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Ontogenetic development underlies population response to mortality

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Understanding demographic responses to mortality is crucial to predictive ecology. While classic ecological theory posits reductions in population biomass in response to extrinsic mortality, models containing realistic developmental change predict the potential for counterintuitive increase in stage-specific biomass, i.e. biomass overcompensation. Patterns of biomass overcompensation should be predictable based on differences in the relative energetic efficiencies of juvenile maturation and adult reproduction. Specifically, in populations where reproduction is the limiting process, adult-specific mortality should enhance total reproduction and thus juvenile biomass. We tested this prediction by inducing an array of stage-specific harvesting treatments across replicate populations of *Daphnia pulex*. In accordance with reproductive regulation, the greatest biomass response occurred in the juvenile *Daphnia* stage and this response occurred most strongly in response to adult mortality. Nevertheless, we failed to detect significant biomass overcompensation and instead report largely compensatory effects. In total, our work demonstrates that knowledge of population structure is necessary to accurately predict population dynamics, but cautions that further research is needed to illuminate the factors generating over-compensatory versus compensatory responses across natural populations.

Keywords: biomass overcompensation, *Daphnia pulex*, development, harvesting, ontogenetic asymmetry, stage-structure

Introduction

Understanding how populations respond to perturbations, such as changes in mortality or resource productivity, is central to predicting ecological dynamics (Nicholson 1957, Turchin 2003, Yang et al. 2008). Our understanding of such responses traditionally derives from unstructured models which assume reproduction and mortality are the only processes underlying population change. Yet, ontogenetic development is ubiquitous within natural populations (Werner and Gilliam 1984, Miller and Rudolf 2011, Rudolf and Lafferty 2011), and both reproductive output and mortality risk change as individuals grow (i.e. size- or stage-dependent life history traits; de Roos and Persson 2013). Accordingly, theory incorporating size or stage-dependence produces an array of population dynamic patterns not possible in analogous unstructured

models, including biomass overcompensation, cohort cycles, emergent facilitation and alternative stable community states (de Roos et al. 2003, Miller and Rudolf 2011, de Roos and Persson 2013, Huss et al. 2013, Schröder et al. 2014). While developmental variation has been incorporated into increasingly complex community models (van de Wolfshaar et al. 2006, Ohlberger et al. 2012, Leeuwen et al. 2014, Toscano et al. 2017), some of the most fundamental and far-reaching predictions of this new theory, such as population responses to perturbation, still require focused experimental testing. These tests are necessary to revise our understanding of the basic processes driving population and community dynamics.

Perhaps the most important and far-reaching way in which unstructured and structured model predictions diverge is regarding population responses to extrinsic mortality (de Roos et al. 2007), a widespread natural perturbation. Unstructured models generally predict reductions in population density and biomass due to mortality, and this basic prediction is implicit in the vast majority of classical ecological theory (but see Abrams 2009, Schröder et al. 2014 for scenarios in which unstructured models can produce positive responses to mortality, or 'hydra effects'). In contrast, structured models containing a realistic representation of the developmental process predict, counterintuitively, that biomass can increase in response to mortality (de Roos and Persson 2013, Schröder et al. 2014). The ultimate cause of such positive biomass responses is ontogenetic asymmetry, or differences in the relative energetic efficiencies of developmental stages (de Roos et al. 2007). For example, for fish populations, competition is generally more intense within the reproductive adult stage versus within the juvenile stage (i.e. juveniles use energy more efficiently), a scenario termed reproduction regulation (Persson and de Roos 2006, Schröder et al. 2009). Here, biomass builds up within the adult stage due to a reproductive bottleneck at normal background mortality levels, and increasing adult mortality is expected to enhance juvenile fish biomass via increased reproduction. More generally, mortality acts to release the energetically less efficient (i.e. regulatory) developmental stage from intra-stage competition, thereby enhancing the biomass production of this stage. Thus, positive effects of mortality on stage-specific biomass should be predictable based on the ontogenetic asymmetry of a population (Persson and de Roos 2013). Empirical tests of such predictions can illuminate the importance of developmental variation in governing natural population dynamics.

The magnitude of positive biomass responses to mortality within structured populations should further depend on the stage-specificity of mortality. Using a general structured consumer–resource model, de Roos et al. (2007) show that stage-specific biomass overcompensation is a widespread response to mortality in populations characterized by ontogenetic asymmetry: overcompensation occurs in response to both stage-independent and stage-specific mortality, even when stage-specific mortality is induced within the non-regulatory stage (i.e. the stage exhibiting

overcompensation). Nevertheless, the magnitude of overcompensation varies across these different types of mortality: overcompensation is greatest when mortality targets the regulatory stage, intermediate when mortality is stage-independent, and least when mortality targets the non-regulatory stage (de Roos et al. 2007). Though empirical studies testing for biomass overcompensation are still rare (Schröder et al. 2014), only one study to our knowledge has induced (stage-specific) mortality across different stages while monitoring biomass, rather than density, responses (Schröder et al. 2009, 2014). Such studies are ultimately necessary to pinpoint ontogenetic asymmetry in energetics as the property that allows for biomass overcompensation (Schröder et al. 2014).

The goal of our study was to test for the occurrence of biomass overcompensation, a key population dynamic phenomenon, as driven by developmental change. To accomplish this, we harvested different developmental stages within lab populations of *Daphnia pulex* while monitoring stage-specific and total population biomass and density responses over seven *Daphnia* generations. Evidence for ontogenetic asymmetry within *Daphnia* enables us to make clear predictions regarding the effects of developmental variation on population dynamics. Intra-stage competition appears stronger within adult versus juvenile *Daphnia* (i.e. reproductive regulation: present study, Nilsson et al. 2010) and given this adult-dominated population structure, we hypothesized that positive biomass responses to mortality would occur in the juvenile, rather than the adult *Daphnia* stage. Furthermore, we predicted overcompensation would be strongest in response to adult, rather than juvenile *Daphnia* mortality, because adult mortality most directly reduces competition within this regulatory stage (de Roos et al. 2007). While our results support the predicted stage-specificity of positive biomass responses, we detected largely compensatory rather than overcompensatory biomass responses. Thus, our study demonstrates the importance of ontogenetic development in population responses to perturbation but cautions that more work is needed before biomass overcompensation is presumed a general phenomenon in nature.

