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Population structure determines functional differences among species and ecosystem processes

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Linking the structure of communities to ecosystem functioning has been a perennial challenge in ecology. Studies on ecosystem function are traditionally focused on changes in species composition. However, this species-centric approach neglects the often dramatic changes in the ecology of organisms during their development, thereby limiting our ability to link the structure of populations and communities to the functioning of natural ecosystems. Here we experimentally demonstrate that the impact of organisms on community structure and ecosystem processes often differ more among developmental stages within a species than between species, contrary to current assumptions. Importantly, we show that functional differences between species vary depending on the specific demographic structure of predators. One important implication is that changes in the demography of populations can strongly alter the functional composition of communities and change ecosystem processes long before any species are extirpated from communities.

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Natural communities are increasingly altered by anthropogenic factors[1]. Unfortunately, the ways ecosystems respond to these community changes often cannot be predicted by classical theory[2]. For instance, although size-selective harvesting of predatory fishes in the Atlantic has not changed their total biomass or diversity for decades, it has dramatically altered community structure and many ecosystem properties[3]. This suggests that current approaches miss important information necessary to predict how changes in the structure of communities will alter the functioning of natural ecosystems.

Part of the problem may arise from the coarse resolution of community structure that stems from current species-centric approaches. When facing complex systems like natural communities, the challenge is to identify the scale with which we need to resolve the system to make accurate predictions. Mounting evidence indicates that the ecological differences among organisms in the community (that is, functional diversity) are more important than taxonomic diversity to link community structure and ecosystem functioning[4–9]. Functional diversity, however, is still estimated based on the average traits of a species. A drawback of this species-centric approach is the assumption that the functional role of a species is fixed within a community[4–7,10], which also implies that functional differences within species are negligible compared with differences among species. This, however, ignores the potential importance of ecological variation among individuals within a species[2,11,12], thereby precluding our ability to link changes in population and community structure to ecosystem functioning[13].

By far, the largest source of this intraspecific variation stems from differences in body size and developmental stage[14–17]. Indeed, ecological differences (for example, diet, habitat use) among stages within species can rival or even exceed differences between species[18–20]. Given such differences among stages, the demographic structure of populations should strongly determine the functional roles of species, thereby influencing ecosystem functioning[21]. Yet, there is considerable debate about whether and how it should be incorporated into current ecological models and conservation efforts[2,22–25] because it is generally assumed that ontogenetic functional variation within species is insignificant compared with functional variation among species at the ecosystem level, although this remains to be tested.

To predict how ontogenetic functional diversity influences ecosystem functioning requires an understanding of the magnitude of functional differences among stages, how this compares with functional differences across species, and given that multiple stages commonly co-occur, we need to know whether effects of stages are independent of each other to predict the realized effect of a species on the ecosystem and long-term dynamics.

Here we present a novel experiment that manipulates the stage structure of populations for two key predator species in complex experimental communities to examine how changes in the structure of populations are linked to the functional role of a species, functional differences among species and ultimately the structure and processes of complex ecosystems. By measuring nine key ecosystem properties, and identifying, counting, measuring and weighing >35,000 individuals from >65 morphospecies in total, we demonstrate that changes in the demographic (stage) structure of populations scale up to alter the structure and functioning of complex ecosystems, and that functional differences between species are determined by their respective demographic structure. These results indicate that changes in the demography of populations can strongly alter the functional composition of communities and change ecosystem processes long before any species are extirpated from communities.

Results

General patterns. We found striking differences among treatments across all measured ecosystem properties, ranging from community composition (Fig. 1) and biomass of functional

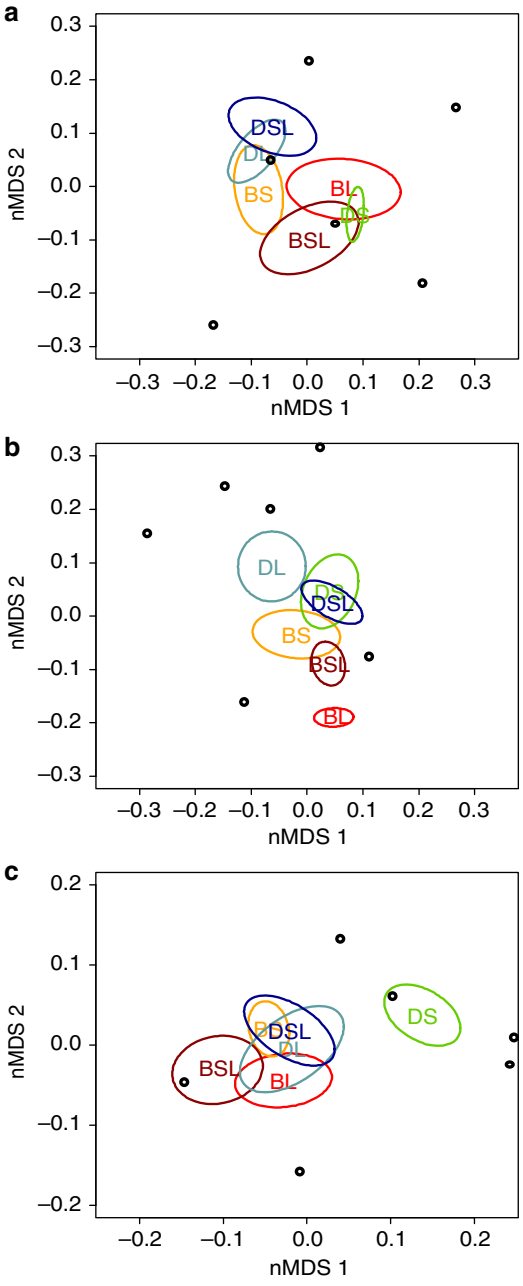

Figure 1 | Differences in animal community structure. Two non-metric multidimensional scaling (nMDS) axes of (**a**) proportional abundance and (**b**) proportional biomass of all animal species, and (**c**) proportional abundance of different macroinvertebrate size classes for different predator treatments. The ellipse shows the mean (centre of ellipse) ± 1 s.e.m. of the community structure for each treatment ($n = 6$ per treatment). Letters within an ellipse indicate predator treatments; the first letter indicates the species identity of the predator (D = Dragonfly (*Anax*), B = Beetle (*Cybister*), subsequent letters indicate what stages of that predator are present: S = small, L = large and SL = small + large). Black circles indicate individual mesocosm communities in the predator-free control. nMDS two-dimensional stress levels for (**a**) = 0.2, (**b**) = 0.15 and (**c**) = 0.11. See Table 1 for statistical analysis.

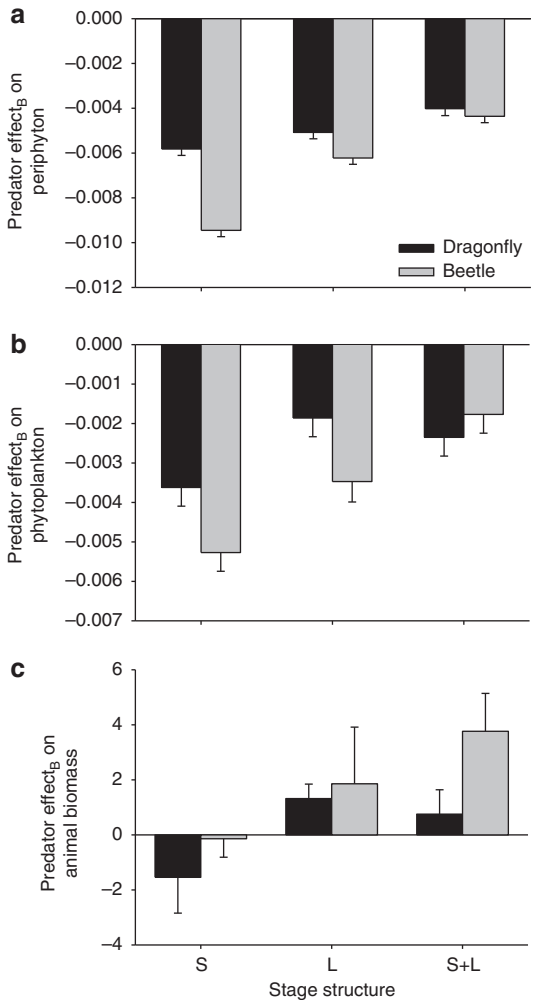

Figure 2 | Differences in primary producer and animal biomass. Bars indicate treatment means + 1 s.e.m., stage structure indicates what stage of a predator species is present: S = small stage, L = large stage, S + L = both stages co-occur. Effect$_B$ indicates the standardized per-unit biomass effect (see Supplementary Methods for details) of predators within a given treatment on the respective ecosystem trait relative to the predator-free control. (**a**) and (**b**) indicate the proportional change in respective primary producer biomass over the duration of the experiment based on chlorophyll-*a* relative to the control. (**c**) Dry biomass of all macroinvertebrates and amphibians at the end of the experiment relative to the control. Each treatment was replicated six times. See Table 1 for statistical analysis.

