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Altering aquatic food webs with a global insecticide: arthropod–amphibian links in mesocosms that simulate pond communities

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Abstract. Pesticides play a critical role in maximizing yields of economically important crops and minimizing the human health threats of disease-carrying pests, but they often have collateral effects on nontarget species. We used a mesocosm study to address how the most commonly used insecticide in the USA, malathion, applied at low, ecologically relevant concentrations (20 and 110 μg/L) affects species interactions in aquatic communities. Unlike many community ecotoxicology studies, our study assessed how malathion affects both consumptive and nonconsumptive effects of predators. We also considered how the vertical distribution of predator cues and malathion (caused by potential stratification) affects species interactions. We found no evidence for vertical stratification of malathion, a result suggesting that exposure to the pesticide was uniform throughout the water column. Malathion was lethal to some primary consumers (cladocerans) at both concentrations and to top predators (dragonflies) at the highest concentration (110 μg/L). These lethal effects initiated density-mediated indirect effects in both cases. Malathion also may have decreased dragonfly foraging efficiency, resulting in increased tadpole survival (trait-mediated indirect effect), which decreased the resources used by tadpoles (periphyton). Collectively, our results show that malathion alters species interactions. However, we suggest that the degree to which pesticides affect aquatic communities will depend strongly on the species composition of communities. Therefore, the community-level consequences of pesticide exposure are likely to vary across the ecological landscape.

Key words: pesticide, phytoplankton bloom, malathion, stratification, amphibian declines.

Pesticides help maintain productive yields of economically important crops and prevent outbreaks of disease-carrying pests. However, pesticides can have unintended consequences for nontarget organisms because they attack evolutionarily conserved physiological processes (e.g., acetylcholinesterase inhibitors, endocrine disruptors, photosynthesis inhibitors). At lethal concentrations, pesticides reduce population size, which may cause deterioration of the genetic quality of populations and increase the risk of local extinctions (Shaffer 1981, Nunney and Campbell 1993, Lynch et al. 1995). Pesticide-induced reductions in population size also may have community-level consequences (Relyea and Hoverman 2006, Rohr et al. 2006). In aquatic communities, arthropods have diverse roles in food webs and tend to be very sensitive to low, ecologically relevant concentrations of insecticides. In these communities, insecticide-induced reductions in arthropod populations can initiate strong density-mediated indirect effects (Relyea and Hoverman 2006, 2008, Relyea and Diecks 2008). Thus, the distribution of sensitive taxa across a food web may be a critical determinant of the degree to which pesticides perturb an ecological community.

Pesticides also can have strong effects on communities at sublethal concentrations if they affect traits that are important in species interactions. Pesticides cause such effects by interfering with the nervous systems of organisms, resulting in changes in behavior. These behavioral changes can have immediate and long-term fitness consequences. For example, pesticides can affect behaviors that are used to avoid predators and find mates, leading to reduced survival and lower reproductive success (Park et al. 2001, Tietjen 2006). Moreover, pesticides can decrease foraging activity (Bridges 1997, 1999, Relyea and Mills 2001, Rohr et al. 2003, Broomhall 2004, Punzo 2005), which may decrease growth rates (Relyea and Mills 2001, Relyea 2004, Gurushankara et al. 2007, Shenoy et al. 2009). Pesticide-induced reductions in foraging

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and assimilation efficiency are likely to have strong effects on the development of many traits.

The spatial scale at which pesticides affect communities has received little attention. Thermal stratification of aquatic habitats can lead to decreased mixing of substances (Wetzel 2001), including pesticides (Sudo et al. 2004, Jones et al. 2010, 2011). Thus, pesticide exposures can vary even at very local scales (within a wetland or small pond). Moreover, the solubility of the pesticide in water will affect the vertical distribution of the pesticide. For example, chlorpyrifos, which is not very soluble in water (2 μg/L at 2–35°C), stratifies in water. Initially, it is more concentrated at the surface (first 24 h), but eventually, it becomes concentrated in sediments (Crum and Brock 1994). In contrast, the herbicide linuron, which is more soluble in water (75 μg/L), does not stratify in aquatic systems (Crumrkz et al. 1998). Thus, level of exposure to pesticides may not be homogenous within wetlands (Clark et al. 1993), a situation that can lead to spatial variation in their effects on populations and species interactions.

We assessed how uncaged predators and spatially distributed caged predator cues interact with a common pesticide (the insecticide malathion) to affect aquatic communities. We constructed communities typical of small ponds. These communities consisted of producers (phytoplankton and periphyton), primary consumers (zooplankton and tadpoles), and secondary consumers (nymphal dragonflies). We considered the possibility that malathion might stratify in mesocosms and designed our experiment such that, if malathion stratified, predator cues would be either present or absent in the areas where malathion concentrations were highest. This design enabled us to ask whether the effects of spatial distribution of pesticides and predators on aquatic communities were interactive.

We hypothesized that malathion would cause both density- and trait-mediated indirect effects in the aquatic community and that the strength of these effects would depend on both the concentration of malathion and the spatial distribution of predator cues. We used taxa that vary in their sensitivity to malathion (cladoceran zooplankton [most sensitive], nymphal dragonflies [sensitive], and tadpoles [least sensitive]) (Relyea 2004, Relyea and Diecks 2008, Relyea and Hoverman 2008, Relyea and Edwards 2010). We predicted that higher concentrations of malathion would kill zooplankton and dragonflies but would have sublethal effects on tadpoles. We expected lower concentrations to have lethal effects on zooplankton but sublethal effects on dragonflies and tadpoles. Consequently, we predicted that even the lowest concentration of malathion would initiate a phytoplankton bloom by killing zooplankton (density-mediated indirect effect), which would cause a decrease in periphyton (competitor with phytoplankton for light and nutrients) and, thus, would result in less food for tadpoles. We used uncaged and caged dragonfly predators to examine how pesticides influenced consumptive and nonconsumptive effects of dragonflies on tadpoles. We predicted that pesticide-induced density-mediated indirect effects via dragonflies would be strongest at high concentrations of malathion because this concentration should be lethal to dragonflies (Relyea and Edwards 2010). At lower concentrations, we predicted trait-mediated indirect effects caused by changes in tadpole and dragonfly behavior (Relyea and Edwards 2010). In mesocosms with uncaged predators, we expected dragonflies to consume fewer tadpoles in the presence of malathion than in the absence of malathion, leading to more periphyton and, thus, more food for individual tadpoles in mesocosms with malathion. Last, we predicted that if malathion stratified in the water column, it would alter habitat use by tadpoles and dragonflies.

Malathion

Malathion is used globally and is the most commonly used insecticide in the USA (10–15 million kg were applied in the USA in 2001; Kiely et al. 2004). It is an organophosphate insecticide that binds irreversibly to acetylcholine esterase and inhibits neurotransmitter function. Malathion has a relatively short half-life, ranging from 2 d at pH = 8 to 26 d at pH = 6 (Guerrant et al. 1970, Wang 1991), but its effects on the nervous system can persist for weeks because new acetylcholine esterase must be synthesized to recuperate from exposure (Chambers et al. 2002, Ferrari et al. 2004). Recent concerns over a possible link between pesticides and population declines of the California red-legged frog (*Rana aurora draytonii*) have led to efforts to predict expected environmental concentrations (EECs) of pesticides in lentic systems based on application frequency (every 2–14 d), application rates, and proportion of expected drift (Odenkirchen and Wente 2007). Based on applications to treat a variety of terrestrial crops (50+ crops), malathion is expected to occur at 9 ± 27 μg/L (mean ± 95% CI) in lentic habitats. Applications near water, either to treat aquatic crops or to kill disease-carrying mosquitoes, result in substantially higher EECs (539–1797 μg/L; Odenkirchen and Wente 2007).

Methods

We conducted our experiment during summer 2008 at the University of Pittsburgh's Pymatuning

Laboratory of Ecology in northwestern Pennsylvania (USA). We used a completely randomized, factorial design with 3 nominal pesticide (malathion) concentrations (0, 100, and 500 μg/L) and 4 predator (*Anax junius*) treatments (no predator, 2 caged predators near the surface of the mesocosm, 2 caged predators near the bottom, and 2 uncaged predators) and examined effects on tadpoles of green frogs (*Rana clamitans*), gray treefrogs (*Hyla versicolor*), and bullfrogs (*Rana catesbeiana*). We used concentrations of malathion that were well below the range that produces 50% mortality of tadpoles of these species in 16-d exposures ($LC50_{16\text{-}d}$; 1500–4100 μg/L; Relyea 2004). Moreover, 1000 μg/L of malathion applied for 16 d does not decrease survival of green frogs and gray treefrogs relative to water controls and causes only an 18% reduction in bullfrogs (Relyea 2004). Therefore, the concentrations we used were unlikely to have direct lethal effects on tadpoles, but were within the EECs found in lentic habitats (Odenkirchen and Wente 2007). We replicated controls and caged-predator treatments 3× and uncaged-predator treatments 4× (39 experimental units).

Experimental units were 1300-L polypropylene cattle tanks (1.68 m diameter × 0.47 m deep) that served as pond mesocosms. Each mesocosm was covered with a 60% shade cloth lid to prevent colonization by insects and escape of amphibian metamorphs. We filled each mesocosm with ~1000 L of well water between 18–20 June (pH = 8). On 23 June, we added 300 g of air-dried oak leaves (*Quercus* spp.), 0.7 L of pond water (a mixture from 3 nearby ponds), and 25 g of commercial rabbit chow to each mesocosm. The oak leaves provided nutrients and structure for benthic organisms; the pond water provided an inoculum of phytoplankton, periphyton, zooplankton, and microbes; and the rabbit chow served as a source of nutrients for algae and microbes. We scanned the pond water visually and removed invertebrate predators before adding it to the mesocosms. On 26 June, we added another 0.7 L of pond water (a mixture from nearby ponds) and 2 unglazed clay tiles (15 × 15 cm) for sampling periphyton to each mesocosm. We allowed periphyton, phytoplankton, and zooplankton populations to develop in the mesocosms for 12 d before adding amphibians and applying treatments.

