of this ontogenetic diversity for the dynamics of communities and ecosystem processes is critical to understand the functional role of species in natural communities.

Predicting whether and how changes in the size structure of a species affect the structure of communities and ecosystem processes is not straightforward. For instance, per capita consumption rates (and thus interaction strength) are often expected to increase with size (Yodzis and Innes 1992, Emmerson and Raffaelli 2004). Large stages often have broader diets than small stages (with diets of small stages frequently nested within diets of large stages) and face lower predation risks (reviewed in Woodward et al. 2005). In addition, in predatory species, larger stages also typically have a higher trophic position, and top predators often have disproportionally strong effects on the community (Power et al. 1996). All of these relationships would suggest that the largest stage has the strongest and broadest impact on the ecosystem (thus largely determining the functional role of a species in the ecosystem). On the other hand, per-unit-biomass consumption rates and population density are often expected to decrease with size (Reiss et al. 2011). If strong enough, this negative relationship between body size and per-biomass consumption rate could weaken or even reverse the relative impact of stages on the ecosystem, with smaller stages having the strongest impact on the system. In both scenarios, the functional differences among the stages scale positively with differences in size. However, if ontogenetic niche shifts in species interactions occur (e.g., small stages may interact with a completely different part of the community than large stages), we may not see a clear relationship (positive or negative) between size and relative impact on the ecosystem. In the latter scenario, the functional role of a species would show clear shifts with changes in the size structure.

Here we take an experimental approach to examine whether and how stages differ in their functional role by manipulating the size structure of a keystone predator, the larval stage of the dragonfly *Anax junius*, in experimental pond communities. In particular we test (1) whether changes in the population size structure of a keystone species alter its functional role in the ecosystem, (2) whether these changes scale up to alter community structure and ecosystem processes, and (3) how functional differences scale with relative differences in size among stages.

Methods

Focal organisms

Several aspects of larvae of the dragonfly *Anax junius* (see Plate 1) create an excellent system for studying the ecological consequences of ontogenetic functional diversity. First, this generalist predator is one of the most abundant and widespread dragonflies in North America and is known to strongly impact the structure of fishless pond communities (Van Buskirk 1988, Wilbur and Fauth 1990, McPeek 1998). Secondly, individuals increase more than 17-fold in length during development and the stage structure of the population changes during the course of a season (Wissinger 1992; V. H. W. Rudolf, *personal observation*). Third, our preliminary stable isotope analysis of natural populations indicates that its trophic position strongly increases with size (V. H. W. Rudolf, *unpublished data*).

Experimental design

Detecting functional differences among very different-sized predators is challenging because individuals differ substantially in their biomass. In traditional designs, predator densities or biomass are often standardized. However, when predators differ substantially in size, such substitutive designs lead to dramatic differences in either biomass or density and conclusions about identity effects are likely to be confounded by these differences (Chalcraft and Resetarits 2004, O'Connor et al. 2008). Such confounding effects can be even more amplified when individual growth rates (and mortality rates) are higher in smaller individuals (as in our study species), because treatments would rapidly diverge through time. Furthermore, neither density nor total biomass is ever constant across stage classes within in our study species (Wissinger 1992; V. H. W. Rudolf, *unpublished data*), and keeping total biomass constant would lead to unrealistically high densities of small stages and experimental artifacts. Consequently, in systems where

the effects of individuals on the ecosystem are largely driven by body size, there is a high risk of misinterpreting results when using traditional substitutive designs (Chalcraft and Resetarits 2004, Schneider et al. 2012). Therefore, we refrained from keeping initial biomass or density constant and followed previous suggestions that recommend using natural size–abundance relationships (Chalcraft and Resetarits 2004, O'Connor et al. 2008). This approach allowed us to estimate the actual impact of each size class in natural populations and the relative impact of each size class by separating quantitative differences from qualitative differences among size classes.

The experiment consisted of four treatments, each replicated six times ($n = 24$): ponds received 18 small *A. junius* (head width (HW) 2.85–3.3 mm, body length (BL) 8.9–11.7 mm, dry mass (DM) 0.026–0.04 mg), or 6 medium *A. junius* (HW ~4.5 mm, BL ~16 mm, DM ~0.24 mg), or 3 large *A. junius* (HW ~6.9 mm, BL ~32 mm, DM ~0.61 mg), or none of these stages (control). We collected all *A. junius* larvae from one local pond. Size classes were chosen based on the size structure of the natural population and to maximize differences among size classes while reducing the risk of early metamorphosis of the largest size class. Density and relative abundance of each size class were a direct reflection of the relative size–abundance relationship of the natural *A. junius* population (V. H. W. Rudolf, *unpublished data*). Importantly, differences in initial total biomass across treatments strongly declined during the course of the experiment (due to natural differences in growth rates among stages), and there were no significant differences among average biomass in small and large predator treatments (see Appendix B for details). The experiment was terminated after three weeks when the first *A. junius* larvae started to emerge as adults.

Experimental pond communities

The experiment was carried out in experimental outdoor ponds that closely mimicked the structure and complexity of local fishless ponds. Full details of the experimental procedures are given in Appendix A. In brief, experimental ponds were established in 1200-L plastic stock tanks set up on an open field in a randomized complete-block design three months before the start of the experiment. Each tank was fitted with a 50% shade cloth lid and received 220 g wet mass of macrophytes and 2.5 kg dry mass of mixed leaf litter. Tanks were fertilized once before experiment initiation with nitrogen (10.4 g $NaNO_3$ per tank) and phosphorus (0.33 g NaH_2PO_4 per tank) to increase initial primary productivity. To establish a complex pond community, we stocked tanks repeatedly before the start of the experiment with concentrated zooplankton and 1 L of a highly concentrated and diverse mix of small invertebrates (benthic and pelagic organisms) sifted from the vegetation and sediment of two local fishless ponds. In addition, we added a diverse range of larger invertebrates (including other predatory insects; see Supplement) and tadpoles from five anuran species collected from four local ponds (299 *Bufo nebulifer*, 75 *Rana clamitans*, 157 *Hyla* (*versicolor* and *cinerea*) per tank). Together with natural colonization of tanks (mostly beetles and chironomids), this created a highly diverse community with >60 morpho-species of vertebrates and invertebrates across all tanks (see Supplement). The experiment started on 3 June when the different *A. junius* stages were added to the tanks and ended after three weeks when the first *A. junius* larvae started emerging from the tanks.

Ecosystem functions and properties

To quantify treatment effects on the ecosystem functioning, we measured a range of ecosystem processes and different aspects of community structure (see Appendix A for full details). We calculated the decomposition constant (k values) from the exponential decay curve model estimated from the dry mass lost per day from leaf litter bags that were added at the beginning of the experiment. We also estimated net primary productivity (NPP) and respiration (R) calculated from diurnal oxygen cycles (Wetzel and Likens 2000, Downing and Leibold 2002). We estimated weekly changes in biomass for two dominant forms of primary producer in our ponds, periphyton (benthic algae) and phytoplankton (pelagic algae), from extracted chlorophyll *a* following standard procedures (see Appendix A for full details).

We monitored tanks daily and collected all emerging invertebrates and vertebrates. At the end of the experiment, we sampled the pond communities using the following approach. We first used depth-integrated tube samplers to collect zooplankton samples. Then we took standardized subsamples of macro-invertebrate communities from the vegetation and benthos. Finally, we collected all macro-invertebrates (>4 mm) and amphibians remaining in the tanks. We then quantified animal biomass and community structure (benthic, vegetation, total) by counting, measuring, and weighing >35 500 individuals from >65 species (see Supplement).

Community structure was analyzed for both the abundances and total biomass of species (see Appendix A for details). The goal of this analysis was to detect if *A. junius* stages have different effects on the community structure rather than the total abundance of species. Thus we used relative biomass or density of each species (i.e., proportion of total community biomass or density of an experimental pond) to test for differences in community structure across treatments. Finally, we analyzed changes in the size structure of the macro-invertebrate community by comparing square root-transformed abundance of individuals within $\log_{10}$ size classes based on dry mass. Analyses using different bin sizes gave similar results (not shown here), indicating that the analysis is robust to changes in bin size.

Statistical analysis

Algae biomass samples were analyzed using repeated-measures ANOVA with the *A. junius* size class treatment as a fixed effect. The appropriate covariance structure for the repeated-measures analysis ("type = arh(1)" for phytoplankton and "type = un" for periphyton) was determined using the fitted model with the lowest AIC value (Littell et al. 1998). NPP and respiration were analyzed using one-way ANOVA with treatment as a fixed effect and change (final sample − first sample) in NPP or respiration as the dependent variable to account for variation in initial conditions among tanks. Decomposition rates (k) were analyzed with one-way ANOVA with treatment as a fixed effect and average leaf litter mass loss per day as the dependent variable. We used a one-tailed log-likelihood ratio χ^2 test to test for random block effects (Littell et al. 2006). When block effects were not significant, block degrees of freedom were pooled with the error term degrees of freedom for the final analysis. Because of the large variation in primary producer abundance among tanks at the beginning of the experiment, some tanks were significant outliers (based on Studentized residual test with Bonferroni adjustments and interquartile range tests) and thus were removed from the analysis. To account for the missing replicates in analyses, the degrees of freedom were adjusted using the Satterthwaite procedure (Littell et al. 2006). All data met normality and heteroscedasticity assumptions and were carried out using the "proc mixed" procedure in SAS 9.3 (Littell et al. 2006).

We analyzed differences in the structure of communities among treatments using nonparametric, permutational multivariate statistics based on Bray-Curtis similarity metrics using the software PRIMER 6 (Clarke and Gorley 2006). First, we tested whether the variability in community structure (i.e., dispersion) differed among treatments using PERMDISP (Anderson 2006). Secondly, if treatments met the assumption of similar variances, we tested whether communities differed significantly among treatments using permutational multivariate analysis, PERMANOVA (Anderson 2001, McArdle and Anderson 2001). When block effects were not significant, block degrees of freedom were pooled with the error term degrees of freedom for the final analysis. Both permutation analyses were carried out using 999 permutations and based on centroids. Community structures were visualized with nonmetric multidimensional scaling plots (nMDs) using PRIMER 6. In general, there were no significant differences between the control and any of the *Anax* size treatments, largely because of the substantial variation in the control. Visual inspection of nMDS plots revealed that the control was typically at the center and spread evenly over the different size treatments. Thus, we focus our analyses here on our main question, i.e., whether *Anax* size classes differed in their effect on community structure; we provide the control only as a reference. We used structural equation modeling to look at relationships among ecosystem response variables. Finally, to test whether potential differences in average biomass among predator treatments were important in explaining the observed results, we first tested whether average *Anax* biomass differed among treatments and then tested whether the per-unit-biomass effect size differed among predator size treatments for all ecosystem processes (NPP, R, K) and animal and plant biomass (see Appendix B for details).