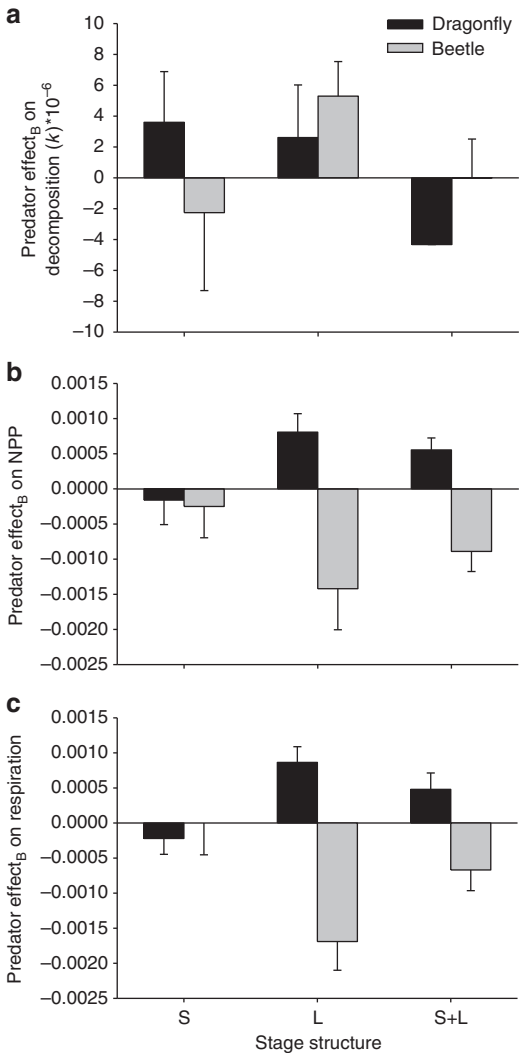

Figure 3 | Differences in ecosystem processes. Bars indicate treatment means + 1 s.e.m., size structure indicates what stage of a predator species is present (see Fig. 2). Effect$_B$ indicates the standardized per-unit biomass effect (see Supplementary Methods for details) of predators within a given treatment on the respective ecosystem trait relative to the predator-free control. (**a**) Decomposition constant (k). (**b**,**c**) proportional change in net primary productivity (NPP) and R based on diurnal cycles of dissolved oxygen over the duration of the experiment. Each treatment was replicated six times. See Table 1 for statistical analysis.

groups (Fig. 2) to ecosystem processes (Fig. 3; Table 1). Functional differences among populations with different stage structures often rivalled or even exceeded differences among species. Importantly, functional differences among species were typically dependent on the specific stage structure within species (Table 1, species × stage). Consequently, the majority of ecosystem properties were jointly determined by the stage and species' identity of focal predators (Table 1).

Effects on community structure. The impact of a predator on community composition was contingent on its stage and species identity regardless of whether community structure was analysed based on biomass or density indices (Fig. 1; Table 1). This interaction was caused by several factors. First, the magnitude of stage-specific differences in community composition varied between species (for example, difference in L versus S for dragonfly versus beetle, Fig. 1a,b). Second, the difference in community composition among treatments with different predator species was stage specific (Fig. 1a,b). As a consequence, communities could differ more among treatments that received different stages of the same species than treatments with different species (Fig. 1). For instance, communities with large and small dragonfly predators differed much more from each other (based on density indices) than from communities with predatory beetle stages (Fig. 1a). In addition, differences among species did not consistently scale with size. For instance, communities with large dragonflies were more similar (based on biomass indices) to communities with small beetles compared with communities with large beetles (Fig. 1b), indicating that the size of individuals cannot be used as a surrogate for species either.

Table 1 | Effects of species identity and stage structure on ecosystem properties.

	Community structure					
Source of variation	**Biomass**		**Density**		**Body size**	
Species	$F_{1,29}$	3.9**	$F_{1,25}$	1.5	$F_{1,25}$	3.47*
Stage	$F_{2,29}$	0.9	$F_{2,25}$	0.7	$F_{2,25}$	2.04
Species × stage	$F_{2,29}$	1.9*	$F_{2,25}$	1.7*	$F_{2,25}$	1.09†
	Biomass of trophic functional group					
Source of variation	**Periphyton**		**Phytoplankton**		**Animal**	
Species	$\chi^2_{1,29}$	52.9****	$\chi^2_{1,29}$	5.2*	$\chi^2_{1,30}$	2.6
Stage	$\chi^2_{2,29}$	143.2****	$\chi^2_{2,29}$	27.4****	$\chi^2_{2,30}$	6.8*
Species × stage	$\chi^2_{2,29}$	35.7****	$\chi^2_{2,29}$	7.2*	$\chi^2_{2,30}$	1.0
	Ecosystem rate					
Source of variation	**NPP**		**R**		**Decomposition (*k*)**	
Species	$\chi^2_{1,29}$	32.3****	$\chi^2_{1,29}$	24.4****	$F_{1,22}$	0.02
Stage	$\chi^2_{2,29}$	1.8	$\chi^2_{2,29}$	1.5	$F_{2,22}$	1.72
Species × stage	$\chi^2_{2,29}$	18.2***	$\chi^2_{2,29}$	22.9****	$F_{1,22}$	1.34

Community structure was analysed with PERMANOVA and reported test statistics are pseudo-F values. Degrees of freedom were adjusted when block effects were included in the analysis and for missing replicates because of removal of one significant outlier. Community structure was analysed based on proportional biomass and abundances of species or size classes (that is, per capita biomass of invertebrates), and thus indicates true structural differences that correct for variation in total biomass or density of predators or prey. Analysis of biomass and ecosystem rates were based on standardized per-unit biomass effects of predators on ecosystem traits relative to the control and corrected for potential differences in predator biomass. *P*-values for biomass and ecosystem rates are based on general linear models with F-statistic or likelihood-ratio χ^2-statistics, and are corrected for missing replicates (see Supplementary Table S1 for details).
†$P<0.1$
*$P<0.05$
**$P<0.01$
***$P<0.001$
****$P<0.0001$

Table 2 | General relationships among ecosystem properties.

	NPP†	**Periphyton biomass**	**Phytoplankton biomass**	**nMDS1‡**
NPP	–	–	–	–
Periphyton biomass	0.011	–	–	–
Phytoplankton biomass	0.170	−0.080	–	–
nMDS1‡	−0.537*	−0.190	0.187	–
nMDS2‡	−0.215	−0.288**	0.414***	−0.195

Values indicate partial correlation coefficients based on Pearson statistics.
†Both NPP and respiration were highly correlated ($r=0.98$) and correlation coefficients were essentially identical. Consequently only values for NPP are represented here for simplicity. ‡Non-metric multidimensional scores for community structure based on proportional (relative) biomass of species within tanks (see Supplementary Methods for details).
*$P<0.1$
**$P<0.05$
***$P<0.01$

Community composition was analysed based on the relative abundance/biomass of species within a community, and therefore was corrected for any natural differences in total biomass or density across predator treatments (which was analysed separately, Supplementary Methods). Consequently, the observed differences in community structure represent true changes in species composition, indicating that stages and species interacted (directly or indirectly) with different components of the food web. Such qualitative differences would not be expected if stages share species interactions and if foraging rates simply scale with differences in predator biomass or size (stage). Interestingly, these differences in species composition across treatments were not reflected in the size structure of the community itself. That is, the relative abundance of different-sized individuals in a community remained comparable across treatments (Table 1; Fig. 1c). This suggests that change in species composition did not merely result from smaller predator stages consuming more small prey than large predator stages[26]. Given that differences in food web structure were significantly correlated with changes in all ecosystem processes (Table 2), this indicates that changes in the population stage structure of a keystone species translated into changes in the relative abundance of different functional groups within a community.

Effects on functional groups. Besides differences in relative community composition, treatments also differed in total biomass across trophic functional groups even after accounting for potential differences in predator biomass across treatments. The per-unit biomass effect of predators on periphyton (benthic algae) and phytoplankton (pelagic algae) biomass was driven by the interaction of species identity and stage structure of populations: although beetles generally had a stronger per-unit biomass effect than dragonflies, the differences were stage specific and disappeared when two stages were present together (Table 1; Fig. 2). In contrast, final animal biomass was only affected by predator stage regardless of predator identity (Table 1; Fig. 2). Interestingly, small predator stages had a stronger per-unit biomass effect (Fig. 2), and generally reduced total primary producer and animal biomass in mesocosms more than large stages (Supplementary Fig. S1). This occurred despite total biomass of keystone predators in treatments with large predators being similar or slightly higher (Supplementary Methods).

However, the differences in animal and phytoplankton bio mass were significantly correlated with differences in species composition (Table 2), indicating that predator-mediated changes in functional composition of communities were primarily responsible for the observed differences among predator stages.