Methods

Study system

We tested population responses to stage-specific mortality using replicate lab populations of *Daphnia pulex*. *Daphnia* (Order: Cladocera) is a key herbivore and important prey for size-selective predators within freshwater food webs (Brooks and Dodson 1965, Sarnelle 1993, Boersma et al. 1996, Wagner et al. 2004). A number of studies have explored the role of developmental processes in generating *Daphnia*'s well-known cyclic population dynamics (McCauley et al. 1990b, 2008), establishing this genus as an important model regarding the population dynamic consequences of ontogenetic stage structure.

We collected *Daphnia* from a small fishless pond in Huntsville, Texas and maintained populations under high food conditions in the lab for several months prior to experiments. Reproductively mature, female *Daphnia* produce clonal female offspring when environmental conditions are favorable and ephippia (haploid eggs that are fertilized by males) when resources are scarce. *Daphnia* size at reproductive maturity depends on food availability and temperature, among other environmental variables (Gurney et al. 1990, McCauley et al. 1990a, Stibor 1992). Within the present study (i.e. our lab environment), *Daphnia* reached reproductive maturity (i.e. began producing eggs) as small as 0.9 mm. Thus, we considered *Daphnia* below this size threshold as juveniles and *Daphnia* above this size threshold as adults, though our findings do not depend on the precise threshold value (Supporting information).

Treatments

Daphnia populations within our experimental setup are dominated by adult biomass in the absence of extrinsic mortality (Fig. 2A, control treatment; Nilsson et al. 2010). For reasons explained in the Introduction, mortality inflicted on adults (relative to mortality targeting juveniles or stage-independent mortality) should produce the greatest positive biomass response because it most directly reduces competition within the adult stage (de Roos et al. 2007). Accordingly, our experimental design focused on adult harvesting.

We applied four treatments to replicate *Daphnia* populations (n = 4 replicates per treatment): 1) a high level of adult-specific harvesting (instantaneous adult mortality rate: 0.07 per day); 2) a low level of adult-specific harvesting (instantaneous adult mortality rate: 0.04 per day); 3) a high level of juvenile-specific harvesting (instantaneous juvenile mortality rate: 0.07 per day); and 4) a no-harvest control. Overcompensation occurs as a hump-shaped response between biomass and mortality (de Roos et al. 2007, Nilsson et al. 2010), and thus we harvested adult *Daphnia* at two different levels to increase our chances of capturing this hump-shaped region. Furthermore, a previous study on *Daphnia* biomass overcompensation induced a single high level of stage-independent mortality (instantaneous total mortality rate: 0.2 per day), which the authors suggested could have overwhelmed any positive biomass response (Nilsson et al. 2010). Again, due to the hypothesized reproduction regulation of *Daphnia* populations, we excluded a low-level juvenile-specific harvesting treatment from our design because we expected juvenile mortality to induce the weakest biomass response.

Experimental setup

We ran the experiment in a semi-chemostat system. Our system used peristaltic pumps to deliver algae continuously to microcosms housing replicate *Daphnia* populations. Each cylindrical microcosm (3 l volume) featured four 63 μ nylon mesh-covered outflow holes at the water surface to retain

Daphnia but allow suspended particulate matter to pass through. This flow-through setup creates semi-chemostat resource dynamics, an assumption of models in which the conditions for biomass overcompensation have been explored (de Roos et al. 2008, Nilsson et al. 2010, Huss and Nilsson 2011). Microcosms (n = 16) were arranged in two adjacent spatial blocks and treatments were assigned randomly within each spatial block (two replicates per block). Microcosms within each spatial block shared the same peristaltic pump, algae stock tank and drainage system.

We used a 3:1 mixture of natural and simulated pond water as the experimental medium because our previous work has shown this mixture effective in maintaining *Daphnia* populations over multiple generations. Natural pond water was collected monthly from a small pond in Sam Houston National Forest, Texas. This pond water was then stored in a refrigerated room prior to preparation for use in the experiment. Pond water preparation followed a 2-step purification process including 10 μ vacuum filtration and 3 h autoclave sterilization. Artificial pond water was created following the 'animal medium' recipe in Wyngaard and Chinnappa (1982). All experiments were conducted in a temperature-controlled room (set at 22°C) under a 12:12 dark-light cycle.

Algae culture and delivery to microcosms

The flagellated green alga *Chlamydomonas reinhardtii* was cultured in the lab and used as the food resource for *Daphnia*. Algae (CC-1010 wild type mt+ [UTEX 90], *Chlamydomonas* Resource Center) was grown in flasks containing TAP (Tris-Acetate-Phosphate) growth medium under continuous light. Algae was harvested near peak density and centrifuged at 5000 rpm for 8 min. After centrifugation, TAP medium was discarded and replaced with simulated pond water. We then measured algae cell density using a hemocytometer and added this concentrated algae to stock tanks containing 16 l of the experimental medium. We adjusted the volume of algae added to stock tanks to maintain a constant resource level (157 480 cells ml^{-1}) entering microcosms throughout the experiment. Stock tanks containing magnetic stir bars were continuously mixed over stir plates to keep algae suspended. Peristaltic pumps drew diluted algae from stock tanks and delivered it (using 1.42 mm ID Tygon tubing) to experimental microcosms (0.945 ml min^{-1} inflow rate, 0.019 h^{-1} dilution rate) housing *Daphnia* populations. Algae stock tanks that fed microcosms were cleaned and replenished with fresh algae and experimental medium every 48 h throughout the duration of the experiment.

Experimental harvesting

Daphnia were introduced to microcosms (50 individuals per microcosm) on 11 December 2017 and populations were allowed to increase and enter a regular pattern of cycling before the start of harvesting treatments. *Daphnia* populations were harvested once per week from 16 January 2018 until 27 March 2018 (11 total harvesting events). *Daphnia*

generation time within our semi-chemostat system is roughly two weeks and thus our experiment allowed for approximately seven *Daphnia* generations.