Results

Decomposition rates

Decomposition rates (k values) did not differ among size treatments ($P = 0.921$, $F_{3,20} = 0.16$). For large *Anax* (AL), $k = 0.0138 \pm 0.0009$ mg/d (mean ± SE); for medium *Anax* (AM), $k = 0.0109 \pm 0.0003$ mg/d; for small *Anax* (AS), $k = 0.0108 \pm 0.0009$ mg/d; for the control, $k = 0.0107 \pm 0.0006$ mg/d).

Ecosystem productivity and respiration

Net primary productivity (NPP).—NPP significantly increased during the experiment in all treatments (all $P < 0.003$), but the relative increase differed significantly among treatments (Table 1, Fig. 1). NPP increased three times as much in ponds with large and medium-sized *Anax* compared to ponds with small *Anax* (post hoc comparison: AL vs. AS, $P = 0.0082$; AM vs. AS, $P = 0.0147$). The increase in NPP was, on average, slightly higher in the control compared to ponds with small *Anax*, but this was not significant ($P = 0.4696$)

Respiration.—The change in respiration rates during the experiment differed significantly among treatments (Table 1) and showed a pattern similar to that for NPP (Fig. 1). Respiration rates increased seven and five times more in ponds with large and medium *Anax*, respectively, compared to ponds with small *Anax* (post hoc comparison: AL vs. AS, $P = 0.002$; AM vs. AS, $P = 0.0094$). Ponds with small *Anax* were also the only ponds that showed no significant increase in respiration rates over the experiment ($t_{1,19} = 0.86$, $P = 0.4020$; Fig. 1).

Primary producer biomass

Phytoplankton.—Over the whole experiment (census 1 vs. census 3), phytoplankton biomass in ponds with small *Anax* decreased three times more than in ponds with large *Anax* ($t = 2.10$, $P = 0.0504$) and 10 and 16 times more than in ponds with medium-sized *Anax* and the control respectively (AS vs. AM, $t = 2.60$, $P = 0.0150$; AS vs. Control, $t = 2.72$ $P = 0.0139$). However, how phytoplankton biomass changed over time also differed significantly among treatments (Table 1). Phytoplankton biomass did not change significantly over time in the control or in treatments that had received medium-sized predators (*Anax*) (planned contrasts; control, all $P > 0.6$; AM, all $P > 0.3$) (Fig. 2A). In contrast, phytoplankton biomass declined significantly between each census period in treatments with small *Anax*

TABLE 1. Treatment effects on ecosystem processes, with size indicating the main treatment that manipulated presence of three size classes of the keystone predator, larvae of the dragonfly *Anax junius*.

Process and effect	*F*	df	*P*	Treatment ranks
Decomposition rate				
Size	0.16	3, 20	0.921	NS
Change in respiration				
Size	5.75	3, 19	0.0057	L ≥ M > C ≥ S
Change in NPP				
Size	4.24	3, 19	0.0188	L ≥ M > C ≥ S
Change in phytoplankton				
Size	1.88	3, 22.5	0.1620	S > L > M ≥ C
Time	6.19	2, 26.4	0.0062	
Size × Time	3.17	6, 26.5	0.0178	S > L > M ≥ C
Change in periphyton				
Size	0.76	3, 19.4	0.5320	S > L > C ≥ M
Time	26.92	2, 17.7	<0.0001	
Size × Time	3.02	6, 17.6	0.0325	S > L > C ≥ M

Notes: Time indicates changes in the respective response variable across three sampling periods over the course of the experiment, and it may interact with size. Decomposition rate was estimated for the whole duration of the experiment. Phytoplankton and periphyton biomass were analyzed using repeated-measures ANOVA with the Satterthwaite procedure to adjust df to account for missing replicates. Change in respiration, NPP, periphyton, and phytoplankton indicates the change in the respective rate or biomass over the entire duration of the experiment. Treatment ranking indicates relative differences in average values among size classes of *A. junius* (small, S; medium, M; large, L) and the control (C).

(planned contrasts; all $P < 0.01$), whereas biomass only declined significantly between the second and third census period in treatments with large *Anax* (planned contrasts; first vs. second, $P = 0.989$; second vs. third, $P = 0.011$).

Periphyton.— The change in periphyton biomass over time differed significantly among treatments (Table 1, Fig. 2B). Periphyton biomass only declined significantly during the first and second census period in treatments with small *Anax* (planned contrasts; first vs. second, $P = 0.0002$; second vs. third, $P = 0.2897$), whereas it declined significantly between each census period in all other treatments including the control (planned contrasts; all $P < 0.024$). Furthermore, the magnitude in decline also varied among treatments during the first and second census period: phytoplankton declined twice as much in treatments with large and small *Anax* compared to treatments with medium *Anax* or the control (Fig. 2B). In general, these differences in temporal dynamics of phytoplankton and periphyton indicate that not only the quantitative but also the qualitative impact of *Anax* on both types of primary producer biomass differed significantly among size classes.

Vertebrate and invertebrate biomass

The final biomass of animals (vertebrates plus invertebrates) did not differ among ponds with small (1991 ± 315.5 mg, mean ± SE) and medium-sized (1942.6 ± 3.15.5 mg) *Anax*, but both had ~30% lower biomass than ponds with large *Anax* (2689.2 ± 223.1 mg) (AL vs. AM, $P = 0.03$; AL vs. AS, $P = 0.04$; Fig. 3A). A separate analysis for habitat-specific biomass of macro-invertebrates (see *Methods*) revealed that *Anax* size classes also differed in their habitat-specific effects. Invertebrate biomass in the vegetation differed significantly ($F_{3,20} = 6.38$, $P = 0.003$) among *Anax* size treatments (Fig. 3B). Average invertebrate biomass was 3.3 and 2.6 times lower in ponds with small *Anax* compared to ponds with large *Anax* or medium *Anax*, respectively. Interestingly, invertebrate biomass was two times higher in the large-*Anax* treatment compared to

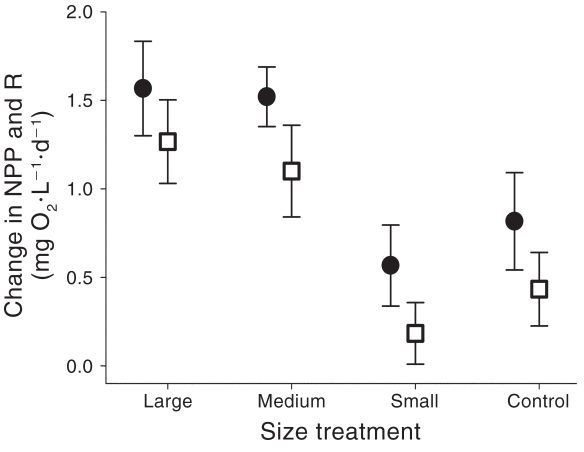

FIG. 1. Treatment effects on change (final minus initial; mean ± SE) in ecosystem net primary productivity (NPP, solid circles) and respiration (R, open squares) during the experiment. Control indicates that no *Anax junius* dragonfly larvae were added; large, medium, and small indicate the respective size class of the predator *A. junius* that was added to experimental ponds.

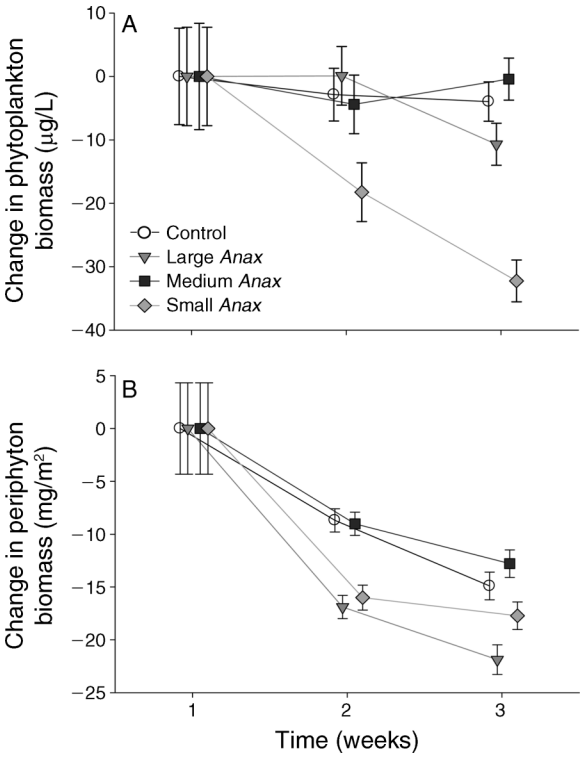

FIG. 2. Relative change in (A) phytoplankton biomass and (B) periphyton biomass measured from chlorophyll *a* extractions across treatments over three weeks. Values are standardized by subtracting initial measurements at the beginning of the experiment from all subsequent observations of a given tank. Control indicates no added *A. junius*; large, medium, and small *Anax* indicate the respective size class of *A. junius* that was added to experimental ponds. Symbols indicate model estimated means (± SE) that account for missing values and repeated measures of ponds.

the control ($P = 0.0085$), whereas small *Anax* treatments had half the invertebrate biomass observed in the control, although this difference was only significant when the significant outlier was removed from the small *Anax* treatment ($P = 0.0166$). Conversely, there were no differences among treatments in biomass for benthic invertebrates ($F_{3,15} = 0.33$, $P = 0.803$). In general, this indicates that size classes of *Anax* differed in their microhabitat-specific effects on lower trophic levels.

Community structure

The overall community structure differed significantly among size treatments when analyzed based on abundance and biomass. Analyses based on abundance revealed that ponds with medium-sized *Anax* varied significantly more than ponds with small *Anax* (PERMDISP; $P = 0.005$; Fig. 4A), whereas there were no differences among the other treatments (all $P > 0.155$). The variance was lowest for small *Anax*, suggesting that they had the most consistent impact on community structure. In addition, the overall community structure differed significantly among ponds with small vs. large *Anax* (PERMANOVA; pseudo-$F_{1,10} = 2.606$, $P = 0.008$; Fig. 4A). An analysis based on relative biomass showed no significant differences in variation in community structure among treatments (PERMDISP; all $P > 0.331$). However, community structure of ponds also differed significantly among size class treatments (PERMANOVA; pseudo-$F_{2.15} = 1.8518$, $P = 0.041$), but in paired comparisons only the difference between medium and small *Anax* was significant (AM vs. AS, $P = 0.011$; AM vs. AL, $P = 0.570$; AL vs. AS, $P = 0.210$; Fig. 4B). A separate analysis on only the zooplankton community structure showed similar patterns (not shown here). Because these analyses were based on proportional abundance (which corrects for differences in total densities or biomass), these results clearly indicate that different size classes of *Anax* had qualitatively different effects on the structure of the community, even after correcting for differences in effect size.