Effects on ecosystem rates. Ultimately, stage-specific differences in trophic cascades among keystone predators also resulted in differences in all measured ecosystem processes. Total decomposition rates were only different among mesocosms with different predator stages, regardless of their species identity (Supplementary Table S1; Supplementary Fig. S2). However, there was no significant difference in per-unit biomass effect among predator treatments, suggesting that this difference was largely driven by differences in predator biomass across stage treatments (Table 1; Fig. 3). The change in net primary productivity (NPP) and respiration (R) during the experiment was affected by the interaction of species identity and stage of the predator treatment (Table 1; Supplementary Table S1) and closely followed changes in community composition (Fig. 1; Table 2). In both predator species, small stages had significantly different per-unit biomass effects on NPP and R rates than treatments with large stages (all $P<0.05$, *post-hoc* test; Fig. 3). However, relative differences among stage treatments were opposite in beetle versus dragonfly treatments: in dragonfly treatments, small stages had lower effects on R and NPP, whereas the opposite was true for beetle treatments. Furthermore, small stages of both species had similar effects, whereas large stages had different and even opposite effects across species (Fig. 3). Importantly, this implies that whether species were functionally different or redundant[27] changed depending on which stages are compared. This clearly emphasizes the importance of accounting for stage structure within species when making inferences about a species' functional role and functional differences among species.

Non-additive effects of consumer stages. Given that multiple stages co-occur in many species, an important unresolved question is whether the combined effects of different stages on the ecosystem can be predicted by their individual effects or whether indirect interactions among co-occurring stages lead to non-additive effects within species (Supplementary Methods). In general, we detected non-additive effects of stages for both species for three out of the six ecosystem properties (Supplementary Table S2; Supplementary Figs S1 and S2). The reduction in phytoplankton and decomposition rates in treatments where both stages co-occurred was generally less than expected based on a null model assuming independent (additive) effects of both stages, whereas periphyton biomass was generally larger than expected by the null models. Although such non-additive effects can arise through a range of complex interactions[28], a more detailed analysis suggests that they probably result from a combination of consumptive (that is, cannibalism) and behavioural mediated interactions among predator stages (Supplementary Table S2). Importantly, this evidence of non-additive, diversity effects within species indicates that we typically cannot average across stages to predict the impact of a species on the functioning of ecosystems.

Discussion

Studies on the relationship between biodiversity and ecosystem functions traditionally focus on species diversity, and implicitly assume that ecological variation within species has little consequences at the ecosystem scale and is insignificant compared with interspecific differences[29]. Our results revealed functional differences among stages at the ecosystem level that rivalled and frequently exceeded differences among species. Furthermore, functional differences among species were contingent on the specific stage structure of the species. These findings indicate that the functional role of a species in a community is not fixed as commonly assumed[4–7,10] but is instead dynamic and varies with changes in the stage/size structure of populations. Given that the majority of animal and plant species (in terrestrial and aquatic systems) experiences shifts in ecological interactions during their ontogeny[2,16,30,31], our findings likely apply to a broad range of species and systems. Importantly, this implies that changes in the population structure of species can alter the functional composition of communities and lead to concordant changes in ecosystem functioning long before any species are extirpated. This prediction is consistent with observational data on whole ecosystems, where long-term changes in the size/stage structure of predatory fish were correlated with relative changes in the biomasses of lower trophic levels, even when the total biomass of predatory fish remained unchanged[3,32]. It also suggests that natural ecosystems are much more sensitive to human disturbances (for example, size-selective harvesting) than previously thought and emphasizes the importance of the demographic structure of populations for conserving ecosystem processes.

Identifying functional differences among demographic stages within species is challenging because it requires experiments that are short enough to avoid major stage transitions yet long enough to determine effects on ecosystem processes. Consequently, the duration of this experiment had to be shorter than the generation time of the focal species. However, this is a common scenario in studies on biodiversity and ecosystem function when analysing dynamics across trophic levels, which often vary in generation times, and it does not limit the relevance and importance of the results for predicting the long-term effects on the ecosystem. The stable stage structure of populations is dynamic and varies with natural environmental changes (in space and time)[24,33,34]. In addition, anthropogenic disturbances, such as size-selective harvesting, habitat fragmentation, invasive species and climate change, have already led to long-term changes in the stage and size structure of many animal and plant species worldwide[3,24,32,35]. Our results indicate that to understand how a species influences long-term dynamics of communities and ecosystems, we need to know how individual stages contribute to the functional role of a species in an ecosystem, and whether there are indirect interactions among stages leading to non-additive (non-independent) effects.

In our system, we found dramatic differences in the functional role among stages in both species. In addition, we found that the combined effects of stages are often not independent (that is, not additive), largely because of cannibalistic interactions but also because of other potential non-consumptive indirect interactions. Together, this indicates that any short- or long-term changes in the population stage structure of species will also result in short- and/or long-term changes in ecosystem properties in our and other systems.

One of the central challenges in biodiversity research is to identify the appropriate scale at which to resolve communities[2,29]. Classical approaches have typically focused on species and estimated functional differences among species based on the average traits of species. Although this work has greatly improved our understanding of the relationship between biodiversity and ecosystems functioning[36], this approach implicitly assumes that the functional roles of species and thus functional differences among species are fixed traits within a community. Our results

are in sharp contrast to this assumption by demonstrating that functional role within and functional differences among species changed during the ontogeny. In some instances, small stages of both predator species had similar effects and large stages differed dramatically (for example, had opposite effects relative to the control), whereas for others small stages were more dissimilar than large stages (for example, Figs 1 and 3). This ontogenetic change in species-specific differences could stem from a number of factors that differ among both species, including morphology, behaviour and how gape limitation scales with size (Supplementary Methods). There is also no reason to expect that differences among species remain constant over ontogeny. For instance, dragonfly larvae in our system switch in their microhabitat use (from vegetation to leaf litter) during ontogeny[21], although we did not find a clear indication that this also occurs for beetle larvae. Such differential shifts in microhabitat use could explain why functional differences between species changed during ontogeny. However, predicting the exact underlying mechanisms is challenging because of the complexity (that is, >65 morphospecies) of the system. However, regardless of the exact mechanisms, our results indicate that functional differences among species did not simply scale linearly with size, indicating that we cannot average across stages to estimate functional differences among species.

The ontogenetic shifts in functional differences among species highlight a new problem for biodiversity studies: how can we predict ontogenetic shifts in the functional differences among species? Our results clearly indicate that we cannot simply use size (or biomass) as a surrogate for species to predict the functional role of individuals in complex communities. However, there is no *a priori* reason to assume that functional differences within or among species would scale with size. The impact of individuals on the ecosystem depends on how it alters the functional composition of the community, that is, its direct and indirect interactions. Although interaction strength typically scales with consumer and resource size ratios, the presence/absence of ecological interactions also change with size during ontogeny. In complex communities, such ontogenetic niche shifts can quickly deteriorate the ability of size-differences to explain the impact of individuals on communities and ecosystem processes. Indeed, we found that stages interacted (directly and indirectly) with different components of the food web, indicating ontogenetic niche shifts in ecological interactions that lead to concurrent changes in ecosystem processes. Importantly, the changes in community structure could not be explained by simple shifts in the size structure of the community. This indicates that shifts in ecological interactions did not follow simple size-scaling feeding relationships but instead indicates ontogenetic shifts in behaviour and/or microhabitat use. Such ontogenetic shifts have been recorded in a large variety of vertebrate and invertebrate taxa[16,24]. However, although individuals within species clearly can change dramatically in their ecology during ontogeny[16,17,24], these changes are still constrained by the basic traits (for example, morphology) of the species itself. For instance, although small stages of both species should be more similar in the size range of prey they consume compared with large stages of both species, what individuals eat will depend on a variety of factors, such as their microhabitat they use, feeding mode and behaviour, all of which will be constrained by their morphologies. As a consequence, similar-sized individuals from different species could have very different effects on community structure as was the case in our study. Thus, future studies that identify how ontogenetic niche shifts in species interactions are constrained by species traits are needed to develop a new general framework that links functional variation within and between species to predict the relationship between community structure and ecosystem processes.

Methods

Experimental design. Larvae of the dragonfly *Anax junius* and the diving beetle *Cybister fimbriolatus* were collected from two local ponds in eastern Texas, and stage-specific densities represent natural densities. Although both species are known to be important apex predators (in their final stage) structuring fishless pond communities, they differ in many morphological and behavioural traits and how their morphology changes during development (see Supplementary Methods). Both species increase by more than 15-fold in length (mass) during their development, and the population structure of both species varies considerably across seasons and populations.

Identifying 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[37,38]. Moreover, neither density nor total biomass are ever constant across stage classes within our study species[39], 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[37,40]. Therefore, we refrained from keeping initial biomass or density constant and followed previous suggestions and designs that recommend using natural size–abundance relationships[21,37,38,40]. 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 seven treatments, each replicated six times ($N=42$ ponds) that manipulated the presence and absence of small (S; initial per capita dry mass $\sim$0.026–0.06 mg) and large (L; initial per capita dry mass $\sim$0.6 mg) stages of *A. junius* and *C. fimbriolatus* separately, resulting in a 2 (species) $\times$ 3 (stage combinations: S, L, S + L) factorial design plus one predator-free control treatment. Mesocosms either received 18 small *A. junius* or 3 large *A. junius* or both, or 18 small *C. fimbriolatus* or 3 large *C. fimbriolatus* or both, or none of these stages (control). The differences in stage structure (one or two stages present) reflect natural differences among populations (ponds) within a time period or seasonal changes within a population. Size classes were chosen based on the size structure of the natural population and to keep total predator biomass (larvae of *A. junius* or *C. fimbriolatus*) within a size class-treatment constant across species, while reducing the risk of early metamorphosis of the largest size class. Thus, density and per capita biomass of individual stages were similar across species and represented natural size–abundance relationships for each species (Supplementary Methods). This design allowed us to determine (i) the individual effect of each stage on the ecosystem, (ii) whether effects of stages within species were independent and (iii) whether functional differences among species changed with changes in stage structure within species.

