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A suite of prey traits determine predator and nutrient enrichment effects in a tri-trophic food chain

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Abstract. Predation, predation risk, and resource quality affect suites of prey traits that collectively impact individual fitness, population dynamics, and community structure. However, studies of multi-trophic level effects generally focus on a single prey trait, failing to capture trade-offs among suites of covarying traits that govern population responses and emergent community patterns. We used structural equation models (SEM) to summarize the non-lethal and lethal effects of crayfish, *Procambarus fallax*, and phosphorus (P) addition, which affected prey food quality (periphyton), on the interactive effects of behavioral, morphological, developmental, and reproductive traits of snails, *Planorbella duryi*. Univariate and multivariate analyses suggested trade-offs between production (growth, reproduction) and defense (foraging behavior, shell shape) traits of snails in response to non-lethal crayfish and P addition, but few lethal effects. SEM revealed that non-lethal crayfish effects indirectly limited per capita offspring standing stock by increasing refuge use, slowing individual growth, and inducing snails to produce thicker, compressed shells. The negative effects of non-lethal crayfish on snails were strongest with P addition; snails increased allocation to shell defense rather than growth or reproduction. However, compared to ambient conditions, P addition with non-lethal crayfish still yielded greater per capita offspring standing stock by speeding individual snail growth enabling them to produce more offspring that also grew faster. Increased refuge use in response to non-lethal crayfish led to a non-lethal trophic cascade that altered the spatial distribution of periphyton. Independent of crayfish effects, snails stimulated periphyton growth through nutrient regeneration. These findings illustrate the importance of studying suites of traits that reveal costs associated with inducing different traits and how expressing those traits impacts population and community level processes.

Key words: geometric morphometrics; indirect effects; non-lethal; nutrients; predator-prey.

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INTRODUCTION

Developmental and behavioral systems are integrated to yield suites of covarying traits that respond through trait plasticity to biotic and abiotic environmental cues. Community ecologists are increasingly appreciative of the cascading consequences of trait-level responses to predators and food resources that jointly impinge on prey populations, contributing to community dynamics (Abrams 1991, Power 1992, Werner and Peacor 2006, Peckarsky et al. 2008). Predators reduce prey density, and their chemical and visual cues alter expression of prey traits, with consequences for fitness and prey population growth (Lima and Dill 1990, Kats and Dill 1998,

Tollrian and Harvell 1999, Murdoch et al. 2003, Werner and Peacor 2003, Preisser et al. 2009). Consequently, both lethal and non-lethal impacts of predators cascade to resources eaten by prey producing indirect effects (e.g., trophic cascades) across trophic links (Turner and Mittelbach 1990, Schmitz et al. 2000, Werner and Peacor 2003, Wojdak and Luttbeg 2005). However, most of this work has focused on individual traits in consumers rather than the suites of prey traits that characterize plastic responses to environmental cues in these interaction webs (but see: Brönmark et al. 2012, Hoverman and Relyea 2012). Because traits may be negatively correlated leading to trade-offs, consideration of single traits in isolation may fail to reveal the constraints or synergies of environmental drivers on primary consumers that determine indirect effects within a food web.

High quality primary food resources (i.e., relatively high chlorophyll *a* concentrations and low C:P ratios, Sterner and Elser 2002) are predicted to increase the negative non-lethal effects of predators on prey growth for at least two reasons. First, fearful prey seek refuge and forage less, causing them to miss out on the opportunity for rapid growth they could enjoy by consuming high quality resources (Abrams 1991, Peacor 2002, Turner 2004). Second, predators can cause prey to invest scarce energy into production of costly defensive traits, yielding a phenotypic trade-off between life-history (growth, reproduction) and defensive traits (behavior, morphology) (Anholt and Werner 1995, Van Buskirk 2000, Relyea 2002, Brönmark et al. 2012). Despite non-lethal predators that strongly reduce prey growth when or where food quality is high, population growth may still exceed that of growth in low food quality environments and mitigate the non-lethal effects of predators because prey are consuming high-quality food. Additionally, resource quality can influence how lethal and non-lethal effects of predators alter community structure through trophic cascades (Werner and Peacor 2006). Combined, this literature suggests the importance of resource quality in predicting the results of top-down effects in communities. However, no study has considered how a suite of prey traits (e.g., behavioral, developmental, and reproductive) responds to the lethal and non-lethal effects of predators at different levels of resource quality to influence population and community level processes. We propose that ignoring phenotypic trade-offs among suites of prey traits diminish the ability to predict prey population growth and community structure along nutrient and predator density gradients. Understanding the consequences of these gradients is critical to quantifying the many ways that humans impact ecosystems through nutrient enrichment and removal of apex predators (Schmitz 2010).

Structural equation modeling (SEM) is a powerful tool for quantifying the effects of multiple interdependent factors in a network to reveal the importance of each and partition direct and indirect effects. SEM treats variables in a network, such as suites of traits or food webs, as both dependent and independent variables. Traditionally, SEM has been used with non-experimental data; however, there are several compelling reasons to use SEM for investigating outcomes with experimental data including the advantage of considering system effects rather than individual treatment effects (Grace 2006). For example, when SEM has been applied to experimental results, interesting insights into system behavior emerged that were not apparent from typical univariate and multivariate analyses (Rohr et al. 2008, Scherber et al. 2010). In this study, we used SEM to parameterize three models that integrated the individual and interactive responses of multiple prey traits to the lethal and non-lethal effects of predators at different nutrient levels using an experiment with crayfish predators (*Procambarus fallax*), snail prey (*Plaborbella duryi*), and periphyton resources from the Everglades. Our models and predictions were based on theoretical and empirical studies of predator-prey interactions and the cascading effects of predators on the food prey consume at different resource levels (Abrams 1991, Turner 2004, Wojdak and Luttbeg 2005, Werner and Peacor 2006).

For snail population growth we hypothesized that non-lethal crayfish would stimulate snails to express defensive traits (behavior, shell shape, and thickness) that would slow investment in production (growth, offspring) traits (h1; Fig. 1A). Conversely, lethal crayfish effects would compensate for non-lethal effects by decreasing competition among remaining snails (h2). Add-

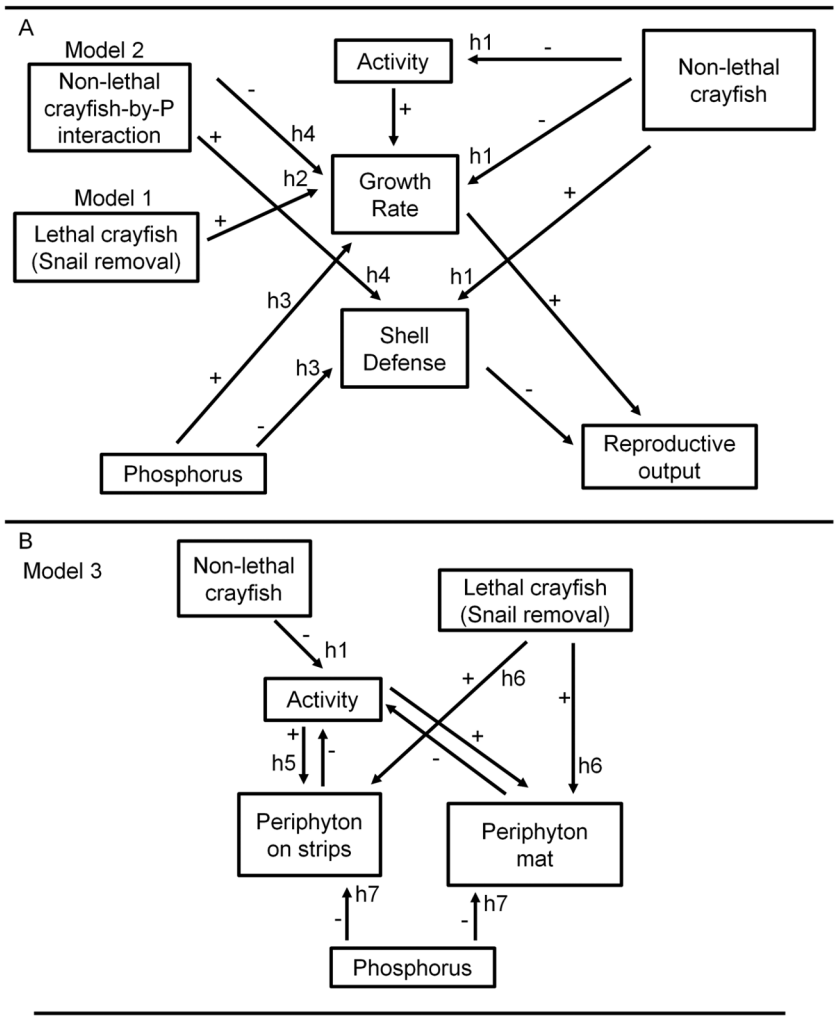

Fig. 1. Three conceptual models illustrating how non-lethal and lethal effects of predators, and P addition are hypothesized to influence *P. duryi* traits that impact their reproductive output (A) and cascade to alter the food snails consume (B). The first model (A) tested for the relative importance of non-lethal and lethal effects, while the second model (A) examined how P addition modified the non-lethal effects of crayfish. The third model (B) examined the relative importance of lethal, non-lethal, and P addition effects on periphyton growth and spatial distribution.

ing phosphorus would counteract the negative effects of non-lethal crayfish on production traits (h3) and adding P with non-lethal crayfish (interactive effect) would stimulate snails to allocate more resources toward defensive traits (h4). In regards to community structure, we predicted that non-lethal crayfish should reduce snail activity and concentrate their grazing in protected areas to produce spatial variation in periphyton distribution (h5; Fig. 1B; i.e., a non-lethal trophic cascade). Reducing snail density via thinning would relax grazing on periphyton and result in an increase in total periphyton biomass (h6; i.e., lethal trophic cascade). Finally, P additions will cause periphyton mat to disintegrate leading to a reduction in periphyton mat standing crop and a shift in taxonomic composition (h7; see *Methods: Study system* below).