Macro-invertebrate community size structure

The size structure of the macro-invertebrate community differed significantly among *Anax* size class treatments (PERMANOVA; overall including block, pseudo-$F_{2,10} = 2.79$, $P = 0.038$; see Appendix C). The difference was largest among communities with large vs. small *Anax* (PERMANOVA; $P = 0.048$), mostly because

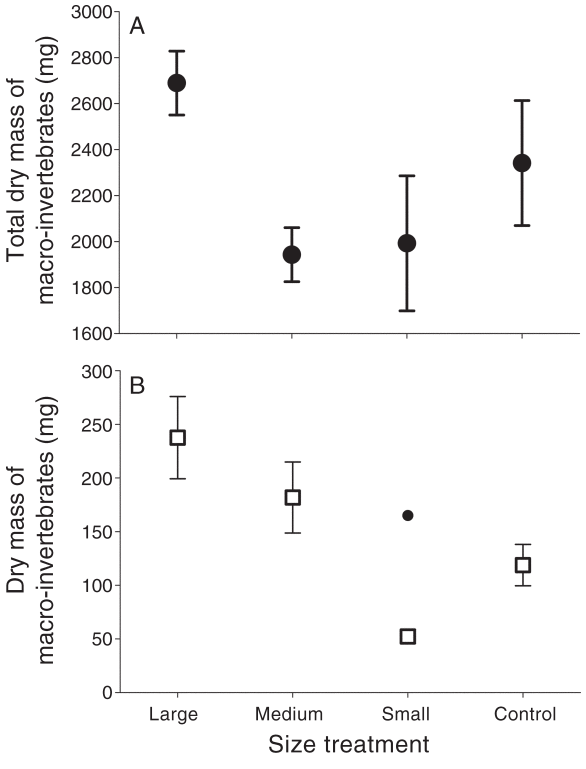

FIG. 3. (A) Total dry mass of macro-invertebrates and (B) biomass in the vegetation subsample in ponds without added *A. junius* (control) or with large, medium, or small stages of *A. junius*. All values are means ± SE. The small solid circle in panel (B) indicates a significant outlier.

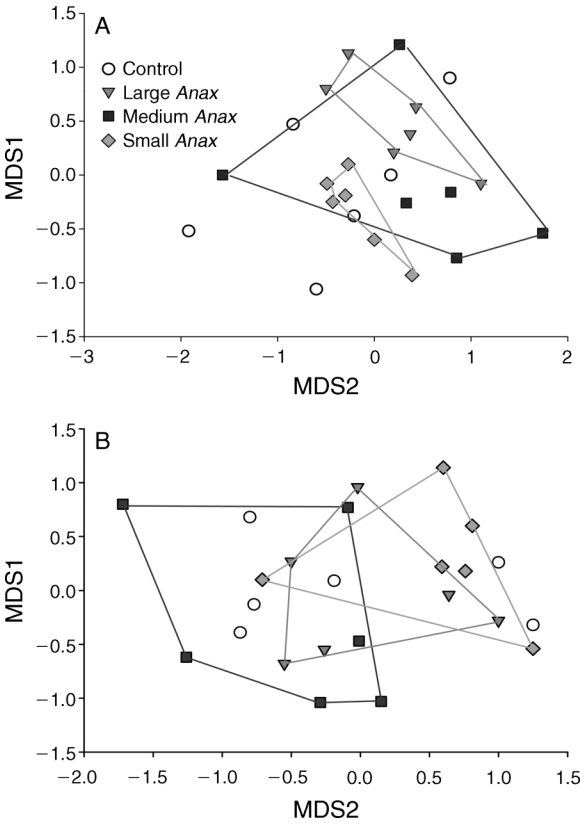

FIG. 4. Differences in community structure among ponds without added *A. junius* (control) or with large, medium, or small stages of *A. junius*. The figure presents nonmetric multidimensional scaling (nMDS) plots of species' relative (A) densities and (B) biomass (i.e., proportional abundance within a pond) in control and different *Anax* treatments. Points represent individual replicates of a treatment, and polygons contain all replicates within a treatment. The nMDS stress is: (A) 0.19, (B) 0.16.

abundance within the smallest macro-invertebrate size class (0.001–0.01 mg) was fourfold lower in small-*Anax* compared to large-*Anax* ponds. The size structure of communities with medium-sized *Anax* was intermediate between communities with large or small *Anax* (AM vs. AL, $P = 0.266$; AM vs. AS, $P = 0.063$). There was no difference in the variance of community structure among treatments (PERMDISP; $P > 0.328$).

Relations among response variables

To get more insight into the relationships among response variables, we carried out several analyses using structural equation modeling that connected relative changes in the community structure (based on two nMDS scores from Fig. 4) with changes in primary producer biomass and changes in NPP and respiration (see Appendix D for details). The analyses revealed that regardless of how community structure was analyzed (relative densities or relative biomass of species), one nMDS score was significantly negatively related to changes in periphyton biomass whereas the other was positively related to changes in phytoplankton biomass. A more detailed analysis suggests that the change in periphyton biomass was largely driven by changes in amphibian community structure (i.e., relative density of *Hyla*) among treatments. We suspected that differences in zooplankton community structure could be responsible for changes in phytoplankton biomass, but no simple relationships could be identified, suggesting that more complex interactions were responsible for this. Changes in NPP and respiration, in turn, were largely driven by changes in periphyton biomass, whereas changes in phytoplankton biomass or community structure had no significant effects. In general this indicates that the stage-specific effects of *A. junius* on primary producer biomass and ecosystem processes were not driven by direct effects, but rather by a range of complex indirect interactions (e.g., trophic cascades).

Per-unit-biomass effect of predator stages

It is possible that some of the observed quantitative differences in ecosystem properties (NPP, respiration, primary producer and animal biomass) among treatments could arise at least partially due to differences in predator biomass across treatments. To account for this potential confounding effect, we calculated average *Anax* biomass for a given predator stage treatment and used these estimates to calculate the per-unit-biomass effect of predator stages on these ecosystem processes (see Appendix B for details).

We found that the average *Anax* biomass across the experiment did not differ significantly (unequal variance *t* test; $P = 0.29$) between small (226.8 ± 15.4 mg, mean ± SE) and large predator stages (264.3 ± 22.2 mg), but both had 1.3–1.5 times lower *Anax* biomass (unequal variance *t* test; AM vs. AL, $P = 0.035$; AM vs. AS, $P < 0.001$) than treatments with medium-sized *Anax* (352.5 ± 20.6 mg).

Overall, we found the same pattern for per-unit-biomass effects of predator stages as in our analyses of the untransformed data (see Appendix B for details). Per-unit-biomass effect on NPP, respiration, periphyton, phytoplankton and animal biomass, and macroinvertebrate biomass in the vegetation all differed significantly among size treatments, whereas decomposition and benthic macro-invertebrate biomass did not differ among treatments (Appendix B: Table B1, Figs. B1–B3). Again, the largest differences were observed between the per-unit-biomass effects of small and large predator stages, and the effect size often differed not only in magnitude but also direction (i.e., positive vs. negative effect relative to the control; see Appendix B: Table B1, Figs. B1–B3).

DISCUSSION

Ontogenetic niche shifts are ubiquitous in natural communities (Rudolf and Lafferty 2011), but little is known about whether and how they scale up to influence the structure and functioning of complex ecosystems.

Our results clearly show that the impact of a predator on the structure of complex communities and ecosystem processes depends upon the ontogenetic stage of the predator. Importantly, these differences remained unchanged even after accounting for potential differences in predator biomass across treatments. In general this indicates clear functional differences among stages within a keystone species that can scale up to alter the functioning of entire ecosystems. Furthermore, the stage-specific effects did not simply scale with size or biomass of the predator, indicating ontogenetic niche shifts in species interactions. These results emphasize the importance of accounting for ontogenetic functional diversity within species for predicting how natural and anthropogenic changes alter natural ecosystems.

Size, biomass, and functional differences among ontogenetic stages

We found that altering the size structure of our focal species changed all aspects of the ecosystem, including ecosystem processes, primary producer and animal biomass, and community structure. This strong effect is even more impressive given the considerable natural variation in initial primary producer biomass and invertebrate colonization among experimental ponds. It is possible that simple biomass or size scaling relationships could create differences among predator stages in community- and ecosystem-level effects. Because we used natural densities for each stage, initial predator biomass was positively related with size, but biomass differences strongly attenuated over the experiment (because predator growth rates and mortality rates decreased with body size). As a consequence, average biomass of *Anax* during the experiment was not significantly different between large and small predator stages, but both had ~1.3 times lower biomass than treatments with medium-sized *Anax* (see Appendix B for details). However, small individuals have higher metabolic rates and thus higher per-biomass consumption rates. For example, in an aquatic shredder guild, per-unit-biomass consumption was higher when it was composed of small stages compared to large stages (Reiss et al. 2011). Therefore, even though total predator biomass was similar in treatments with small and large predator stages, the potentially high per-biomass consumption rates of small predators could have resulted in effects comparable to or stronger than those of predators in the medium-sized and large *Anax* treatments.

Several patterns in our data suggest that observed differences cannot be explained by simple size-scaling relationships of consumption (metabolic) rates of the predator but instead indicate ontogenetic niche shifts in ecological interactions in a keystone predator. First, even after accounting for differences in predator biomass, we found that the per-unit-biomass effect differed among predator stages. Second, neither large nor small stages consistently had the strongest effect on all ecosystem response variables. For instance, although large stages had the strongest impact on NPP and respiration, small stages had up to 10-fold stronger effects on primary producer biomass and reduced total animal biomass significantly more than large stages. Third, the ranking of size classes based on effect size did not consistently scale with size (positively or negatively) and varied depending on the response variable. For example, medium-sized stages had the weakest effect on primary producer biomass (despite having the highest predator biomass of all treatments) while small stages had the strongest effect. Finally, large and small stages often had opposing effects relative to the control, indicating clear qualitative differences in the nature of the effect. Similarly, the temporal dynamics of the biomass of both types of primary producers differed significantly among stages even after accounting for differences in total biomass of primary producers, again indicating clear qualitative differences. These qualitative differences, opposite effects, and changes in effect size ranking among stages are not predicted from simple differences in foraging or metabolic rates that scale with size, biomass, or a combination of the two factors.