During each harvesting event we first stirred microcosm contents to homogenize contents. Using a 50 ml Hensen Stempel zooplankton sampling pipette, we then removed either 50% of the microcosm volume (1500 ml, high level harvesting treatments) or 25% of the microcosm volume (750 ml, low level harvesting treatment), depending on the assigned treatment. For the control treatment we removed 750 ml of medium containing *Daphnia* and followed the process for removing adults (explanation below) but returned all contents to control microcosms (i.e. a sham treatment).

To remove adult *Daphnia*, water from microcosms was filtered through 530 μ mesh that retained adults but allowed juveniles to pass through. This mesh was then rinsed thoroughly to capture adult *Daphnia*. The water containing any *Daphnia* that passed through the mesh was returned to microcosms. To remove juveniles, water was first filtered through 530 μ mesh. This mesh was rinsed to capture adult *Daphnia*, which were returned to microcosms. The water containing juvenile *Daphnia* that passed through the 530 μ mesh was next filtered through 153 μ mesh that retained juvenile *Daphnia* but allowed algae and resting eggs to pass through (also returned to microcosms). This 153 μ mesh was then rinsed to capture juvenile *Daphnia*. All *Daphnia* removed from microcosms were processed, as detailed below, to estimate the amount and stage-specificity of *Daphnia* biomass removed by harvesting treatments.

Sampling

We sampled microcosms twice per week throughout the duration of the experiment. Sampling events occurred on the first and third days after harvesting. For each sample, microcosm contents were stirred, and 200 ml was removed using a 50 ml Hensen Stempel zooplankton sampling pipette. We measured the total length of the first 30 *Daphnia* individuals (or fewer if there were less than 30 individuals) within each 200 ml sample and counted eggs, young in brood pouches and resting eggs for reproductive females. Any additional *Daphnia* (> 30) within each 200 ml sample were counted. These samples were then returned to microcosms (i.e. non-destructive sampling).

We applied length-weight regressions to derive *Daphnia* biomass densities. If the total number of individuals per sample was less than 30, we applied a length–weight relationship (Nilsson et al. 2010) to individual length measurements to estimate juvenile, adult and total biomass per 200 ml sample. If the total number of *Daphnia* per sample was greater than 30 individuals, we used the size distribution from the 30 measured individuals to estimate the biomass of unmeasured individuals and combined these biomasses to yield biomass estimates per sample. To do this, we first calculated the proportion of individuals within 0.1 mm size classes (ranging from 0.3 to 1.2 mm) out of the 30 measured individuals within each sample. We then multiplied these proportions

times the total number of unmeasured individuals, rounding down to the nearest individual. Assuming that unmeasured individuals within each size class were of the mean length (e.g. 0.45 mm in the 0.4–0.5 mm size class), we used these lengths to calculate the biomass of unmeasured individuals. We applied this same basic scaling procedure to derive estimates of stage-specific population densities per sample. Individuals ≥ 0.9 mm were considered adults and individuals < 0.9 mm were considered juveniles in all calculations.

Analysis

Our experiment consisted of 11 weeks of treatments and 27 sampling events between 5 January 2018 and 13 April 2018. Two of the sampling events were conducted before treatments began and four were conducted after treatments ceased. Analyses covered the 21 sampling events during treatments and two sampling events after treatments had concluded to capture the effects of the final harvesting event.

We tested stage-specific harvesting treatment effects on: 1) the amount of stage-specific biomass removed from populations; 2) stage-specific and total population biomass and density; and 3) reproductive responses. We examined the amount of stage-specific biomass removed from populations to test the efficacy of harvesting treatments. We further tracked population density responses, in addition to biomass responses, to provide deeper insight into population responses to mortality (Schröder et al. 2014). Treatment effects on reproductive responses were examined to explore potential mechanisms behind biomass and density responses to mortality. We calculated two reproductive response variables: average clutch size (a per capita measure) and total reproductive output (a population-level measure). Average clutch size was calculated by dividing the total number of eggs + offspring in brood pouches per sample by the number of ovigerous individuals per sample. Total reproductive output was calculated by multiplying average clutch size × the proportion of ovigerous adults per sample × total adult density per sample.

Auto-correlation function (ACF) plots indicated significant temporal autocorrelation in our response variables (measured as time series), violating the assumption of independence. To address this, we followed recommendations by (Zuur et al. 2009) to model autocorrelation structure within generalized least squares (nlme package (Pinheiro et al. 2018) in R software). For each response variable (i.e. each generalized least squares model), we utilized the most flexible autocorrelation structure: auto-regressive moving average, ARMA(p, q). We fit this autocorrelation model at the microcosm level (form = time|microcosm) within generalized least squares, obviating the need to model microcosm as a random effect. We further fit ARMA models to individual time series independently of generalized least squares to confirm these models did a good job of capturing *Daphnia* cycling.

All generalized least squares models included harvesting treatment, time and a treatment × time interaction as fixed effects. Biomass response variables were log-transformed, and density response variables were square-root transformed

prior to model fitting. Our approach to finding an adequate ARMA(p, q) structure was to fit models with 1–7 total parameters (all possible combinations of p and q: 35 different structures) while retaining all fixed effects and select the model with the lowest AIC. When the best-fitting models were similar in AIC values (< 2 ΔAIC), we chose the model with the fewest number of combined p, q parameters to maximize parsimony. We note that according to (Zuur et al. 2009), finding an adequate autocorrelation structure is sufficient to account for non-independence of data, with relatively little to be gained from finding a 'perfect' fit. Here, the 'adequate' p and q structure was the final autocorrelation structure for each response variable that captured most of the variance explained by temporal autocorrelation.

Each model was subjected to this same procedure to determine and incorporate the best fit ARMA(p, q) autocorrelation structure. Once an appropriate ARMA(p, q) structure was identified, we tested the overall significance of fixed effects by dropping these terms from models and comparing nested models using likelihood ratio tests. We tested for differences among harvesting treatment levels using least-squares means contrasts (lsmeans package (Lenth 2016) in R software).