Methods

Study system.—The Everglades is a karst

freshwater system with historically very low levels of waterborne P. Phosphorus levels have increased in many parts of the Everglades during the last century from agricultural runoff facilitated by canal dredging (Davis and Ogden 1994). Adding P to karst ecosystems results in the decline of periphyton standing crop, organic matter, and chlorophyll *a* (Turner et al. 1999, Rejmankova and Komarkova 2000, Gaiser et al. 2005). The mechanism for this counterintuitive response remains unclear, but likely involves increased bacterial production and changes in competitive interactions of periphyton mat components (Gaiser et al. 2011). Despite the decline in periphyton standing crop and chlorophyll *a*, studies show that adding P increases secondary consumer abundance (e.g., small fish and macroinvertebrates) indicating that the quality of primary food resources improved and that high quality food moved up the food web stimulating consumer growth (Turner et al. 1999, Geddes and Trexler 2003, Sargeant et al. 2010).

Experimental design.—We conducted a three-factor experiment with two levels of predator (non-lethal crayfish or absent), two levels of P (ambient or added), and three levels of snail density (non-removal, removal scaled to field predation rates to simulate lethal effects, or absent) in a factorial randomized block mesocosm experiment with three replicates that lasted 41 days (Appendix: Table A1). The mesocosm array consisted of thirty-six rectangular concrete tanks (2.2 × 1 × 1 m, L × W × H) arranged in a 6 by 6 tank grid at the Daniel Beard Research Center, Everglades National Park (25°23′17″ N, 80°40′58″ W). Each tank was equipped with a screened standpipe that regulated water depth and a shade-cloth cover (50% light penetration) that prevented animals from colonizing or escaping. We attached five black plastic strips (30 cm × 3 cm, L × W) to the tank floor to measure periphyton colonization and snail grazing; snails in tanks with non-lethal crayfish were observed using them as refuge by hiding under the strips. Tanks were filled to 30 cm (volume: 660 l) with well water on 30 May 2007; the next day, we used a 2000-ml graduated cylinder modified with drain holes to add a 900-ml aliquot of fresh benthic periphyton to each tank collected from a nearby marsh and sorted to remove large mat-dwelling invertebrates and macrophyte stems. Following prior Everglades research (Liston 2004, Gaiser et al. 2005), we added 0.061 g P/m^2/day (0.00197 mol P/m^2/day) in the form of NaH_2PO_4 dissolved in a 100 ml of well water to P addition tanks for a total delivery of 0.85 g P/m^2 over 14 days to allow assimilation by periphyton.

Twenty-five snails, chosen to attain a biomass of 1,703 ± 20 mg of shell-free wet tissue mass per tank with a variation in snail size of 40 mg, were added to non-removal and removal tanks on 23 June from stock populations that originated from multiple locations in the Everglades. To obtain the targeted size variation (40 mg), we chose many small snails and three or four larger snails to mimic Everglades' snail populations, which are multivoltine (Ruehl 2010). A locally derived shell length (0.01 mm) to shell-free wet-mass (0.1 mg) regression was used to estimate snail biomass (Obaza and Ruehl, *in press*). All tanks received a screen-covered (plastic hardware cloth) predator holding cage (150 cm long, 74 cm diameter). In non-lethal tanks, we added one crayfish (25.21 ± 0.62 mm carapace length, mean ± 1 SE) to the holding cages on 23 June. Every other day, we crushed two snails in the tank and fed them to the crayfish to provide chemical cues from crayfish, cues from dying snails, and cues from crayfish eating and processing snail tissue (Crowl and Covich 1990, Alexander and Covich 1991, Covich et al. 1994, Turner et al. 1999, Turner et al. 2000, Bernot and Turner 2001). Throughout the paper, we refer to this treatment as "non-lethal crayfish."

We simulated lethal effects of crayfish with a 6% daily removal rate of snails over the course of the experiment to match field mortality estimated by tethering studies (Ruehl 2010). This removal rate led to a goal of 88% (12% remaining) lower snail density by the end of the experiment. To accomplish this, we removed 6, 4, 5, 3, 2 and 2 snails on day 4, 7, 12, 20, 26 and 35 of the experiment to produce an exponential decline in density. We used an exponential decline to provide a strong test of the lethal effects of predators on snails and their resources by removing most of the snails during the first half of the experiment. Snails were selected for removal by projecting a grid on each tank and randomly choosing locations to collect snails until the target number for that day was reached.

We concluded the experiment on 3 August 2007, 41 days after treatments began.

Snail responses.—We measured five snail traits: activity, shell thickness, shell shape, growth rate (F0), and per capita offspring (F1) standing stock, known to vary in response to non-lethal predators. Activity (burrowing, Snyder 1967) was assessed twice at the beginning (days 5 and 6), middle (days 16 and 18), and end (days 38 and 40) of the experiment by removing covers, and counting the number of visible snails while walking slowly around the tank. We considered visible snails as active; those not observed were considered inactive (i.e., in refuge). The proportion of active snails was averaged within each period (e.g., days 5 and 6) and served as the dependent variable. We based proportions on the final number of snails for non-removal treatments and the estimated number at the time of the trial in removal treatments. We measured shell thickness to 0.01 mm at the apex of the aperture with calipers and calculated size-free shell thickness as the residuals from a regression of log shell thickness, as the dependent variable, by shell ash-free dry mass (AFDM) and shell-free wet-tissue mass. We characterized aperture shape with geometric morphometrics by digitizing 2 landmarks and 12 semi-landmarks on digital images of shell aperatures using tpsDIG (Bookstein 1991, Rohlf and Marcus 1993, Rohlf 2008, Fig. 2A). Landmark configurations were adjusted for position, orientation, and scale by generalized Procrustes analysis using tpsRegr (Rohlf 2011). We took tank means of superimposed coordinates and distilled shape variation into fewer variables with a principal components analysis; the principal components served as dependent variables. Mean individual growth rate (mg/day/tank) of F0 snails (snails initially added) was assessed on experiment day 20 by measuring 8 snails (collected as above) from each tank. Growth rate during this period served as the dependent variable because it represents the peak period of growth rate for *P. duryi* in laboratory populations (Ruehl, *personal observation*). These snails were returned to tanks, except for those scheduled for removal. At the conclusion of the experiment, we identified F1 individuals (offspring from F0 individuals) based on size. *Planorbella duryi* held at constant temperature and density mature around 11 mm shell length (Ruehl, *personal observation*). Total F1 biomass from each tank was calculated using the length-mass regression described above by measuring the shell length of individuals or using an average shell length based on a representative subset applied to the total count when there were greater than 20 F1 snails. We estimated per capita F1 standing stock (mg/tank) by dividing total F1 biomass by the sum of the number of F0 snails collected at the end of the experiment and those removed previously. The removed snails were included because they potentially contributed to F1 standing stock prior to removal. Per capita F1 standing stock served as the dependent variable.

Periphyton responses.—We measured four characteristics of the periphyton mat: dry mass (mg), C:P ratio, chlorophyll *a* concentration (μg/mg dry mass), and algae composition (biovolume/ml) of each group. These characteristics served as dependent variables. We gathered 30-ml periphyton mat samples three times (days 0, 20, 41) by using a net to collect portions of benthic periphyton from different areas of the tank chosen at random. Samples were frozen until processing. Thawed samples were weighed and periphyton was separated from non-periphyton material (e.g., remaining macrophyte fragments) that was dried and weighed to subtract it from periphyton mass estimates. Periphyton was blended, diluted to a known volume, and four measured sub-samples were removed. One sub-sample was dried (70°C) to a constant mass and weighed to estimate dry mass. A second sub-sample was dried to a constant mass and analyzed for total C:P. Total Carbon was determined with duplicate samples using an elemental analyzer (Fisons Instruments NA1500NCS); total P was measured on duplicate samples using the dry-oxidation, acid hydrolysis method (Solorzano and Sharp 1980). The third sub-sample was diluted 100 fold and a 1-ml aliquot was filtered onto a 2.5-cm glass-fiber filter that was frozen and kept in the dark to prevent degradation; chlorophyll *a* was extracted using 90% acetone and read fluorometrically within 24 hours. The final sub-sample was frozen for quantifying periphyton taxonomic composition in tanks (day 41). Sub-samples were thawed and then diluted to a known volume and a homogenized 1-ml aliquot was spread onto a cover slip. The