Overall, our results indicate that stages differed significantly in their effect on community composition (for both biomass and densities). Because this analysis focused on proportional differences, significant treatment effects indicate that stages did not only alter the absolute abundance of species but also altered the relative abundance of species within a community. This clearly indicates that stages differed in their direct and indirect interactions within the food web, leading to a structural change in the community composition. Results from the path analysis suggest that these differences among stages in interactions indirectly led to concurrent changes in ecosystem processes such as NPP, respiration, or biomass of trophic levels.

The ontogenetic shift in species interactions in our system is consistent with diet shifts observed in other species of dragonfly larvae (e.g., Woodward and Hildrew 2002, Rudolf and Armstrong 2008), predatory invertebrates, and vertebrates (e.g., Polis 1984, Munoz and Ojeda 1998, Woodward and Hildrew 2002, Rudolf 2006). Such diet shifts are often correlated with ontogenetic shifts in microhabitat use (e.g., Persson and Eklov 1995, Rudolf and Armstrong 2008) and preference for different-sized prey (e.g., large predators often consume larger prey; Woodward et al. 2005), which could explain why small and large stages differed in their microhabitat-specific effects (i.e., invertebrate biomass in the vegetation) and effects on size structure of the macro-invertebrate community, respectively. However, our results extend these diet observations by demonstrating that differences among ontogenetic stages can lead to changes in the structure of complex communities and qualitatively different effects on other ecosystem properties. Thus, the functional role of a species is not constant, but instead changes with shifts in

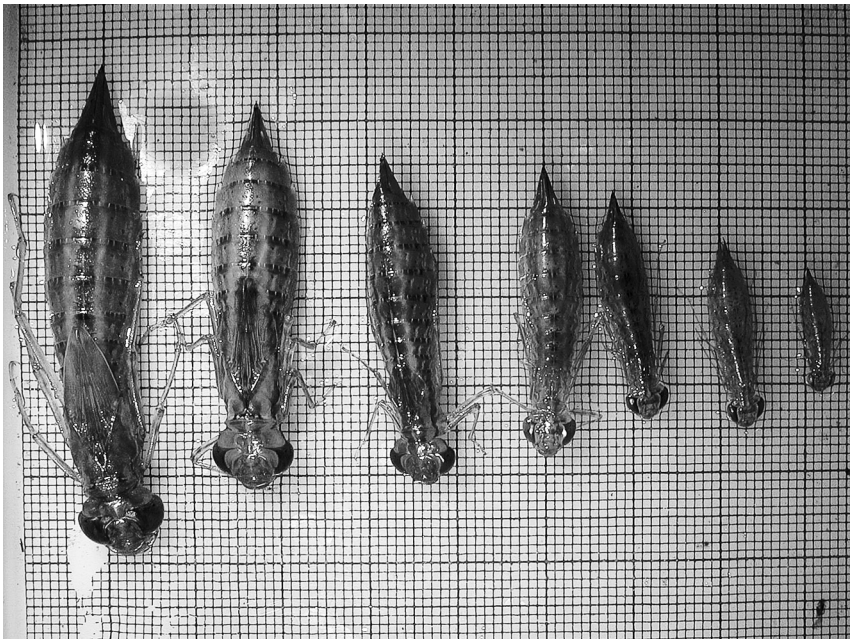

PLATE 1. Example of size differences among successive developmental stages of the focal species *Anax junius*. The picture only shows the seven (out of 13) largest developmental stages, as the smallest self-feeding (youngest) stage have a body length of only a few millimeters. Photo credit: V. H. W. Rudolf.

the size structure of the species. Given that populations often consist of multiple stages, the next intriguing question is whether in such scenarios the functional role of a species is simply the sum of its individual components (stages), or whether more complex effects arise.

The results indicate that ontogenetic functional diversity is important at the community and ecosystem scale, but how can we predict the changes in community- and ecosystem-level processes that occur with changes in the ontogeny of organisms if they do not scale in a simple way with body size? Given the ontogenetic shifts in species interactions, it is not surprising that body size was a poor predictor for how individuals differed in their effect on the ecosystem. Body size arguably determines the strength of species interactions (e.g., an increase in predator–prey size ratio generally increases the effect of the predator on its prey), but differences in body size also lead to differences in presence/absence of species interactions (e.g., if the predator–prey size ratio increases enough, the predator might switch from one prey species to another). Thus, the potential for body size (mass) differences to predict differences in the net effect of individuals on complex community and ecosystem processes will decrease rapidly with increasing differences in interaction among individuals. Thus, we need a framework to predict how species interactions change over ontogeny. Recent studies suggest that optimal foraging theory can be used (to some extent) to predict the diet of consumers (and thus the structure of food webs) based on the relative body size differences of individuals within a community. In combination with a size-based interaction strength, a similar optimal foraging approach potentially could be used within species to predict ontogenetic diet shifts, providing a more general framework to predict how individuals (among and within species) influence ecosystems. However, the question is whether this approach can be extended to explain intra- and interspecific functional differences, or whether we also need to account for the identity or traits of species (e.g., due to morphological constraints). Thus, future studies that examine whether functional differences among size classes are consistent across species and how ontogenetic functional diversity relates to species traits are needed to identify general patterns and develop a general predictive framework to ontogenetic functional diversity into community ecology.

Ontogenetic functional diversity and natural ecosystems

Although increasing evidence indicates that ontogenetic niche shifts can have important effects on the dynamics of species interactions (reviewed in Miller and Rudolf 2011), they have received surprising little attention at a community or ecosystem scale (but see Rudolf and Lafferty 2011). Our results indicate that ontogenetic niche shifts in a keystone predator can lead to functional differences among stages within populations. As a consequence, changes in the size structure of predator populations can result in concordant changes in ecosystem structure and processes. Given that ontogenetic niche shifts are ubiquitous in natural communities (Rudolf and Lafferty 2011), this has several important implications. First, our results indi-

cate that the classical approach of assuming an average functional role of a species can be misleading, because its role is dynamic and will change with shifts in the size structure of the species. Secondly, it emphasizes the importance of accounting for bio- (functional) diversity below the species level to predict how natural and anthropogenic changes impact natural ecosystems.

While, to the best of our knowledge, our results are the first experimental demonstration of clear functional differences among ontogenetic stages at the ecosystem level, these findings are consistent with observational data from other systems. For example, in shallow lakes, dramatic shifts in the size structure of the top predator, perch, were correlated with shifts in the relative abundance of zooplankton and phytoplankton (Persson et al. 2003). Although this change in perch size structure was probably driven by size-structured interactions among stages within the population (cannibalism), many other natural or anthropogenic factors can also lead to shifts in the size structure of populations. For example, harvesting in fisheries is extremely size selective and has led to global decline in the average body size among top predatory fish (e.g., Shackell and Frank 2007, Swain et al. 2007). Recent studies indicate that although biomass remained largely unchanged, this decline was correlated with changes in abundances of lower trophic levels (Shackell et al. 2010). Both observations are consistent with our experimental findings that ontogenetic shifts in the functional role of species can alter the structure of communities. Our study provides an important next step by demonstrating that such shifts can also lead to changes in ecosystem processes. Given the high frequency of ontogenetic niche shifts in natural communities (Rudolf and Lafferty 2011), this emphasizes the importance of accounting for functional biodiversity below the species level (i.e., ontogenetic functional diversity) to predict how natural and anthropogenic changes alter the structure and functioning of natural ecosystems.

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Supplemental Material

Appendix A

Detailed description of the experimental methods (*Ecological Archives* E094-093-A1).

Appendix B

Detailed description of biomass-corrected analyses and results (*Ecological Archives* E094-093-A2).

Appendix C

Average abundance–biomass relationships in different predator treatments (*Ecological Archives* E094-093-A3).

Appendix D

Results of structural equation modeling indicating relationships among treatments and ecosystem variables (*Ecological Archives* E094-093-A4).

Supplement

Treatment means and standard errors for densities and biomass of community members at the end of the experiment, categorized by taxa (*Ecological Archives* E094-093-S1).

Ecological Archives

Volker H. W. Rudolf, Nick L. Rasmussen Year of publication. Ontogenetic functional diversity: Size-structure of a keystone predator drives functioning of a complex ecosystem. *Ecology* VOL:pp–pp.

Appendix A. Detailed description of experimental methods.

Method details

Experimental pond communities

The experiment was carried out in experimental ponds that closely mimicked the structure and complexity of local fishless ponds that are dominated by invertebrate predators. Experimental ponds were established in 1200 L plastic stock tanks set up in a randomized complete block design at Rice University's South Campus experimental facility, Houston, TX. All tanks were filled with water March 16th, 2009, three months before the start of the experiment. All tanks were covered with 50% shade cloth lids which provided natural shading levels and allowed a variety of small to medium-sized invertebrates to colonize the tanks while keeping large predators out of and metamorphs (insects and amphibians) in the tanks. After one month (April 23) we added 2.5 kg (air dried) of mixed leaf litter to each pond. Leaf litter represented a random mixture of leaves (mostly pine and oak) that were collected from the border of two local ponds. The next day, each tank received 500 ml of concentrated zooplankton and phytoplankton collected from two local fishless ponds. We fertilized tanks once before experiment initiation with nitrogen (10.4g $NaNO_3$ per tank) and phosphorus (0.33g NaH_2PO_4 per tank) to increase initial primary productivity. On May 14th, we added 220g (wet mass) of macrophytes collected from one local pond to each tank (mostly *Potamogeton sp* and *Najas sp*). Finally, to establish a natural, complex community we added to each tank a total of 1 L of highly concentrated and diverse mix of small invertebrates (benthic and pelagic organisms) sifted from the vegetation and

sediment of two local fishless ponds. In addition we collected a diverse range of larger invertebrates (including other predatory insects, see Appendix Table A1) from several local ponds, which were added in equal numbers to all tanks (from May 22nd – June 1st). This approach also inevitably resulted in the random addition of a few recently hatched (1st instar) *A. junius* to tanks that were too small to be detected during our screening process. Each tank also received an equal number of tadpoles (mix of hatchlings and medium-sized tadpoles) from five anuran species collected from four local ponds (299 *Bufo nebulifer*, 75 *Rana clamitans*, 157 *Hyla* (*versicolor* & *cinerea*) per tank). Together with natural colonization of tanks (mostly beetles and chironomids) this created a highly diverse community with >60 morpho-species of vertebrates and invertebrates across all tanks (see Appendix Table A1). The experiment started on June 3rd when the different *A. junius* stages were added to the tanks.