Results

Harvesting treatment efficacy

Stage-specific harvesting treatments were largely successful in removing the targeted *Daphnia* developmental stage throughout the duration of the experiment (Fig. 1A). Though adult harvesting treatments (low-adult, high-adult) did remove some juvenile biomass, this was significantly less than that removed by the high-juvenile harvesting treatment (contrast, pooled adult harvesting treatments versus high-juvenile: p < 0.001; Fig. 1B). As intended, the high-adult harvesting treatment removed approximately twice the adult biomass of the low-adult harvesting treatment (pairwise contrast: p < 0.001), while adult biomass removal in the high-juvenile treatment was negligible (Fig. 1C). These treatment effects on juvenile and adult biomass removal were consistent throughout the duration of the experiment (likelihood ratio tests of treatment × time interactions: p > 0.05; Fig. 1A).

Reproduction regulation

In support of reproduction-regulation, adult *Daphnia* comprised 75% of total population biomass in the absence of harvesting (i.e. the control) when averaged over the duration of the experiment (Fig. 2A). In contrast, juveniles dominated total density, comprising 63% of all *Daphnia* in the control on average (Fig. 2B).

Juvenile responses

As hypothesized, juveniles exhibited stronger positive (though statistically insignificant) biomass and density responses to

mortality compared to adults (biomass: Fig. 2C versus E; density: Fig. 2D versus F). We detected compensation, i.e. the lack of significant differences between harvesting treatments and the control, in both juvenile biomass (contrast, pooled harvesting treatments versus control: p = 0.718; Fig. 2C, 3A–B) and juvenile density (contrast, pooled harvesting treatments versus control: p = 0.612; Fig. 2D). While the high-adult harvesting treatment increased juvenile biomass by 12% and density by 13% relative to the no-mortality control (Fig. 2C–D), these differences were not statistically significant (biomass, pairwise contrast: p = 0.258; density, pairwise contrast: p = 0.178). The only significant differences between harvesting treatment levels occurred between high-adult and high-juvenile treatments (biomass, pairwise contrast: p = 0.009; density, pairwise contrast: p = 0.096), which produced the most positive and most negative juvenile responses to harvesting, respectively (Fig. 2C–D). All treatment effects on juvenile biomass and density were consistent throughout the duration of the experiment (likelihood ratio tests of treatment × time interactions: p > 0.05; Fig. 3A–B).

Adult responses

In contrast, adult *Daphnia* biomass was significantly reduced by harvesting treatments when compared to the control (contrast, pooled harvesting treatments versus control: p = 0.005; Fig. 2E, 3C–D). Reductions in adult biomass occurred across all harvesting treatment levels, including the treatment targeting juveniles (pairwise contrasts: p < 0.05). Similar reductions due to harvesting occurred with *Daphnia* density (Fig. 2F), though this effect was not significant (contrast, pooled harvesting treatments versus control: p = 0.114, Fig. 2B). These treatment effects on adult *Daphnia* biomass and density were consistent throughout the duration of the experiment (likelihood ratio tests of treatment × time interactions: p > 0.05, Fig. 3C–D).

Total population responses

Because total *Daphnia* biomass was dominated by adults (Fig. 2A), total biomass responses to harvesting mirrored that of adult biomass: total biomass was significantly reduced by harvesting treatments when compared to the control (contrast, pooled harvesting treatments versus control: p = 0.022, Fig. 2G). Total density was dominated by juveniles (Fig. 2B), and accordingly, showed compensation in response to harvesting treatments (contrast, pooled harvesting treatments versus control: p = 0.450, Fig. 2E). Treatment effects on total *Daphnia* biomass and density were consistent throughout the duration of the experiment (likelihood ratio tests of treatment × time interactions: p > 0.05, Fig. 3E–F).

Reproductive responses

Daphnia responded to harvesting by increasing mean clutch size (per capita reproductive output) relative to the control (contrast, pooled harvesting treatments versus control:
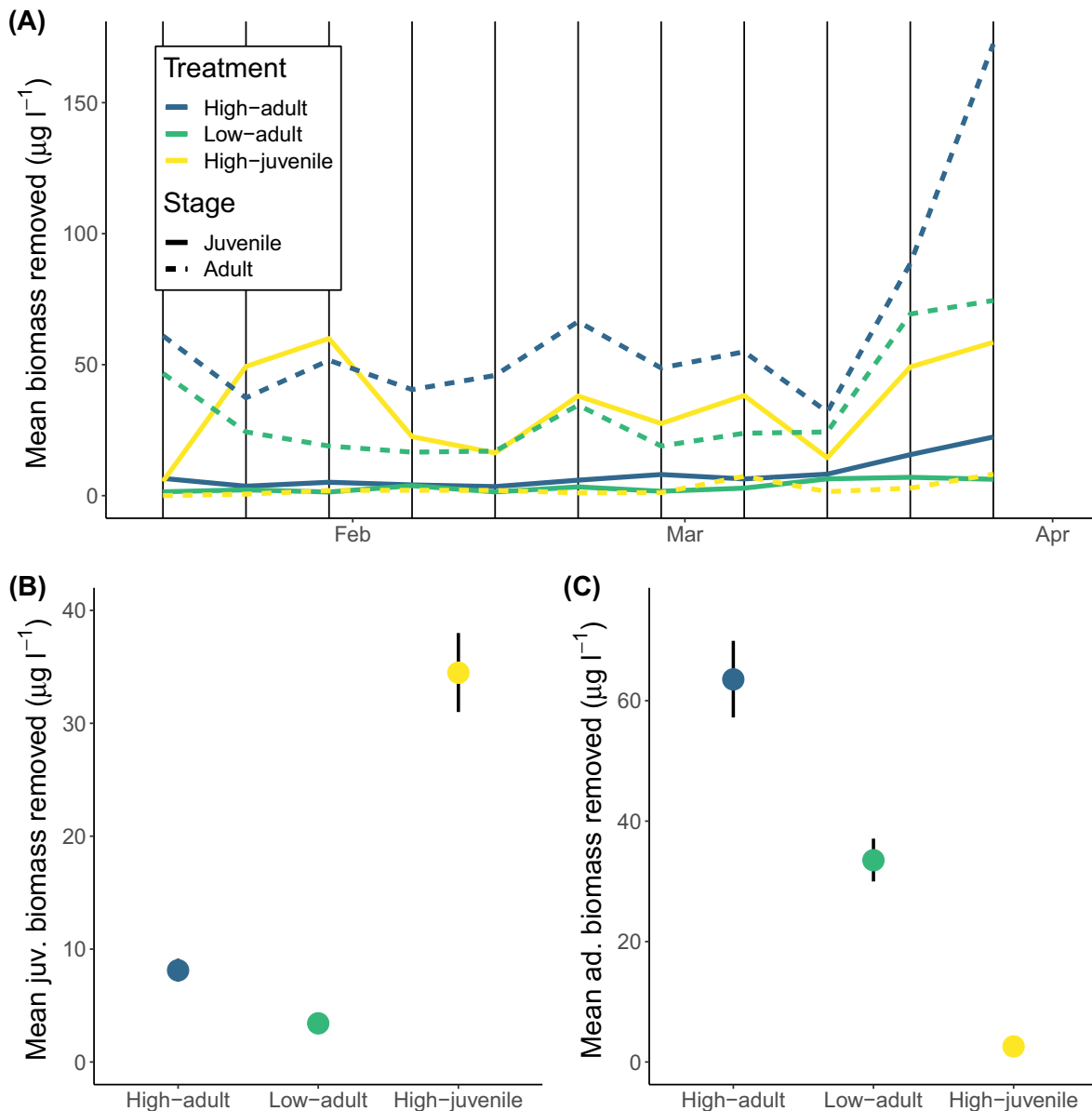
Figure 1. *Daphnia* biomass removed by harvesting treatments. (A) Time-series of mean juvenile and adult biomass removed (averaged across treatment replicates) over the duration of the experiment. Vertical black lines indicate the days on which *Daphnia* were harvested. (B) Mean juvenile biomass removed per harvesting event ± 1 SE over the duration of the experiment (n = 11 harvesting events total). (C) Mean adult biomass removed per harvesting event ± 1 SE over the duration of the experiment.