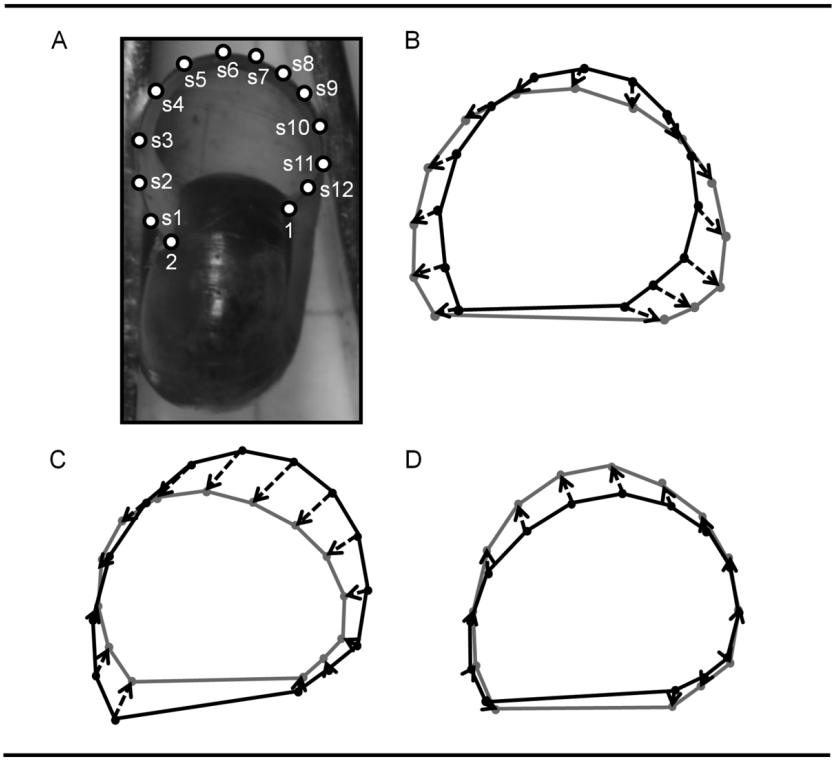

Fig. 2. Landmarks and semi-landmarks (s) used in geometric morphometric analysis of *P. duryi* aperture shape (A). Landmark configuration illustrating the mean shape in the absence of non-lethal crayfish (solid black outline) with vectors (dashed) pointing to gray outline representing the mean shape with non-lethal crayfish as a deformation from the crayfish-absent treatment (B). Shape change along principal component 1 (PC1) (C) and 2 (PC2) (D). For both components, positive values are in black and negative values are in gray. Negative values on PC1 were characteristic of shell shapes induced by non-lethal crayfish, while positive values are without crayfish; conversely, for PC2 positive values are indicative of shell shape induced by non-lethal crayfish and negative values are without crayfish. The magnitude of change induced by non-lethal crayfish, based on Procrustes distance using all shape variables, was 0.03 ± 0.0003 estimated with a 1000 bootstraps of pairwise distances.

dried cover slip was placed on a microscope slide with a drop of water and sealed with nail polish. At least 500 cells were counted along 0.36-μm transects using a compound light microscope with a 60× objective with a total magnification of 600×. Cells were grouped into coccoid blue green (CBG), filamentous blue green (FBG), coccoid green (CG), filamentous green (FG), 3 size classes of diatoms (DIA), and 3 size classes of desmids (DES; Gaiser et al. 2006). Biovolume of each group was estimated by approximating cells to geometric shapes.

We quantified total periphyton volume in tanks on the final day of the experiment (day 41) by loading wet periphyton into a 2000-ml graduated cylinder modified with drain holes. Using the final volume and the initial volume, we interpolated total periphyton volume in tanks halfway through the experiment by calculating a linear rate of change between the beginning and end of the experiment for each tank. Periphyton mat dry mass and chlorophyll *a* concentration were scaled up to represent the total periphyton volume in a tank at the beginning, middle, and end of the experiment using the initial, estimated, and final periphyton volumes.

We measured three characteristics of periphyton growing on plastic strips: chlorophyll *a* density (μg/mm^2), periphyton production of each group (biovolume/cm^2/day), and total periphyton production (total biovolume/cm^2/day). These served as dependent variables in statistical

models. Three 4-cm^2 plastic strips were chosen haphazardly for collection from the tank floor during the experiment (days 0, 20, 41). We gently cut the remaining edge of the plastic strip (a 2-cm cut was made every 2 cm on all strips prior to the experiment) with scissors and carefully transferred them to a plastic bag with forceps to prevent the loss of loosely attached periphyton. The plastic squares were frozen until processing. In the lab, periphyton was scraped from the squares and the scraped surface area (mm^2) recorded. The resulting periphyton was diluted to a known volume and homogenized. We filtered a 1-ml sub-sample onto a 2.5-cm glass-fiber filter for chlorophyll *a* analysis following procedures above. Taxonomic composition was quantified for the final day of experiment as above, but biovolume was expressed as production providing insight into changes in both composition and standing crop.

Analysis.—Analyses tested for the effects of non-lethal crayfish (h1, h5), lethal crayfish (snail removal, h2, h6), P addition (h3, h7), and associated interactions (h4, Fig. 1). In all cases, block and block-by-treatment were treated as random effects; non-significant block effects were dropped from final models. Repeated measures analysis of variance (RANOVA) was used when data were collected multiple times and analysis of variance (ANOVA) tested for treatment effects when data were collected once. We used the Satterthwaite correction to estimate denominator degrees of freedom because there were unequal numbers of snails due to the removal treatment. Significant main effects with more than two levels (e.g., snail density) and significant interaction terms were investigated using Tukey's post-hoc tests. All transformations were made to meet homogeneity of variance according to Levene's test. The following variables were log transformed: proportion active snails, per capita F1 standing stock, periphyton dry mass, proportion of chlorophyll *a* per dry mass, chlorophyll *a* density on plastic strips, periphyton mat composition for each algal group, periphyton production for each algal group on strips, and total periphyton production on strips. We excluded one tank (non-lethal crayfish, no removal, P addition) from all analyses because most snails died within the first 20 days of the experiment for an unknown reason. Snail survivorship was high for all other tanks.

We analyzed snail aperture shape with multivariate analysis of covariance (MANCOVA) using the first three principal components (PC) of shape that accounted for 93% of shape variation as dependent variables. In addition to the base model, we included centroid size, a multivariate measure of size, as a covariate to account for multivariate allometry; significant centroid size-by-treatment interaction terms were retained when significant to account for heterogeneity of slopes. Effect sizes were reported as Wilks' partial-eta squared (η_p^2). We quantified the magnitude of shape difference with Procrustes distance, calculated as the Euclidean distance between the average superimposed coordinates of non-lethal and no-crayfish treatments (Bookstein 1996); the mean and standard error of shape distance was estimated with 1000 bootstraps. We presented shape change as deformation vectors from the consensus using the design matrix of the statistical model and the mean superimposed shape coordinates with tpsRegr (Rohlf 2011, e.g., Ruehl et al. 2011). Because PC1 and PC2 were used in SEM, we also depicted shape change along these axes for comparison to the overall deformation vectors to provide insight into the direction of change from negative to positive along each axis.

Algal taxonomic composition for periphyton mat (biovolume for each group) and strips (production for each group) were analyzed with multivariate analysis of variance (MANOVA) testing for effects using the model described above; effect sizes were reported as Wilks' partial-eta squared (η_p^2). We visualized significant effects for periphyton mat composition (biovolume/ml) using canonical axes derived from the MANOVA. We investigated total periphyton production on plastic strips with ANOVA. Significant variation in periphyton composition and production on plastic strips was reported as the average production for each taxonomic group by treatment.

We used SEM to integrate the different response variables from the experiment into analyses that examined the independent and interactive effects of top-down and bottom-up effects on individual traits that influenced reproductive output and community structure (Fig. 1). For an intuitive sense of trade-offs among snail

traits, we first examined trait correlations with Pearson's r. For SEM, non-lethal crayfish, lethal crayfish (snail removal), P addition, and the non-lethal-by-P addition interaction served as exogenous variables. In the first two models, shell defense was modeled as a composite of PC1 and PC2 of aperture shape (combined 88% of variation) and size-free shell thickness. Following Grace (2006), we set the variance of the composite variable to zero and identified it by fixing one unstandardized path coefficient (PC2 of shape) to one. We used the dependent variables from the above ANOVA and the average of the repeated measures variables from RANOVA analyses to parameterize the remaining factors in the models. Covariances between PC1 of shape and growth rate, and between PC2 of shape and size-free shell thickness improved model fit. In the third model, periphyton growing on plastic strips and periphyton mat were modeled as latent variables that were indicated by chlorophyll *a* density on strips and chlorophyll *a* concentration in periphyton mat taken on the final day of the experiment; activity was modeled as above. Reciprocal paths between both periphyton latent variables and snail activity captured both directions of causality. Covariances between periphyton on strips and periphyton mat, and between snail activity and lethal effects (snail removal) improved model fit. We assessed model fit using the Bollen-Stine bootstrap χ^2 test (2000 bootstraps) and estimated path coefficients using maximum likelihood estimates (Grace 2006). We used an Information Theoretic approach to determine which of the first two models fit best; models were considered equivalent if their AICc scores differed by ≤ 4; AICc was used because of small sample size (Anderson 2008). Statistical analyses were conducted with SAS 9.2 (SAS Institute, Cary, NC) and AMOS 17 (AMOS Development Corp., Crawfordville, FL); SEM was repeated with M-plus6 to verify results (Muthén and Muthén 1998).