We collected newly oviposited egg clutches from nearby ponds and allowed the eggs to hatch in 200-L wading pools (30 clutches of gray treefrogs collected between 25 May and 4 June, 15 clutches of green frogs collected between 27 May and 4 June, and 15 clutches of bullfrogs collected on 6 June). We fed tadpoles rabbit chow ad libitum until 7 July, when we allocated tadpoles to the mesocosms. We added 60 individuals of each species to each mesocosm (mean initial mass [mg] ± SE: gray treefrogs = 81 ± 6, green frogs = 27 ± 2, bullfrogs = 22 ± 2) to yield 180 tadpoles/mesocosm (27 individuals/m^2 for each species). These tadpole densities were well within natural hatchling densities for each species (E. Werner, University of Michigan; RAR; K. Yurewicz, Plymouth State University; D. Skelly, Yale University; M. Bernard, Case Western Reserve University; C. Davis, University of Michigan; unpublished data). For each species, we set aside samples of 20 tadpoles in well water to assess 24-h survival subsequent to handling stress. All 3 species had 100% survival.

We equipped each mesocosm with 4 predator cages, 2 at the top and 2 at the bottom of the water column (1 each on the east and 1 each on the west side of the mesocosm). We constructed predator cages from plastic drainage pipe (8-cm length × 12-cm diameter) capped with fiberglass screen. We used a small piece of polystyrene foam to float cages at the water surface and a small rock to anchor cages at the bottom of the water column. This procedure allowed us to produce predator cues that were more concentrated at the top or the bottom of the mesocosm. We also included a treatment with uncaged dragonflies to assess community responses to direct consumption of tadpoles.

On 11 July (day 0 of the experiment), we added 2 nymphal dragonflies (*Anax junius*) to mesocosms assigned to caged- and uncaged-predator treatments (mean ± SD head width [mm]: 6.1 ± 0.7). We fed each dragonfly 300 mg of gray treefrog tadpoles before adding them to the mesocosms to produce predator cues. In the uncaged-predator treatment, we housed dragonflies in floating cages for 24 h to release predator cues into the mesocosm before releasing them to allow tadpoles to respond behaviorally to the presence of the dragonflies. We fed caged dragonflies 300 mg of tadpoles every 2 to 3 d to maintain chemical cues in the mesocosms (the tadpole species used was alternated at each feeding). This feeding regimen produced large reductions in activity and increased hiding behavior in tadpoles in earlier studies (Van Buskirk and Relyea 1998, Schoeppner and Relyea 2008, 2009). We lifted cages above the water surface to feed dragonflies. We equalized this disturbance across mesocosms by lifting cages in uncaged-predator and predator-free mesocosms briefly above the water surface.

We applied pesticide treatments on the day that we added predators to the mesocosms. We used a commercial formulation of malathion with 50% active ingredient (Malathion Plus; Ortho Corporation,

Marysville, Ohio). Our use of a commercial formulation meant that we also added undisclosed, inert ingredients to the mesocosms. We acknowledge that these ingredients also might affect aquatic communities. We added 1.025 mL or 0.205 mL of formulated product to the mesocosms to achieve the nominal concentrations of 100 and 500 μg/L. We used a 4-L watering can (filled with 4 L of charcoal-filtered, ultraviolet [UV]-irradiated water) to apply the pesticide. We designed our applications to mimic overspray, which could lead to stratification of pesticides in lentic habitats (Jones et al. 2010, 2011). We applied malathion across the surface of the mesocosm from a height of ~18 cm. We applied 4 L of charcoal-filtered, UV-irradiated water to mesocosms that did not receive malathion. We used a pipe sampler to collect 77 mL of water from the top 5 cm and bottom 5 cm of each mesocosm ~1 h after applying malathion. We pooled samples by pesticide treatment and location (top or bottom), mixed them thoroughly, and split the pooled mixture into 2 samples. We placed each sample in a precleaned, 500-mL amber glass jar and stored them at 2°C for subsequent chemical analysis by an independent laboratory (Mississippi State Chemical Laboratory, Mississippi State, Mississippi; lower detection limit = 0.2 μg/L). We applied malathion again on day 12 and used the same protocol to measure concentrations. This application frequency is within the standard range for malathion (2–14 d; Odenkirchen and Wente 2007).

Water analyses indicated that our actual concentrations were 20% of nominal concentrations for both pesticide treatments, which is common in mesocosm studies (Brock et al. 2000). For the 500-μg/L treatment, samples taken at the surface had measured concentrations of 117 and 114 μg/L on days 0 and 12, respectively, and samples taken from the bottom had measured concentrations of 101 and 122 μg/L on days 0 and 12, respectively. For the 100-μg/L treatment, samples taken at the surface had measured concentrations of 11 and 24 μg/L on days 0 and 12, respectively, and samples taken at the bottom had measured concentrations of 18 μg/L on both days. We found no evidence of stratification in the mesocosms and no concentration differences between application dates. Therefore, we report the average concentration for the 4 samples taken per treatment when referencing malathion treatments (nominal 100 μg/L = 20 μg/L, and nominal 500 μg/L = 110 μg/L). For the day-0 dosing, we also analyzed top and bottom water samples from no-malathion treatments. We detected very low concentrations of malathion in these mesocosms (0.16 and 0.09 μg/L in top and bottom water samples, respectively).

Response variables

We measured several abiotic and biotic response variables to assess how aquatic communities respond to pesticides and spatially variable predator stress. For variables that were measured twice during the experiment, we spaced our sampling effort such that the 1st measurement was taken before the 2nd pesticide application. Our 2nd set of measurements was taken several days after the 2nd pesticide application. On days 6 and 18, we used a calibrated digital water meter (WTW, Wareham, Massachusetts) to measure pH and dissolved O_2 at the center of each mesocosm near the surface and bottom. We used a separate water meter to measure temperature. Preliminary readings showed that temperature at the bottom and top of the mesocosm were very similar (within 1°C). Therefore, we measured temperature at mid-depth in the center of each tank. On day 18, we used an underwater quantum sensor (LI-COR, Lincoln, Nebraska) to measure light attenuation in each mesocosm to assess whether a phytoplankton bloom had occurred. We measured light attenuation on day 18 because if the phytoplankton were to bloom, they would have done so by this date (Relyea and Diecks 2008, Relyea and Hoverman 2008). We measured light intensity 2.7 and 23.5 cm below the surface of each mesocosm and calculated the decay rate of light with increased water depth (K) as

$$K = \ln(L_{2.7}/L_{23.5})/d$$

where $L_{2.7}$ and $L_{23.5}$ are the intensity of sunlight at 2.7 and 23.5 cm, respectively, and d is the difference in depth between the 2 measurements. Collectively, these data gave us a picture of the abiotic conditions in the mesocosms early (day 6) and late (day 18) in the experiment. Furthermore, these data provided information on how quickly the pesticide might be expected to degrade because the breakdown of malathion is dependent on temperature and pH.

We sampled zooplankton, phytoplankton, and periphyton twice. We sampled zooplankton with a 0.2-L tube sampler at 5 standardized locations in each mesocosm on days 10 and 20. We pooled the 5 samples and filtered them through a 62-μm Nitex screen (Nitex, Sofia, Bulgaria). We preserved samples in 70% ethanol and subsequently counted the cladocerans and copepods in each pooled sample. We used higher levels of taxonomic resolution rather than species-level resolution because the dominant species in each zooplankton group show similar responses to malathion (Relyea and Diecks 2008).

We assessed phytoplankton biomass by collecting 500-mL water samples from the middle of the water

column in the center of each mesocosm on days 12 and 20. We vacuum-filtered each sample through a Whatman GF/C filter, wrapped the filters in aluminum foil, and stored them at –20°C for subsequent chlorophyll *a* analysis. We used the methods given by Arar and Collins (1997), including the acidification step, to extract chlorophyll *a*. We measured the concentration of chlorophyll *a* (μg/L) in each sample with a calibrated fluorometer (model TD-700; Turner Instruments, Sunnyvale, California).

We assessed periphyton biomass on days 11 and 19. We removed a tile from each mesocosm and scrubbed the periphyton growing on one side of the tile (the side exposed to the sun) in a plastic tub containing filtered well water. We vacuum-filtered this water through a preweighed Whatman GF/C filter that had been dried at 80°C for 24 h. We dried the filter at 80°C for 24 h and reweighed it to determine the biomass of periphyton on each tile.

The day after the 1st pesticide application, we began daily predator health assessments. We scanned each uncaged-predator mesocosm for dead predators and checked the health of caged predators. Dead dragonflies were immediately removed and replaced to maintain predator cues in mesocosms. For each mesocosm, we recorded the number of dragonflies that died during the experiment.

Starting on day 3, we made daily behavioral observations of tadpole hiding behavior and activity. On each sampling date, we scan-sampled (Altmann 1974) each mesocosm and recorded the number of individuals that were visible 12 cm above the bottom (indicated by cube-shaped projections that were distributed equidistantly along the bottom of the mesocosm) of the mesocosm and the number of individuals observed that were moving. Each scan sample lasted only a few seconds (enough time to look over the entire tank). These methods allowed us to assess how behavioral traits (low activity and hiding in the benthos) that decrease predation risk were affected by our treatments (Peacor and Werner 1997, Relyea 2002a, b, Schoeppner and Relyea 2005, 2008). We did not differentiate the species during these observations, but all 3 species demonstrate similar predator-induced changes in behavior in response to *Anax* (Relyea and Werner 1999, Schoeppner and Relyea 2005, 2008). The phytoplankton blooms that occurred in some of the tanks did not hinder these observations.