Ecosystem functions and properties

Decomposition

Each tank received two bags (15 x 20 mm, mesh size: 3.5mm^2) filled with 2500mg of the same random mixture of leaf litter added to the ponds. Leaves were dried for 48h at 60°C, weighed, and then pre-soaked in reverse osmosis (RO) water for two days before they were added to tanks one day before the start of the experiment. At the end of the experiment, all leaf litter bags were removed from the tanks, soaked for two days in RO water and carefully rinsed to remove algae and invertebrates from the samples. Leaf litter bags were then dried for 48h at 60°C and reweighed. Decomposition rates (*k*) were calculated from exponential decomposition decay curve model with $M_t = M_0 \exp(-k\ t)$, where M_0 indicates the initial mass of leaf litter bags, M_t the average final leaf litter mass, and t is duration of the experiment.

Ecosystem productivity and respiration

We estimated net primary productivity (NPP) and respiration (R) calculated from diurnal oxygen cycles (Wetzel and Likens 2000, Downing and Leibold 2002). We measured Dissolved Oxygen (DO) with an oxygen probe (YSI, Professional Plus) three times within 24h: at sunrise, sunset, and the next sunrise. NPP is given by the increase in DO between first sunrise and sunset, and respiration is given by the decrease in DO between sunset and the second sunrise (e.g. Wetzel and Likens 2000, Downing and Leibold 2002, Harmon et al. 2009). All measurements were taken weekly during the experiment (n = 3).

Primary producer biomass

We estimated biomass for two dominant forms of primary producer in our ponds, periphyton (benthic algae) and phytoplankton (pelagic algae). We estimated standing biomass of periphyton weekly from three glass microscope slides per tank (0.74cm x 0.25cm) that were propped at an angle against the side of the tank above the leaf litter layer. After seven days, glass slides were removed for processing and replaced with a set of new slides. Periphyton from both sides of all three slides from the respective sample period was combined for the analysis. Phytoplankton biomass was estimated from 250-ml water samples collected at mid water level weekly from each tank. Periphyton concentration and phytoplankton concentration were then determined fluorometrically (AquaFluor, Turner Designs) through chlorophyll-a extraction in 95% Ethanol following standard protocols (Eaton et al. 2005). The first sample was taken one week after the start of the experiment and then every week for three consecutive weeks.

Community structure

During the experiment we monitored tanks daily for emerging insects and amphibians. Amphibian metamorphs were weighed after tail absorption and released at the origin of capture. Metamorph wet mass was converted into dry mass using our previously established, species-specific wet to dry mass conversion relationships for amphibians, and a subset of emerged invertebrates were used to calculate species specific averages for dry mass. At the end of the experiment we first took six zooplankton samples per tank (total 2.5L) using a depth integrated tube sampler. The samples were filtered through an 80µm Nytex mesh, combined and preserved in 75% ethanol. We then sub-sampled the floating vegetation and benthos community with a fine mesh (500-micron mesh Nytex) D-net (30.5cm wide). Vegetation samples included several sweeps that removed the entire floating vegetation. Benthos samples were taken with two perpendicular sweeps across the full diagonal of the tank. Vegetation and benthos samples were then carefully rinsed, filtered, and the content preserved in 75% ethanol. Finally, we destructively sampled all macro-invertebrates (≥ 4 mm long) and amphibians until no individuals were left in the tank (hereafter referred to as "final samples"). All animals were initially preserved in 75% ethanol and stored at -25°C until further analysis.

We quantified the structure of zooplankton, amphibian, and invertebrate (benthic, vegetation, and total) communities by counting, measuring, and weighing over 35,500 individuals from >65 species (see Appendix Table A1). We calculated benthos, vegetation, and total (vegetation+ benthos + final samples) dry biomass of invertebrates, and dry mass of tadpoles grouped by species after drying samples at 60°C for 48h. Species specific invertebrate dry masses were calculated by measuring body length and/or head width of individuals using image analysis (Image J) and converting them into dry mass using our own and published (Benke et al. 1999) length-mass regressions. The obtained estimates of invertebrate dry mass

closely followed the pattern of actual weighed dry mass without treatment bias, although it consistently underestimated total dry mass. Zooplankton community structure was determined by counting all individuals within a sample. Larvae and adults of invertebrate species with complete metamorphosis were analyzed separately because of their functional differences (e.g., beetle larvae vs. adults).

Community structure was analyzed for both the abundances and total biomass of species. The goal of this analysis was to detect if *A. junius* stages have different effects on the community structure rather than the total abundance of species. Thus we used relative biomass or density of each species (i.e., proportion of total community biomass or density of an experimental pond) to test for differences in community structure across treatments. For the biomass analysis we scaled zooplankton subsamples up to the volume of the whole tanks. We did not scale the densities of subsampled zooplankton up to whole tank volume since this would have resulted in zooplankton species being up to10,000 more abundant than any other species. While the scaling also resulted in significant differences in whole community structure among size treatments, the differences were completely dominated by zooplankton species (accounting for 80-90% of differences among tanks even after fourth root transformation). Thus, we used densities from our actual zooplankton subsamples instead, which were within the range of all other vertebrate and invertebrate densities. Overall, both types of analysis (scaled or not scaled densities) led qualitative similar results so we only report the latter analysis. Finally, we analyzed changes in the size-structure of the macro-invertebrate community by comparing square-root transformed abundance of individuals within $\log_{10}$ size classes based on dry mass. Analyses using different bin sizes gave similar results (not shown here) indicating that the analysis is robust to changes in bin size.

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Ecological Archives

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Appendix B. Detailed description of biomass corrected analyses and results.

Biomass corrected analysis

Methods

In our experiment we used natural densities of each stage. While this is the recommended design when predators differ substantially in size to avoid many experimental artifacts that would be caused by using traditional designs that keep biomass or densities of predators constant (Chalcraft and Resetarits 2004, O'Connor et al. 2008), it inevitably also results in differences in biomass among predator treatments. To test whether these potential differences among predator treatments were important in explaining the observed results, we first tested whether average *Anax* biomass differed among treatments and then tested whether the per-unit biomass effect size differed among predator size treatments.

We calculated the average *Anax* biomass in a given treatment over the duration of the experiment assuming an exponential model, where the biomass at time, B_t ,is given by $B_t = B_0 * e^{(g*t)}$, with B_0 indicating the initial biomass within a tank, g = biomass growth rate, and t = time. g was calculated by setting B_t equal to the final biomass, t equal to the duration of the experiment, and solving the equation for g. Based on this model average *Anax* biomass was highest in medium sized predator treatments (mean = 352.5 mg, SE= ±20.6), which was 1.3 times higher than the biomass in treatments with large predators (mean = 264.3 mg, SE = ±22.2) (unequal variance t-test: P = 0.035) and 1.5 times higher than biomass in treatments with small predators (mean =

226.8 mg, SE = ±15.4) (unequal variance t-test: P = 0.0006). Average predator biomass did not differ between small and large predator treatments (unequal variance t-test: P= 0.29). The biomass estimate for large predator treatments likely represents an underestimation as this estimate includes a fraction of individuals which reached metamorphosis during the last days of the experiment and dragonflies lose some weight during metamorphosis. While this model is the most biological realistic scenario, we also estimated biomass assuming a linear increase to test how robust our analysis was to specific model assumptions. Different model assumptions led to qualitatively similar results as the relative differences in biomass among treatments remained largely constant regardless of model specifications. Consequently we only present analyses based on the most realistic model (exponential) here. We calculated the biomass corrected effect of different predator size classes on ecosystem response variables as $X_B = (X_{JP} - X_C)/B_J$, where X_{JP} indicates the value of a given response variable for pond P in size-treatment J (small, medium or large), X_C indicates the average of the respective response variable in the control, and B_J is the average biomass in predator treatment J. Positive values of X_B indicate that the respective ponds had larger values than the control and negative values the opposite. The biomass corrected ecosystem responses were then analyzed with the same statistics as described in the methods in the main text. For the repeated measures analysis of periphyton and phytoplankton biomass we analyzed the data in two ways. We either simply divided the respective primary producer biomass for each time step by the average predator biomass for the respective treatment, or we divided it by the time specific average biomass for a given time interval which we estimated using the biomass equation model above. The general results were largely the same regardless of how predator biomass was incorporated into the repeated measures analysis. Because we believe

that the latter most likely represents the natural scenario, we only present the results of this analysis here.

Results

The biomass corrected analysis (Table A4) shows the same general qualitative patterns observed with the untransformed data (see Table 1 in main text) and confirms that predator size classes differed qualitative in their effects on the ecosystem.

Decomposition

Per unit biomass effect on decomposition rates did not differ among predator stages ($F_{2.15}$ =0.11, P = 0.8959) (large *Anax* (AL) = 0.005075 ±0.006746 SE, medium *Anax* (AM) = 0.001813±0.002490 SE, small *Anax* (AS) = 0.001806 ±0.01481 SE).

Ecosystem productivity and respiration

Net primary productivity (NPP) – Per unit biomass effect on the relative change in NPP during the experiment differed significantly among size treatments (Table A4, Fig. A5). Importantly, while effect size did not differ between treatments with large or medium sized *Anax* (P = 0.5319), both differed significantly from treatments with small *Anax* (AL vs AS P = 0.0071, AM vs AS P = 0.0332). Importantly, there was a clear qualitative difference: the effect size for NPP was positive for AL and AM treatments relative to the control, while it was negative in AS (Fig. A5).

Respiration – Per unit biomass effect on the relative change in respiration differed significantly across size treatments and followed the same patterns as for NPP. AL and AM treatment did not significantly differ from each other (P = 0.3003), while both differed from AS treatments (AL vs

AS P = 0.0019, AM vs AS P = 0.0229). Again, the effect was positive in AL and AM relative to the control, while AS treatments exhibited the opposite trend.

Primary producer biomass

Phytoplankton – When corrected for predator biomass, the relative change in phytoplankton biomass over time was significantly influenced by the interaction of time and size treatment (Table A4). Similar to the analysis of the raw data, this interaction was largely driven by the significant higher but decreasing effect size in treatments with small predators over the first two time periods (both $P<0.038$), while treatments with large and medium sized *Anax* were not different from the control (all $P>0.23$) at any point in time (Fig. A6).