Results

Snail responses.—Experiment-wide survivorship of F0 *P. duryi* averaged 0.80 ± 0.03 (mean ± SE, n = 11) for the non-removal treatment and 0.55 ± 0.07 (mean ± SE, n = 12) for the remaining snails in the removal treatment. Including removed snails in survivorship calculations as consumed, resulted in an average of 0.07 ± 0.01 (mean ± SE, n = 12) survivorship, lower than our goal of 12% (100–88% survivorship) for the removal treatment. By simulating mortality, we created a relatively high snail density treatment (non-removal) and a declining snail density treatment (removal), in addition to the no-snail control during the 41-day experiment.

Snails were 53% less active (i.e., increased refuge use) with non-lethal crayfish than without (h1; $F_{1,12} = 29.22$; $P < 0.001$). In tanks with non-lethal crayfish, *P. duryi* were commonly found under plastic strips and periphyton mats. The effect of non-lethal crayfish on snail activity was consistent over time ($F_{2,24.3} = 0.73$; $P = 0.491$) and activity did not vary in response to other treatments or their interactions ($P > 0.05$, in all cases).

Snails produced 134% thicker shells with non-lethal crayfish compared to tanks without crayfish (h1; $F_{1,13.2} = 14.25$; $P = 0.002$) and 111% thinner shells in removal compared to non-removal treatments ($F_{1,13.2} = 5.37$; $P = 0.037$). Adding P led to snails producing 104% thinner shells compared to ambient conditions, but was not significant (h3; $F_{1,13.2} = 4.07$; $P = 0.065$). In addition to these main effects, the three-way interaction between non-lethal crayfish, P, and removal was significant ($F_{1,13.2} = 14.11$; $P = 0.002$). Snails from tanks without crayfish, receiving P, with snail removal, produced 104% thinner shells compared to the average thickness of the other treatments (Tukey < 0.05).

Non-lethal crayfish induced aperture shape variation after controlling for multivariate allometry and allometric differences among treatments (h1; $F_{3,11} = 5.52$; $P = 0.015$; $\eta_p^2 = 0.601$; Procrustes distance = 0.028 ± <0.001, mean ± 1 SE). Shape did not vary with other treatments or their interaction ($P > 0.05$, in all cases). Deformation vectors revealed that snails experiencing non-lethal crayfish developed apertures that were compressed near the apex compared to those in tanks without crayfish (Fig. 2B). PC1 accounted for 62% of shape variation and snails that developed compressed apertures in tanks with non-lethal crayfish were arbitrarily assigned negative values (Fig. 2C), while PC2 described 26% of shape variation and snails from non-lethal crayfish tanks had positive values (Fig. 2D).

Snail daily growth rate averaged 2.72 ± 0.25

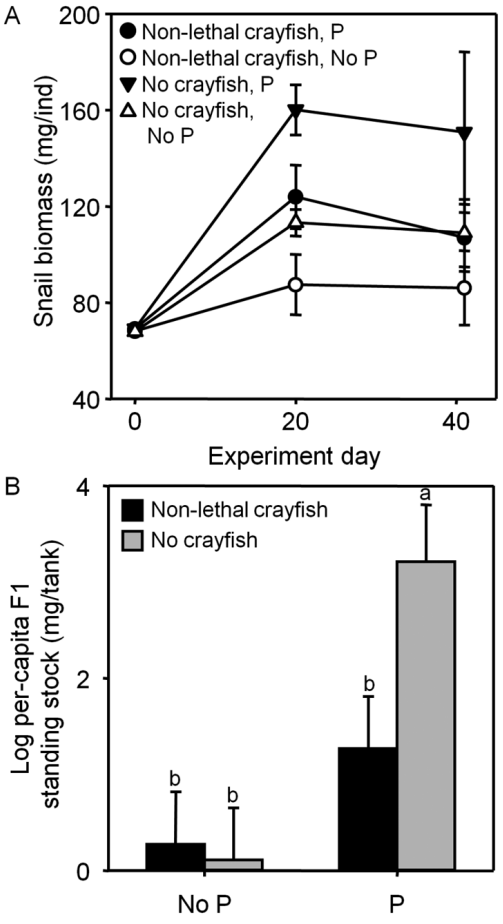

Fig. 3. Snail biomass (mean ± SE) (A) and per capita F1 standing stock (mean ± SE) (B) for *P. duryi* in response to non-lethal crayfish and phosphorus additions; removing snails had no effect and is not shown. For analyses, growth rate (body mass/time) during the first 20 days of the experiment served as the dependent variable because F0 snails began to senesce as the population turned over during the second half of the experiment. Different letters indicate significant differences using Tukey's post-hoc test ($P < 0.05$).

mg/day (mean ± 1 SE) over the 41 day experiment, but growth primarily occurred during the first 20 days (5.97 ± 0.37 mg/day, mean ± 1 SE) before the majority of snails began allocating resources toward reproduction and then senescing (Fig. 3A). During this rapid phase of growth, the presence of non-lethal crayfish slowed growth by 22% compared to no-crayfish treatments (h1; $F_{1,13.1} = 8.58$; $P = 0.012$), while P additions stimulated growth by 29% compared to ambient conditions (h3; $F_{1,13.1} = 15.73$; $P = 0.002$); however there was no interaction (h4; $F_{1,13.1} = 0.20$; $P = 0.665$).

Per capita F1 *P. duryi* production (no./adult/tank) averaged 2.02 ± 1.10 (mean ± 1 SE) resulting in an average standing stock (mg/adult/tank) of 13.87 ± 6.83 (mean ± 1 SE), but a range of 0 to 23.42 individuals/tank (0–129.93 mg/tank). Per capita F1 standing stock was 54% lower when non-lethal crayfish were present, but the effect was not significant ($F_{1,11.4} = 3.87$; $P = 0.073$; Fig. 3B). Adding P increased per capita F1 standing stock 91% compared to ambient conditions ($F_{1,4.21} = 10.20$; $P = 0.031$). The magnitude of per capita F1 standing stock reduction by non-lethal crayfish depended on P addition (non-lethal crayfish-by-P: $F_{1,11.4} = 5.46$; $P = 0.039$). In tanks with P addition, non-lethal crayfish reduced per capita F1 standing stock by 60% compared to tanks without crayfish (Tukey, $P < 0.05$; Fig. 3B). Reducing snail density by simulating lethal predator effects (snail removal) failed to affect individual growth rate of remaining snails or per capita F1 standing stock after accounting for removed snails (h2; $P > 0.05$, in all cases).

Periphyton responses.—Phosphorus additions lowered periphyton C:P ratio by 39% compared to ambient conditions ($F_{1,16.9} = 3356.50$; $P < 0.001$). The periphyton mat gradually disintegrated in P addition tanks resulting in 78% lower periphyton mat dry mass compared to ambient tanks by the end of the experiment (h7; P-by-day: $F_{2,27.5} = 15.50$; $P < 0.001$). Concurrent with periphyton mat decline during the experiment, chlorophyll *a* concentrations in the mat decreased by 53% with P additions compared to ambient tanks (P-by-day: $F_{2,30.9} = 7.16$; $P = 0.003$; Fig. 4A). Chlorophyll *a* density on plastic strips revealed the same general trend with a 40% decline in chlorophyll *a* with P addition by the conclusion of the experiment (P-by-day: $F_{2,28.4} = 10.66$; $P < 0.001$; Fig. 4C). Interestingly, chlorophyll *a* density on strips declined in tanks with non-lethal crayfish during the experiment (non-lethal crayfish-by-day: $F_{2,28.4} = 4.13$; $P = 0.03$; Fig. 4D), resulting in 55% less chlorophyll *a* on strips in tanks with non-lethal crayfish compared to tanks without crayfish (h5; Tukey < 0.05). There was a similar trend for periphyton mats, but the effect was not significant (non-lethal crayfish-by-day:

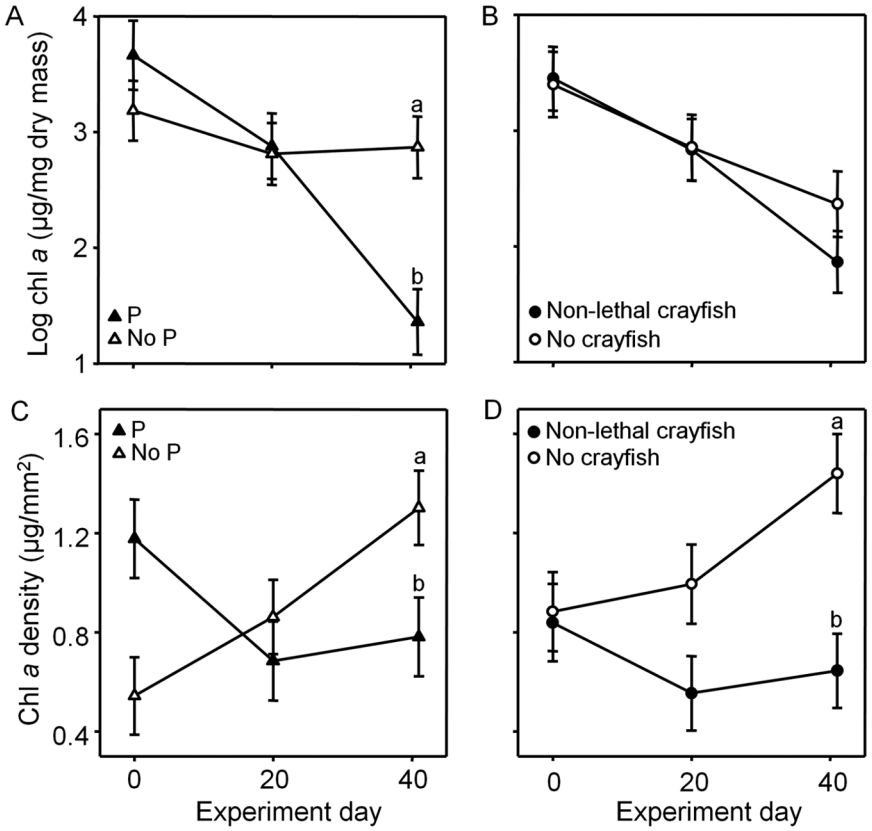

Fig. 4. Chlorophyll *a* concentration (mean ± SE) in periphyton mat (A and B) and chlorophyll *a* density (mean ± SE) on plastic strips (C and D) over the 41-day experiment in response to the addition of P (A and C) or a non-lethal crayfish (B and D). Different letters indicate significant differences within panels using Tukey's post hoc test ($P < 0.05$).

$F_{2,29.8} = 0.59$; $P = 0.56$; Fig. 4B). We found no evidence that lethal effects (snail removal) influenced periphyton chlorophyll *a* on strips or in periphyton mat (h6; $P > 0.05$ in all cases).

At the end of the experiment, periphyton mat composition was affected by P addition (h7; P: $F_{6,18} = 20.87$; $P < 0.001$; $\eta_p^2 = 0.874$), snail density ($F_{12,36} = 2.54$; $P = 0.015$; $\eta_p^2 = 0.706$), and their interaction (P-by-snail: $F_{12,36} = 2.82$; $P = 0.008$; $\eta_p^2 = 0.734$). Phosphorus addition had the strongest effect on periphyton mat composition leading to a shift from diatoms (DIA) and coccoid blue green algae (CBG) to filamentous blue green (FBG) and filamentous green (FG) algae (Fig. 5A). The magnitude of P addition effects on periphyton mat composition depended on the presence of snails. Composition shifted toward FBG and FG in tanks lacking *P. duryi*, while treatments with *P. duryi*, regardless of density, were more similar to ambient P tanks characterized as having more DIA and CBG algae. Plastic strips measured the change in periphyton composition and production on a standard substrate. Composition on strips was affected by P addition (h7; $F_{6,17} = 25.38$; $P < 0.001$; $\eta_p^2 = 0.900$) and non-lethal crayfish (h5; crayfish: $F_{6,17} = 5.52$; $P = 0.002$; $\eta_p^2 = 0.661$), but there was no effect of snails on composition or evidence of interactions ($P > 0.3$ in all cases). Similar to periphyton mat, P addition had the strongest effect, shifting composition away from CBG and DIA and toward FBG and FG taxa (Fig. 5B). Non-lethal crayfish also shifted composition away from CBG and DIA and toward FBG and FG algal groups (Fig. 5B).

Total periphyton production on strips (biovolume/cm^2/day) was 25% lower in tanks with P addition compared to those without P addition

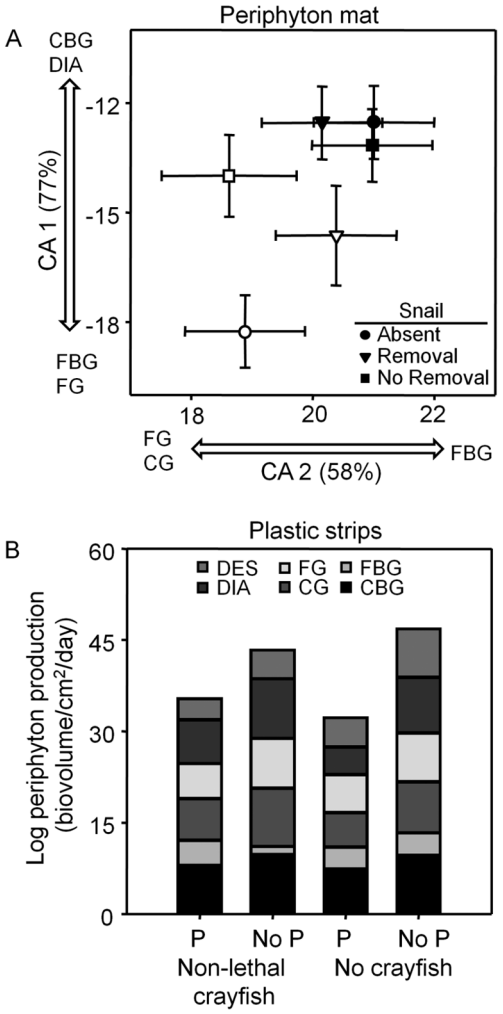

Fig. 5. Canonical axes (mean ± 95% CI) derived from a MANOVA of soft algae taxonomic groups quantified from periphyton mat (biovolume/ml) (A) and periphyton production on plastic strips (B). Compositional changes in periphyton mat to P addition (open symbols) depended on snail density; tanks with a few snails (Removal) and many snails (No Removal) more closely resembled ambient P conditions (filled symbols). Production on plastic strips was lower with P addition, while composition shifted toward FBG and FG without crayfish or with P addition. Soft algae group abbreviations are: DES = desmids, DIA = diatoms, FG = filamentous green, CG = coccoid green, FBG = filamentous blue green, and CBG = coccoid blue green.

Table 1. Pearson's r correlation table for the suite of *P. duryi* traits used in SEM. Traits are grouped into production (growth, per capita F1 standing stock) and defense (activity, shell thickness, and shape PCs). Shell thickness (SF Shell thickness) represents the size- and mass-free residuals from regression. PC1 and PC2 accounted for 88% of shell shape variation. Inspection of shape change along PC1 indicated that shapes induced by non-lethal crayfish were arbitrarily assigned negative values. Therefore, we switched the sign to provide an intuitive sense of trait correlations and labeled it INV PC1 for inverse PC1.

Trait	Growth Rate	Log per capita F1 standing stock	Log activity	SF Shell thickness	INV PC1
Log per capita F1 standing stock	0.70				
Log activity	0.60	0.49			
SF Shell thickness	−0.42	−0.29	−0.47		
INV PC1	−0.52	−0.52	−0.31	0.19	
PC2	−0.12	−0.24	−0.34	0.59	0.00

(h7; $F_{1,21} = 57.93$; $P < 0.001$). Snail density treatments (non-removal, removal, absent) did not alter periphyton production on strips (h6; $F_{2,21} = 2.90$; $P = 0.077$) despite 8% more production in tanks without snails compared to non-removal snail tanks; removal tanks were intermediate in production. Non-lethal crayfish or interactions between treatments did not affect total periphyton production on strips ($P > 0.3$ in all cases).

Correlated trait responses and structural equation models.—We found numerous negative correlations among traits indicating snails traded-off resource allocation among traits (Table 1). Growth rate and reproduction were negatively correlated with defense traits. Because non-lethal crayfish-induced shell shapes were arbitrarily assigned negative values on PC1 (Fig. 2C), we flipped the sign of PC1 and labeled it INV PC1 in the correlation table and SEMs so the sign of correlations among other traits would be intuitive.

Chlorophyll *a* concentrations in periphyton mat and chlorophyll *a* density of periphyton on plastic strips were strongly correlated with the major groups of soft algae in the periphyton mat, the canonical axes derived from the MANOVA of periphyton mat composition, the periphyton

Table 2. Pearson's r correlation table of the relationship between, chlorophyll *a* concentration from periphyton mat (Log chl *a* peri mat) and chlorophyll *a* density on plastic strips (Log chl *a* strips) used in SEM and *P. duryi* activity (Log activity), periphyton mat C:P ratios (Log C:P ratio peri mat), periphyton mat composition for each group (peri mat), periphyton composition on plastic strips for each group (strips), the two canonical axes (CA1 and CA2 peri mat comp) derived from the snail-by-nutrient effect of the MANOVA on composition, or total periphyton production on strips (Log prod strips). Soft algae group abbreviations are: DIA = diatoms, CBG = coccoid blue greens, CG = coccoid greens, DES = desmids, FBG = filamentous blue green algae, and FG = filamentous greens.

