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Life in a contaminant milieu: PPCP mixtures generate unpredictable outcomes across trophic levels and life stages

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Abstract. Nearly all aquatic ecosystems are affected by sublethal levels of anthropogenic chemical contamination, but other agents of large-scale anthropogenic disruption of ecosystems have received more attention. Consequently, ecologists do not fully appreciate how sublethal contaminant exposure affects ecosystems. Sublethal contaminants can affect ecological systems directly via their impacts on an organism's fitness or indirectly by changing the strengths of species interactions. This study investigated how an emerging class of contaminants—pharmaceuticals and personal care products (PPCPs)—influences food webs by affecting the biology of organisms and by interfering with predator–prey interactions. Specifically, we investigated how three common PPCPs—caffeine, DEET (*N*, *N*-diethyl-meta-toluamide), and triclosan—affect the strength of the interaction between a common mosquito predator (i.e., mosquito fish) and mosquito larvae as well as how these PPCPS affect mosquito survival, life history traits, and oviposition site choices. We found that all three PPCPs, individually and combined as a mixture, reduced predator consumption rates. Relative to a contaminant-free control, the presence of predator cues reduced mosquito oviposition and larval abundance for all PPCP treatments except for DEET. Predator cues reduced mosquito adult emergence across PPCP treatments; however, mosquitoes that were exposed to caffeine did not emerge as adults even in the absence of predator cues. This study shows that the effects of PPCPs are diverse and can interact with mosquitoes and their predators in ways that cannot be predicted by their individual effects. In a contaminated world, ecologists need to better understand how sublethal concentrations of ubiquitous, biologically active pollutants might challenge what we think we know about how ecological systems function.

Key words: ecotoxicology; habitat selection; mosquito; oviposition site choice; personal care products; pharmaceuticals; predator–prey; wetland.

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Introduction

Nearly all aquatic ecosystems are affected by anthropogenic chemical contamination (Gessner and Tlili 2016), but other agents of large-scale anthropogenic disruption of ecosystems (e.g., rising CO_2 concentrations, biodiversity loss, nutrient pollution) have received more attention (Bernhardt et al. 2017). While there is extensive toxicological research focused on identifying the molecular mechanisms through which chemical contaminants influence model organisms, the results of these studies do not directly translate into insights on how pollutants affect natural

ecosystems (Rosi-Marshall and Royer 2012, Bernhardt et al. 2017). Studies on non-model and wild species have shown that pollutants, at environmentally realistic concentrations, are sublethal and can alter the physiology, development, and behavior of organisms rather than directly reducing their abundance through mortality (Halling-Sørensen et al. 1998). Consequently, it can be difficult to scale the results of classic toxicological approaches focused on investigating lethality or other individual-level effects to understand how contaminants will affect population and community dynamics (Rosi-Marshall and Royer 2012, Bernhardt et al. 2017).

To understand how contaminants influence the complex dynamic interactions within ecological systems, we must determine how these chemicals affect the ecological mechanisms that control community structure and function (e.g., primary productivity, decomposition, trophic interactions; Relyea and Hoverman 2006, Relyea and Edwards 2010). To a considerable extent, community structure and function is defined by trophic interactions which are influenced by the ability of individuals to detect and respond to one another and their surroundings (Laska and Wootton 1998). Abiotic factors, both synthetic and natural, frequently alter the interaction strength between species due to their impact on organismal detection and subsequent behavioral responses (Dunson and Travis 1991). Pollutants (i.e., pesticides, metals, hydrocarbons) have been shown to hinder organismal chemosensory functions (Secondi et al. 2009, Tierney et al. 2010, Brönmark and Hansson 2012). For instance, crayfish exposed to the herbicide metolachlor showed a substantially weakened response to food odors, suggesting that pollutants can cause info-disruption in consumption and/or predation (Wolf and Moore 2002). Pesticides and metals can also modify the ability for prey to detect or respond to predator cues which could disrupt trophic interactions (reviewed in Lürling and Scheffer 2007, Van Donk et al. 2016).

The effects of chemical contaminants on the processes that control predation strength may be impactful for communities since predators play a vital role in community dynamics (Schmitz et al. 2004, Knight et al. 2005, Estes et al. 2011). Predators shape community structure by altering the abundance, distribution, and behavior of their prey via consumptive and nonconsumptive mechanisms. Consumptive effects refer to the direct consumption of prey which leads to changes in prey abundance (Peckarsky et al. 2008). Nonconsumptive effects refer to the nonlethal impacts that predators have on food webs by changing aspects of the prey's phenotype or behavior. For instance, even in the absence of prey consumption, the visual or chemical cues of predators can induce changes in prey foraging rates, hiding behavior, morphology, dispersal, and/or habitat selection (Schmitz et al. 2004, Peckarsky et al. 2008). These effects of predators on prey can cascade through the food web and influence other trophic levels (Schmitz et al. 2004, Heithaus et al. 2008). For this reason, it is paramount that we understand how ecologically relevant concentrations of contaminants affect both the strength of predation and the responses of organisms to the threat of predation (Clements and Newman 2003).