Experimental communities and ecosystem properties. Mesocosms were constructed in 1,200-l cattle-watering tanks, filled with leaf litter, macrophytes, well water and nutrients. Complex communities were established using a combination of natural colonization of mesocosms and stocking animals from local pond communities. Community structure was estimated from counting, measuring and weighing over 35,500 individuals from >65 species, and included all invertebrates and vertebrates that emerged during the experiment, estimates from subsamples of zooplankton communities and whole-pond samples of all macroinvertebrates and vertebrates that were destructively collected at the end of the experiment (Supplementary Table S4). We also measured total animal biomass, primary producer biomass of benthic (periphyton) and pelagic (phytoplankton) algae using chlorophyll-*a* extractions, net primary productivity (NPP), respiration (R), and decomposition rates (k). Full description of methods is given in Supplementary Methods. All procedures were in compliance with ethical guidelines for animal use and approved by the Institutional Animal Care and Use Committee (IACUC Protocol no. A09022601).

Statistical analyses. Community structure was analysed using permutational multivariate statistics based on Bray–Curtis similarity metrics, with relative biomass or density of each species (that is, proportion of total community biomass or density of an experimental pond) as dependent variable. This allowed us to isolate treatment effects on community structure after correcting for potential differences in absolute biomass or density across treatments. All other ecosystem properties were analysed using generalized linear models with appropriate error structure in two ways. First, we analysed treatment effects based on untransformed values for all ecosystem properties. Second, to account for any potential differences in biomass effects among predator treatments, we estimated the per capita biomass effect ($=\text{effect}_B$) for each predator treatment (for details, see Supplementary Methods). Finally, we tested whether stages had independent (additive) effects when they co-occurred using three different null models that assumed additive, multiplicative

or biomass-corrected-independent effects of stages. A detailed description of statistical analysis is available in Supplementary Methods.

References

1. Sala, O. E. *et al.* Biodiversity - global biodiversity scenarios for the year 2100. *Science* **287,** 1770–1774 (2000).
2. Rudolf, V. H. W. & Lafferty, K. D. Stage structure alters how complexity affects stability of ecological networks. *Ecol. Lett.* **14,** 75–79 (2011).
3. Shackell, N. L., Frank, K. T., Fisher, J. A. D., Petrie, B. & Leggett, W. C. Decline in top predator body size and changing climate alter trophic structure in an oceanic ecosystem. *Proc. R. Soc. Lond. B* **277,** 1353–1360 (2010).
4. Hillebrand, H. & Matthiessen, B. Biodiversity in a complex world: consolidation and progress in functional biodiversity research. *Ecol. Lett.* **12,** 1405–1419 (2009).
5. Wright, J. P. *et al.* Conventional functional classification schemes underestimate the relationship with ecosystem functioning. *Ecol. Lett.* **9** (2006).
6. Tilman, D. *et al.* The influence of functional diversity and composition on ecosystem processes. *Science* **277,** 1300–1302 (1997).
7. Petchey, O. L. & Gaston, K. J. Functional diversity: back to basics and looking forward. *Ecol. Lett.* **9,** 741–758 (2006).
8. Griffin, J. N., Mendez, V., Johnson, A. F., Jenkins, S. R. & Foggo, A. Functional diversity predicts overyielding effect of species combination on primary productivity. *Oikos* **118,** 37–44 (2009).
9. Hooper, D. U. *et al.* Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* **75,** 3–35 (2005).
10. Stouffer, D. B., Sales-Pardo, M., Sirer, M. I. & Bascompte, J. Evolutionary conservation of species' roles in food webs. *Science* **335,** 1489–1492 (2012).
11. Bolnick, D. I. *et al.* Why intraspecific trait variation matters in community ecology. *Trends Ecol. Evol.* **26 (2011).**
12. Harmon, L. J. *et al.* Evolutionary diversification in stickleback affects ecosystem functioning. *Nature* **458,** 1167–1170 (2009).
13. Luck, G. W., Daily, G. C. & Ehrlich, P. R. Population diversity and ecosystem services. *Trends Ecol. Evol.* **18,** 331–336 (2003).
14. Benton, T. G., Plaistow, S. J. & Coulson, T. N. Complex population dynamics and complex causation: devils, details and demography. *Proc. R. Soc. Lond. B* **273,** 1173–1181 (2006).
15. Persson, L. Trophic cascades: abiding heterogeneity and the trophic level concept at the end of the road. *Oikos* **85,** 385–397 (1999).
16. Werner, E. E. & Gilliam, J. F. The ontogenetic niche and species interactions in size structured populations. *Annu. Rev. Ecol. Syst.* **15,** 393–425 (1984).
17. De Roos, A. M., Persson, L. & McCauley, E. The influence of size-dependent life-history traits on the structure and dynamics of populations and communities. *Ecol. Lett.* **6,** 473–487 (2003).
18. Munoz, A. A. & Ojeda, F. P. Guild structure of carnivorous intertidal fishes of the Chilean coast: implications of ontogenetic dietary shifts. *Oecologia* **114,** 563–573 (1998).
19. Polis, G. A. Age structure component of niche width and intraspecific resource partitioning: can age groups function as ecological species? *Am. Nat.* **123,** 541–564 (1984).
20. Woodward, G. & Hildrew, A. G. Body-size determinants of niche overlap and intraguild predation within a complex food web. *J. Anim. Ecol.* **71,** 1063–1074 (2002).
21. Rudolf, V. H. W. & Rasmussen, N. L. Ontogenetic functional diversity: size-structure of a keystone predator drives functioning of a complex ecosystem. *Ecology* **94,** 1046–1056 (2013).
22. Cohen, J. E. *et al.* Improving food webs. *Ecology* **74,** 252–258 (1993).
23. Gilljam, D. *et al.* in *Adv. Ecol. Res. Vol.* 45, 67–133 (Academic Press, 2011).
24. Miller, T. E. X. & Rudolf, V. H. W. Thinking inside the box: community-level consequences of stage-structured populations. *Trends Ecol. Evol.* **26,** 457–466 (2011).
25. Ings, T. C. *et al.* Ecological networks–beyond food webs. *J. Anim. Ecol.* **78,** 253–269 (2009).
26. Otto, S. B., Rall, B. C. & Brose, U. Allometric degree distributions facilitate food-web stability. *Nature* **450,** 1226–1229 (2007).
27. Lawton, J. H. & Brown, V. K. in *Biodiversity and ecosystem function* (Schulze, E. D. & Mooney, H. A. eds) 255–270 (Spinger, 1993).
28. Cardinale, B. J. *et al.* Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* **443,** 989–992 (2006).
29. Reiss, J., Bridle, J. R., Montoya, J. M. & Woodward, G. Emerging horizons in biodiversity and ecosystem functioning research. *Trends Ecol. Evol.* **24,** 505–514 (2009).
30. Yang, L. H. & Rudolf, V. H. W. Phenology, ontogeny, and the effects of climate change on the timing of species interactions. *Ecol. Lett.* **13,** 1–10 (2010).
31. Boege, K. & Marquis, R. J. Facing herbivory as you grow up: the ontogeny of resistance in plants. *Trends Ecol. Evol.* **20 (2005).**
32. Persson, L. *et al.* Gigantic cannibals driving a whole-lake trophic cascade. *Proc. Natl Acad. Sci. USA* **100,** 4035–4039 (2003).
33. Rudolf, V. H. W. Seasonal shifts in body size diversity and trophic interactions in size-structured predator-prey systems. *J. Anim. Ecol.* **81,** 524–532 (2012).
34. Urban, M. Predator size and phenology shape prey survival in temporary ponds. *Oecologia* **154,** 571–580 (2007).
35. Persson, L. *et al.* Culling prey promotes predator recovery - alternative states in a whole-lake experiment. *Science* **316,** 1743–1746 (2007).
36. Cardinale, B. J. *et al.* Biodiversity loss and its impact on humanity. *Nature* **486,** 59–67 (2012).
37. Chalcraft, D. R. & Resetarits, W. J. Metabolic rate models and the substitutability of predator populations. *J. Anim. Ecol.* **73,** 323–332 (2004).
38. O'Connor, N. E., Grabowski, J. H., Ladwig, L. M. & Bruno, J. F. Simulated predator extinctions: Predator identity affects survival and recruitment of oysters. *Ecology* **89,** 428–438 (2008).
39. Wissinger, S. A. Niche overlap and the potential for competition and intraguild predation between size-structured populations. *Ecology* **73,** 1431–1444 (1992).
40. Schneider, F. D., Scheu, S. & Brose, U. Body mass constraints on feeding rates determine the consequences of predator loss. *Ecol. Lett.* **15,** 436–443 (2012).