p = 0.025), with the high-juvenile harvesting inducing the greatest increase in clutch size (contrast, high-juvenile treatment versus control: p = 0.027) (Fig. 4A). The proportion of reproductive adults responded similarly to harvesting treatments, though the effect of harvesting was marginal (contrast, pooled harvesting treatments versus control: p = 0.0581; Fig. 4B). These reproductive responses, when multiplied times the number of adults, resulted in compensation in total reproductive output across treatments (contrast, pooled harvesting treatments versus control: p = 0.790; Fig. 4C). These treatment effects on *Daphnia* reproductive responses were consistent throughout the duration of the experiment (likelihood ratio tests of treatment × time interactions: p > 0.05).

Discussion

While it is generally assumed that extrinsic mortality (e.g. predation or disease) should reduce population density and thus biomass, this prediction derives from models that overlook a fundamental feature of natural populations: developmental variation. New theory incorporating food-dependent development instead predicts biomass increase in response to mortality (i.e. biomass overcompensation) (Schröder et al. 2014), and biomass overcompensation underlies much of the higher-order, community-level consequences of developmental variation (Roos et al. 2008, Huss and Nilsson 2011, de Roos and Persson 2013, Huss et al. 2014). *Daphnia* biomass
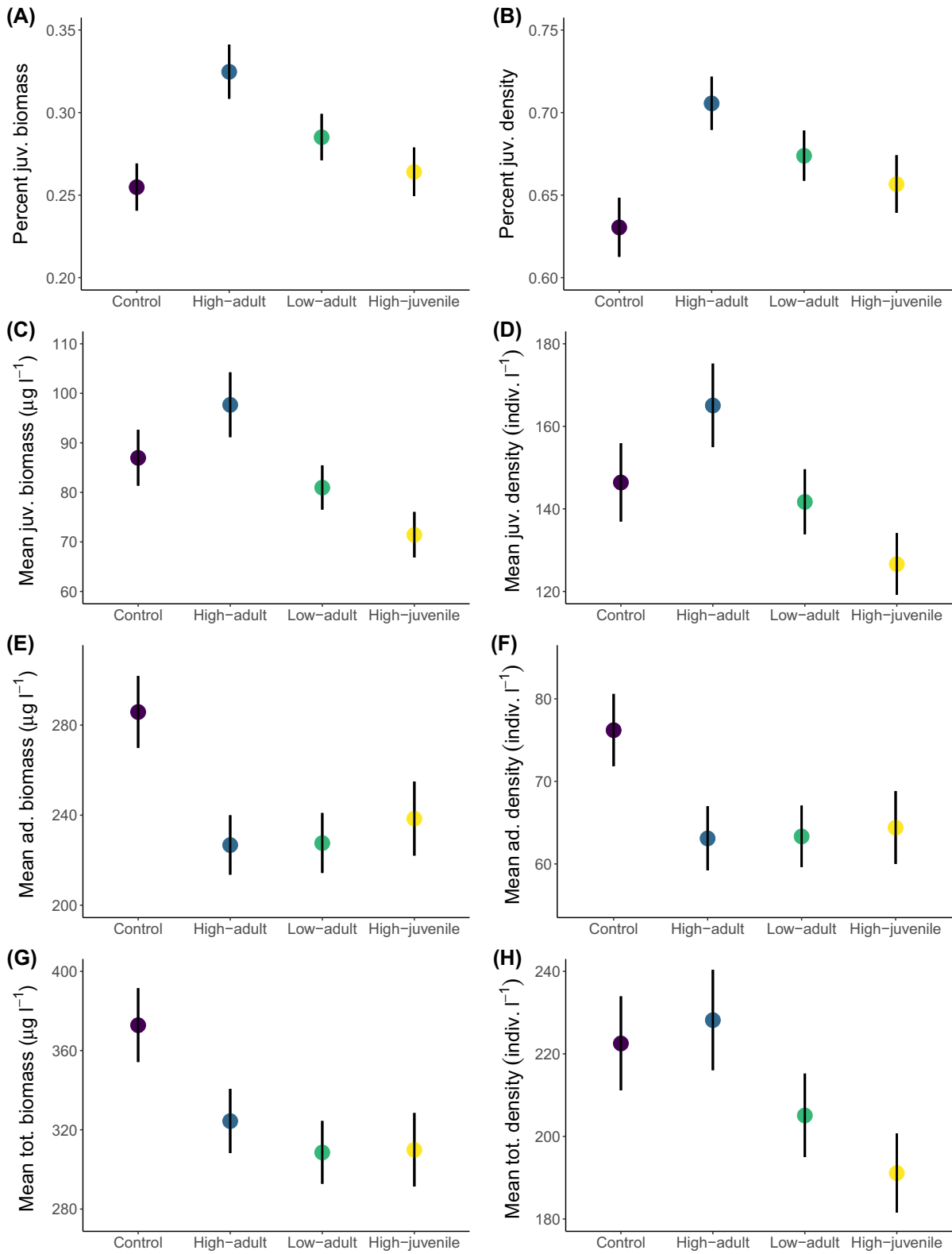
Figure 2. *Daphnia* population metrics (mean ± 1 SE) averaged over the duration of the experiment for control and harvesting treatments. (A) Mean percent juvenile biomass. (B) Mean percent juvenile density. (C) Mean juvenile biomass. (D) Mean juvenile density. (E) Mean adult biomass. (F) Mean adult density. (G) Mean total biomass. (H) Mean total density.
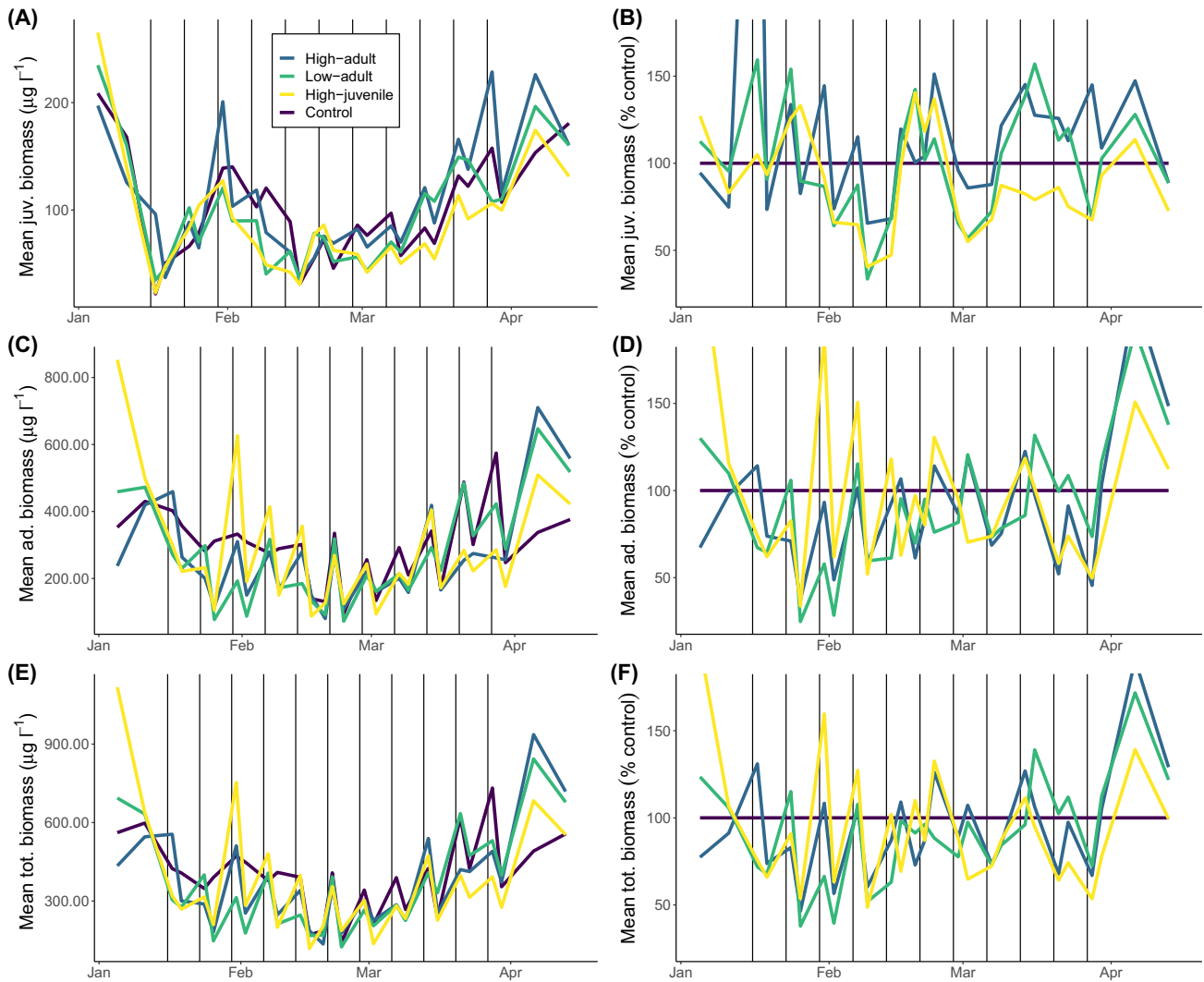
Figure 3. Time-series of mean *Daphnia* biomass for control and harvesting treatments (A, C, E) and mean *Daphnia* biomass normalized as percent of control biomass (B, D, F) over the duration of the experiment. Vertical black lines indicate the days on which *Daphnia* were harvested. Error bars omitted for clarity.

responses to mortality in our study were largely consistent with those predicted by reproductively-regulated population theory, yet the magnitude of these effects was lower than expected (de Roos et al. 2007, Schröder et al. 2009). Specifically, juvenile *Daphnia* exhibited compensatory, but not over-compensatory, biomass responses to adult mortality (Nilsson et al. 2010). Thus, our work demonstrates that ontogenetic stage-structure mediates population response to extrinsic mortality, but cautions that further research is necessary to illuminate the factors underlying the occurrence of biomass overcompensation across natural populations.