There are millions of anthropogenically generated chemicals that are commonly introduced into natural systems (Gessner and Tlili 2016); yet, we know relatively little about how they affect the strength of species interactions or other ecological processes that control community structure and function. For example, the effects of ubiquitous contaminants, like pharmaceuticals and personal care products (PPCPs), on species interactions and other ecosystem processes are largely unknown. Yet, PPCPs are comprised of a large range of compounds used for human and/or animal personal care and/or health. In addition, their effects may be more problematic than other types of pollutants (e.g., seasonally administered chemicals) because many PPCPs are continuously introduced into aquatic systems (Schwarzenbach et al. 2006, Rosi-Marshall and Royer 2012).

The concentrations of PPCPs detected in aquatic environments are typically low, but low concentrations can induce strong effects, especially for PPCPs because these chemicals are specifically designed to elicit biological effects at low (i.e., sublethal) concentrations (Halling-Sørensen et al. 1998, Brausch and Rand 2011). Like pesticides, many PPCPs (e.g., antibiotics, disinfectants, and insect repellents) are designed to kill or hinder growth of organisms and/or interfere with organismal behavior and biochemistry, thus

heightening the potential for these micro-pollutants to pose risks to waterways and the aquatic organisms that inhabit them (Brausch and Rand 2011, Cizmas et al. 2015, Bernhardt et al. 2017). Indeed, sublethal effects of PPCPs (e.g., changes in behavior) have been found in a variety of organisms exposed to environmentally relevant concentrations (Fong and Molnar 2008, Brodin et al. 2013, 2014).

However, as with other chemical pollutants, most research on PPCPs has been devoted to evaluating how individuals or populations of a single species respond to the exposure of a single PPCP (Rosi-Marshall and Royer 2012, Bernhardt et al. 2017). Little toxicological work has focused on the combined effects of PPCPs as mixtures (Backhaus 2014) or on how the strength of species interactions, like predation, is affected by ecologically relevant levels of these toxicants. If pollutant mixtures are altering biological processes that control community structure and function, then we must consider how these pollutants are altering our understanding of ecology.

Caffeine (a central nervous system stimulant), DEET (an insect repellant), and triclosan (an antimicrobial disinfectant) are among the most frequently detected PPCPs in natural systems and can sometimes be found in relatively high concentrations in the effluent of wastewater treatment plants (Hedgespeth et al. 2012, Luo et al. 2014). In this study, we investigate the effects of these three common PPCPs and their combination—at environmentally relevant concentrations—on the strength of direct and indirect interactions between mosquito fish predators and mosquitoes. Mosquitoes and their predators are an ideal model system for investigating the ecological effects of PPCPs because mosquitoes are often considered a nuisance species, are vectors of pathogens, and are prominent members of many contaminated aquatic ecosystems (Hedgespeth et al. 2012, Walton 2012, Luo et al. 2014). Moreover, mosquito fish are also common inhabitants of contaminated ecosystems, and they are often used as a biological control agent to reduce mosquito populations (Walton 2007). Within aquatic systems, like wetlands, mosquito fish can influence mosquito populations by consuming larvae and/or by influencing habitat selection and adult mosquito oviposition behavior (Vonesh and Blaustein 2010). Therefore, we ask the following: (1) Is the consumptive effect (i.e., functional response) of mosquito fish predation on mosquito larvae influenced by these PPCPs? (2) Is mosquito oviposition site selection affected by PPCP contamination and predator cue presence? (3) Does the presence of PPCPs and predator cues affect mosquito larval abundance and adult emergence?