Periphyton –The relative change in periphyton biomass over time differed significantly among predator treatments after correcting for differences in predator biomass (Table A4, Fig. A6). While the effect on change in periphyton biomass was not significantly different from the control in treatments with large predators ($P>0.12$), both treatments with medium and small *Anax* decrease significantly more than the control during the second census period (AM: $P=0.0115$, AS: $P = 0.0005$).

Total vertebrate and invertebrate biomass

After accounting for differences in predator biomass, total animal biomass in treatments with large (1.319, ±0.5265) *Anax* differed from total biomass in treatments with medium (-1.1321 ±0.5942) or small *Anax* (-1.544 ±1.2992)(AL vs AM: $P = 0.0385$, AL vs AS: $P = 0.0567$), while the latter two did not differ from each other (AM vs AS: $P = 0.8408$). Importantly, there were clear qualitative differences: total biomass increased in treatments with large *Anax* relative to the

control but decreased in treatments with small and medium sized *Anax* (Fig. A7).

There were no significant differences in standardized biomass of benthic macro-invertebrates ($F_{2,15} = 0.39$, $P = 0.6857$). However, standardized biomass of macro-invertebrates in the vegetation differed significantly among treatments ($F_{2,15} = 8.86$, $P = 0.0029$) (Fig. A7): treatments with large and medium sized predators had a higher biomass than the control and did not differ from each other ($P = 0.1055$), but both differed significantly from treatments with small predators (AL vs AS: $P = 0.0008$, AM vs AS: $P = 0.0263$) which had significantly lower biomass than the control. This pattern held true regardless of whether the outlier was included or not.

Literature

Chalcraft, D. R. and W. J. Resetarits. 2004. Metabolic rate models and the substitutability of predator populations. Journal of Animal Ecology **73**:323-332.

O'Connor, N. E., J. H. Grabowski, L. M. Ladwig, and J. F. Bruno. 2008. Simulated predator extinctions: Predator identity affects survival and recruitment of oysters. Ecology **89**:428-438.

Table B1: Per unit biomass effects of different predator size treatments on ecosystem processes. See Table 1 in main text for details on statistical tests and variables.

Effect	F value	P value	Treatment ranking
Decomposition rate			
Size	$F_{2,15}$= 0.11	0.8959	NS
Change in Respiration			
Size	$F_{2,14}$ = 7.61	0.0058	L≥M> S
Change in NPP			
Size	$F_{2,14}$ = 5.44	0.0179	L≥M> S
Change in Phytoplankton			
Size	$F_{2,16.4}$ = 3.73	0.0462	S>L=M
Time	$F_{2,15.4}$ = 2.49	0.1156	
Size*Time	$F_{4,15.5}$ = 3.22	0.0418	
Change in Periphyton			
Size	$F_{2,14.9}$ = 0.95	0.4104	S>M≥ L
Time	$F_{2,14.1}$ = 32.61	<0.0001	
Size*Time	$F_{4,14}$ = 4.96	0.0106	

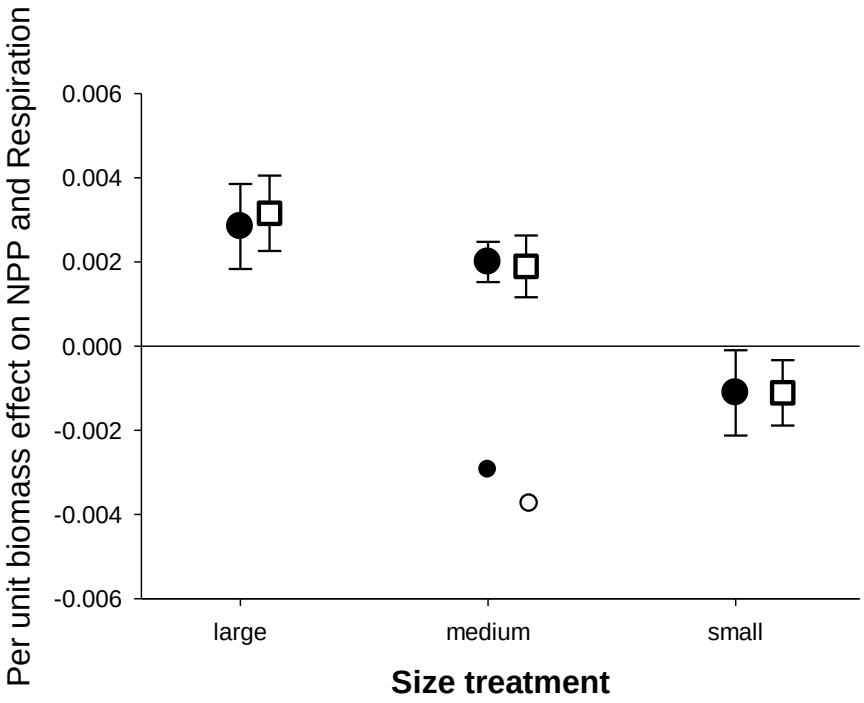

Fig. B1: Per unit biomass effect on mean (±1 SE) change (= final - initial) in ecosystem net primary productivity (NPP, filled circles) and respiration (R, open squares) during the experiment. Values represent the per unit biomass effect of the respective predator treatment relative to the control (see methods for details). The outliers for NPP and respiration are indicated by small filled and open circles respectively. Large, medium and small indicate the respective size class of the predator *A. junius* that was added to experimental ponds.

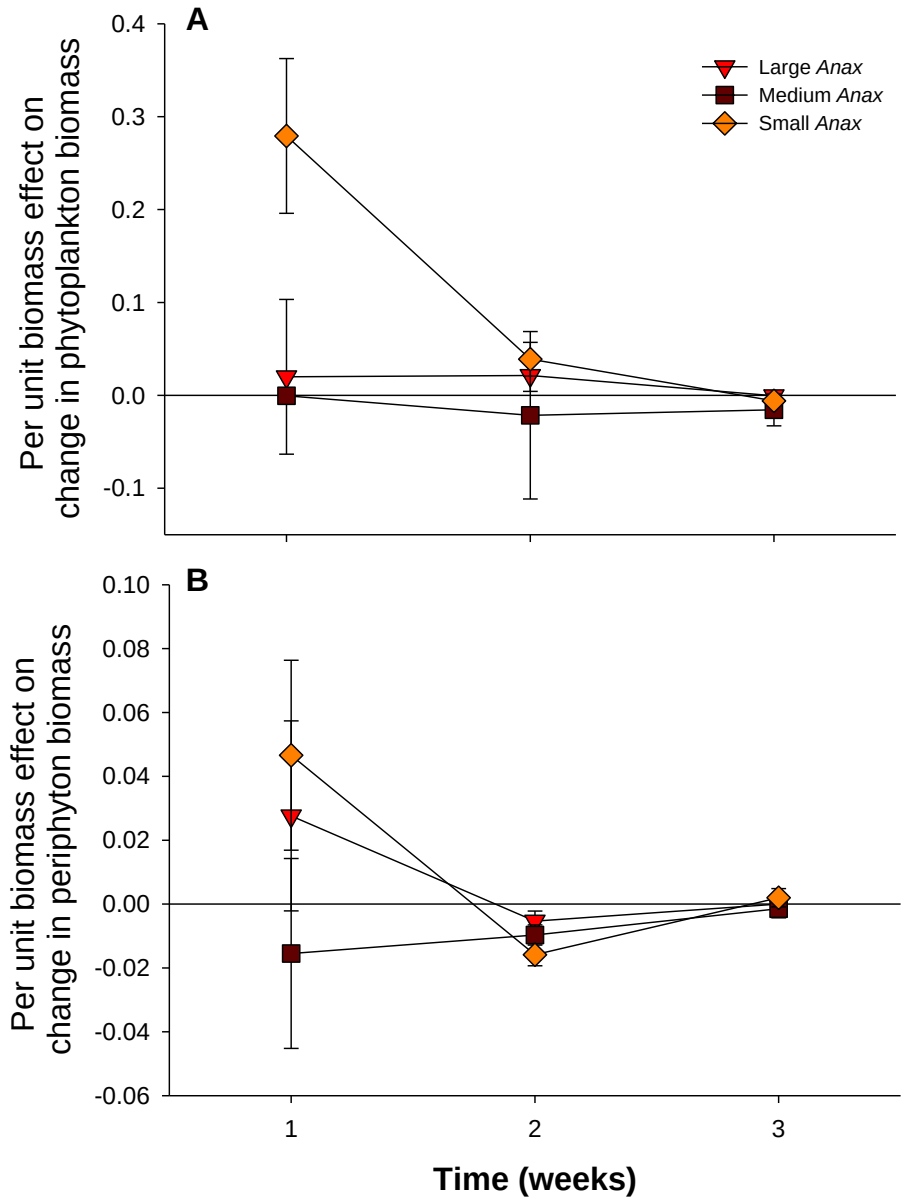

Fig. B2: Per unit biomass effect on relative change in **A**) phytoplankton biomass and **B**) periphyton biomass measured from chlorophyll-a extractions across treatments. Values represent the per unit biomass effect of the respective predator treatment relative to the control (see methods for details). Large, medium and small indicate the respective size class of the predator *A. junius* that was added to experimental ponds. Symbols indicate model estimated means that account for missing values and repeated measures of ponds (±1 SE).