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Author contributions

V.H.W.R. and N.L.R. performed the experiment and wrote the manuscript, V.H.W.R. conceived, designed and analysed the experiment.

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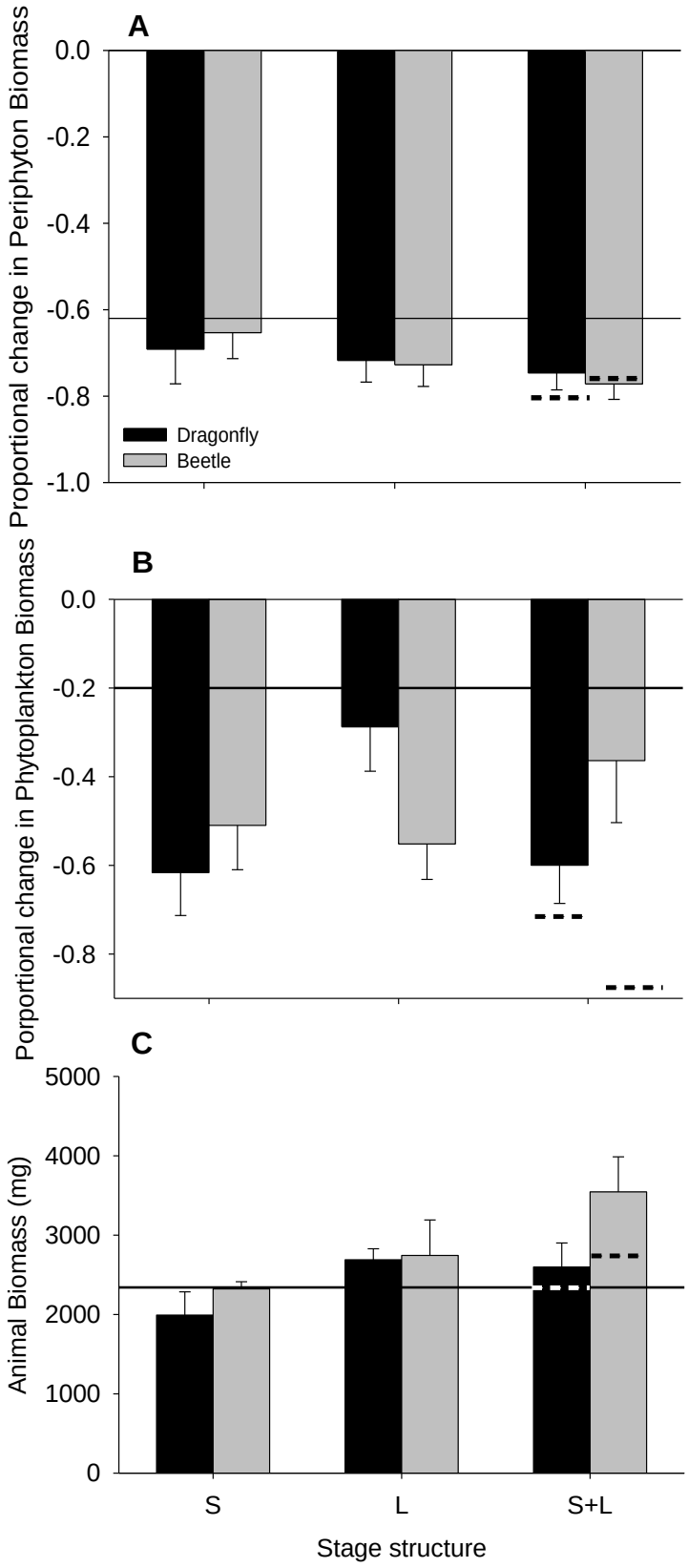
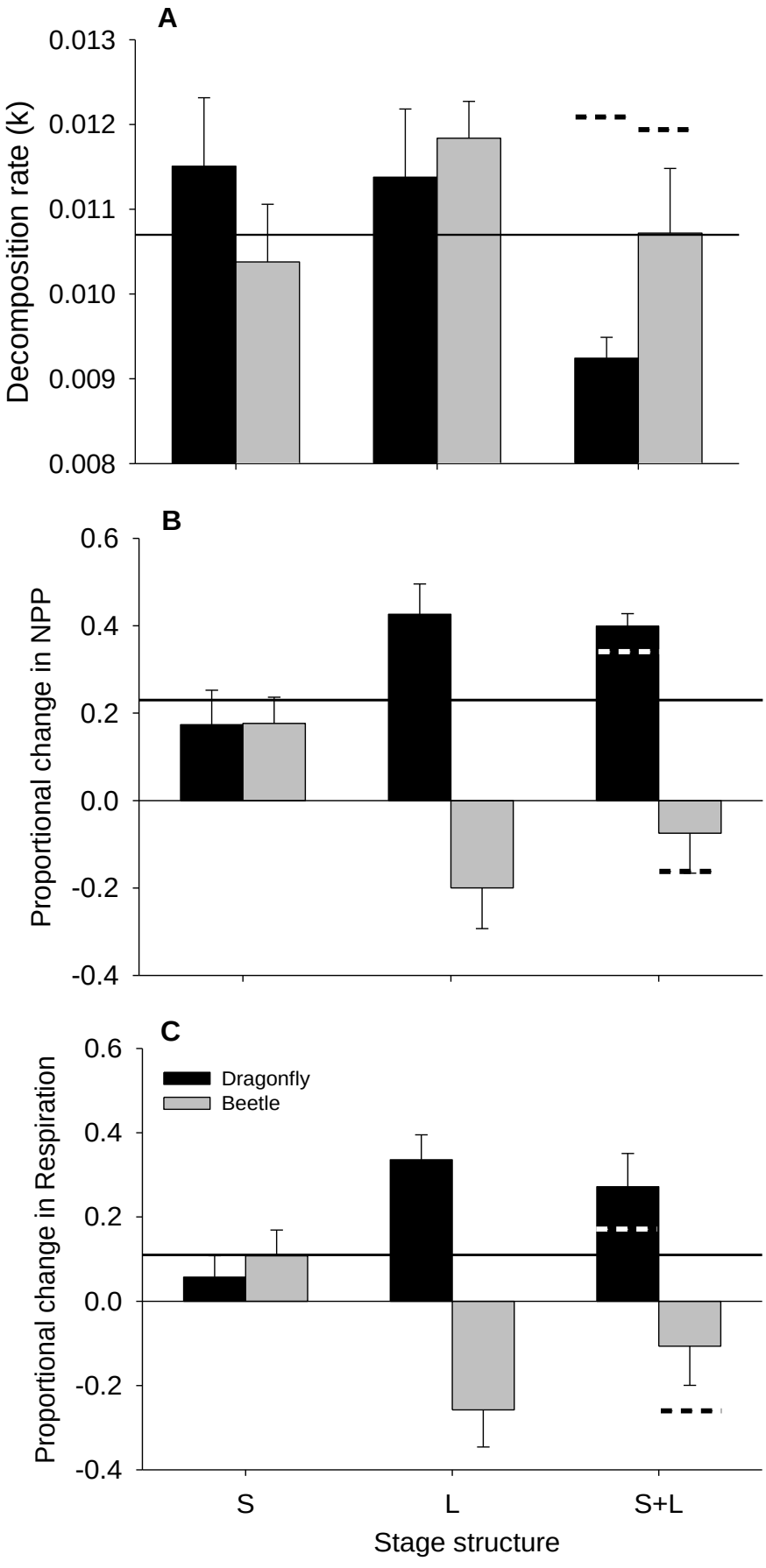
Supplementary Figure S1: Differences in primary producer and animal biomass. Bars indicate treatment means ±1SE, stage structure indicates what stage of a predator species is present: S = small stage, L=large stage, S+L= both stages co-occur. Solid lines indicate the mean observed in controls. Dashed lines indicate expected values for the respective response variable when both stages of a species co-occur based on additive effects model assuming independent effects of each stage (see S7 for details). (**a**) and (**b**) indicate proportional change in respective primary producer biomass over the course of the experiment based on chlorophyll-a concentrations. (**c**) Dry biomass of all macro-invertebrates and amphibians at the end of the experiment.


A
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Dragonfly
Beetle
Proportional change in Periphyton Biomass
B
0.0
-0.2
-0.4
-0.6
-0.8
Porportional change in Phytoplankton Biomass
C
5000
4000
3000
2000
1000
0
Animal Biomass (mg)
S
L
S+L
Stage structure

Supplementary Figure S2: Differences in ecosystem processes. Bars indicate treatment means ±1SE, size structure indicates what stage of a predator species is present (see Fig. 2). Solid lines indicate the mean observed in controls. Dashed lines indicate expected values for the respective response variable when both stages of a species co-occur based on additive effects model assuming independent effects of each stage (see S1 for analysis details). (**a**) Leaf litter decomposition rate (k) (see Supplement methods for details). (**b**) and (**c**) indicate proportional change in net primary productivity (NPP) and respiration (R) based on diurnal cycles of dissolved oxygen over the duration of the experiment (see S1 for details).