Testing theory: the juvenile response

Our study explored biomass overcompensation within reproductively regulated *Daphnia* populations, which allowed us to test clear hypotheses regarding the importance of food-dependent development in mediating population response to mortality. Theory predicts that

when populations are regulated by reproduction, adult mortality should enhance total reproduction and thus juvenile biomass because it reduces competition among adults. In line with this prediction, we found that juvenile *Daphnia*, the non-regulatory stage, exhibited the greatest compensation in biomass in response to mortality, with the strongest response induced by mortality of regulatory adults. As a consequence of compensation in juvenile biomass, mortality elicited a shift in the stage structure of *Daphnia* populations: The percentage of total biomass made up by juveniles increased from 25% (no extrinsic mortality control) to 32% (high adult harvesting treatment) and the percentage of juvenile individuals increased from 63% (no extrinsic mortality control) to 71% (high adult harvesting treatment). All of these juvenile biomass and density responses were at least partially driven by a positive effect of mortality treatments on per capita adult *Daphnia* clutch size, leading to compensation in total reproductive output
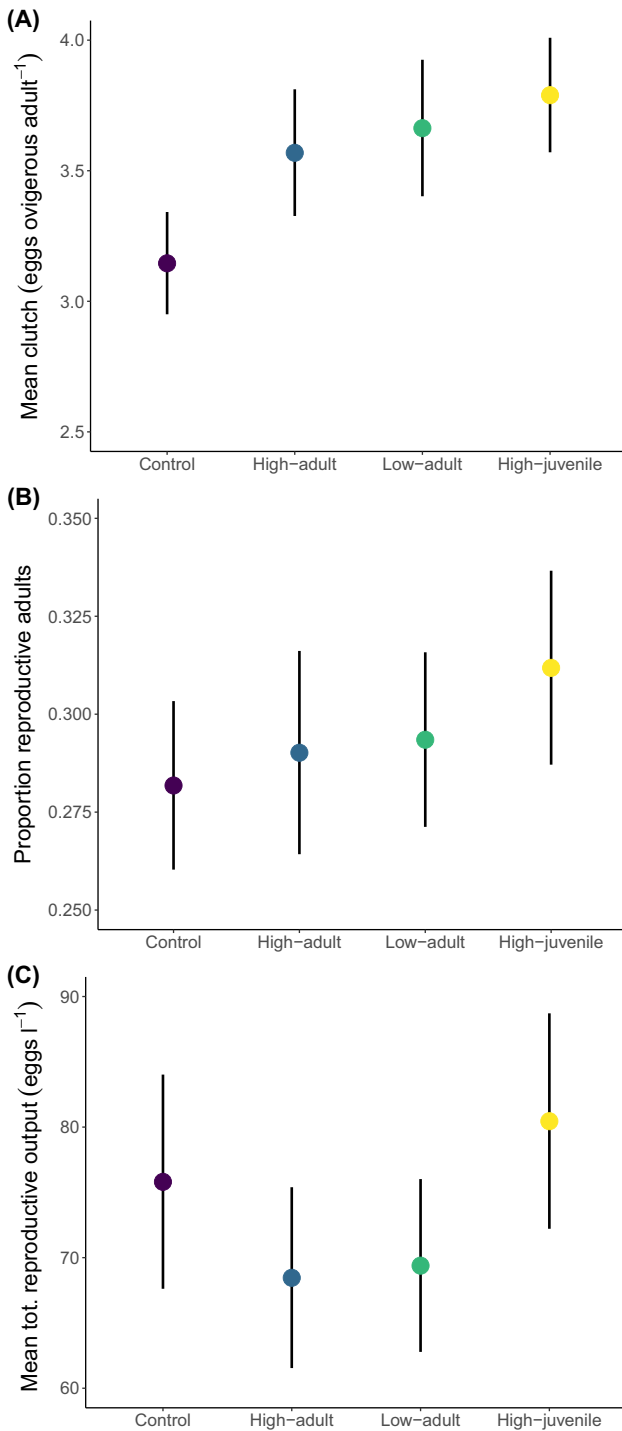
Figure 4. *Daphnia* reproductive responses to harvesting treatments averaged over the duration of the experiment. (A) Mean clutch size ± 1 SE (a per capita measure). (B) Mean proportion of reproductive (ovigerous) adults ± 1 SE. (C) Mean total reproductive output ± 1 SE (a population-level measure). Total reproductive output was calculated by multiplying mean clutch size × the proportion of ovigerous adults per sample × total adult density per sample.

across treatments. Thus, we see broad correspondence between compensation in adult reproductive output and compensation in juvenile biomass and density

in our study, though our analysis failed to detect differences in reproductive responses among mortality treatment levels.

While experiments inducing different types of stage-specific mortality and measuring biomass responses are still exceedingly rare, our findings are consistent with other data from reproductively-regulated populations (Schröder et al. 2009, 2014, Nilsson et al. 2010). More specifically, the patterns we recovered regarding different types of stage-specific mortality match the most complete biomass overcompensation test to date on reproductively regulated poeciliid fishes (Schröder et al. 2009). Yet our findings differ from previous work in one main way: these studies detected stage-specific biomass overcompensation in juveniles (Schröder et al. 2009, 2014), while we detected biomass compensation (Nilsson et al. 2010). Notably, we detected juvenile biomass compensation across all mortality treatments, even when mortality was induced in the juvenile stage. We note that while biomass overcompensation in our experiment was statistically insignificant, high-level adult *Daphnia* harvesting did increase juvenile biomass by 12% and juvenile density by 13% relative to the no-mortality control. Regardless, while our experiment recovered patterns consistent with reproductively regulated population theory, we conclude that our effects are somewhat weak.