Materials and Methods

Predation experiment

Determining the strength of consumptive effects in predator–prey interactions can be accomplished by characterizing predator functional response curves (Holling 1959, 1961). A predator's functional response curve describes consumption rate, F, as a function of prey density, N, and thus links predator–prey population dynamics (Holling 1959). Predator functional responses typically take one of three forms: Type I, linear with a ceiling ($h = 0$ and $\nu = 1$); Type II, saturating ($h > 0$ and $\nu = 1$); and Type III, sigmoidal (Eq. 1, $h > 0$ and $\nu > 1$; Holling 1959, 1961, Real 1977).

$$F = \frac{\alpha N^{\nu}}{1 + \alpha h N^{\nu}}. \quad (1)$$

In all three functional response forms, prey consumption at low densities is determined by the predator's attack rate, α (rate at which predators encounter and capture prey), and the density of prey, N (Eq. 1). The Type II and III forms saturate at a maximum consumption rate that is set by a handling time parameter ($h > 0$) or the amount of time spent finding and consuming prey (Holling 1959). The Type III functional form includes a third parameter ($\nu > 1$) that depicts low consumption or infrequent encounters at low prey abundances. Because they explicitly link predator and prey population dynamics, predator functional responses provide a useful framework for investigating how extrinsic factors affect the strength of predator–prey interactions (Abrams 1990).

We conducted an experiment from 11 June to 15 September 2017 to determine whether PPCPs affect the shape of the functional response for mosquito fish (female *Gambusia holbrooki*) foraging on mosquito larvae (*Culex quinquefasciatus* Say). Specifically, we crossed five different density treatments (10, 25, 50, 100, and 200 larvae

per 8 L of artificial lake water) of mosquito larvae with five ecologically relevant PPCP treatments: (1) 40 μg/L caffeine, (2) 15 μg/L DEET, (3) 5 μg/L triclosan, (4) a mixture of all three at the same concentrations, and (5) a solvent control (reverse osmosis water with evaporated ethanol; see Appendix S1 for chemical preparation).

The experiment was replicated in five different temporal blocks (duration of each block was 5 h) in a temperature- and light-controlled room (12-h light–dark cycle) and utilized 25 aquaria (51 × 25 × 30.5 cm) that were randomly assigned to each of the 25 treatments (5 prey density treatments × 5 PPCP treatments). The tanks were filled with 8 L of artificial lake water, where reverse osmosis water was used instead of tap water (Provasoli et al. 1957). Female *G. holbrooki* (mass 0.78 g on average, snout to caudal fin length 4.3 cm on average) used in this study were collected from a permanent freshwater pond (35°36′9.8″ N, 77°21′36.4″ W). Fish acclimated to the tanks and artificial lake water for at least 24 h prior to being assigned to a treatment and were starved for at least 72 h prior to mosquito larval addition. *Culex quinquefasciatus* (Sebring strain) larvae were reared in a controlled laboratory setting and were fed both liver powder/yeast (2:1) and Spirulina fish flakes ad libitum until they reached the third or fourth instar—the stage in which they were used in the experiment. Mosquito larvae were then counted and placed into their respective density treatments. At the end of each experimental replicate (temporal block), the number of larvae consumed in a 5-h period was quantified.

The mosquitoes (i.e., *Cx. quinquefasciatus*) used in this experiment are known to be sensitive to insecticides. To ensure that the PPCP treatments did not cause larval mortality, we placed 10 larvae into 300-mL glass bowls (Pyrex 10-oz Custard Cup, Corelle Brands, Charleroi, PA, USA) that were filled with 200 mL of artificial lake water and the same five PPCP concentrations as listed above. To determine whether incidental mosquito mortality might influence *G. holbrooki* foraging, we also tested to see whether *G. holbrooki* would consume dead mosquito larvae by placing one *G. holbrooki* (0.691 g, 4.5 cm) that was starved for at least 72 h in a control treatment with 10 dead larvae and quantified consumption.

Oviposition experiment

To determine how the response to the threat of predation is altered by PPCPs, we conducted an oviposition site choice experiment in outdoor mesocosms between 8 August and 7 September 2016 at East Carolina University's West Research Campus (35°37′51″ N, 77°29′9″ W). We used a 2 × 5 factorial experimental design where the presence or absence of chemical cues from a predator (i.e., cues of predation from female *G. holbrooki*) was crossed with the same five PPCP manipulations described in the predation experiment.

The experiment was conducted in three spatial blocks each consisting of 10 plastic stock tanks (63.5 × 89 × 30.5 cm) randomly assigned to one of the predator cue treatments (cue presence or absence) and one of the five PPCP manipulations for a total of 10 treatment combinations. The experiment was replicated six times: three spatial replicates of each treatment at two different time points. Each spatial block was situated at least 25 m apart, and mesocosms within a block were spaced at least 10 m apart to prevent local contagion effects (Resetarits and Silberbush 2016). Each simulated wetland pool was filled with 50 L of rainwater and contained 10 g of organic matter (16% protein rabbit feed). Mesocosms were located near known ephemeral wetlands and were placed ~110 cm from the tree line.