We collected the 1st gray treefrog metamorph on day 12. On that day and thereafter until the end of the experiment, we did daily searches for metamorphs. We removed metamorphs from mesocosms when both forelimbs had emerged and tail resorption was complete (stage 46; Gosner 1960). For each metamorph, we recorded the number of days that elapsed between the start of the experiment and development to Gosner stage 46. We euthanized each metamorph in 2% MS-222, preserved it in 10% formalin, and later weighed it. We calculated the proportion of gray treefrogs that metamorphosed.

We terminated the experiment on 1 August, 21 d after treatments were applied to mesocosms. We collected all remaining tadpoles and dragonflies from each mesocosm. We euthanized tadpoles in 2% MS-222 and preserved them in 10% formalin. We preserved dragonflies in 70% ethanol. We calculated % survival for each anuran species, and we calculated growth rates as (log[final mass] – log[initial mass])/24 d for green frog and bullfrog tadpoles. We did not calculate tadpole growth rates for gray treefrogs because most of the animals had metamorphosed (mean ± SD; 68% ± 19) or were nearing metamorphosis by the end of the experiment. As amphibians approach metamorphosis, growth trajectories first level off and then start to decline (Wilbur and Collins 1973). Therefore, we used mass at metamorphosis as our size-based performance measure for gray treefrogs. For all amphibian response variables, we used the mean response from each mesocosm.

Statistical analyses

We used general linear models (GLMs) to analyze response variables. For response variables that violated the assumption of homogeneity of variances, we attempted either log(x) or arcsine(x) (for proportion responses) transformations to equalize variances. If transformations were unsuccessful, we rank-transformed the data. After running GLMs, we used multiple comparisons to assess which treatments were responsible for significant effects. For data that did not require transformation, log(x)-, and arcsine(x)-transformed data, we used Tukey's Honestly Significant Difference multiple comparison procedure. For rank-transformed data, we used Dunn's test in place of Tukey's test (Glantz 1997).

Abiotic responses.—Abiotic response variables provided insights into how quickly malathion should degrade and the productivity of the aquatic environment (e.g., higher levels of DO and pH are indicative of higher primary productivity). We used a repeated-measures multivariate analysis of variance (rm-MANOVA) to assess how time, predator, and pesticide treatments affected temperature, DO, and pH.

Primary producers and zooplankton abundance.—Insecticides typically initiate a trophic cascade that operates through the sensitivity of zooplankton.

Therefore, we used a multivariate analysis to assess how predator treatments and pesticide concentrations affected these taxa. We used an rm-MANOVA with time as a within-subject factor and predator treatments and pesticide concentration as between-subject factors. We analyzed responses by 2 zooplankton groups (cladocerans and copepods) and 2 groups of primary producers (phytoplankton and periphyton). We used an analysis of variance (ANOVA) to compare light attenuation (greater light attenuation is indicative of high phytoplankton levels) on day 18 across predator and pesticide treatments to establish whether a phytoplankton bloom occurred in mesocosms where zooplankton populations were reduced.

Anuran survival, growth, and behavior.—We used a MANOVA to assess how predator treatments and malathion concentration affected anuran growth and survival. Amphibians from 2 mesocosms were accidently mixed while we were taking down the experiment. We excluded these replicates from this analysis.

We used an ANOVA to assess the effects of treatments on the proportion of gray treefrogs that metamorphosed. For mesocosms that produced metamorphs, we used a MANOVA to determine whether treatments affected time to and mass at metamorphosis.

For tadpole behavior data, we compared the proportion of individuals observed higher in the water column (from 12 cm above the bottom of the mesocosm to the surface) and the proportion of individuals observed that were active across treatments. Our measures of tadpole behavior varied widely among sampling dates, probably because of variation in the time of day when we made observations and variation in weather conditions over the course of the experiment. Inspection of behavioral data over time revealed no strong treatment × time interactions for either measure of tadpole behavior. Therefore, we analyzed 18-d averages of proportion of tadpoles observed and the proportion of tadpoles active.

To determine the proportion of tadpoles observed, we divided the number of tadpoles observed in each mesocosm by the estimated number (using a negative exponential mortality curve) of stocked individuals remaining after subtracting the cumulative number of metamorphs collected from the mesocosm on the observation date. We used a negative exponential mortality curve because *Anax* predation on tadpoles typically results in such a function (Van Buskirk and Yurewicz 1998, Relyea 2002b). We measured tadpole activity as the proportion of observed tadpoles that were moving during an observation. We used a MANOVA to compare antipredator responses (hiding behavior and changes in activity) across predator and malathion treatments.

Anax *survival.*—For treatments with predators, we assessed whether predator location in the mesocosm (caged top, caged bottom, and uncaged) and pesticide concentration affected predator survival. We used a nonparametric Kruskal–Wallis test because transformation of data did not resolve unequal treatment variances.

Results

Abiotic responses

The rm-MANOVA on abiotic response variables (temperature, DO, and pH) revealed significant predator (Pillai's Trace 1.037, $F_{15,75} = 2.641$, $p = 0.003$), pesticide (Pillai's Trace 0.817, $F_{10,48} = 3.315$, $p = 0.002$), and predator × pesticide (Pillai's Trace 1.379, $F_{30,135} = 1.714$, $p = 0.02$) effects. It also revealed significant time (Pillai's Trace 0.947, $F_{5,23} = 82.706$, $p < 0.001$), time × pesticide (Pillai's Trace 0.908, $F_{10,48} = 3.989$, $p = 0.001$), and time × predator (Pillai's Trace 0.82, $F_{15,75} = 1.88$, $p = 0.039$) effects, but the 3-way interaction was not significant (Pillai's Trace 0.896, $F_{30,135} = 0.982$, $p = 0.501$). We used univariate tests and multiple comparisons to determine the sources of these multivariate responses.

Dissolved O_2.—The time × pesticide interaction was significant (Table 1). On day 6, treatment effects on DO were similar between the tops and bottoms of mesocosms (Fig. 1A). DO was higher in mesocosms dosed with malathion than in controls. DO did not differ between mesocosms dosed with 20 and 110 μg/L malathion. On day 18, treatment effects on DO were similar between the tops and bottoms of mesocosms (Fig. 1B). Uncaged predators caused an increase in DO when malathion was absent, but this effect diminished as malathion concentration increased. The DO data suggested that the mesocosms were not stratified.

pH.—The within-subjects interactions (time × pesticide and time × predator; Table 1) were significant. On day 6, pH was similar between the tops and bottoms of mesocosms (Fig. 1C). pH was higher in the 2 pesticide treatments than in the controls. On day 18, pH was similar between the tops and bottoms of mesocosms (Fig. 1D). Uncaged predators caused an increase in pH when pesticide was absent, but this effect diminished as pesticide concentration increased. DO and pH responses reflected changes in primary productivity.

Temperature.—Temperature increased over the experiment, but time × treatment interactions were not significant (Table 1). Averaged across treatments, temperature was higher on the 2nd than on the 1st

TABLE 1. Analysis of variance table showing *F*-values and significance levels for the effects of predators, pesticide (malathion), and their interaction on dissolved O_2 (DO), pH, and temperature across time. Within-subjects effects (time and time × treatment interactions) and between-subjects effects are provided. Bold indicates significant effects. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

		DO		pH		
Source	df	Bottom	Top	Bottom	Top	Temperature
Within-subjects effects						
Time	1,27	**164.201*** **	**193.708*** **	**90.013*** **	**132.377*** **	**303.116*** **
Time × pesticide	2,27	**28.338*** **	**34.611*** **	**22.964*** **	**30.824*** **	1.069
Time × predator	3,27	2.059	0.976	**4.326***	**3.82***	0.201
Time × pesticide × predator	6,27	0.965	0.598	0.594	0.343	2.153
Between-subjects effects						
Pesticide	2,27	1.094	0.706	1.119	0.651	**13.466*** **
Predator	3,27	**8.762*** **	**8.001*** **	**6.532****	**7.264*** **	0.316
Pesticide × predator	6,27	**3.131***	**3.528****	**2.606***	**2.675***	0.931

sampling date (day 6: 25.4 ± 0.5°C [mean ± SD], day 18: 26.9 ± 0.6°C). Averaged across sampling dates, the effect of pesticide was significant, but the predator and malathion × predator interaction effects were not significant (Table 1). A small increase in temperature occurred in pesticide treatments relative to in controls (control: 25.8 ± 0.3°C, 20 μg/L: 26.2 ± 0.3°C, 110 μg/L: 26.5 ± 0.4°C; Tukey's tests, all $p \leq 0.03$).

Primary producers and zooplankton abundance

The rm-MANOVA on primary producers and zooplankton revealed significant predator (Pillai's Trace 0.84, $F_{12,78} = 2.53$, $p = 0.007$) and pesticide (Pillai's Trace 1.31, $F_{8,50} = 11.88$, $p < 0.001$) effects, but the predator × pesticide effect was not significant (Pillai's Trace 0.81, $F_{24,108} = 1.14$, $p = 0.32$). The time (Pillai's Trace 0.83, $F_{4,24} = 29.84$, $p < 0.001$) and time × pesticide (Pillai's Trace 0.55, $F_{8,50} = 2.38$, $p = 0.03$) effects were significant, but time × predator (Pillai's Trace 0.42, $F_{12,78} = 1.06$, $p = 0.41$) and the 3-way interaction effect (Pillai's Trace 0.6, $F_{24,108} = 0.79$, $p = 0.74$) were not. To determine the sources of these multivariate responses, we subsequently used univariate tests and multiple comparisons.

Cladocerans.—The time and time × treatment interactions were not significant (Table 2). Pesticide affected abundance, but the predator and pesticide × predator effects were not significant (Table 2). Mesocosms with malathion had virtually no cladocerans compared to controls (Dunn's tests, all $p < 0.01$; Fig. 2A).