Several explanations exist for the lack of significant biomass overcompensation. First, *Daphnia* populations undergo multiple types of stage-driven population cycles, the physiological mechanisms behind which remain debated (McCauley et al. 2008, Martin et al. 2013, van der Meer 2016). Intrinsic *Daphnia* population cycles were clearly observed in our study, and most apparent in time-series of the adult stage. While our analysis approach allowed us to account for intrinsic *Daphnia* cycling at the microcosm-level, such strong and complex temporal autocorrelation within *Daphnia* population dynamics could still obscure treatment effects. Second, while adult *Daphnia* in our study were reproductively mature, they could also continue to grow (Schröder et al. 2009). Thus adult *Daphnia* energy could be allocated to growth rather than reproduction, reducing the magnitude of any positive reproductive response (Ohlberger et al. 2011). However, we compared detailed *Daphnia* size distributions across treatment levels and found no evidence for more subtle shifts in size structure (e.g. within juvenile and adult stages; Supporting information). Third, it is possible that *Daphnia* do not exhibit overcompensation, or do so only under limited conditions. While earlier studies suggested biomass overcompensation in cladocerans (Slobodkin and Richman 1956, Edley and Law 1988), a more recent study (Nilsson et al. 2010) instead showed juvenile biomass compensation in response to stage-independent (i.e. random) mortality. Lack of overcompensation in this study (Nilsson et al. 2010) could have occurred due to: 1) stage-independent mortality, which is expected to elicit weaker effects than stage-specific mortality (de Roos et al. 2007); or 2) the use of a single high mortality level that overwhelmed a potential overcompensatory response. We designed our experiment to include both low and high levels of stage-specific adult mortality in hopes

of capturing a positive biomass response, and yet failed to do so. We did however find that juvenile biomass was 16% greater at the high versus low adult mortality level (though this effect was statistically insignificant), suggesting that an even higher mortality level could have captured a potential biomass response peak.

Still, both Nilsson et al. (2010) and the present study failed to demonstrate biomass overcompensation in *Daphnia*, raising the question of whether overcompensatory responses only exist within a limited parameter space (e.g. range of mortality levels) for certain taxa. Because overcompensation induces cascading effects of developmental variation on whole communities (de Roos and Persson 2013), weak effects might limit the importance of these responses for natural population dynamics and species interaction webs. Indeed, biomass overcompensation is the necessary prerequisite for several population and community effects of ontogenetic development (Roos et al. 2008, Huss and Nilsson 2011, de Roos and Persson 2013, Huss et al. 2014), including emergent facilitation between predators (Roos et al. 2008, Huss et al. 2014) and alternative stable community states (van de Wolfshaar et al. 2006, Gårdmark et al. 2015, Toscano et al. 2016). Clearly, more empirical studies testing for biomass overcompensation in response to stage-specific mortality are needed before biomass overcompensation is presumed a general phenomenon in nature. Understanding the empirical factors that limit the magnitude of biomass overcompensation in natural systems and incorporating these factors into theory is a major research priority moving forward.

Testing theory: adult response

Consistent with other experiments on reproductively-regulated populations (Schröder et al. 2009, Nilsson et al. 2010), adult biomass declined with mortality in our study. Thus, biomass compensation in our study was a stage-specific phenomenon exhibited only by juveniles. Still, we detected an interesting lack of treatment effects on adult biomass. From theory, we expected that: 1) high adult harvesting would reduce adult biomass the most; 2) high juvenile harvesting would reduce adult biomass the least, and; 3) low adult harvesting would have an intermediate effect on adult biomass. While our low adult harvesting treatment was successful in removing exactly half the adult biomass of the high adult harvesting treatment, we found no difference in long-term adult biomass between these treatments. Furthermore, harvesting juveniles produced the same reduction in adult *Daphnia* biomass, despite the juvenile mortality treatment being extremely accurate in capturing juveniles and not adults. One potential contributing factor to these results is that our adult harvesting treatments also removed some juvenile biomass. Regardless, these findings show that very different types of stage-specific mortality can produce a similar reduction in adult biomass. Thus, while juvenile biomass responses in our study were largely in line with reproductive regulation (de Roos et al. 2007, Schröder et al. 2009), the mechanisms underlying observed adult biomass responses require further study.

Relevance for natural dynamics

Our findings suggest that *Daphnia*, a key primary consumer within freshwater systems, exhibit remarkable resistance to extrinsic mortality. Despite inducing substantial stage-specific mortality (instantaneous mortality rate in high harvesting level treatments: 0.07 per day, or 50% of stage-specific biomass removed per week), we found compensation in total *Daphnia* population density, suggesting that the increase in juveniles was roughly equal to the loss of adults through harvesting. While total *Daphnia* biomass was reduced by mortality due to the loss of large adults, juvenile biomass compensation suggests that size-selective predators feeding on adult *Daphnia* can help support predators feeding on juveniles (Brooks and Dodson 1965, Huss and Nilsson 2011). Testing this prediction under natural conditions could provide new insight into the role of zooplankton in supporting diverse predator guilds, and more broadly how ontogenetic development mediates energy flow through food webs (Reichstein et al. 2015).

Conclusions

Unstructured theory assuming individual equivalence has long dominated our understanding of population dynamics, yet ontogenetic variation is widespread and structured theory predicts fundamentally different responses to perturbation (de Roos and Persson 2013, Persson and de Roos 2013). At the most basic level, positive biomass responses arise due to the size-scaling of biomass production (e.g. via rates of food consumption, metabolism or mortality) (Peters 1983, Werner and Gilliam 1984, de Roos et al. 2007). Thus while our study used mortality to shift the adult-juvenile competitive balance, our findings extend to any type of perturbation that modifies ontogenetic asymmetry within populations (Schröder et al. 2014). Because such responses have clear applied relevance to the management of exploited populations (de Roos and Persson 2002, Gårdmark et al. 2015), as well as major implications for species interactions and community dynamics (Roos et al. 2008, Huss and Nilsson 2011, de Roos and Persson 2013, Huss et al. 2014), future experiments inducing a range of stage-specific mortality and measuring biomass responses are crucial. Such work could help to determine whether biomass overcompensation as a result of food-dependent development should be incorporated as a core (i.e. default) feature of population and community theory moving forward.

Data availability statement

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.vt4b8gtr4> (Toscano et al. 2020).

Author contributions

Benjamin J. Toscano: Conceptualization (equal); Formal analysis (lead); Investigation (supporting); Writing – original draft (lead); Writing – review and editing (lead). **Alexandra S. Figel**: Conceptualization (equal); Formal analysis (supporting); Investigation (lead); Writing – original draft (supporting); Writing – review and editing (supporting). **Volker H. W. Rudolf**: Conceptualization (equal); Formal analysis (supporting); Writing – original draft (supporting); Writing – review and editing (supporting).

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