Gambusia holbrooki used in this experiment were collected from a nearby permanent freshwater pond (35°33′28.4″ N, 77°20′54.7″ W). To extract predator cues, four female *G. holbrooki* were housed in an aquarium (51 × 25 × 30.5 cm) filled with 28 L of rainwater and were fed Spirulina fish flakes every other day. For the predator cue treatment, 250 mL of water from the aquarium with the fish predators was added to the mesocosms every 2 d. The same quantity of rainwater was added to the non-cue treatments on the same days that predator cues were added to the predator cue treatment tanks.

For each treatment, we quantified mosquito: (1) egg raft deposition, (2) larval abundance, (3) larval species identity, (4) adult emergence, and (5) adult species identity. To quantify egg raft deposition, egg rafts were counted daily until 6 d post-initial oviposition per block (time at which emergence traps were placed on top of the tanks). Egg deposition was only quantified for

egg raft-laying mosquitoes (i.e., *Culex* spp. and other mosquitoes). Other mosquitoes (e.g., *Anopheles* and *Aedes* spp.) lay their eggs singly above the water or on the surface of the water (Service 2008) and could not be individually counted in this study. Larval abundance was measured via a standardized dipnetting protocol (a single figure-eight sweep around the perimeter and across the center of the tank) using different brine shrimp dipnets (101.6 × 10.8 × 33 cm) to avoid cross-contamination; tanks were dipnetted daily starting on day 3 post-initial oviposition per block until emergence traps were placed over the entire tank on day 6 post-initial oviposition. When breaking down each experimental block (on days 10 and 11 post-initial oviposition), an additional 15 standardized dipnet sweeps were performed to get a more complete assessment of final larval abundance. Collected larvae were then raised to the fourth instar, in a controlled laboratory setting, for species identification (Harrison et al. 2016). Emergence traps were placed over the entirety of the tanks on day 6 post-initial oviposition and were left on the tanks until days 10 and 11 (three blocks went for an additional day due to severe weather). Emergence traps covered the entire tank and consisted of polyethylene screen (mosquito netting), a rope to secure netting, a garden stake to hold the netting upright, a Nalgene bottle to collect emerging adults, and rubber bands to secure the bottle to the garden stake. Nalgene bottles were checked daily for adult emergence, and bottles with captured adults were placed into a −4°C freezer to allow adult emergence to be quantified and species to be identified (adult females were identified using the identification guide by Harrison et al. 2016).

Statistical analysis

All analyses were conducted using the R statistical programming environment (R Core Team 2016). For the predation experiment, functional response curves were fit using a flexible trait approach (McCoy and Bolker 2008, Okuyama 2012) which allows for quantification of key functional response parameters (such as prey handling time or attack rate) to differ as a result of some biological or environmental factor (presence of PPCPs in this study). Based on the findings of prior studies and an initial assessment of the data, we fit a Type II functional response using Rogers random predator model since it accounts for changes in prey abundance, due to predation over the course of the experiment, when estimating parameters (Rogers 1972, Juliano 2001, Bolker 2008, McCoy and Bolker 2008, McCoy et al. 2011).

Inferences about the effects of PPCPs on the predator's functional response were based on a model comparison approach in which we compared the relative explanatory power of (1) a model that estimated attack rates and handling times for the five PPCP treatments independently, which tests whether PPCPs affect both the attack rate and handling time of prey; (2) a single estimate of attack rate, but separate estimates of handing times, which tests whether PPCPs affect *G. holbrooki* feeding rates; (3) a single estimate of handling time, but separate estimates of attack rates, which tests whether PPCPs affect the likelihood of attack by *G. holbrooki*; and (4) a random model that fits a single estimate of attack rate and handling time, which acts as the null model. We assessed model fits and based inferences about the effects of PPCPs on the interaction between *G. holbrooki* and *Cx. quinquefasciatus* larvae on sample size-corrected Akaike information criterion (AIC_c) values (Bozdogan 1987).

For the oviposition experiment, we used the R library lme4 (Bates et al. 2015) to conduct generalized linear mixed-effects models (GLMMs) to test the effects that different PPCPs have on the nonconsumptive effects of predators. For mosquito egg raft deposition, larval abundance, and adult emergence, we specified PPCP and predator cues as fixed effects and experimental block as a random effect. Model assumptions were evaluated via visual inspection of residual and quantile–quantile plots and tested for over-dispersion where appropriate. Inferences from GLMMs were based on likelihood ratio tests by comparing models with and without parameters of interest. Inferences about differences among levels within treatments are based on 95% confidence intervals.