Copepods.—Univariate tests revealed a time × pesticide effect (Table 2). Therefore, we examined treatment effects within sampling dates. On day 10, pesticide affected copepod abundance, but predator and the pesticide × predator interaction did not (Table 2). Across all predator treatments, copepod abundance was similar between the 0 μg/L and 20 μg/L treatments (Tukey's test, $p = 0.085$) but was significantly lower in the 110 μg/L treatment (Tukey's test, 110 μg/L vs 20 μg/L: $p = 0.046$, 110 μg/L vs 0 μg/L: $p < 0.001$; Fig. 2B). On day 20, predator affected copepod abundance, but pesticide and the predator × pesticide interaction did not (Table 2). Across all malathion treatments, copepods were more abundant in mesocosms with *Anax* caged at the top of mesocosms than in all other predator treatments (Tukey's test, $p < 0.03$; Fig. 2B).

Phytoplankton.—The rm-ANOVA revealed an increase in phytoplankton over time but no time × treatment effect (Table 2). Pesticide and predator, but not their interaction, affected phytoplankton (Table 2). Averaged across all predator treatments, mesocosms containing 0 μg/L malathion had less phytoplankton than mesocosms with 20 μg/L or 110 μg/L malathion (Tukey's tests, both $p < 0.001$; Fig. 2C). Averaged across all malathion treatments, mesocosms containing uncaged predators had less phytoplankton than mesocosms containing caged or no predators (Tukey's tests, all $p \leq 0.02$; Fig. 2C).

Effects on phytoplankton biomass were consistent with light decay results. Pesticide treatment affected light decay rates ($F_{2,27} = 8.96$, $p = 0.001$), but predators ($F_{3,27} = 2.05$, $p = 0.13$) and the pesticide × predator interaction ($F_{6,27} = 1.48$, $p = 0.22$) had no effect. Light decay rates were 15% higher in mesocosms with 20 μg/L malathion (Tukey's test, $p = 0.03$) and 22% higher in mesocosms with 110 μg/L malathion than in controls (Tukey's test, control: 0.0079 ± 0.0002 [mean K ± SE], 20 μg/L: 0.0091 ± 0.0005, 110 μg/L: 0.0097 ± 0.0002, $p = 0.001$). Light decay rates did not differ between mesocosms with 20 and 110 μg/L malathion (Tukey's test, $p = 0.294$).

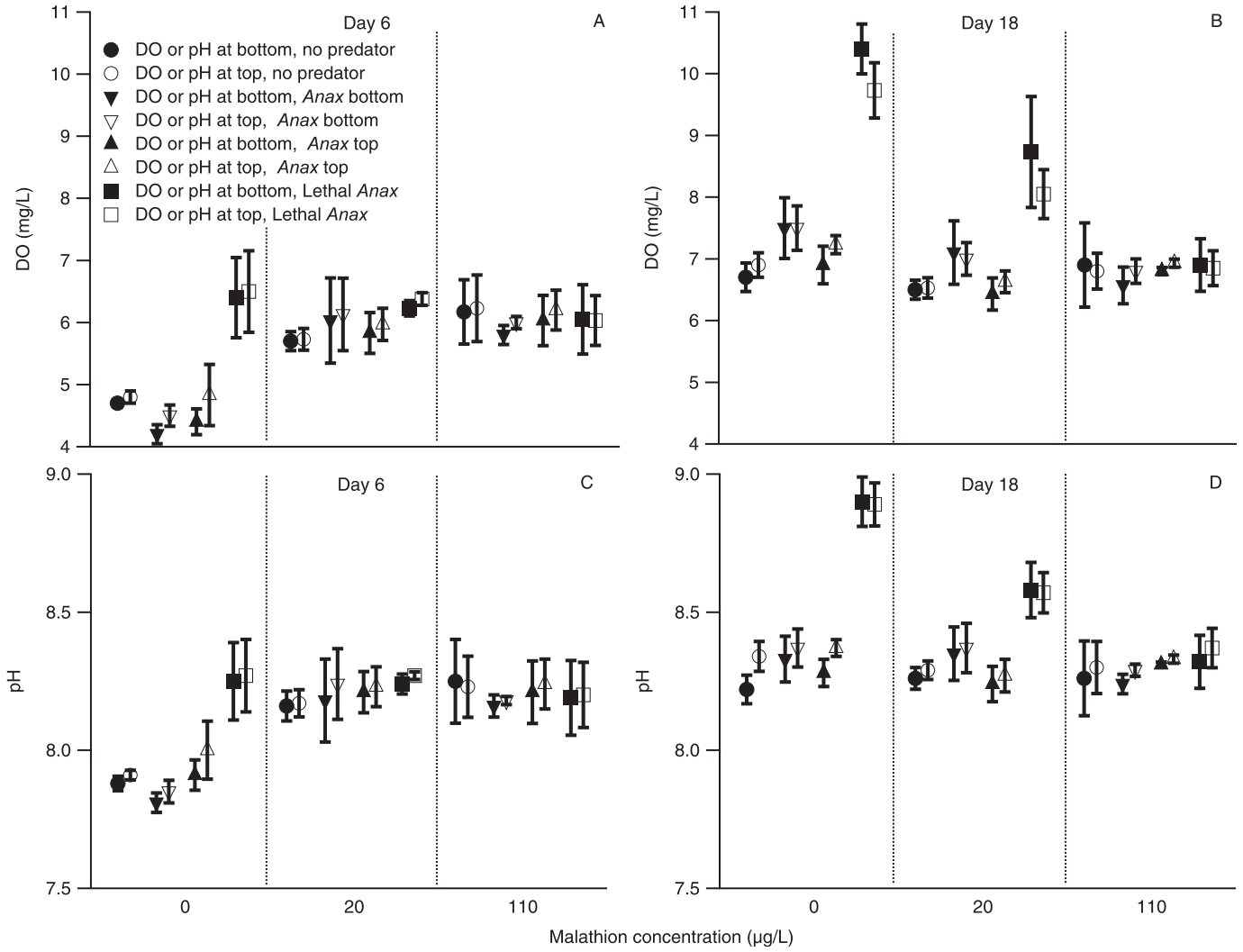

FIG. 1. Mean (±1 SE) dissolved O_2 (DO) on days 6 (A) and 18 (B) and pH on days 6 (C) and 18 (D) in predator and malathion concentration treatments. *Anax* top = predator caged at the top of the mesocosm, *Anax* bottom = caged predator at the bottom of the mesocosm, lethal *Anax* = uncaged predator.

Periphyton.—Periphyton biomass increased over time, but time × treatment interactions were not significant (Table 2). Periphyton was affected by pesticide and predators, and the pesticide × predator interaction was marginally significant (Table 2). The interaction was caused by a diminishing increase in periphyton with higher concentrations of malathion in mesocosms with uncaged predators. Across sampling dates, in the absence of malathion, periphyton biomass was 169% higher in mesocosms with uncaged predators than in mesocosms with no predators. In the presence of malathion, periphyton biomass increased only 74% in mesocosms with 20 µg/L malathion and did not increase in mesocosms with 110 µg/L malathion (Fig. 2D).

Anuran antipredator behavior

Tadpole behavior was affected by predator (Pillai's Trace = 1.04, $F_{6,50} = 9.06$, $p < 0.001$) and the predator × pesticide interaction (Pillai's Trace = 0.84, $F_{12,50} = 3.01$, $p = 0.003$), but not by pesticide (Pillai's Trace = 0.22, $F_{4,50} = 1.51$, $p = 0.21$). Predator effects on the number of tadpoles observed depended on malathion concentration (predator × malathion interaction: $F_{6,25} = 4.39$, $p = 0.004$). In the absence of malathion, caged predators at the top and bottom of mesocosms reduced the number of observed tadpoles by 24 and 41%, respectively, whereas uncaged predators caused a 68% reduction. A similar pattern was observed in mesocosms with 110 µg/L malathion. At 20 µg/L, the number of

TABLE 2. Analysis of variance table showing *F*-values and *p*-values (in parentheses) for the effects of time, pesticide (malathion), and predators on biotic response variables. Within-subjects effects (time and time × treatment interactions) and between-subjects effects are provided. Bold indicates significant effects.

Source	df	Cladocerans	Copepods	Phytoplankton	Periphyton
Within-subjects effects					
Time	1,27	0.62 (0.44)	3.53 (0.07)	**44.83 (<0.001)**	**65.81 (<0.001)**
Time × pesticide	2,27	0.03 (0.97)	**10.74 (<0.001)**	0.98 (0.39)	1.78 (0.19)
Time × predator	3,27	0.87 (0.47)	2.39 (0.09)	0.41 (0.75)	0.87 (0.47)
Time × pesticide × predator	6,27	0.56 (0.76)	0.49 (0.81)	1.9 (0.12)	1.14 (0.37)
Between-subjects effects					
Pesticide	2,27	**56.89 (<0.001)**	**day 10: 11.48 (<0.001)** day 20: 0.04 (0.96)	**97.36 (<0.001)**	**16.35 (<0.001)**
Predator	3,27	0.04 (0.99)	day 10: 0.35 (0.79) **day 20: 5.67 (0.004)**	**7.85 (0.001)**	**10.01 (<0.001)**
Pesticide × predator	6,27	0.03 (1.0)	day 10: 0.48 (0.82) day 20: 0.67 (0.67)	2.02 (0.1)	2.38 (0.06)

tadpoles observed was highest in the no-predator controls and lowest in the uncaged-predator treatments. However, no difference between the top and bottom cage predator treatments was found (Fig. 3A). Tadpole activity was affected by predators ($F_{3,27} = 29.37$, $p < 0.001$) but not by pesticide ($F_{2,27} = 1.64$, $p = 0.21$) or the pesticide × predator interaction ($F_{6,27} = 2.04$, $p = 0.095$). Tadpole activity was lower in mesocosms with uncaged predators than in mesocosms with caged predators and no-predator controls (Tukey's tests, all $p < 0.001$). However, the effect of uncaged predators was weaker when mesocosms were dosed with 110 μg/L of malathion (Fig. 3B).