A
0.013
0.012
0.011
0.010
0.009
0.008
Decomposition rate (k)
B
0.6
0.4
0.2
0.0
-0.2
-0.4
Proportional change in NPP
C
Dragonfly
Beetle
0.6
0.4
0.2
0.0
-0.2
-0.4
Proportional change in Respiration
S
L
S+L
Stage structure

Supplementary Table S1: Effects of stages and species on ecosystem processes

	Biomass of trophic functional group					
Source of variation	Periphyton		Phytoplankton		Animal	
Species	$\chi^2_{1,29}$	0.0	$\chi^2_{1,29}$	0.1	$\chi^2_{1,30}$	$3.0^{\dagger}$
Stage	$\chi^2_{2,29}$	2.4	$\chi^2_{2,29}$	2.2	$\chi^2_{2,30}$	9.5^{**}
Species*Stage	$\chi^2_{2,29}$	0.3	$\chi^2_{2,29}$	6.9^{*}	$\chi^2_{2,30}$	1.6
	Ecosystem rate					
Source of variation	NPP		R		Decomposition (k)	
Species	$F_{1,29}$	41.9^{****}	$F_{1,29}$	31.7^{****}	$\chi^2_{1,22}$	0.4
Stage	$F_{2,29}$	0.9	$F_{2,29}$	0.6	$\chi^2_{2,22}$	10.5^{**}
Species*Stage	$F_{2,29}$	22.8^{****}	$F_{2,29}$	24.0^{****}	$\chi^2_{1,22}$	6.7^{*}

All analyses are based on raw data without correcting for potential differences in biomass across predator treatments. See Table 1 in main text for analyses of per-unit biomass effects of predators. Degrees of freedom were adjusted for block effects and missing replicates because of removal of significant outliers. (Note that removal of outliers did not alter general patterns). P values are based on general linear models (with corresponding F- or likelihood-ratio χ^2-statistics), and corrected for missing replicates (see Analysis for details). †P<0.1, ‡P<0.06, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Supplementary Table S2: Differences in expected vs. observed effects on ecosystem responses for treatments where small and large

	Multiplicative model*		Additive model*		Biomass corrected model*	
	Anax	*Cybister*	*Anax*	*Cybister*	*Anax*	*Cybister*
Ecosystem properties & functions						
Animal biomass	t_5=0.80, P = 0.458	t_5=1.86, P = 0.120	t_5=0.66, P = 0.539	t_5=2.61, **P = 0.047**	t_5=0.85, P = 0.433	t_5=1.95, P = 0.109
Change in periphyton	t_4=1.15, P = 0.300	t_5=0.24, P = 0.815	t_4=1.00, P = 0.37	t_5=0.388, P = 0.714	t_4=12.63, **P = 0.0002**	t_5=28.3,1 **P <0.0001**
Change in phytoplankton	t_5=3.25, **P = 0.023**	t_5=7.25 **P = 0.0008**	t_5=1.18, P = 0.297	t_5=3.5, **P = 0.017**	t_4=1.27, P = 0.261	t_5=5.4, **P = 0.003**
Decomposition rate	t_5=4.90, **P = 0.004**	t_4=1.57, P = 0.191	t_5=5.69, **P < 0.003**	t_4=1.60, P = 0.186	t_4=1.60, P = 0.186	t_4=1.60, P = 0.186
Change in respiration rate	t_5=1.78, P = 0.292	t_5=1.61, P = 0.168	t_5=0.17, P = 0.871	t_5=1.608, P = 0.169	t_5=1.608, P = 0.169	t_5=1.608, P = 0.169
Change in net primary productivity	t_5=0.92, P = 0.401	t_5=0.993, P = 0.366	t_5=0.26, P = 0.807	t_5=1.765, P = 0.138	t_5=1.765, P = 0.138	t_5=1.765, P = 0.138

stages co-occur.

* type of models used for estimating expected values (see Supplementary Methods “Intraspecific diversity effects” for details).

Statistics are based on two-tailed t-tests; differences in df are due to removal of significant outliers.

Supplementary Methods

Focal organisms

Larvae of the dragonfly *Anax junius* and the diving beetle *Cybister fimbriolatus* are wide-spread species in North America. We chose both species for several reasons. Final instars of both species are top predators in fishless ponds, and both species are known to strongly determine the structure of these pond communities [41-46]. Both species increase by more than 15 fold in length (mass) during their development. Depending on the season, a population can consist of a single cohort composed entirely of either small- or large-bodied larvae (beginning vs. end of reproductive season), or it can be strongly size-structured when multiple cohorts overlap (e.g., middle of breeding season) [39,46]. In south east Texas, similar differences in stage structure can also occur within a season across populations (ponds) because of differences in the onset of reproduction across populations. In addition, our preliminary stable isotope analysis indicates that both species show significant changes in trophic position (measured with relative differences in $\delta^{15}N$) with size during their ontogeny. However, although both species occupy a seemingly similar ecological role, they differ substantially in key aspects of their morphology (e.g. gills vs. breathing air, chewing mouth parts vs. piercing mouth parts and poison), development, and changes in gape width over ontogeny (*Anax*: 11-13 instars with different gape width vs. *Cybister:* 3 instars with different gape width), and ecology (e.g. habitat use, hunting mode). Given these differences both species are expected to show some differences in their functional role and the relative importance of size and strength of ontogenetic niche shifts (i.e. functional differences among stages).

Experimental design

Detecting functional differences among predators is challenging when predators differ substantially in their biomass. Traditional designs typically standardize either predator biomass or density among treatments using additive or substitutive designs. While such designs may be appropriate for systems where predators differ little in size, it leads to dramatic differences in density or biomass when predators differ in size. As a consequence, conclusions about identity effects are inevitably confounded by biomass or density [37,38]. Recent studies indicate that density-dependent effects cause population effects to differ from that expected based on allometric (or biomass) scaling relationships [40]. Consequently, the null models underlying traditional designs that keep density or biomass constant are likely to be incorrect. For systems in which the functional role of individuals largely depends on body size (mass), there is a high risk of misinterpreting results when using traditional additive or substitutive designs [37-40]. Furthermore, neither biomass nor density are ever constant across stages/size classes in natural populations of our study species [39], and keeping total biomass constant would lead to unrealistically high densities and experimental artifacts with little relevance to natural systems. Thus, we refrained from keeping biomass or density constant. Instead, we followed suggestions from previous studies [37-40] and used natural mass- abundance relationships based on field densities. 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 from qualitative differences among size classes. Future studies that adjust densities based on metabolic demands could provide additional mechanistic insight into how potential stage specific differences in metabolic rates influence a species functional role, but this was outside of the scope of this study.

The experiment consisted of seven treatments each replicated six times (N = 42 ponds) that manipulated the presence/absence of two size classes of either *A. junius* or *C. fimbriolatus.*

Mesocosms either received 18 small *A. junius* (head width (HW): 2.85-3.3 mm, body length (BL): 8.9-11.7 mm, dry mass (DM): 0.026-0.05 mg)), or 3 large *A. junius* (HW: ~6.9 mm, BL: ~32 mm, DM: ~0.61 mg) or both, or 18 small *C. fimbriolatus* (HW: 1.9-2.1 mm, DM: 0.027-0.06 mg) or 3 large *C. fimbriolatus* (HW: ~5.2, DM: ~0.64 mg) or both, or none of these stages (control). Such differences in stage-structure (one or two stages present) reflect natural differences among populations (ponds) within a time period or seasonal changes within a population. Size classes were chosen based on the size structure of the natural population and to keep total predator biomass (larvae of *A. junius* or *C. fimbriolatus*) within a size class treatment constant across species, while reducing the risk of early metamorphosis of the largest size class. We collected all *A. junius* and *C. fimbriolatus* larvae from two local ponds.

Experimental communities

The experiment was carried out in mesocosms that closely mimicked the structure and complexity of local fishless ponds that are dominated by invertebrate predators. Mesocosms 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 seven weeks 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. On May 14th, we added to each tank 220 g (wet mass) of macrophytes collected from one local pond (mostly *Potamogeton sp.* and *Najas sp.*). To establish a natural complex community, one week before the start of the experiment 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. 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. In addition we collected a diverse range of larger invertebrates (including other predatory insects, see Supplementary Table S3) from several local ponds, which were added in equal numbers to all tanks (from May 22nd – June 1st). 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 (Supplementary Table S3). The experiment started on June 3rd when the different *Anax* and *Cybister* stages were added to the tanks. The experiment was terminated after three weeks when the first *Anax* started to emerge.

Ecosystem functions and properties

Decomposition

We estimated decomposition rates within a pond from a mixture (mostly oak) of dried leaves over the entire duration of the experiment. Each pond received two leaf litter bags (15 x 20 mm, mesh size: 3.5 mm^2) each filled with 2500 mg of oven-dried leaves (48 h at 60°C) from the same

random mixture of leaf litter added to the ponds. Decomposition rates (*k*) were calculated from the 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 took weekly measurements to estimate net primary productivity (NPP) and respiration (R) calculated from diurnal oxygen cycles [47,48]. We measured Dissolved Oxygen (DO) with an oxygen probe (YSI, Professional Plus) three times a day: at sunrise (t_0), sunset (t_1), and the following sunrise (t_2). NPP is given by the increase in DO_{t1-t0}, and R by the decrease in DO_{t1-t2}.