For *Culex* egg raft deposition, we included time (days post-initial oviposition) as a fixed effect in addition to PPCP and predator cues. For count data (i.e., mosquito egg raft deposition, larval abundance, and species abundance), analyses

were performed assuming a Poisson error distribution or a negative binomial error distribution when over-dispersed. For analyses of larval abundance, we included the number of egg rafts counted in each tank as an offset to control for differences in inputs on larval recruitment rates. However, adult emergence was highly zero-inflated; therefore, we analyzed these data using a hurdle model. This type of model incorporates two models: (1) a binomial logistic regression, which models presence or absence of emerging adults, and (2) a truncated Poisson distribution, which models positive counts. For this analysis, we could not control for the number of egg rafts due in part to the zero inflation; however, there was also no correlation between inputs and adult emergence.

Results

Predation experiment

In the absence of predators, all larvae survived in the caffeine, triclosan, control, and mixture treatment after 5 h, and nine out of the 10 larvae in the DEET treatment survived which suggests that the PPCP concentrations used in this study did not induce immediate mortality. We also determined that female *G. holbrooki* readily consume dead mosquito larvae as all 10 larvae were consumed within 30 min of being added to the tank.

Consumption of mosquito larvae by *G. holbrooki* was estimated by assuming a Type II functional response, according to Rogers random predator model, but allowing model parameters to vary by treatment (McCoy and Bolker 2008). The model with the highest explanatory power, based on AIC_c values, included separate estimates of attack rates and handling times for each of the five PPCP treatments suggesting that different PPCPs have different effects on the predator's probability of attack and on the time spent handling captured prey or searching for new prey (Fig. 1a). Predators that were exposed to PPCPs had slower attack rates (>61.5% lower) and longer handling times (>34.6% longer) than the control (Fig. 1a).

Predators that were exposed to a mixture of all three PPCPs had the lowest attack rate when compared to each PPCP individually (>71.2% slower than PPCP treatments, Fig. 1b). Alternatively, fish exposed to caffeine had the longest handling time (Fig. 1b) which resulted in predators in the caffeine group having the lowest predation rate overall (>27.7% lower than DEET, triclosan, and control, 9.3% lower than the mixture treatment, Fig. 1a). Interestingly, predators exposed to DEET and triclosan had similar attack rates and handling times to one another (Fig. 1b), resulting in similar functional response curves (Fig. 1a).

Oviposition experiment

Egg raft deposition and larval abundance.—The tanks received a total of 246 *Culex* egg rafts (~4 total egg rafts per tank on average). To account for over-dispersion, mosquito egg raft deposition and larval abundance were analyzed assuming a negative binomial distribution. Neither the three-way interaction between time, PPCP treatment, and predator cue ($\chi^2 = 3.6996$, df = 4, $P = 0.4482$) nor the two-way interaction between time and PPCP treatment ($\chi^2 = 4.0703$, df = 4, $P = 0.3966$) were significant predictors of egg raft accumulation over time. The two-way interactions between time and predator cue ($\chi^2 = 3.8476$, df = 1, $P = 0.04982$) and PPCP and predator cue ($\chi^2 = 16.471$, df = 4, $P = 0.002448$) were significant in the final simplified model. Specifically, in the absence of predator cues, *Culex* egg rafts accumulated at a slower rate (>55% slower) in all of the PPCP treatments than they did in the control. More *Culex* egg rafts accumulated (53.7%) in pools without predator cues than pools with predator cues, except for DEET. Interestingly, there were 13.8% fewer egg rafts in the DEET with no predator cues than DEET with predator cues (Fig. 2a). In the presence of predator cues, eggs accumulated 55.1% more in the DEET treatment than in the control (Fig. 2a).

There were no significant differences in the number of *Culex* mosquito larvae collected among treatments (interaction between PPCP and predator cues $\chi^2 = 2.2953$, df = 4, $P = 0.6816$; presence of predator cue $\chi^2 = 2.8418$, df = 1, $P = 0.09184$; PPCP $\chi^2 = 1.1824$, df = 4, $P = 0.881$). However, consistent with the total number of egg rafts, there was a general decline in larval abundance in the presence of predator cues (Fig. 2b, 57.2% fewer larvae in pools with