Anuran survival and growth

We found a multivariate effect of pesticide (Pillai's Trace = 0.91, $F_{10,44} = 3.68$, $p = 0.001$), predator (Pillai's Trace = 1.13, $F_{15,69} = 2.78$, $p = 0.002$), and their interaction (Pillai's Trace = 1.42, $F_{30,125} = 1.65$, $p = 0.03$) on the survival of all 3 anuran species and the growth rate of bullfrog and green frog tadpoles. Therefore, we proceeded with analysis of univariate effects.

Green frog survival.—Predator and pesticide but not the predator × pesticide interaction affected green frog survival (Table 3). Survival was high in mesocosms with no predators or caged predators (>80%) but was lower in mesocosms with uncaged predators (Tukey's tests, all $p < 0.001$). The pesticide main effect was driven by higher survival in mesocosms dosed with 110 μg/L than those dosed with 20 μg/L malathion (Tukey's test, $p = 0.01$; Fig. 4A).

Bullfrog survival.—Predators affected bullfrog survival, but pesticide and the predator × pesticide interaction did not (Table 3). Bullfrog survival was lower in mesocosms with uncaged predators than in no-predator controls (Tukey's tests, all $p < 0.001$; Fig. 4B).

Gray treefrog metamorph and tadpole survival.—Predators and the predator × pesticide interaction affected gray treefrog metamorph and tadpole survival, but the effect of pesticide was marginally nonsignificant (Table 3). Uncaged predators reduced gray treefrog survival in the absence of malathion, but this effect weakened with increased malathion (Fig. 4C).

Green frog and bullfrog growth.—Predators, pesticide, and predator × pesticide affected green frog growth rate. The most striking pattern was a much higher growth rate in the uncaged-predator treatment than in other predator treatments in mesocosms with 20 μg/L malathion. This pattern also occurred in mesocosms with 0 μg/L malathion, but it was much weaker. In mesocosms with 110 μg/L malathion, growth rates were comparable among predator treatments (Table 3, Fig. 5A). Similar patterns were found for bullfrogs (Table 3, Fig. 5B).

Gray treefrog metamorphosis

Gray treefrog mass at metamorphosis was significantly affected by predators (Pillai's Trace = 0.7, $F_{6,52} = 4.67$, $p = 0.001$) and the predator × pesticide interaction (Pillai's Trace = 0.68, $F_{12,52} = 2.21$, $p = 0.03$), but not by pesticide (Pillai's Trace = 0.17, $F_{4,52} = 1.23$, $p = 0.31$) (Table 4). Metamorph mass was higher in uncaged-predator mesocosms than in other predator treatments, but this effect decreased as malathion concentration increased (Fig. 5C). Predator, pesticide, and the predator × pesticide interaction did not affect time to metamorphosis (all gray treefrogs metamorphosed, on average, after 21 d; Table 4).

The percentage of gray treefrogs that metamorphosed was affected by predator and the predator × pesticide interaction, but not pesticide (Table 4). The predator × pesticide interaction was driven by an

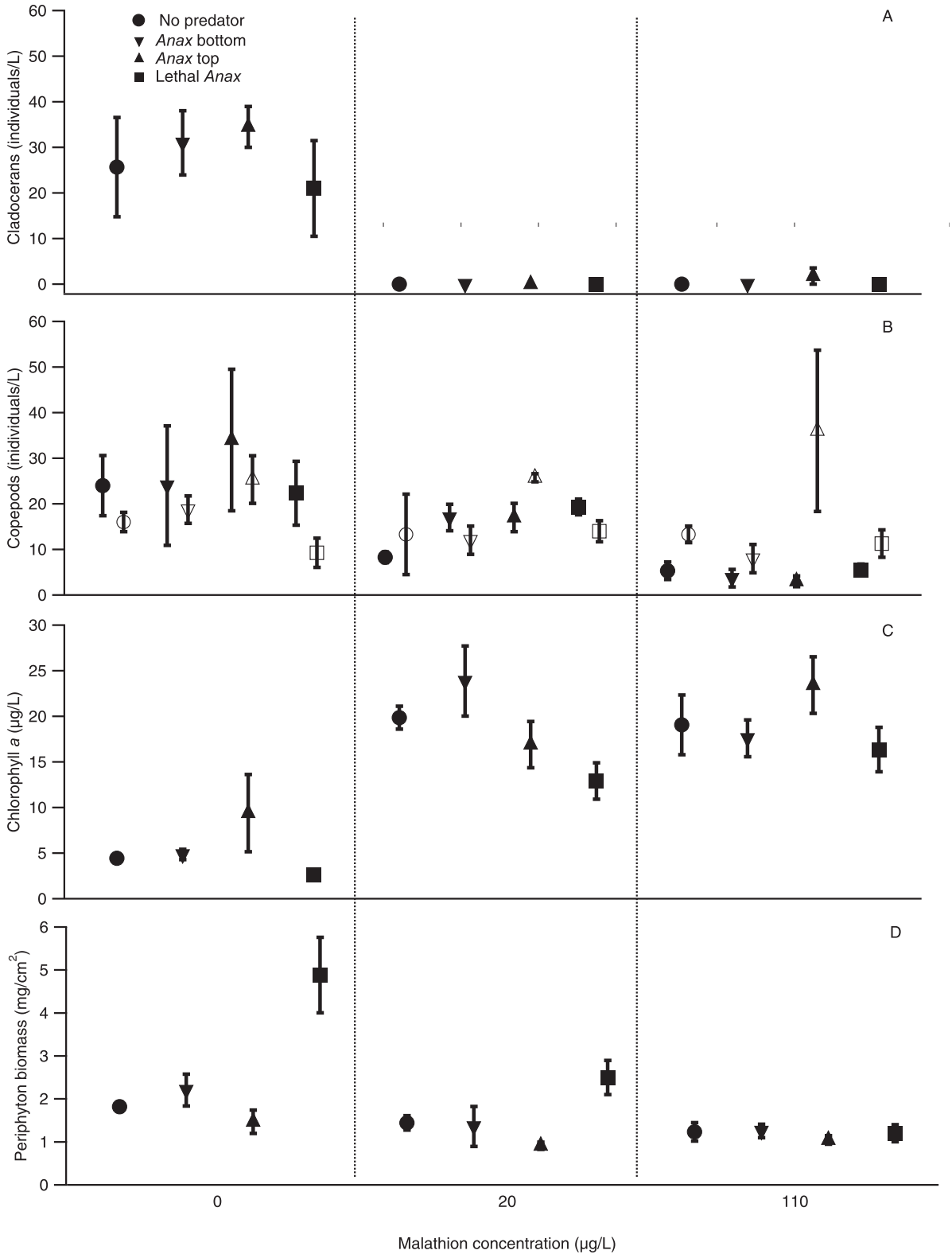

FIG. 2. Mean (±1 SE) density of cladocerans (A) and copepods (B), concentration of chlorophyll *a* (C), and periphyton biomass (D) in predator and malathion concentration treatments. *Anax* top = predator caged at the top of the mesocosm, *Anax* bottom = caged predator at the bottom of the mesocosm, lethal *Anax* = uncaged predator. For copepods, the filled symbols represent day 10 and the open symbols day 20.