Periphyton measure	Log Snail activity	Log C:P ratios peri mat	CA1 peri mat comp	CA2 peri mat comp	Log chl *a* peri mat	Log chl *a* strips	Log prod strips
Log C:P ratios peri mat	−0.23						
CA1 of peri comp	−0.24	0.76					
CA2 of peri comp	−0.11	0.31	−0.02				
Log chl *a* peri mat	−0.04	0.47	0.44	0.26			
Log chl *a* strips	0.2	0.3	0.17	0.12	0.63		
Log prod strips	−0.43	0.78	0.57	0.49	0.6	0.52	
Log DIA peri mat	−0.2	0.85	0.86	0.39	0.57	−0.12	0.75
Log CBG peri mat	0.06	−0.76	−0.67	−0.41	−0.22	0.66	−0.59
Log CG peri mat	0.24	0.31	0.13	0.02	0.19	0.28	0.26
Log DES peri mat	0.08	−0.15	−0.27	0.39	−0.06	0.14	−0.03
Log FBG peri mat	−0.13	0.36	0.03	0.91	0.21	−0.14	0.48
Log FG peri mat	0	0.15	0.24	0.48	0.18	0.71	0.32
Log DIA strips	−0.5	0.62	0.58	0.1	−0.03	−0.12	0.56
Log CBG strips	−0.32	0.73	0.43	0.49	0.73	0.66	0.92
Log CG strips	0.3	0.67	−0.46	0.08	0.28	0.14	−0.08
Log DES strips	−0.65	0.13	0.5	0.34	−0.17	−0.14	0.83
Log FBG strips	−0.1	−0.43	0.14	0.06	−0.08	0.28	0.07
Log FG strips	−0.19	0.47	0.49	0.22	0.61	0.71	0.8

composition on plastic strips, and the total production of periphyton on plastic strips (Table 2). Both chlorophyll *a* density on strips and periphyton production on plastic strips had strong positive correlations with C:P ratios consistent with univariate analyses above showing that P addition, which lowered C:P ratios, reduced chlorophyll *a* concentrations in periphyton mat and periphyton production on plastic strips (Table 2). Chlorophyll *a* density on strips was positively correlated with snail activity, while total periphyton production on strips was negatively correlated with snail activity. Inspecting correlations among individual algal groups revealed that coccoid green algae (Log CG strips) was the only group positively correlated with snail activity suggesting that this group was responsible for the increase in chlorophyll *a* on strips. The increase of chlorophyll *a* on strips with increased snail activity supports the notion that active snails spent less time grazing near strips. These lines of evidence indicate that the use of chlorophyll *a* measures in SEM was representative of periphyton responses to treatments.

All three SEMs were adequately fit by our data (Bollen Stine $\chi^2 > 0.05$). However, between the two models considering snail traits, the model with the non-lethal crayfish-by-P interaction fit better than the model with lethal effects (AICc: Non-lethal crayfish-by-P model = −1033.4, Lethal (snail removal) model = −1026.1, Δ of 7.3). Removing snails had little influence on snail growth and weak indirect effects on reproductive output (Appendix: Fig. A1, Table A2). Non-lethal crayfish reduced snail growth (h1) directly and indirectly by reducing snail activity (h1; i.e., foraging behavior) that combined to limit reproductive output (Fig. 6A). Independent of growth, non-lethal crayfish stimulated production of shell defenses (h1; shell shape and thickness) that also depressed reproductive output. Comparing the strengths of these three paths revealed that producing defensive structures had the largest negative effect on reproductive output (product of coefficients: Activity = −0.04, Growth = −0.06, Defense = −0.23; Table 3). Conversely, P addition independently increased snail growth (h3) and reduced production of shell defenses (h3) that separately stimulated reproductive output. Like

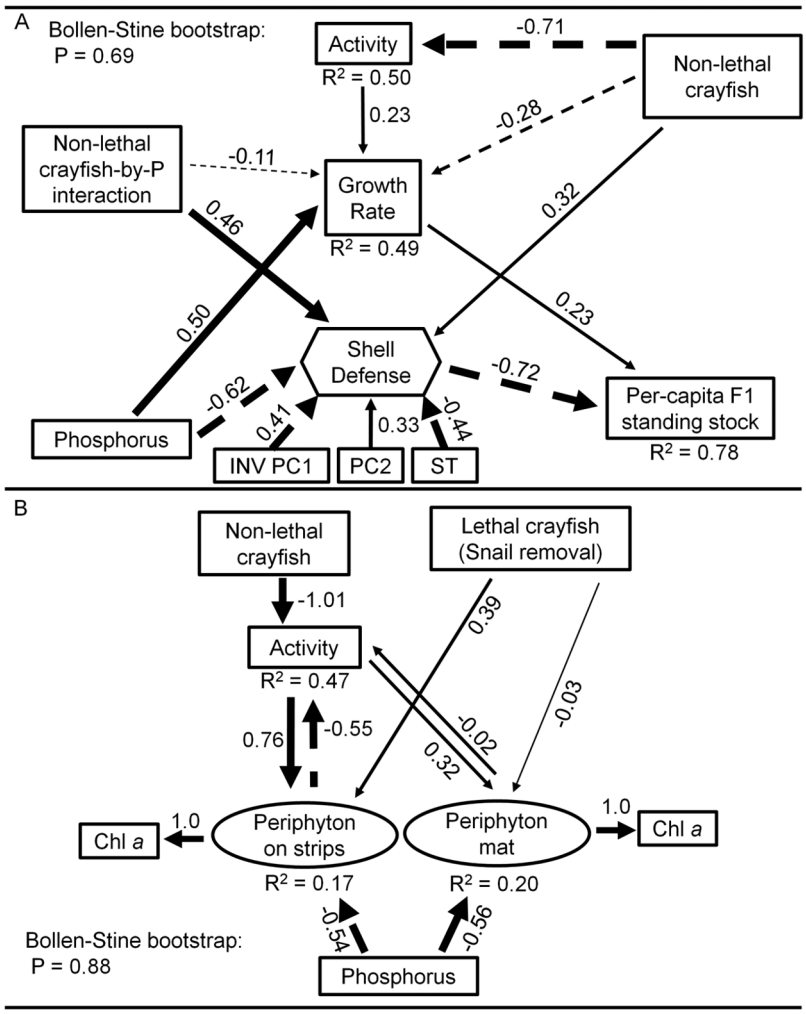

Fig. 6. Structural equation model results for the second and third conceptual models testing for non-lethal, P-addition, and their interactive (non-lethal × P addition interaction) effects on a suite of *P. duryi* traits (A) and the non-lethal and lethal (snail removal) indirect effects on periphyton (B). Squares are observed, circles are latent, and hexagons are composite variables; solid arrows are positive effects and dashed lines are negative effects. Numbers associated with arrows are standardized path coefficients representing the strength of the relationship between variables and were used to determine line thickness. R^2 values represent the proportion of variance explained by each endogenous variable. In A, negative values along PC1 of shape were indicative of those induced by non-lethal crayfish; thus, for clarity we switched the sign and labeled the box INV PC1. Shell defense traits were positively correlated (Table 2). Shell defense was a composite variable of the inverse of principal component 1 (INV PC1) and component 2 (PC2) of shell shape, and size-free shell thickness (ST). The variance of the shell defense variable was set to zero and the unstandardized path coefficient of shape PC2 was fixed to one in order to identify the variable. Thus, there is no R^2 for the composite variable. In B, the periphyton on plastic strips and periphyton mat latent variables were indicated by chlorophyll *a* concentrations.

non-lethal crayfish effects, the path from P addition to reproductive output (per capita F1 standing stock) through shell defense was larger than the path through growth (product of coefficients: Growth = 0.11, Defense = 0.45).

The non-lethal crayfish-by-P interaction variable modeled how P addition affected the strength of non-lethal crayfish effects on snail

Table 3. Direct, indirect, and total effects calculated from the standardized path coefficients from the SEM examining the effects of P addition, non-lethal crayfish, and their interaction on a suite of *P. duryi* traits. Values are in standard deviation units. Note that the total effect of P addition on per capita F1 standing stock, a measure of population growth, was much larger than the non-lethal effect of crayfish. SF Shell thickness = size-free shell thickness, INV PC1 = the inverse of principal component 1 of shape. See text for additional detail.

Variable	Non-lethal crayfish	P addition	Non-lethal crayfish × P addition	Log activity	Growth rate	SF Shell thickness	INV PC1	PC2
Direct effects								
Log activity	−0.71	...	...	...	...	...	...	...
Growth rate	−0.28	0.50	−0.11	0.23	...	...	...	...
Shell defense	0.32	−0.62	0.46	...	...	−0.44	−0.41	0.33
Log per capita F1 standing stock	...	...	...	...	0.23	...	...	...
Indirect effects								
Log activity	...	...	...	...	...	...	...	...
Growth rate	−0.17	...	...	...	...	...	...	...
Shell defense	...	...	...	...	...	...	...	...
Log per capita F1 standing stock	−0.33	0.56	−0.36	0.05	...	0.32	0.30	−0.24
Total effects								
Log activity	−0.71	...	...	...	...	...	...	...
Growth rate	−0.44	0.50	−0.11	0.23	...	...	...	...
Shell defense	0.32	−0.62	0.46	...	...	−0.44	−0.41	0.33
Log per capita F1 standing stock	−0.33	0.56	−0.36	0.05	0.23	0.32	0.30	−0.24

traits that influenced reproductive output. Adding P increased the negative effect of non-lethal crayfish on individual snail growth (h4) and increased resource allocation to shell defenses (h4) that separately reduced reproductive output. Similar to the main effect variables, the path from the interaction variable through shell defense had a larger effect on reproductive output than the path through individual growth (product of coefficients: Growth = −0.02, Defense = −0.33; Fig. 6A). Overall, the total positive effect of P addition on reproductive output was larger than the total negative effect of non-lethal crayfish (P = 0.56 vs. Non-lethal crayfish = −0.33), even though P additions resulted in larger negative effects of non-lethal crayfish on reproductive output (Non-lethal crayfish × P = −0.36; Table 3).