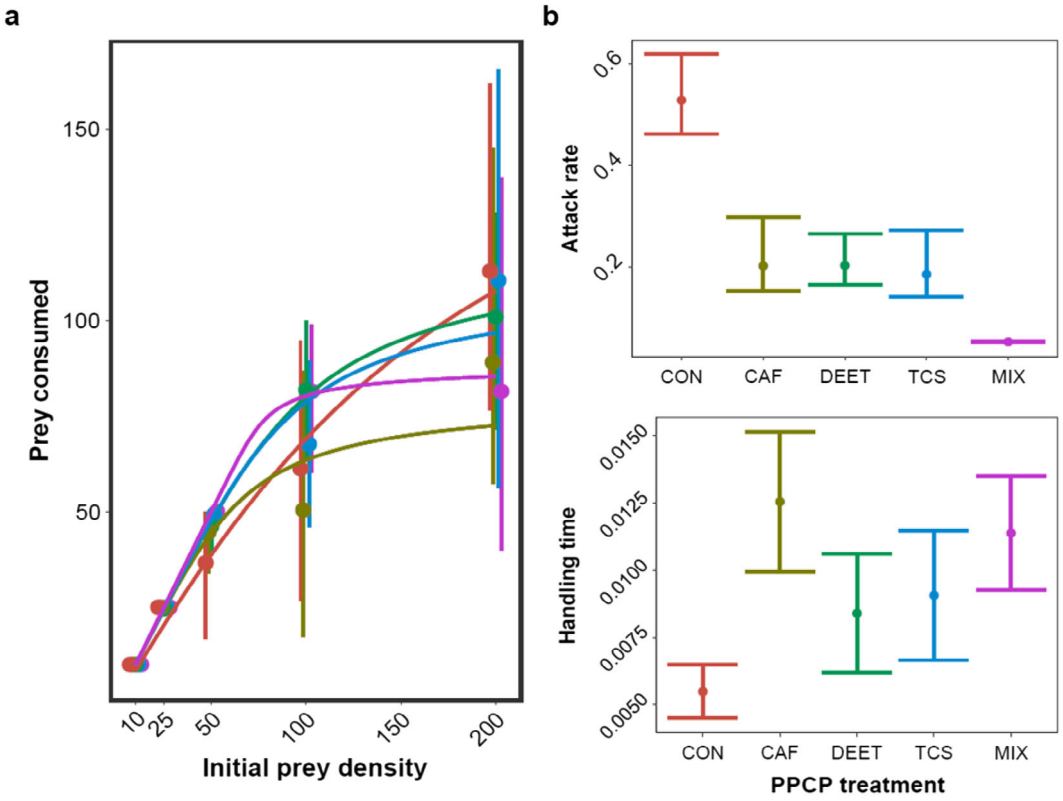

Fig. 1. (a) Amount of prey consumed by predators over a 5-h period using five increasing densities. Lines represent predator (*Gambusia holbrooki*) consumption of prey (*Culex quinquefasciatus*) in the control (CON, red), caffeine (CAF, tan), DEET (green), triclosan (TCS, blue), and mixture (MIX, purple) treatments. Mean estimates of prey consumed for each treatment are indicated by points with 95% confidence intervals (n = 5 trials). Attack rates (α) and handling times (h) used to generate lines in were obtained from the model with the highest explanatory power. (b) Points represent means of attack rates and handling times, and error bars represent 95% confidence intervals. Labels represent the control (CON, red), caffeine (CAF, tan), DEET (green), triclosan (TCS, blue), and mixture (MIX, purple) treatment.

predator cues). In fact, there were over two times (2.34) more larvae in pools without predator cues than with predator cues (Fig. 2b).

Larval and adult species diversity.—Species richness (alpha diversity) for both larval and adult diversity was analyzed assuming a Poisson error distribution. There were a total of 1663 *Culex* mosquito larvae identified. Whenever possible, we identified individuals to the level of species, but 165 larval individuals were only identified to the genus (i.e., *Culex* spp.). There were 273 mosquito larvae (i.e., *Anopheles* spp.) that were non-raft-forming mosquitoes and 365 mosquito larvae that were unidentifiable; thus, these individuals were not included in our egg raft mosquito larval species richness analysis. Of nine species of *Culex* that are commonly found in southeast North Carolina (Darsie and Ward 2005), the following were identified: *Cx. pipiens* × *quinquefasciatus* (529), *Cx. restuans* Theobald (722), *Cx. salinarius* Coquillett (203), and *Cx. territans* Walker (44). There were no significant predictors of larval mosquito species diversity (interaction between PPCP and predator cue $\chi^2 = 0.1065$, df = 4, $P = 0.9986$; presence of a predator cue $\chi^2 = 0.1015$, df = 1, $P = 0.7501$; PPCP $\chi^2 = 0.0922$, df = 4, $P = 0.999$). Out of all the *Culex* larvae identified, control treatments without the presence of predator cues had the most *Cx. pipiens* × *quinquefasciatus* (33% of the