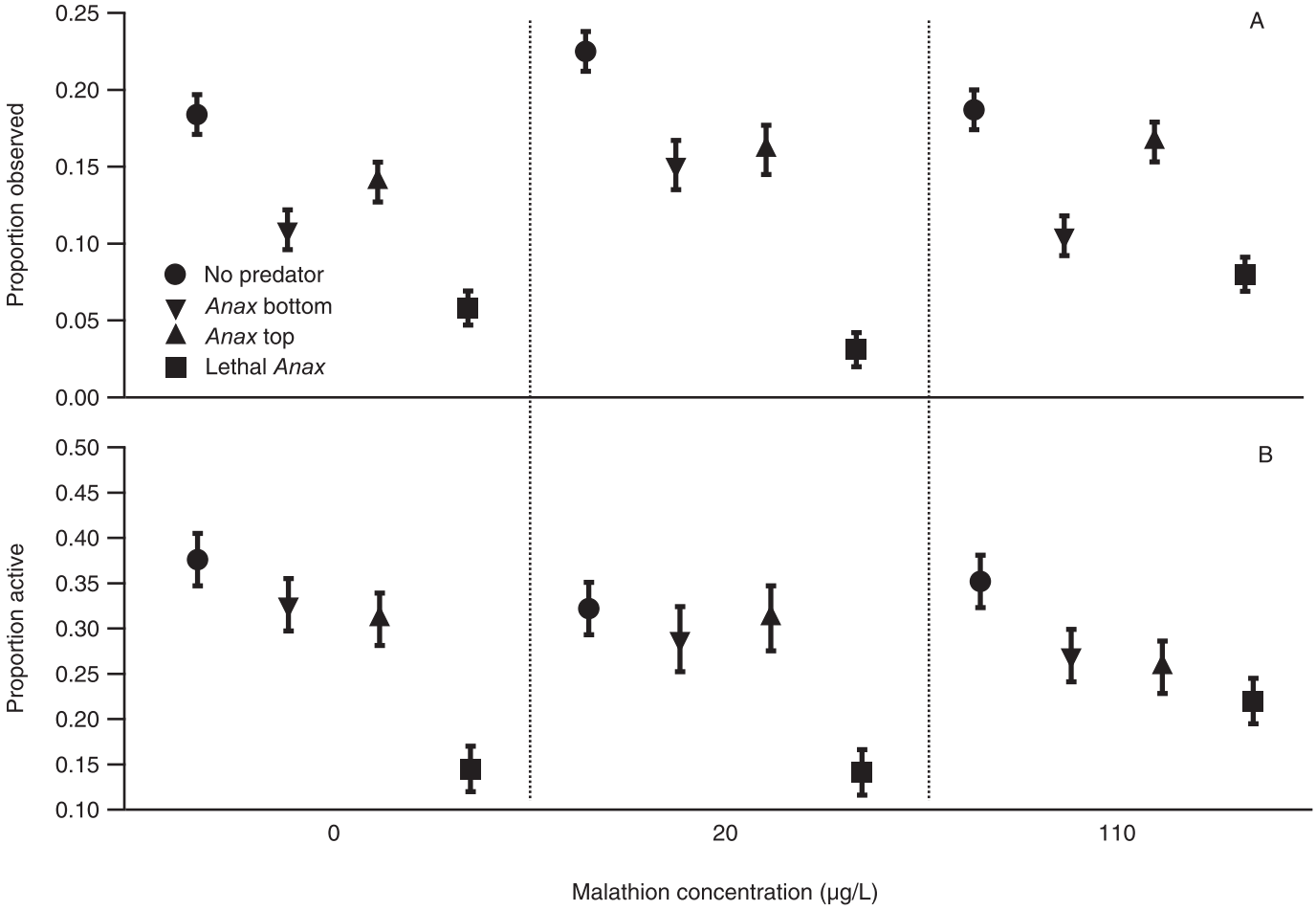

FIG. 3. Mean (±1 SE) proportion of tadpoles observed (A) and that were active (B) in predator and malathion concentration treatments. *Anax* top = predator caged at the top of the mesocosm, *Anax* bottom = caged predator at the bottom of the mesocosm, lethal *Anax* = uncaged predator.

increase in the percentage of gray treefrogs that metamorphosed as malathion concentration increased in uncaged-predator treatments (Fig. 4D).

Predator survival

Predator survival was affected by pesticide (χ^2_8 = 21.03, p = 0.007). Fewer dead dragonflies were replaced in controls and mesocosms dosed with 20 µg/L malathion than in mesocosms dosed with 110 µg/L (median [range], control: 0 [0–2], 20 µg/L: 0 [0–1], 110 µg/L: 4 [2 to 6]; Dunn's tests all $p < 0.01$).

Discussion

Malathion and predators had unique and interactive effects on the aquatic community. Both caused direct lethal effects (e.g., malathion reduced zooplankton populations, whereas uncaged *Anax* decreased anuran populations) and sublethal effects (e.g., predators reduced anuran activity). Malathion and predators initiated density- and trait-mediated indirect effects on the aquatic community. The magnitude and direction of predator effects depended on exposure to malathion. Below, we discuss how the aquatic community responded to combinations of malathion and predators, and we pay special attention to how the 2 interacted to shape species interactions and community structure.

Malathion had lethal effects on zooplankton populations. The concentrations of malathion used in our study (0, 20, and 110 µg/L) are among the lowest concentrations to decrease zooplankton survival under mesocosm conditions (previous studies have used concentrations ranging from 10–460 µg/L; Relyea 2005, 2009, Relyea and Diecks 2008, Relyea and Hoverman 2008). Moreover, our concentrations are environmentally relevant, given average expected environmental concentrations in aquatic habitats of 9 ± 27 µg/L (mean ± 95% CI) when applied to

TABLE 3. Analysis of variance table showing *F*-values and *p*-values (in parentheses) for the effects of pesticide (malathion), predators, and their interaction on amphibian survival and growth. Bold font indicates significant treatment effects.

		Survival			Growth	
Source	df	Green frogs	Bullfrogs	Gray treefrogs	Green frogs	Bullfrogs
Pesticide	2,25	**3.94 (0.033)**	0.22 (0.805)	3.217 (0.057)	**5.33 (0.012)**	**7.19 (0.003)**
Predator	3,25	**21.01 (<0.001)**	**49.17 (<0.001)**	**108.02 (<0.001)**	**10 (<0.001)**	**7.61 (0.001)**
Pesticide × predator	6,25	0.97 (0.464)	1.56 (0.201)	**9.201 (<0.001)**	**4.92 (0.002)**	**3.21 (0.018)**

terrestrial crops and 539 to 1797 μg/L when applied near water to control mosquitoes or pests of aquatic crops (Odenkirchen and Wente 2007). Malathion greatly reduced cladoceran populations at 20 and 110 μg/L, whereas it reduced copepod populations only at the highest concentration. These results are consistent with results of previous mesocosm studies in which cladocerans were generally more sensitive to insecticides (including malathion) than copepods. The difference in sensitivity often resulted in competitive release of copepods (Hanazato and Yasuno 1987, 1989, 1990, Havens 1994, Mills and Semlitsch 2004, Relyea 2005, Relyea and Diecks 2008, Relyea and Hoverman 2008).

Consistent with previous work, the decrease in zooplankton abundance released phytoplankton from herbivory and led to a phytoplankton bloom (Havens 1994, 1995, Boone et al. 2004, Mills and Semlitsch 2004, Relyea and Hoverman 2008). However, in our study, copepods either did not have enough time or were unable to capitalize on the increased phytoplankton biomass and decreased competition from cladocerans. The 8-d interval between the last dose of malathion and zooplankton sampling might have been insufficient for copepod populations to recover from pesticide exposure. Relyea and Hoverman (2008) observed an increase in copepod abundance in mesocosms dosed with 140 μg/L malathion 10 d after dosing. However, in another study, 8 d was not sufficient for copepods to increase in abundance after exposure to 10 μg/L malathion (Relyea and Diecks 2008). Copepods did eventually increase in numbers in pesticide-treated mesocosms relative to controls but only after 22 d. Information on generation times of copepod species is largely missing in the literature, but is needed to predict how quickly tolerant zooplankton species will respond to pesticide-induced increases in food.

The lack of an increase in copepod abundance also could have been the result of the size structure of algal populations that make up the phytoplankton bloom. Copepods generally prefer larger food particles (planktonic algae, protists, and bacteria) than filter-feeding cladocerans (Zöllner et al. 2003). However, larger phytoplankton may be in short supply in algal blooms initiated by loss of top-down control because smaller phytoplankton have a greater surface area to volume ratio and, thus, are superior competitors under these uptake-limited conditions (Verdy et al. 2009). In addition, malathion is more lethal to cladocerans than copepods, but sublethal effects of the pesticide on copepod foraging and reproductive behavior may inhibit population growth. Unfortunately, few data are available on the sublethal effects of pesticides on zooplankton (Gliwicz and Sieniawska 1986, Dodson et al. 1995), and we found no data for copepods. Last, many copepod species are predatory (particularly cyclopoids) and, thus, information on the lethality of pesticides to their prey (primarily rotifers and small cladocerans) is needed (Brandl 2005). Future work in these areas will provide insights into why copepod responses to insecticide-induced changes in resource supply are so variable among mesocosm studies (Hanazato and Yasuno 1987, 1989, 1990, Havens 1994, Mills and Semlitsch 2004, Relyea 2005, 2009, Relyea and Diecks 2008, Relyea and Hoverman 2008).

The pesticide-initiated phytoplankton bloom did not result in a decrease in periphyton, even though this effect has been observed in a number of mesocosm studies with a diverse set of insecticides, including malathion (Havens 1994, 1995, Mills and Semlitsch 2004, Relyea and Diecks 2008, Relyea and Hoverman 2008). However, other investigators also have found no effect of insecticides on periphyton biomass (Relyea et al. 2005) and, in one case, periphyton increased in response to malathion exposure (Relyea 2005). Closer inspection of these studies shows no clear patterns with regard to pesticide concentration. For example, relatively high concentrations of malathion suppressed (nominal concentration 250 μg/L; Relyea and Diecks 2008) or increased (nominal concentration 320 μg/L; Relyea 2005) periphyton. In the study by Relyea (2005), malathion