The third model quantified the cascading effects of our treatments on periphyton, including the indirect effect of non-lethal crayfish on periphyton distributions mediated through snails (h5), the indirect effect of lethal crayfish on periphyton volume (h6; snail removal), and adding P (h7; Fig. 6B). Non-lethal crayfish had a strong negative direct effect (−1.01; Table 4) on snail activity (h1) indicating that snails reduced activity (i.e., increased refuge use around plastic strips). The reciprocal path between activity and periphyton on strips was strong (0.53) compared to the reciprocal path between activity and periphyton mats (0.22). Therefore, the strong negative direct effect of non-lethal crayfish on snail activity cascaded to result in strong negative indirect effects (−0.54) on the periphyton associated with strips, despite the strong positive effect of snail activity (0.76) on periphyton (Table 4). Strong positive effects of activity on periphyton associated with strips indicate that increasing activity (movement away from the plastic strip refuge) facilitated periphyton growth on strips. Phosphorus additions had direct negative effects on periphyton associated with strips (−0.54) and periphyton mat (−0.56), but P indirectly stimulated snail activity (0.22) that increased periphyton growth on strips and in periphyton mat. The latter effect was relatively weak. Reducing snail density had positive direct effects on periphyton associated with strips (0.39) and on periphyton mat (0.03), but these effects, especially those on the periphyton mat, were relatively weak compared to non-lethal effects (Table 4).

Discussion

Structural equation models of a suite of prey traits revealed trade-offs between growth, reproduction, and defense that influenced population and community level effects of predator and

Table 4. Direct, indirect, and total effects calculated from the standardized path coefficients from the SEM examining the effects of P addition, non-lethal, and lethal (snail removal) effects of crayfish on *P. duryi* activity (Log activity) and periphyton mat (Log chl *a* in periphyton mat) or periphyton growing on plastic strips (Log chl *a* in periphyton on strips). Values are in standard deviation units.

Variable	Non-lethal crayfish	Lethal crayfish (Removal)	P addition	Log activity	Periphyton mat	Periphyton on strips
Direct effects						
Log activity	−1.01	...	...	...	−0.02	−0.55
Periphyton mat	...	0.03	−0.56	0.32	...	...
Periphyton on strips	...	0.39	−0.54	0.76	...	...
Log chl *a* in periphyton mat	...	...	...	...	1.00	...
Log chl *a* in periphyton on strips	...	...	...	...	...	1.00
Indirect effects						
Log activity	0.30	−0.15	0.22	−0.30	0.01	0.17
Periphyton mat	−0.22	−0.05	0.07	−0.10	0.00	−0.12
Periphyton on strips	−0.54	−0.11	0.16	−0.23	−0.01	−0.30
Log chl *a* in periphyton mat	−0.22	−0.08	−0.49	0.22	0.00	−0.12
Log chl *a* in periphyton on strips	−0.54	0.27	−0.37	0.53	−0.01	−0.30
Total effects						
Log activity	−0.70	−0.15	0.22	−0.30	−0.01	−0.39
Periphyton mat	−0.22	−0.08	−0.49	0.22	0.00	−0.12
Periphyton on strips	−0.54	0.27	−0.37	0.53	−0.01	−0.30
Log chl *a* in periphyton mat	−0.22	−0.08	−0.49	0.22	1.00	−0.12
Log chl *a* in periphyton on strips	−0.54	0.27	−0.37	0.53	−0.01	0.70

resource manipulations. Defensive shell trait variables were the primary route of non-lethal predator and P treatments on per capita F1 standing stock, a measure of snail population growth. This resulted from a life-history trade-off among growth, defense, and reproduction that favored investment in defense. Similarly, non-lethal predator and P addition indirect effects on periphyton were channeled through snail defensive traits, in this case, refuge use and foraging activity. These results illustrate the benefits of analyzing experimental data with a systems approach in order to understand the individual components of the net top-down and bottom-up factors influencing prey populations and the communities where they reside.

Phosphorus enrichment typically increases system productivity and improves the food quality of primary producers in aquatic systems by increasing periphyton standing crop and decreasing C:P ratios nearer to those of their consumers (Sterner and Elser 2002). Like prior research in the Everglades (Gaiser et al. 2011), in this study, periphyton mat (and production on strips) and chlorophyll *a* concentrations (and chlorophyll *a* density on strips) declined with P additions. Taxonomic composition shifted from diatoms (DIA) and coccoid blue greens (CBG) toward filamentous blue greens (FBG) and filamentous green (FG) algae. Despite declines in periphyton standing crop with P addition, remaining periphyton was higher quality; it had lower C:P ratios and was likely more accessible to grazers without the structural integrity typical of oligotrophic Everglades periphyton mats (Sterner and Elser 2002, Geddes and Trexler 2003).

Predators stress prey they do not kill and surviving prey express anti-predator behavior that often slows energy intake and prompts investment into defensive morphological traits rather than growth and reproduction (Kats and Dill 1998, Tollrian and Harvell 1999). Both of these defensive measures influence prey life-history traits and create trade-offs for the timing of, and size at, first reproduction that influence lifetime fitness and subsequently prey population growth (Crowl and Covich 1990, Chase 1999, Reznick et al. 2000, Sheriff et al. 2009, Hawlena and Schmitz 2010, Brönmark et al. 2012). Variation in food quality often has considerable influence on how non-lethal effects of predators influence prey traits related to fitness (Preisser et al. 2009). For example, Turner (2004) predicted, and found evidence, that predators have the greatest non-lethal effect on individual snail growth at relatively high resource levels because prey spend more time in

refuge instead of foraging. Conversely, Hoverman et al. (2005) and Brönmark et al. (2012) both found that increasing resource levels (or decreasing density) did not influence allocation of resources toward defense traits in snails. Unlike Hoverman et al. and Brönmark et al., we found that increasing resource levels resulted in snails allocating more resources toward defensive traits. In the SEM comparing allocation between defense and life-history traits, the total effect (behavior + shell defense + growth) of non-lethal crayfish on per capita F1 standing stock was strongest in tanks with P additions where per capita F1 standing stock was reduced by 60% compared to tanks with P addition but without caged crayfish. These results suggest that predator induced defense traits could limit prey population growth without killing any prey and the non-lethal effects of predators on prey population dynamics could be exacerbated in times or places with high food quality. Despite the increase in non-lethal crayfish effects with P addition, the total positive effect (shell defense + growth, Table 3) of P addition was much larger than the total negative effect (shell defense + growth + behavior) of non-lethal crayfish on per capita F1 standing stock. Therefore, in the oligotrophic Everglades system, P addition mitigated the negative non-lethal effects of crayfish on snail per capita F1 standing stock. This balance may shift in favor of stronger total non-lethal effects in systems that are naturally more eutrophic or areas that are enriched because of human activities.

Parsing the total effect of non-lethal crayfish on offspring production into the three separate traits (activity, growth, and shell defense) yields insight into the reproductive costs incurred to foil would-be predators. Non-lethal crayfish induced greater changes in behavior (activity) than other traits, but changing behavior had relatively minor impacts on individual growth and offspring production. These results indicate that changing behavior is an inexpensive solution for improving survivorship and probably acts as a first line of defense. Non-lethal crayfish directly reduced individual snail growth that negatively affected per capita offspring production. The mechanism relating the effects of non-lethal crayfish to reduced offspring production directly through growth likely resulted from physiological stress on snails, but the mechanism remains an open question. Although we did not directly measure stress, we did measure shell shape change and found that non-lethal crayfish induced *P. duryi* to build compressed shell apertures. Inducing shell shape change was relatively expensive, in the currency of offspring produced, compared to growth and behavior. This shape change may increase crayfish handling time similar to the elongate shells induced in physid snails (Covich et al. 1994, DeWitt et al. 2000) and appears to be an alternative solution to the tall narrow shells found in *Helisoma*, another ramshorn snail (Hoverman et al. 2005). Like *Helisoma* in the Hoverman et al. study, non-lethal crayfish induced thicker shells in *P. duryi* compared to snails in tanks without crayfish. Additionally, we found that in tanks without crayfish, adding P and removing snails (3-way interaction) resulted in the production of relatively thin snail shells. A recent study in a marine snail found that thick shells were a passive byproduct of slow growth no matter if slow growth resulted from limited resources or threats of predation (Bourdeau 2010). The thinner shelled snails in our study may have resulted from the trend for faster growth in tanks without caged crayfish when high quality food was present and at low snail density. However, snails produced thicker shells in tanks with non-lethal crayfish across other treatment combinations, indicating that snails produce relatively thicker shells regardless of growth rate when predators were present.