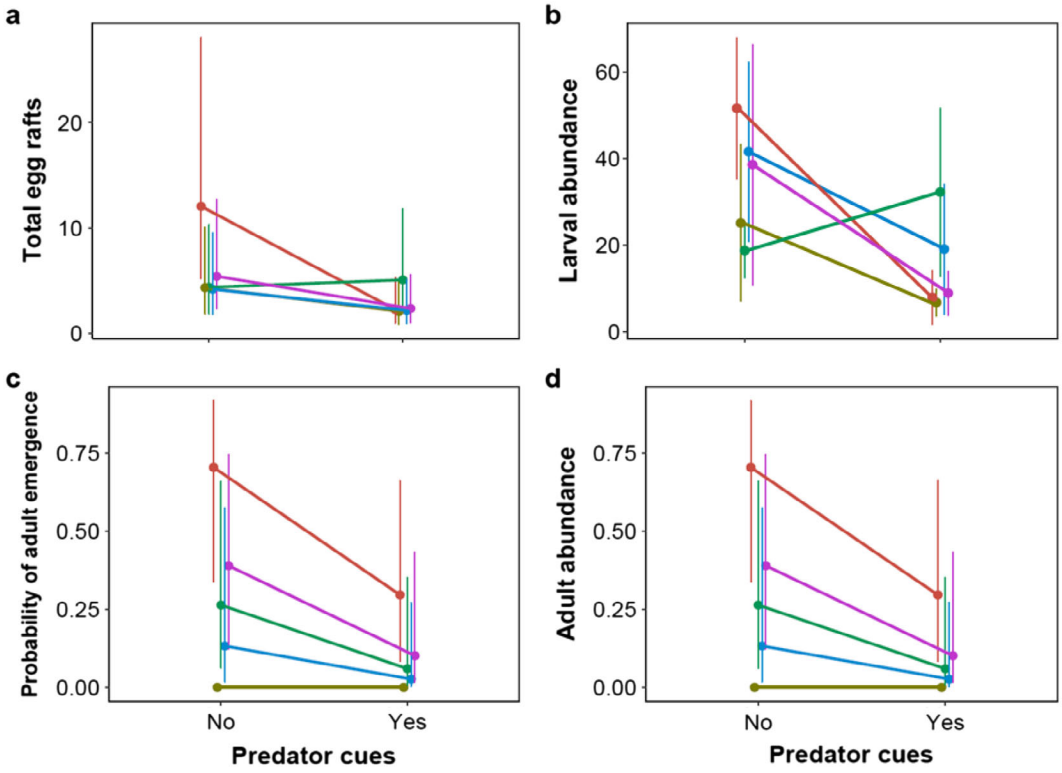

Fig. 2. Interaction plots for *Culex* egg raft deposition (a), larval abundance (b), and adult emergence (c, d). Lines and points for (a) were generated from a negative binomial error distribution where error bars represent 95% confidence intervals (CIs). Lines for (b) were generated from the raw data where points represent means and error bars represent 95% CIs. Lines and points for (c) were generated from a binomial distribution where error bars represent 95% CIs. Lines and points for (d) were generated from a truncated Poisson distribution where error bars represent 95% CIs.

total larvae collected for this species), and the triclosan treatment without predator cues had the most *Cx. restuans* (22% of the total larvae collected for this species).

A total of 99 *Culex* adult mosquitoes were identified. Forty-four specimens were only identified to genus (due to them being male), and the remaining 55 were identified to species: *Cx. pipiens* × *quinquefasciatus* (15) and *Cx. restuans* (40). The two-way interaction between PPCP and predator cue ($\chi^2 = 1.6414$, df = 4, $P = 0.8013$) was not a significant predictor of adult species diversity; however, PPCP ($\chi^2 = 20.674$, df = 4, $P = 0.0003675$) and predator cue ($\chi^2 = 11.645$, df = 1, $P = 0.0006437$) were significant in the simplified model. In the absence of predator cues, the control pools had the largest amount of *Cx. restuans* (42% of the total adults collected for this species) whereas pools containing DEET had the most adults emerge from the species *Cx. pipiens* × *quinquefasciatus* (48% of the total adults collected for this species). In pools containing predator cues, only four individuals were identified. Lastly, since there were no emerging adults from pools that contained caffeine, adult species richness was zero for caffeine treatments.