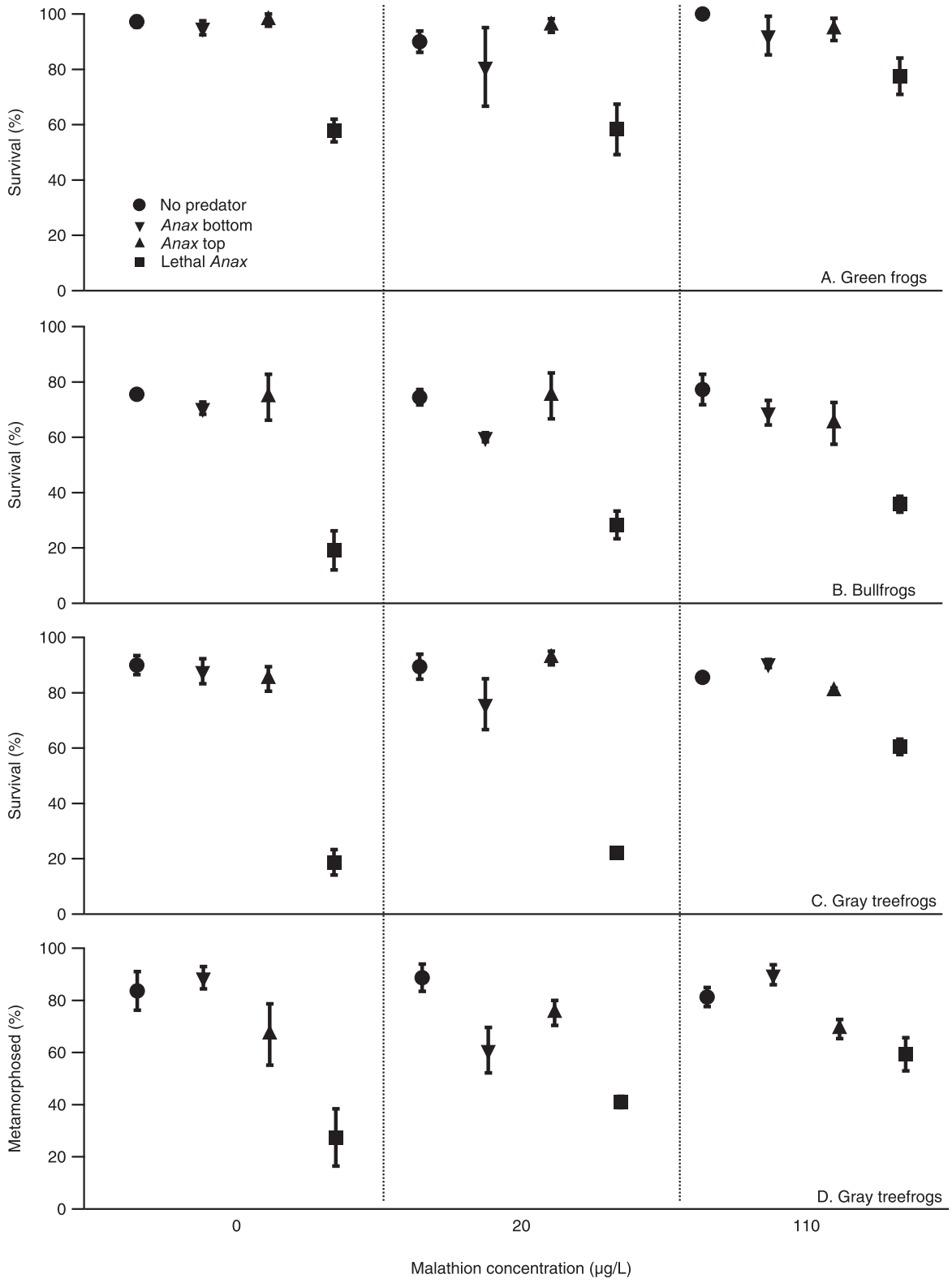

FIG. 4. Mean (±1 SE) % survival of green frogs (A), bullfrogs (B), and gray treefrogs (C), and % metamorphosed gray treefrogs (D) in predator and malathion concentration treatments. *Anax* top = predator caged at the top of the mesocosm, *Anax* bottom = caged predator at the bottom of the mesocosm, lethal *Anax* = uncaged predator.

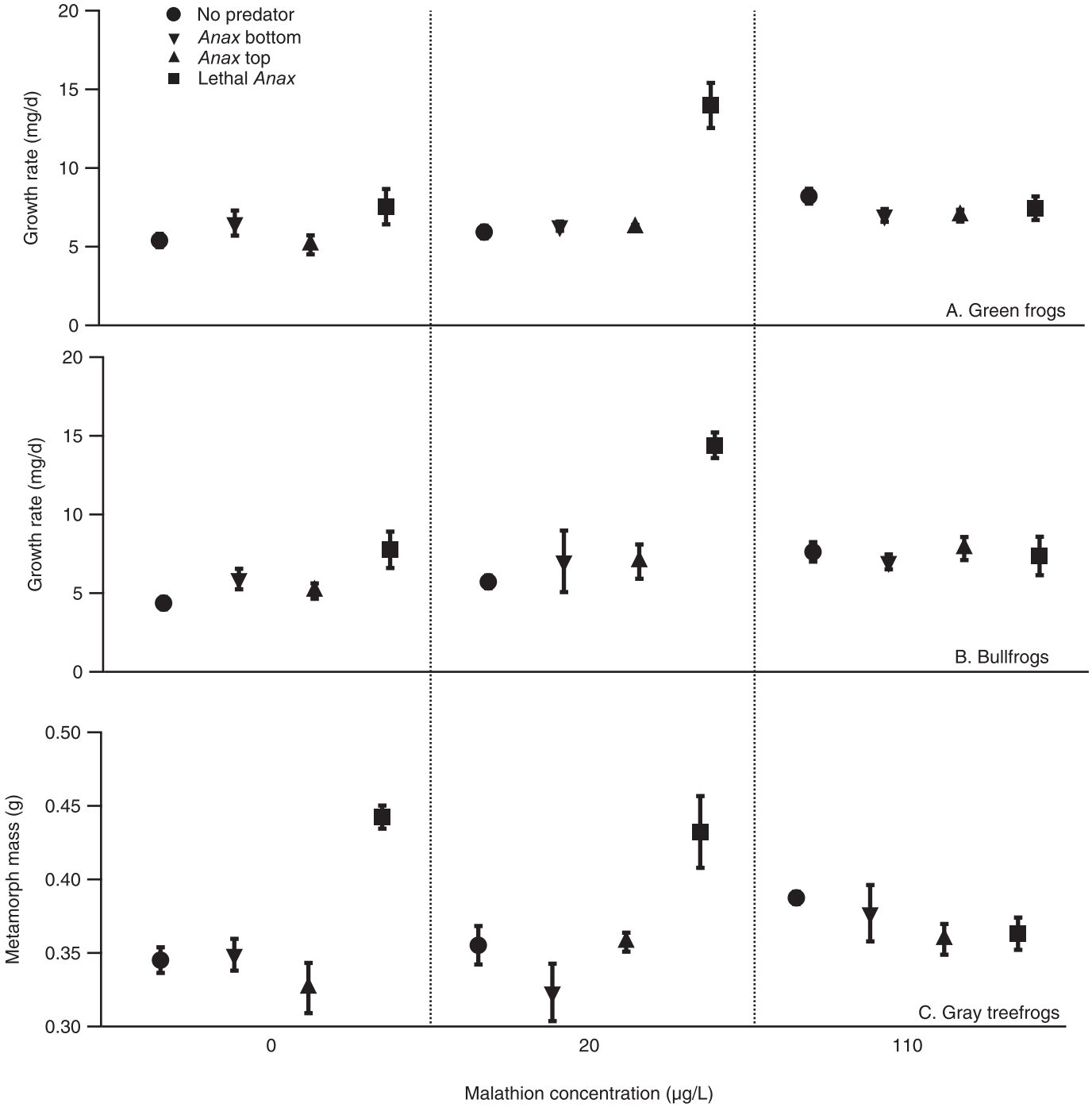

FIG. 5. Mean (±1 SE) growth rates of green frogs (A) and bullfrogs (B) and mass at metamorphosis of gray treefrogs (C) in predator and malathion concentration treatments. *Anax* top = predator caged at the top of the mesocosm, *Anax* bottom = caged predator at the bottom of the mesocosm, lethal *Anax* = uncaged predator.

caused a large reduction in insect predator biomass, an effect that should have initiated a density-mediated indirect negative effect on periphyton caused by the increased survival of herbivorous tadpoles. The paradoxical increase in periphyton might have been the result of a combination of trait-mediated indirect effects via reductions in tadpole foraging behavior and bottom-up effects via nutrients derived from dead predators. Likewise, relatively low concentrations of malathion inhibited (140 µg/L; Relyea and Hoverman 2008) and had no effect (5.8 µg/L; Relyea 2009) on periphyton growth.

TABLE 4. Analysis of variance table showing *F*-values and *p*-values (in parentheses) for the effects of pesticide (malathion), predators, and their interaction on gray treefrog metamorphosis. Bold font indicates significant treatment effects.

Source	df	Mass at metamorphosis	Time to metamorphosis	Proportion metamorphosed
Pesticide	2,25	0.18 (0.835)	2.44 (0.11)	1.87 (0.174)
Predator	3,25	**13.32 (<0.001)**	1.01 (0.4)	**24.38 (<0.001)**
Pesticide × predator	6,25	**5.46 (0.001)**	0.87 (0.528)	**3.21 (0.016)**

Last, sampling periphyton shortly after (21 d, 300 μg/L; Relyea and Hoverman 2008) and well after (44 d, 250 μg/L; Relyea and Diecks 2008) pesticide exposure have both returned results showing a decrease in periphyton in response to pesticides. In all cases, the pesticides reduced at least some zooplankton species. However, periphyton decreased only in cases where phytoplankton blooms were observed. Future studies should address both top-down (e.g., species composition of zooplankton assemblages) and bottom-up forces (e.g., nutrient availability) that may buffer aquatic systems against pesticide-induced phytoplankton blooms.

Tadpole growth rates (green frogs and bullfrogs) and mass at metamorphosis (gray treefrogs) were similar between control and malathion-dosed mesocosms, except for in uncaged-predator treatments (see below for discussion of this effect). Thus, the modest reductions in periphyton observed in malathion-dosed mesocosms were not enough to lead to reduced tadpole performance. Results have been mixed in previous studies of amphibian responses to insecticide-induced reductions in food quantity. For southern leopard frogs (*Rana sphenocephala*), metamorphs took longer to develop and were smaller at metamorphosis when exposed to 2 mg/L of the insecticide carbaryl, but only in the presence of interspecific competition from *Pseudacris crucifer* (Mills and Semlitsch 2004). Relyea and Diecks (2008) found that malathion-induced reductions in periphyton slowed growth of northern leopard frogs (*Rana pipiens*) but had no effect on wood frogs (*Rana sylvatica*). Species-specific patterns were similar in another study using the same pair of amphibians (Relyea and Hoverman 2008). These results may reflect differences in larval period. Species that have longer larval periods (e.g., leopard frogs) are more likely to be affected by pesticide-initiated reductions in periphyton than species with short larval periods (e.g., wood frogs) because the trophic cascade can take several weeks to manifest (Relyea and Diecks 2008).