Non-lethal effects of predators often cascade to positively alter the quantity or affect the spatial distribution of prey resources by altering prey behavior (Werner and Peacor 2003, 2006, Schmitz 2010). Our third SEM quantified the direct and indirect effects of snail behavior (activity/refuge use) on periphyton. Reciprocal paths between periphyton on strips and snail activity indicated dual causation between these variables, but not between periphyton mat and snail activity, because these paths were comparatively weak. This likely resulted because mature Everglades periphyton mats are structurally complex and are not heavily grazed compared to the new growth on artificial substrates (Geddes and Trexler 2003, Chick et al. 2008). The positive direct effect between snail activity and periphyton on strips indicated that increased activity

resulted in *more* periphyton on strips. Increased periphyton growth with increased snail activity suggests grazing facilitated periphyton growth through nutrient regeneration, regardless of crayfish presence (McCormick and Stevenson 1991, Geddes and Trexler 2003). In addition to the positive effect of snails on periphyton, we also found negative effects that were mediated through non-lethal crayfish that altered snail behavior (activity). We observed snails under plastic strips in tanks with caged crayfish and we found 55% lower chlorophyll *a* density on plastic strips in these tanks, compared to other treatments, suggesting that snails seeking refuge from the perceived threat of predation grazed periphyton from the strips. Without non-lethal crayfish, snails actively moved throughout the tank. These observations appeared in the SEM as a large negative direct effect between non-lethal crayfish and snail activity and a positive direct effect between activity and periphyton on strips (refuges) resulting in a negative, non-lethal, indirect effect of crayfish on periphyton in refuges. Thus, non-lethal crayfish indirectly shaped the spatial distribution of periphyton in tanks by directly altering snail behavior (i.e., non-lethal trophic cascade or trait-mediated indirect effect, Abrams et al. 1996, Turner et al. 1999).

Historically, lethal effects were considered the primary way predators influenced prey population growth and community structure (Murdoch et al. 2003). We found little evidence for strong lethal effects of predators by simulating lethal effects in the snail removal treatment that was based on field estimates of snail mortality. Reducing prey density by 60% during the first half of the experiment (88% total) did not influence snail growth, affect reproductive output, or lead to a lethal trophic cascade (i.e., density-mediated indirect effect). Although densities were scaled to field levels and predation rates were based on field estimates (Ruehl 2010), low initial snail densities may have prevented resource limitation at ambient conditions, yielding a weak test for lethal effects (e.g., reduced intraspecific competition). However, snail densities are low in karst wetlands, including the Everglades, compared to other freshwater systems (Ruehl and Trexler 2011), probably because the abundant periphyton mats in these oligotrophic systems have comparatively high C:P ratios (low quality) and are not palatable (Geddes and Trexler 2003, Chick et al. 2008, Sargeant et al. 2011). Therefore, non-lethal effects of predators might be greater than lethal effects in this system because low quality resources limit primary consumer growth. We propose that chronic P enrichment in the Everglades may increase the relative importance of lethal effects along with non-lethal effects of predators because snail densities would likely increase since adding P had the largest effect on snail reproductive output.

Using a multivariate systems approach, we found complex interactive effects between predators and productivity that propagated through trade-offs among a suite of prey traits that, in turn, drove intergenerational dynamics of primary consumers (snails) and cascading effects on algal abundance and spatial pattern. Direct and indirect non-lethal effects of predators were much stronger than simulated lethal effects. Future studies should consider the influence of traits like morphology on fitness, population growth, and community structure in addition to physiology and behavior. A better understanding of such complex effects emerging from life-history trade-offs among traits is needed to manage anthropogenic nutrient enrichment and depletion of apex consumers (Schmitz 2010).

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Supplemental Material

Appendix

Table A1. Experimental treatments used to test for the non-lethal and lethal (Snail removal) effects of a crayfish predator (*P. fallax*) on a snail grazer (*P. duryi*) and the grazer's food (periphyton). A Y indicates the presence of a non-lethal crayfish or snail removal, N indicates no crayfish or no snail removal, and the dash indicates no-snails present. All treatments were tested at ambient and high P levels for a total of 12 treatments and is denoted by the dagger (†) and separate numbers for ambient and high P treatments. Hypotheses are explained in the text and illustrated in Fig. 1. Hypothesis 7 (h7) was tested by comparing tanks with and without P addition. See *Methods* for further detail.

Treatment†	Non-lethal crayfish	Lethal crayfish (Removal)	Effect tested on *P. duryi*	Effect tested on periphyton	Hypotheses
1, 2	Y	Y	Net effect of *P. fallax*	Net direct and indirect effects	
3, 4	Y	N	Non-lethal effect of *P. fallax*	Non-lethal trophic cascade	h1, h3, h4, h5
5, 6	Y	...	...	Control for 1–4	
7, 8	N	Y	Density effect of *P. fallax*	Lethal trophic cascade	h2, h6
9, 10	N	N	Control for 1–4, 7, 8	Control for 1–4, 7, 8	
11, 12	N	...	...	Control for 5, 6	

Table A2. Direct, indirect, and total effects calculated from the standardized path coefficients from the SEM of the first conceptual model examining the effects of P addition, non-lethal, and lethal (snail removal) effects of crayfish on *P. duryi* activity (Log activity), growth rate, size-free shell thickness (SF Shell thickness), and the first two components of shell shape (INV PC1 and PC2). Shape change along PC1 indicated that snails with shapes indicative of those from non-lethal crayfish tanks were arbitrarily assigned negative values so we switched the sign and labeled the axis INV PC1. Values are in standard deviation units.

Variable	Non-lethal crayfish	Lethal crayfish (Removal)	P addition	Log activity	Growth rate	SF Shell thickness	INV PC1	PC2
Direct effects								
Log activity	−0.72	...	...	...	...	...	...	...
Growth rate	−0.32	−0.05	0.50	0.16	...	...	...	...
Shell defense	0.26	0	−0.72	...	...	−0.38	0.53	0.42
Log per capita F1standing stock	...	...	...	...	0.31	...	...	...
Indirect effects								
Log activity	...	...	...	...	...	...	...	...
Growth rate	−0.11	...	...	...	...	...	...	...
Shell defense	...	...	...	...	...	...	...	...
Log per capita F1 standing stock	−0.27	−0.01	0.55	0.05	...	0.21	−0.29	−0.23
Total effects								
Log activity	−0.72	...	...	...	...	...	...	...
Growth rate	−0.43	−0.05	0.50	0.16	...	...	...	...
Shell defense	0.26	...	−0.72	...	...	−0.38	0.53	0.42
Log per capita F1 standing stock	−0.27	−0.01	0.55	0.05	0.31	0.21	−0.29	−0.23

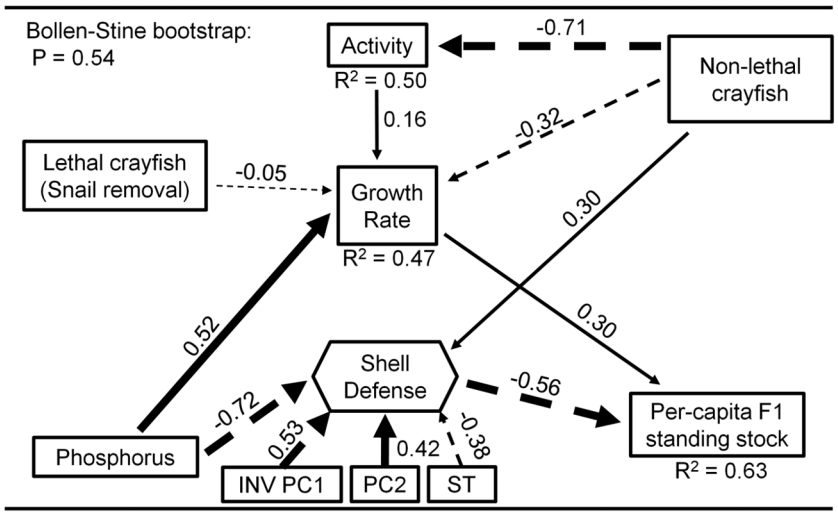

Fig. A1. Structural equation model results for the first conceptual model testing for non-lethal, lethal (snail removal), and P addition effects on different snail traits. Squares symbolize observed variables and hexagons represent composite variables; solid lines are positive effects and dashed lines are negative effects. Numbers associated with paths are standardized path coefficients representing the strength of the relationship between variables and were used to set line thickness. R^2 values represent the proportion of variance explained by each variable. Shell defense was composed of principal component 1 (INV PC1) and component 2 (PC2) of shell shape, and size-free shell thickness (ST). There is no R^2 for the composite variable because we set the variance of the shell defense variable to zero and set the unstandarized path coefficient of shape PC2 to one in order to identify the composite. Inspection of shape change along PC1 revealed that shapes indicative of those induced by non-lethal crayfish were arbitrarily assigned negative values; thus, for clarity we switched the sign and labeled the box INV PC1. Shell shape change and shell thickness traits were positively correlated (Table 2).