Primary producer biomass

We estimated standing biomass of two dominant forms of primary producer in our ponds, periphyton (benthic algae) and phytoplankton (pelagic algae). Standing biomass of periphyton was estimated weekly from three glass microscope slides per tank (0.74 cm x 0.25 cm) that were propped at an angle against the side of the tank above the leaf litter layer for seven days. 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. Biomass of Phytoplankton was estimated from 250-ml water samples collected at mid water level weekly from each tank. Periphyton and phytoplankton concentrations were then determined fluorometrically (AquaFluor, Turner Designs) through chlorophyll-a extraction in 95% Ethanol following standard protocols [49]. The first sample was taken one week after the start of the experiment and then every week for three consecutive weeks.

Community structure

Sampling - We quantified the structure of the zooplankton, amphibian, and macro-invertebrate (benthic, vegetation, and total) community by counting, measuring, and weighing over 35,500 individuals from >65 species (see Supplementary Table S3). We monitored tanks daily for emerging insects and amphibians during the experiment. Amphibian metamorphs were weighed after tail absorption and released at the origin of capture. We converted metamorph wet mass 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 2.5 L) using a depth integrated tube sampler. The samples were filtered through an 80-µm 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 (30.5 cm 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 through the leaf litter layer. Vegetation and benthos sample were then carefully rinsed, filtered, and the contents preserved in 75% ethanol. Finally, we destructively sampled mesocosms and collected 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 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 48 h. Species specific invertebrate and amphibian dry masses (including emerged individuals) 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 [50] length-mass

regressions. The obtained invertebrate dry mass estimates 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).

Quantifying community structure - Community structure was analyzed for both the abundances and total biomass of species. The goal of this analysis was to detect whether species and stages of *A. junius* and *C. fimbriolatus* have different effects on the community structure rather than the total abundance of species (which was analyzed separately). Thus analyses were carried out using relative biomass or density of each species (i.e. proportion of total community biomass or density of an experimental pond) as response variable to test for differences in community structure across treatments. For the biomass analysis we scaled zooplankton samples up to the volume of the whole tanks. We did not scale the densities of zooplankton up to whole tank volume since this would have resulted in zooplankton species being up to10,000 times 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 (results are reported in Table 1 in main text). A separate analysis on only macro-invertebrates without zooplankton species showed a very similar pattern (PERMANOVA Size*Species: $P = 0.045$, Size: $P = 0.603$, Species: $P = 0.112$, Block: $P < 0.001$). In conclusion, regardless of how and whether zooplankton densities were included in the density-based analysis of community structure, statistical analyses

always indicated a significant species* size interaction. Consequently we only show the analysis that includes the rescaled zooplankton densities in the main text as this includes the most information (i.e. species). A full species list with corresponding average densities and biomass for each treatment are given in Supplementary Table S3. Finally, we analyzed changes in the size-structure of the macro-invertebrate community (not including Zooplankton species) by comparing square-root transformed abundance of individuals within $\log_{10}$ size classes based on dry mass. The two focal predator species were never included in any of the analyses on community structure or animal biomass.

Statistical analyses

The goals of this experiment were to determine whether changes in the population (stage) alters **i)** the functional role of a species at the ecosystem level, and **ii)** functional differences among species, and **iii)** whether stages had independent effects (see "Interspecific diversity effects" below for details). To answer i) and ii) we used a 2 (species) x 3 (stage) factorial design to test how changes in stage-structure and species identity influence the respective response variables and whether these effects were independent. A significant interaction (species*stage) indicates that functional differences among species are not constant, but instead change depending on the specific stage-structure of the species. A significant stage effect indicates that the population structure of a species determines its functional role in the ecosystem. To account for any potential differences in biomass effects among treatments, we estimated the per-capita biomass effect (= $\mathbf{effect_B}$) for each predator treatment on all ecosystem processes (see details in section: "*Biomass corrected analysis*") except for community structure because the latter analyses already account for potential biomass differences among predator treatments. Results of the biomass corrected analyses (see Table 1, Fig. 2, 3) are qualitatively similar to the full analyses of

predator effects on the untransformed data which are given in Supplementary Table S1 and Figures S1, S2.

Ecosystem responses

To account for natural variation in initial conditions among tanks we used proportional change [(final sample-first sample)/first sample] in NPP, Respiration and periphyton and phytoplankton biomass. NPP and Respiration, periphyton, phytoplankton, animal biomass, and decomposition rates were analyzed with a general linear mixed model with normal or gamma distributed error terms (depending on the variable) and species, size and their interaction (species*size) as fixed effects and block as a random effect using SAS [51]. When block effects were not significant, block degrees of freedom were pooled with the error term degrees of freedom for the final analysis [51]. One tank was a significant outlier (based on studentized residual outlier test with Bonferroni adjustments and interquartile range detection of outliers), likely because of dramatic differences in community structure (see below), and thus removed from these analyses. To account for differences in sample size all reported test statistics and P values are based on likelihood estimates and type III sums of squares.

Community structure

We analyzed differences in the structure of communities among treatments using non-parametric, permutational multivariate statistics based on Bray-Curtis similarity metrics using PRIMER [52]. First, we tested whether the variability in community structure (i.e. dispersion) differed among treatments using PERMDISP [53]. Secondly, if treatments met the assumption of similar variances (in general we found no significant differences in dispersion among treatments) we tested whether communities differed significantly among treatments using permutational

multivariate analysis (PERMANOVA)[54,55]. 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 using non-metric Multi-Dimensional Scaling plots (nMDs) using the packages "Vegan", "Ecodist", "BiodiversityR", and "Ellipse" implemented in the R-software. One tank was a significant outlier (based on ordered squared robust Mahalanobis distances) and thus removed from the analysis. This was largely driven by the 19-61 times lower biomass of *Hyla* and *Rana* tadpoles compared to the average across all other 41 tanks and corresponding dramatic increase in some zooplankton (in particular Ostracods). It is possible that this was mediated by a disease outbreak in the tadpoles or low water quality with associated bacterial bloom but the exact reasons remained unclear.

Relationships among variables

To identify whether the changes in animal community composition were at least partly responsible for changes in other ecosystem properties, we analyzed the partial correlations among ecosystem properties. Due to the complexity of the pond communities, we used two non-metric multi-dimensional scaling metrics to represent community structure in this analysis. In general, the qualitative relationship between community structure and other ecosystem response variables were similar regardless of whether community structure was based on relative or total biomass or density indices. Because we were most interested in how community composition was related to ecosystem processes we only represent here one example with community structure based on proportional biomass which also provided the best model fit.

Results: Table 1 (in main text) summarizes the partial correlation coefficients. Overall,

we found that community composition (based on relative biomass of species) was indeed strongly correlated with all other ecosystem processes. Interestingly, community structure was more strongly correlated with NPP and respiration than periphyton or phytoplankton. Furthermore, one community composition score was typically associated with NPP and respiration, while the other was strongly correlated with primary producer biomass. In general this is consistent with the hypothesis that predator mediated effects on primary producer and ecosystem rates were indirectly driven by changes in community composition. Since the analyses were based on proportional biomass of species, this suggests that changes in predator stage structure or species identity lead to functional shifts in community composition.

Biomass corrected analyses & results

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 [37,38], it inevitably also results in differences in biomass among stages within predator treatments. To test whether these potential differences among predator stages were important in explaining the observed results, we first tested whether and how average predator (*Anax* or *Cybister*) biomass differed among stage treatments and then tested whether the per-unit biomass effect size (= $\mathbf{effect_B}$) differed among predator size treatments.

Predator biomass across treatments: We calculated the average of the total dry biomass of *Anax* and *Cybister* populations within a pond 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. While the average size of individuals remained significantly different between stage treatments for the duration of the experiment, the average total (population) biomass of *Anax* was not significantly different between treatments with large stages (mean = 264.3 mg, s.e.m. = ±22.2) and small stages (mean = 226.8 mg, s.e.m.= ±15.4) (unequal variance t-test: P= 0.29). Biomass in treatments with both stages (S+L) was significantly lower than expected from the sum of both S and L treatments (expected: 508 mg vs. observed: mean= 341.0 mg, s.e.m.= ±35.8)(P=0.006). Average *Cybister* biomass was significantly higher in treatments with large stages (mean = 217.1 mg, s.e.m.= ±4.4) than in treatments with small stages (mean = 135.7 mg, s.e.m.= ±11.4) (unequal variance t-test: P<0.001). Biomass in treatments with both stages (S+L) was not significantly (P = 0.286) different from biomass expected from the sum of S and L treatments (expected: 352 mg; observed: mean= 320.8 mg, s.e.m. = ±26.8). While this exponential model is the most biologically 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 (exponential) model here.