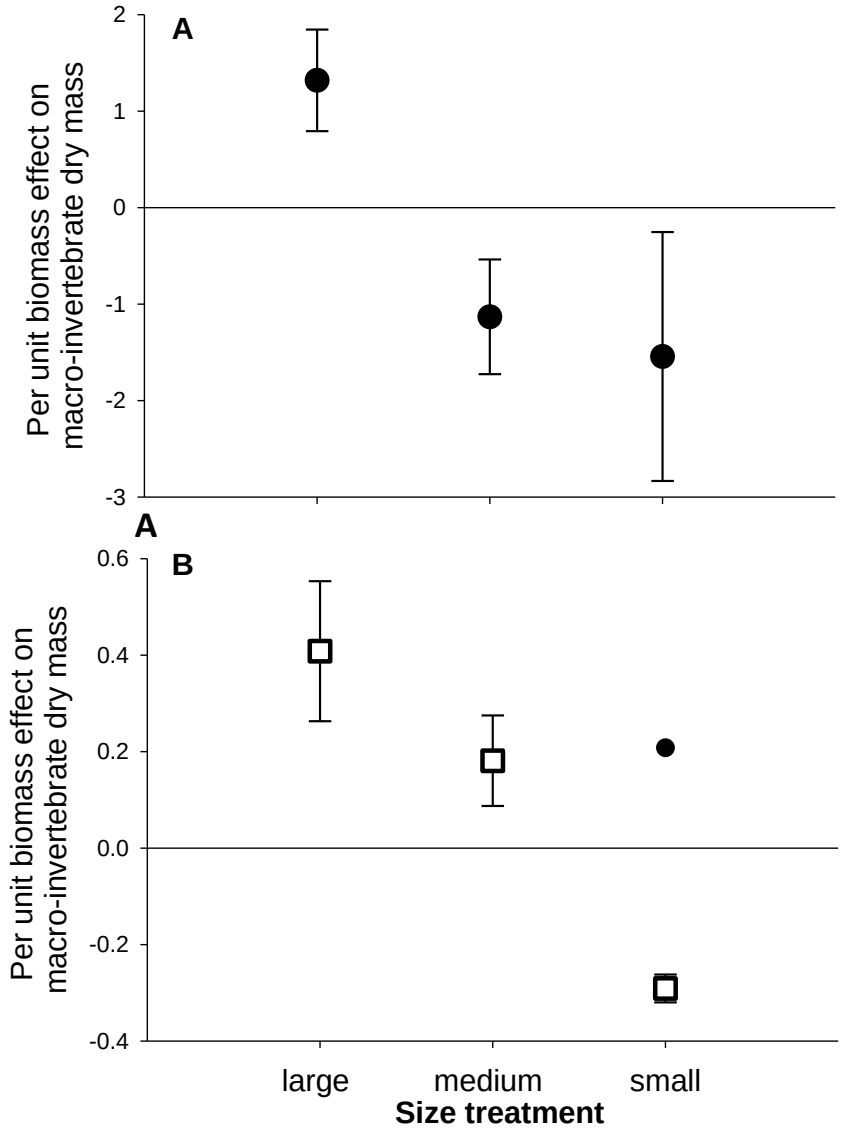

Fig. B3: Per unit biomass effect on average (± 1 SE) dry mass of macro-invertebrates for **A**) total final sample, **B**) vegetation subsample in ponds with large, medium, and small stages of the predator *A. junius*. The filled circle indicates a significant outlier for vegetation biomass. Values represent the per unit biomass effect of the respective predator treatment relative to the control (see methods for details). Large, medium and small indicate the respective size class of the predator *A. junius* that was added to experimental ponds.

Ecological Archives

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Appendix C. Average abundance-biomass relationships in different predator treatments

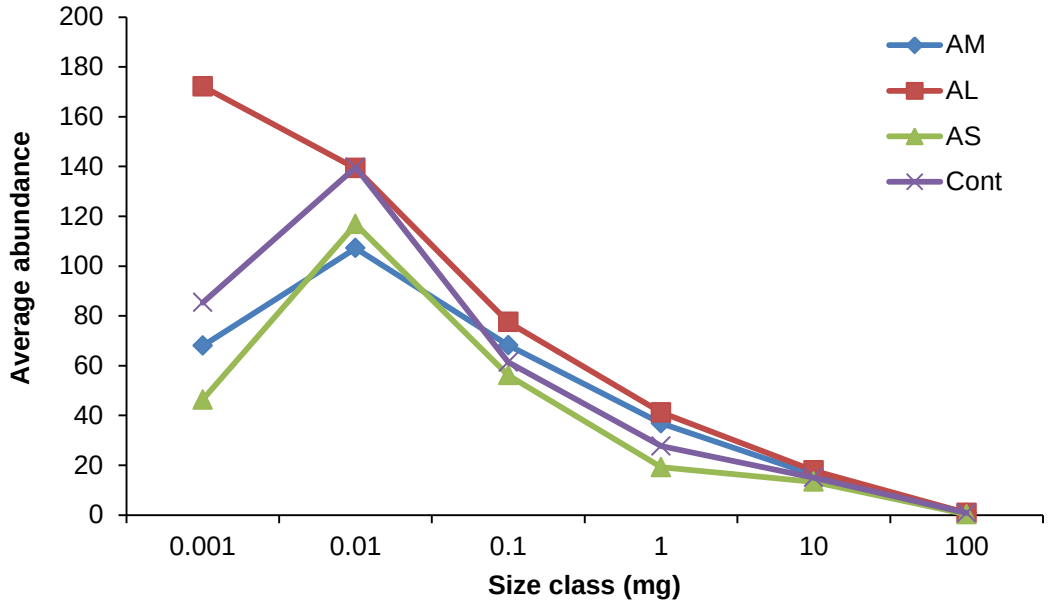

Fig. C1: Average abundance of macro-invertebrates within $\log_{10}$ size classes in ponds with large (AL), medium (AM), small (AS) or no added (Cont) larvae of the predator *A. junius* (n = 6). Error bars are not shown for clarity. Analyses were performed with multi-variate non-parametric permutation tests (PERMANOVA), and indicate significant differences among different *A. junius* size classes (see Results).

Ecological Archives

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Appendix D. Results of structural equation modeling indicating relationships among treatments and ecosystem variables

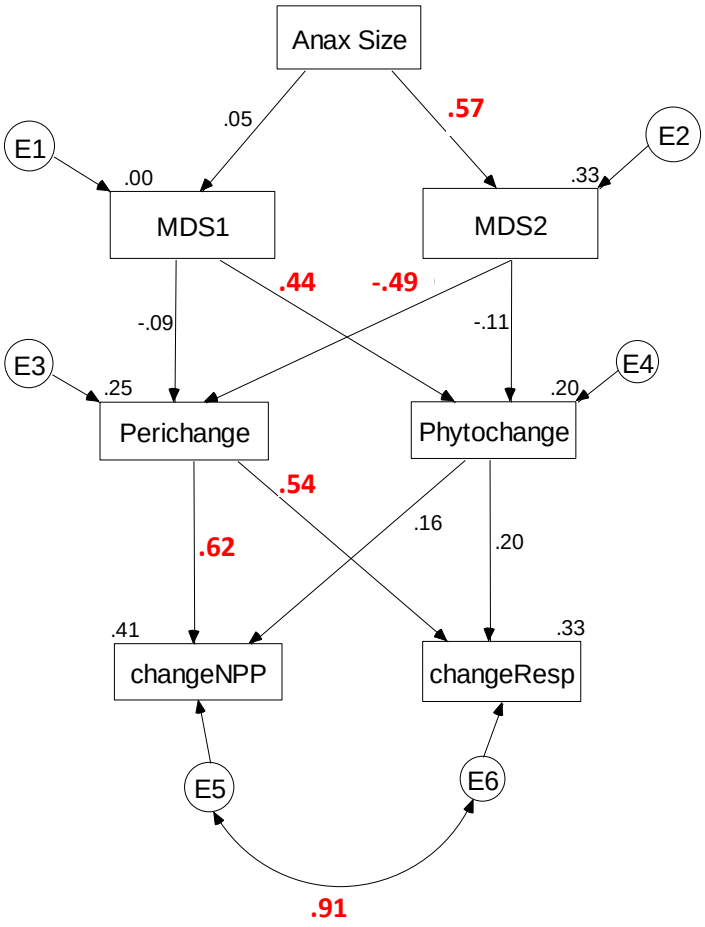

Fig. D1: Structural equation modeling indicating correlations among treatment (Anax size= small, medium or large *A. junius* larvae added) and measured ecosystem response variables. nMDS1 and nMDS2 scores represent the community structure based on relative densities of species (see Fig. 4A in main text), Phytochange, Perichange, changeNPP, and changeResp

indicate the relative change in Periphyton and Phytoplankton biomass, Net Primary Productivity (NPP) and respiration respectively measured within a tank over the course of the experiment. Only the final model with the best fit is indicated. Significant correlations ($P > 0.05$) are in red.

Table: Treatment means and standard errors (se) for densities and biomass of community members at the end of the experiment, categorized by taxa