Ecologically relevant concentrations of malathion altered predator–prey interactions between nymphal dragonflies and amphibians. Uncaged dragonflies reduced anuran survival, but the magnitude of this reduction depended on malathion concentration for all 3 anuran species. Amphibian survival in the presence of uncaged dragonflies improved with increasing malathion concentration. This effect probably was driven by a combination of density- and trait-mediated effects of malathion on dragonfly predators. At 110 μg/L of malathion, dragonfly mortality increased 4× relative to in controls and mesocosms receiving 20 μg/L of malathion. However, improved amphibian survival is unlikely to have been caused by density-mediated effects alone because we inspected the mesocosms daily and replaced any dead dragonflies (i.e., we kept dragonfly density constant). Therefore, reduced foraging efficiency of dragonflies (a trait-mediated effect) also may have improved amphibian survival. Our results are consistent with results of a similar study that used a different suite of amphibians (leopard frogs, wood frogs, and American toads) and higher concentrations (140–460 μg/L) of malathion (Relyea and Hoverman 2008). This pesticide-imposed, density-mediated indirect effect is not specific to malathion. Similar results have been observed in studies with carbaryl, an insecticide with a mode of action similar to that of malathion (Mills and Semlitsch 2004).

In addition to altering patterns of amphibian survival, interactions between predators and pesticides can have sublethal effects that impinge on fitness of both predator and prey (Campero et al. 2007). Sensitivity of growth rate to contaminants may be particularly important given that this trait is tightly correlated with several components of fitness, including postmetamorphic survival and size at reproduction (Berven and Gill 1983, Howard and Kluge 1985, Smith 1987, Newman 1988, Semlitsch et al. 1988). We found strong interactions between malathion treatments and uncaged dragonfly predators on amphibian growth rates. Green frog and bullfrog tadpoles grew 90% faster in mesocosms with uncaged *Anax* than in controls when dosed with 20 μg/L but not when dosed with 110 μg/L of malathion. Growth rate depends on acquisition, assimilation, and allocation of resources, all of which may be affected by predators and pesticides. Decreased competition after thinning by dragonfly predators is unlikely to explain this

pattern because amphibian and dragonfly survival were similar in controls and mesocosms dosed with 20 μg/L of malathion, but growth rates were much higher in the 20-μg/L treatment.

Pesticides and predators also may interact to affect prey behavior and, consequently, prey resource acquisition. A number of investigators have shown that amphibian activity decreases in the presence of predators (Lawler 1989, Tejedo 1993, Relyea and Werner 1999, Relyea 2001, Relyea 2002b) and that this response increases survival at the cost of lower growth rates (Skelly 1992, 1994, Relyea and Werner 1999). Moreover, sublethal concentrations of malathion decrease tadpole activity for all 3 anuran species used in our study (Relyea and Edwards 2010). Perhaps more importantly, the 2 stressors may interact to affect prey physiology and, consequently, prey behavior (Campero et al. 2007). Thus, pesticides and predators together may cause large reductions in amphibian activity, leading to decreased resource consumption. However, in our study, changes in tadpole behavior are not consistent with the higher growth rates observed in mesocosms dosed with 20 μg/L of malathion compared to no-malathion controls in the presence of uncaged predators. Reductions in tadpole activity and increased hiding behavior were similar in the 2 treatments.

Higher green frog and bullfrog tadpole growth rates at 20 μg/L in the presence of uncaged predators may have been caused by an increase in food quality. Both concentrations of malathion resulted in large reductions in cladoceran populations. Cladocerans, and *Daphnia* species in particular, can increase C:P and N:P ratios in systems where P is limiting (Sterner 1990; similar effects have been observed using vertebrate grazers: Knoll et al. 2009). Thus, removal of *Daphnia* decreases P demand, which should increase P availability to periphyton. Moreover, dead *Daphnia* become part of the periphyton and, thus, may directly increase its quality. This increase in quality may especially be the case for tadpoles, which include invertebrates in their diets (Petranka and Kennedy 1999, Schiesari et al. 2009). In mesocosms dosed with 110 μg/L, higher levels of competition because of weaker thinning by dragonflies may have limited the ability of tadpoles to take advantage of increases in food quality. However, if this were the case, size of gray treefrogs at metamorphosis also should have been higher in the 20 μg/L treatment. Future studies are necessary to understand species-specific responses to combinations of pesticides and predators.

Malathion also interacted strongly with nymphal dragonfly predation to affect metamorphic traits in gray treefrogs. Several studies have been done to assess the effect of pesticides on metamorphic traits in amphibians (Mills and Semlitsch 2004, Metts et al. 2005, Teplitsky et al. 2005, LaFiandra et al. 2008, Relyea and Diecks 2008, Brunelli et al. 2009, Distel and Boone 2009, Mackey and Boone 2009, Relyea 2009, Sparling and Fellers 2009), but only a few have examined how pesticides interact with natural stressors to affect this suite of important life-history traits (Mills and Semlitsch 2004, Teplitsky et al. 2005, LaFiandra et al. 2008, Relyea and Diecks 2008). In our study, thinning by uncaged dragonflies increased periphyton availability in no-malathion controls and mesocosms with 20 μg/L malathion and resulted in larger metamorphs. However, at 110 μg/L, this thinning effect was weakened, probably through a combination of increased dragonfly mortality and decreased dragonfly foraging success. Loss of top-down control resulted in greater tadpole density and stronger competition for periphyton, which was reflected in the reduced levels of periphyton in no-predator controls and mesocosms with caged dragonflies. Consequently, smaller metamorphs that were similar in size to individuals from no-predator controls and caged dragonflies were produced in these mesocosms.

Despite strong effects of increased food supply on size at metamorphosis, we found no effect on time to metamorphosis. This result may simply reflect the timing of occurrence of large asymmetries in periphyton among treatments. Periphyton increased in all treatments over the course of the experiment, but mesocosms with low dragonfly mortality had higher levels of DO and higher pH (both indicative of higher primary production) only late in the experiment. Thus, the large disparity in food supply initiated by differential dragonfly predation seems to have occurred coincident with the later stages of tadpole development. A greater effect of increased food supply late in development on size at metamorphosis rather than time to metamorphosis has been reported for 2 other treefrog species (*Hyla gratiosa* and *Hyla cinerea*), a result suggesting that, at least for frogs in this genus, metamorphic traits vary in their sensitivities to changes in resource supply over ontogeny (Leips and Travis 1994, Relyea 2007). Overall, our work points to differential thinning as the primary mechanism driving changes in size at metamorphosis in response to pesticide-induced changes in the intensity of dragonfly predation. However, our study was not specifically designed to address the importance of thinning vs induction. Given that metamorphic traits are often predictive of fitness, future studies should be designed to assess how contaminants affect the relative importance of thinning, induction, and selection in shaping metamorphic traits (Relyea 2007).

Our study also was designed to determine how stratification of pesticides and predator cues interact to affect aquatic communities. We found no evidence that the insecticide malathion stratifies in shallow pond communities, but determining whether stratification of predators and pesticides affects pond communities was important. Very few investigators have examined pesticide stratification in surface waters. Sudo et al. (2004) found that, over much of the year, concentrations of pesticides commonly used in rice production were much higher in surface water (epilimnion) than the deeper hypolimnion of Lake Biwa, Japan. Sudo et al. (2004) suggested that this distribution probably was caused by the sharp thermocline in the lake, which inhibited mixing of warm waters derived from rivers (the source of contaminants) with cooler water in the hypolimnion. However, a few of the pesticides were present at detectable levels in the hypolimnion of the lake, probably because of the long half-life of those pesticides and lake turnover (i.e., mixing) that occurred in winter and early spring. We did not measure temperature at different depths, but the shallow nature of the mesocosms may have resulted in fairly consistent temperatures throughout the water column. Thus, diffusion of malathion to deeper water might have been facilitated. DO concentration was similar at the top and bottom of the tanks, a result suggesting that stratification did not occur in the mesocosms. In contrast to our results, Jones et al. (2010, 2011) found that the herbicide Roundup® did stratify in mesocosms that were smaller in diameter (750 L instead of 1300 L) but similar in depth to those used in our experiment. Thermal stratification was observed in the mesocosms used by Jones et al. (2010, 2011). Water temperatures were 3 to 4°C warmer at the top than at the bottom of the mesocosms. Differences in solubility of pesticides also may explain why stratification is not always observed. For example, Roundup® is more hydrophilic than malathion. Thus, malathion is more likely than Roundup® to precipitate out of the water column and sink to the benthos. Investigators should consider sampling the benthos when assessing the fate of pesticides in aquatic communities.

Conclusions

The application of pesticides has obvious human health and economic benefits. However, recent work in the field of community ecotoxicology has demonstrated that, in addition to having lethal and sublethal effects on nontarget species, pesticides alter species interactions and, thus, how communities function. Our work demonstrates that even at low, ecologically relevant concentrations, the most widely applied insecticide in the US, malathion, has strong lethal and sublethal direct effects at both lower (zooplankton) and higher (dragonfly predators) trophic levels in aquatic food webs. The direct effects initiated by pesticides result in density-mediated indirect effects in aquatic communities. Moreover, because the most sensitive taxa are arthropods (i.e., zooplankton and nymphal dragonflies) that serve as important primary consumers and top predators, many species interactions are disrupted by malathion. Future work should be focused on the mechanisms that drive observed variation in zooplankton and algal community responses across community ecotoxicology studies and the top-down and bottom-up consequences of pesticide-induced perturbations. Furthermore, we used mesocosms to simulate a common and ecologically important type of aquatic community—small ponds (Downing 2010). However, aquatic habitats are extremely diverse, and hydroperiod and other physical features play an important role in shaping the species composition of these habitats (Wellborn et al. 1996). Thus, studies designed to explore how pesticides affect species interactions in suites of species along the hydroperiod gradient will provide a more complete picture of the effects of these chemicals on aquatic communities.

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