Per unit biomass effect of predators: We calculated the biomass corrected effect (**$effect_B$**) of different predator treatments on a given ecosystem response variables (X) as $X_B = (X_{JP} - X_C)/B_J$, where X_{JP} indicates the value of a given response variable for pond P in predator treatment J (AS, AL, ASL, CS, CL, CSL), 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 mesocosms had larger values than the control and negative values the opposite. Results of these analyses are given in Table 1 and Figures 1 & 2 in the main text.

Intraspecific diversity effects – Additive vs. non-additive effects of multiple stages

When both stages of a species are present in the same pond this allows for indirect interactions that can lead to non-additive (diversity) effects. If such non-additive interactions are present, the observed effects in treatments with both stages should be different from expectations calculated from the average effects of individual stage treatments and the control [56]. The biomass corrected effect ($effect_B$) on ecosystem processes (Table 1, Fig. 2-3) already accounts for potential non-additive effects that may stem from predation among functional groups (i.e. cannibalism). Thus, we first used the raw data to calculate the expected effect for S+L treatments on the different ecosystem traits. Given the complexity of the system, it is not clear whether expected effects of both stages should be additive or multiplicative. Therefore, we calculated both types of effects. Given our additive design, multiplicative effects are given by: $x_S * x_L / x_C$, while the additive model is given by: $(x_S - x_C) + (x_L - x_C) + x_C$ (21), where x_S, x_L, and x_C indicate the respective response variable (NPP, R, decomposition rate, periphyton, phytoplankton) of mesocosms with only the small, or large stage, or control respectively. This was done for each species separately. We then compared predictions to observed values of the respective response variables in mesocosms with both stages present using two-tailed t-test since we had no a priori expectations about the directional differences [56]. Because additive and multiplicative models showed similar qualitative results we only present expected values for S+L treatments from additive models (in Supplementary Fig. S1, S2) which were generally more conservative.

Results & *Discussion* – Supplementary Table S2, Figures S1 and S2 summarize the

expected values for six different ecosystem properties and whether they differ significantly from observed values of the respective ecosystem property. At least one null model (additive or multiplicative) indicated "non-additive" (diversity) effects for phytoplankton biomass in dragonfly and beetle treatments. In addition, both models indicated non-additive effects of dragonfly stages on decomposition rates, and there was some support indicating non-additive effects on animal biomass in beetle treatments (Supplementary Table S2). Observed values were lower than expected values for phytoplankton and decomposition rates, while the opposite was true for animal biomass. Combined effects of stages in both species were not significantly different from null models assuming additive effects for NPP, respiration, and periphyton biomass (Supplementary Fig. S1, S1, Table S2). While it may seem somewhat surprising that we observed non-additive effects on phytoplankton biomass but not on NPP and respiration, this can be explained by the fact that phytoplankton biomass was only weakly correlated with NPP and respiration. Instead NPP and respiration were more strongly correlated with the animal community composition (see Table 1). Given the substantial variation in community structure within treatments and the potential for "functional redundancy" (with regards to their impact on NPP and respiration) among individuals and species within the community, this could explain why we did not detect non-additive effects for NPP and respiration.

These non-additive effects indicate the presence of indirect interactions [57,58]. Given the complexity of our pond communities it is difficult to infer the exact nature of these indirect interactions. However, both of our species are highly cannibalistic, and previous studies suggest that cannibalism in a predator can often alter the combined effect of different predator stages on prey survival through consumptive and non-consumptive (behavioral) mediated indirect interactions that could explain the observed non-additive effects of stages [59-61]. For instance, the

presence of large cannibals can reduce the density or foraging rate of small conspecific victims and thereby indirectly increase prey survival. We found that survival of small *Anax* was generally lower (~50%) in the presence of larger conspecifics (unequal variance t-test: $P = 0.011$). We did not find significant differences in survival of *Cybister* larvae among treatments (t-test: $P > 0.55$), but survival was generally lower in these treatments than in *Anax* treatments. Although we cannot directly identify the cause of mortality, we did occasionally observe large larvae of both species (*Cybister* and *Anax*) consume smaller conspecifics during our daily monitoring, suggesting that cannibalism may indeed be responsible for the reduced survival of small *Anax* and *Cybister*. The reduced survival of small stages (at least in *Anax* treatments) in the presence of cannibalistic conspecifics should reduce the combined effect of both stages on their prey and thereby indirectly also ecosystem processes. Indeed, the combined effects of both beetle stages were lower than expected on animal (i.e. their pey) biomass, and decomposition rates were reduced as well, consistent with the presence of a trophic cascade. However, it cannot explain why a similar pattern was observed in beetles, where survival of small stages was not significantly lower in the presence of large stages or why phytoplankton decreased less than expected when both stages were present in both predator treatments.

Cannibalism mediated behavioral interactions among predator stages (i.e. small individuals often alter their foraging behavior or habitat in the presence of large cannibalistic conspecifics) and/or prey (e.g. prey respond differentially to predator stages) could potentially explain some of these patterns [60]. Indeed, previous studies indicate that the indirect effects of behavioral responses can have equal or even larger effects than the consumption (cannibalism) of small predators in some cannibalistic species [61]. To test whether the observed diversity effects were largely driven by the reduction in predator density due to cannibalism, we adjusted our null

models to account for the potential reduction in predator biomass in treatments where both stages are present. In particular, we calculated the expected per-unit biomass effect for S+L treatments as: $(X_S*B_S)+(X_L*B_L)/(B_S+B_L)$, where X_S and X_L represent the per-unit biomass effect for small and large predator stages respectively (for calculation details see section: "Biomass corrected analysis" above) and B_S and B_L B_{S+L} the corresponding average biomass of for a given stage structure treatment. While the non-additive effect for decomposition rates disappeared after accounting for the reduction in predator biomass, we still found non-additive "diversity effects" on primary producer biomass. Thus, the differences between observed and expected effects on ecosystem properties likely was driven by a combination of both consumptive and non-consumptive mediated indirect interactions in our system.

Supplementary References

41. J. E. Fauth, Identifying potential keystone species from field data - An example from temporary ponds. *Ecol. Lett.* **2**, 36 (Jan, 1999).

42. M. A. McPeek, The consequences of changing the top predator in a food web: A comparative experimental approach. *Ecol. Monogr.* **68**, 1 (Feb., 1998).

43. J. Van Buskirk, Interactive effects of dragonfly predation in experimental pond communities. *Ecology* **69**, 857 (Jun., 1988).

44. J. V. Robinson, G. A. Wellborn, Mutual predation in assembled communities of odonate species. *Ecology* **68**, 921 (Aug., 1987).

45. H. M. Wilbur, J. E. Fauth, Experimental aquatic food webs - Interactions between 2 predators and 2 prey. *Am. Nat.* **135**, 176 (FEB, 1990).

46. P. W. Crumrine, Body size, temperature, and seasonal differences in size structure influence the occurrence of cannibalism in larvae of the migratory dragonfly, Anax junius. *Aquat. Ecol.* **44**, 761 (Dec, 2010).

47. A. L. Downing, M. A. Leibold, Ecosystem consequences of species richness and composition in pond food webs. *Nature* **416**, 837 (Apr 25, 2002).

48. R. G. Wetzel, G. E. Likens, *Limnological analyses*. (Springer, New York, ed. 3rd, 2000), pp. 444.

49. A. D. Eaton, L. S. Clesceri, E. W. Rice, A. E. Greenberg, M. A. H. Franson, *Standard Methods for examination of water and wastewater*. (American Public Health Association, American Water Works Association, Water Environment Federation, 2005), pp. 1368.

50. A. C. Benke, A. D. Huryn, L. A. Smock, J. B. Wallace, Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *J. North. Am. Benthol. Soc.* **18**, 308 (1999).

51. R. C. Littell, G. A. Milliken, W. W. Stroup, R. D. Wolfinger, O. Schabenberger, *SAS for mixed models* (SAS Institute Inc., Cary NC, ed. 2nd, 2006), pp. 813.

52. K. Clarke, R. Gorley, *PRIMER v6: User Manual/Tutorial*. (PRIMER-E, Plymouth, 2006).

53. M. J. Anderson, Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* **62**, 245 (2006).

54. M. J. Anderson, A new method for non-parametric multivariate analysis of variance. *Austral. Ecol.* **26**, 32 (2001).

55. B. H. McArdle, M. J. Anderson, Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* **82**, 290 (2001).

56. O. J. Schmitz, L. Sokol-Hessner, Linearity in the aggregate effects of multiple predators in a food web. *Ecol. Lett.* **5**, 168 (Mar, 2002).

57. A. Sih, G. Englund, D. Wooster, Emergent impacts of multiple predators on prey. *Trends Ecol. Evol.* **13**, 350 (Sep, 1998).

58. J. T. Wootton, The nature and consequences of indirect effects in ecological communities. *Annu. Rev. Ecol. Syst.* **25**, 443 (1994).

59. V. H. W. Rudolf, The influence of size-specific indirect interactions in predator-prey systems. *Ecology* **87**, 362 (2006).

60. V. H. W. Rudolf, Consequences of stage-structured predators: Cannibalism, behavioral effects and trophic cascades. *Ecology* **88**, 2991 (2007).

61. V. H. W. Rudolf, The impact of cannibalism in the prey on predator-prey dynamics. *Ecology* **89**, 3116 (2008).