	Absolute Densities								Dry Biomass (mg)							
	Treatment								Treatment							
Taxa	Control		Small Anax		Medium Anax		Large Anax		Control		Small Anax		Medium Anax		Large Anax	
	mean	± se	mean	± se	mean	± se	mean	± se	mean	± se	mean	± se	mean	± se	mean	± se
AMPHIBIA																
Bufo nebulifer	62.5	7.37	48.17	5.42	49.33	5.28	65.83	10.13	445.66	58.66	308.8	38.93	322.12	38.88	516.64	89.7
Hyla cinerea	1.5	0.56	2.17	0.65	1.67	0.49	2.33	0.61	144.63	63.73	124.14	36.07	120.65	36.04	231.14	82.9
Hyla versicolor	16.5	4.49	19.17	4.47	9.17	1.82	16.5	2.63	597.9	181.13	658.8	161.92	285.13	62.64	609.31	96.56
Rana clamitans	22.5	2.36	16.33	4.72	21.5	3.04	27.67	1.17	228.7	17.49	160.55	55.98	321.53	88.52	369.13	87.53
GASTROPODA																
Physidae	2.33	1.96	0.67	0.42	2	0.93	2.17	0.7	0.24	0.06	0.1	0.02	0.13	0.03	0.29	0.07
Planorbidae	3.33	2.06	0.83	0.31	3.67	1.56	4.83	2.37	0.18	0.06	0.19	0.07	0.28	0.2	0.22	0.03
INSECTA																
Coleoptera - adult																
Tiny beetles (< 2.0 mm)	32.17	14.22	16.5	5.12	33.17	11.71	39.5	15.02	6.21	2.74	3.18	0.99	6.4	2.26	7.62	2.9
Liodessus or Neobidessus	31	13.87	16.33	5.16	30	10.05	38.5	14.83	5.98	2.68	3.15	0.99	5.79	1.94	7.43	2.86
Othera	1.17	0.48	0.17	0.17	3.17	2.4	1	0.37	0.26	0.1	0.04	0.04	0.7	0.53	0.22	0.08
Small beetles (2.0 - 3.9 mm)	4.83	1.56	3.17	1.56	4	1.98	6.33	2.99	3.88	0.58	2.83	1.36	5.81	2.75	5.95	3.56
Berosus exiguus	2	0.58	1.83	1.01	0.67	0.49	3.67	1.74	0.92	0.27	0.84	0.47	0.31	0.23	1.68	0.8
Hydrochusb	0.5	0.22	0.5	0.34	1.83	0.91	1	0.63	1.31	0.59	1.31	0.9	4.82	2.39	2.63	1.66
Peltodytes floridensis	0.33	0.21	0.17	0.17	0	0	0.5	0.5	0.73	0.46	0.37	0.37	0	0	1.1	1.1
Otherc	2	1.61	0.67	0.33	1.5	0.96	1.17	0.31	3.2	2.58	1.07	0.53	2.4	1.53	1.87	0.49
Medium beetles (4.0 - 7.9 mm)	8.33	1.09	8.33	2.81	12.5	2.79	13.17	3.8	46.4	8.37	30.37	10.23	55.75	12.62	68.88	11.15
Berosus	0.5	0.22	0	0	0.33	0.21	0.5	0.22	2.1	0.94	0	0	1.4	0.89	2.1	0.94
Copelatusd	1.83	0.48	0.67	0.42	2	0.82	2.5	0.56	22.37	5.82	8.13	5.14	24.4	9.96	30.5	6.87
Coptotomus loticus	0.33	0.21	0	0	0	0	0.5	0.34	5.5	3.48	0	0	0	0	8.25	5.64
Hydrocanthus atripennis	0	0	0	0	0.17	0.17	0	0	0	0	0	0	0.48	0.48	0	0
Laccophilus maculosus	5.67	0.88	7.67	2.69	9.67	2.54	9.67	3.86	16.43	2.56	22.23	7.81	28.03	7.36	28.03	11.2
Othere	0	0	0	0	0.33	0.21	0	0	0	0	0	0	1.43	0.91	0	0
Large beetles (8.0 - 10.9 mm)	1.83	0.54	2.33	0.8	1.83	0.48	1.33	0.8	44.95	13.26	52.4	19.24	38.45	10.38	28	17.17
Thermonectus basillaris	0.83	0.31	0.17	0.17	0	0	0	0	23.25	8.57	4.65	4.65	0	0	0	0
Tropisternus blatchleyi	0.5	0.22	0.67	0.49	0.67	0.21	0.33	0.21	9.65	4.32	12.87	9.54	12.87	4.07	6.43	4.07
Tropisternus collaris	0.5	0.34	1.33	0.61	0.83	0.48	0.67	0.49	12.05	8.23	32.13	14.81	20.08	11.5	16.07	11.92
Tropisternus lateralis	0	0	0.17	0.17	0.33	0.33	0.33	0.21	0	0	2.75	2.75	5.5	5.5	5.5	3.48
Extra large beetles (? 11 mm)	0.33	0.21	0.33	0.33	0.17	0.17	0.33	0.21	23.75	15.66	23.75	23.75	9.02	9.02	6.87	6.87
Acilius fraternus	0.17	0.17	0.17	0.17	0	0	0	0	14.73	14.73	14.73	14.73	0	0	0	0
Graphoderus liberus	0	0	0	0	0	0	0.17	0.17	0	0	0	0	0	0	6.87	6.87
Thermonectus nigrofasciatus	0.17	0.17	0.17	0.17	0.17	0.17	0	0	9.02	9.02	9.02	9.02	9.02	9.02	0	0
Coleoptera - larva																
Berosus	0.5	0.5	0.17	0.17	0	0	0.67	0.33	1.36	1.36	0.45	0.45	0	0	1.81	0.91
Laccophilus	0.83	0.4	0.67	0.33	0.67	0.49	3.33	1.73	0.19	0.09	0.16	0.08	0.16	0.12	0.78	0.4
Peltodytes	6.67	1.38	6	2.1	5.33	2.06	5.83	2.18	3.52	0.73	3.17	1.11	3.58	1.11	3.08	1.15
Thermonectus	0	0	0	0	0	0	0.67	0.67	0	0	0	0	0	0	1.92	1.92
Tropisternus	2.5	1.96	0.83	0.65	0	0	1.33	1.15	6.8	5.34	2.27	1.78	0	0	3.63	3.11
Diptera																
Chironomidae	127	13.25	111.33	20.54	98.5	8.99	126.83	30.16	1.59	0.17	1.39	0.26	1.23	0.11	1.59	0.38
Ephemeropteraf	72	13.4	85.17	7.07	96.33	16.24	66	15.23	1.41	0.59	2.78	1.29	2.6	1.25	0.4	0.17

Hemiptera																
Belostoma	1	0.37	2.33	2.14	0.83	0.17	0.83	0.31	96.78	35.66	40.03	25.34	96.67	19.33	96.67	35.65
Nymph	0.17	0.17	2	2	0	0	0	0	0.11	0.11	1.36	1.36	0	0	0	0
Adult	0.83	0.31	0.33	0.21	0.83	0.17	0.83	0.31	96.67	35.65	38.67	24.45	96.67	19.33	96.67	35.65
Buenoa scimitra	12.33	2.81	6.17	0.95	17.83	4.71	12.17	1.99	28.12	5.72	13.74	2.51	40.05	9.82	26.75	5.64
Nymph	0.5	0.34	0.33	0.33	1	0.63	0.83	0.4	0.24	0.18	0.21	0.21	1.1	0.7	0.92	0.44
Adult	11.33	2.28	5.5	1.06	15.83	3.81	10.5	2.4	27.88	5.6	13.53	2.6	38.95	9.37	25.83	5.92
Hesperocorixa nitida	2.17	0.83	1	0.37	1	0.52	3	0.86	20.15	7.75	9.3	3.4	9.3	4.8	27.9	7.96
Notonecta indica	8.83	2.94	14.5	3.54	14.33	7.06	22.5	9.29	81.67	19.53	67.9	13.15	105.16	39.08	131.55	29.11
Nymph	5.17	2.71	11.33	3.56	10.17	6.44	17.83	9.75	17.03	9.52	12.07	4.67	31.71	25.31	49.28	30.7
Adult	3.67	1.09	3.17	0.6	4.17	1.05	4.67	0.84	64.64	19.13	55.83	10.59	73.46	18.44	82.27	14.87
Notonecta irrorata	0.17	0.17	0	0	0.5	0.5	0	0	6.78	6.78	0	0	20.35	20.35	0	0
Pelocoris femoratus	0.17	0.17	0.5	0.34	0	0	0.17	0.17	0.02	0.02	5.72	5.7	0	0	2.85	2.85
Nymph	0.17	0.17	0.17	0.17	0	0	0	0	0.02	0.02	0.02	0.02	0	0	0	0
Adult	0	0	0.33	0.33	0	0	0.17	0.17	0	0	5.7	5.7	0	0	2.85	2.85
Trichocorixa	0	0	0	0	0	0	0.17	0.17	0	0	0	0	0	0	0.12	0.12
Odonata																
Coenagrionidaeg	22	3.09	17.33	1.43	21.67	5.78	31.83	6	6.03	1.77	4.5	1.11	7.45	3.52	11	3.85
Lestes australis	0	0	0	0	0.17	0.17	0.17	0.17	0	0	0	0	1.62	1.62	0.87	0.87
Libellulidae	16.17	2.02	16.5	1.67	15.67	1.94	16.67	2.46	126.02	29.82	118.48	17.24	139.33	20.57	177.69	30.62
Erythemis simplicicollis	0	0	0.17	0.17	0	0	0.33	0.21	0	0	0.85	0.85	0	0	1.7	1.07
Ladona deplanata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Libellula	0.83	0.31	0.5	0.22	0.67	0.33	0.67	0.21	4.82	1.78	2.89	1.29	2.89	1.97	3.85	1.22
Pachydiplax longipennis	11	0.97	12.67	1.2	11.33	1.38	11.67	1.74	71.69	19.61	85.34	12.31	95.58	14.64	112.65	22.9
Plathemis lydia	3.5	0.89	2.33	0.8	2.5	0.76	3	0.77	44.1	11.15	29.4	10.11	31.5	9.62	37.8	9.76
Tramea carolina	0.83	0.31	0.83	0.31	1.17	0.48	1	0.37	5.42	5.42	0	0	9.36	9.36	21.68	9.85
ZOOPLANKTONh																
Calanoida	1	0.63	0.67	0.33	0.83	0.48	0.83	0.54	0.64	0.41	0.43	0.21	0.54	0.31	0.54	0.35
Ceriodaphnia	144.5	91.7	47.17	15.76	73.83	58.11	32.83	12.6	31.81	20.19	10.38	3.47	16.25	12.79	7.23	2.77
Chydorus	34.17	10.51	21.17	6.05	37.5	19.99	53.17	19.66	9.68	2.98	5.99	1.71	10.62	5.66	15.06	5.57
Cladocera - Immature	1.67	0.67	7.67	5.02	2.5	1.34	3.5	3.3	0.12	0.05	0.54	0.35	0.18	0.09	0.25	0.23
Cyclopoida	134.67	20.74	134.5	24.22	94.33	23.97	86	5.65	111.23	17.13	111.1	20.01	77.92	19.8	71.04	4.67
Kurzia	3.67	3.28	0.5	0.5	3.83	1.96	0.33	0.33	2.15	1.92	0.29	0.29	2.25	1.15	0.2	0.2
Macrothrix	32.67	17.04	19.5	5.95	37.33	21.14	20.5	8.93	113.42	59.16	67.7	20.67	129.62	73.39	71.18	31
Nauplii	81.5	34.11	48.17	9.98	23.17	7.19	28.17	4.64	6.39	2.67	3.78	0.78	1.82	0.56	2.21	0.36
Ostracoda	37	18.69	9.33	2.08	15.67	3.88	14.67	3.54	6.22	3.14	1.57	0.35	2.63	0.65	2.46	0.59
Pleuroxus	0.67	0.21	4	2.29	1.5	1.31	0.33	0.21	0.2	0.06	1.2	0.69	0.45	0.39	0.1	0.06
Scapholeberis	6.67	2.99	0.83	0.4	3	1.61	1.33	0.67	3.42	1.53	0.43	0.21	1.54	0.83	0.68	0.34
Simocephalus	14.67	8.42	19.83	7.31	11.33	4.81	25.67	8.95	80.62	46.29	109.02	40.2	62.3	26.46	141.09	49.2

aParacymus confusus, Desmopachria sp., and many others
bat least two species
cEnochus blatchleyi and others
dat least two species (C. glyphicus and C. debilis)
emultiple unidentified species
fmostly Callibaetis sp. but also some Caenis sp.
glschnura posita, I. hastata, and Enallagma aspersum
hZooplankton densities indicate densities within samples and need to be multiplied by factor of 320 to scale densities up to total tank volume