Adult emergence.—The two-way interaction between PPCP and predator cues ($\chi^2 = 3.0727$, df = 4, $P = 0.5457$) was not a significant predictor for the probability of *Culex* adult emergence. However, PPCP ($\chi^2 = 13.215$, df = 4, $P = 0.01027$) and predator cues ($\chi^2 = 4.8857$, df = 1, $P = 0.02708$) had independent significant effects on adult emergence in the simplified model (Fig. 2c). Specifically, mosquitoes not exposed to predator cues were twice as likely to emerge

relative to those exposed to predator cues, except for mosquitoes in pools that contained caffeine (Fig. 2c). Mosquitoes exposed to the caffeine treatment, with and without predator cues, did not emerge as adults. Interestingly, without the presence of predator cues, mosquitoes that were in the control treatments had a >60% chance of adult emergence compared to all other treatments (Fig. 2c). When adult emergence occurred (Fig. 2d), the interaction between PPCP and predator cues ($\chi^2 = 1.9411$, df = 2, $P = 0.3789$) was not a significant predictor of the number of adults that emerged; yet, there was a significant effect of PPCP ($\chi^2 = 9.5562$, df = 3, $P = 0.02274$) and predator cues ($\chi^2 = 21.374$, df = 1, $P = 3.779e\text{-}06$) in the final simplified model. With the exception of caffeine, there were 11.7 times more mosquitoes emerging as adults in pools with predator cues than without predator cues (Fig. 2d). Although there was a greater probability of *Culex* mosquitoes emerging as adults in the control treatment without predator cues (Fig. 2c), there were 2.3 times more individuals that emerged from pools containing DEET without predator cues than the control (Fig. 2d). There were also fewer individuals (94.7% fewer) that emerged in DEET without predator cues than DEET with predator cues (Fig. 2d), showing an opposite trend to the results for *Culex* egg raft and larval abundance (Fig. 2a, b).

Discussion

Ecological systems are increasingly being modified by an ever-changing plethora of new and emerging contaminant milieus that may change the nature of ecological interactions, yet most ecological theory and predictions about ecological interactions are based on foundational studies that occurred before many modern pollutants were invented. A better appreciation for how biologically active chemical agents, that are now ubiquitous in the environment, are changing the outcome of well-studied ecological processes is needed. In this study, we showed that PPCPs differentially influence survival and development of different life stages of *Culex* mosquitoes as well as the strength of predator–prey interactions by interfering with both the direct (predation and consumptive effects) and indirect (threat of predation and nonconsumptive effects) effects of predators on prey. Moreover, we found that the effects of these chemicals were sometimes different on direct and indirect effects and, in some cases, contraindicative of expectations. For example, DEET reduced mosquito avoidance of predator cues when choosing oviposition site while caffeine reduced predation rates by mosquito fish. Furthermore, we found that the effects of chemical mixtures were not predictable based on their independent effects. In sum, our findings strongly highlight the need to better understand how contemporary changes in ecosystem quality are affecting the nature and outcome of important ecological processes.

Specifically, we found that environmentally relevant concentrations of three PPCPs—caffeine, DEET, triclosan, and a mixture of all three—reduced predation on mosquito larvae by mosquito fish (Fig. 1a) and that the rate at which predators encounter and capture prey (attack rate) was affected differently by these chemicals than the amount of time spent finding and consuming prey (handling time). The attack rate of predators exposed to caffeine, DEET, and triclosan was similar to one another (Fig. 1b) but lower than the attack rates in control treatments; however, maximum consumption was much lower in the caffeine treatments as a result of longer handling times (Fig. 1a, b). One important implication of this caffeine-induced reduction in maximum predation rate is that aquatic systems contaminated with caffeine could produce higher numbers of adult mosquitoes. Indeed, this affect could be significant in areas where fish and other predators are being used for biological control of mosquito populations. However, we found the opposite effect when mixtures of PPCPs were present, even though the mixture contained caffeine. In the PPCP mixture, *G. holbrooki* had higher maximum predation rates as a result of shorter estimated handling times (Fig. 1a), but the mixture of PPCPs also reduced the attack rates compared to all other treatments (Fig. 1b). This result is important for two reasons. First, it suggests that attack rates in mixtures are not easily predicted by the effects of the individual PPCPs in the mixture, which is consistent with other studies on PPCP mixtures (reviewed in Backhaus 2014). Second, our findings signify that density-dependent processes regulating predator–prey dynamics are differentially affected by

different PPCPs and mixtures, which obscures the ability to predict how the effects of these chemicals will influence food webs. These results reinforce a growing literature showing that the effects of PPCPs are chemical-, mixture-, and species-dependent (Brodin et al. 2014) and further highlight the need for more research across taxa and systems.

While predator consumption rate is clearly important for understanding the strength of predator–prey interactions, other processes (e.g., prey behavior and life history decisions) can have important effects that can scale up to affect community structure and function as well (Benard 2004, Schmitz et al. 2004, Orrock et al. 2008, Peckarsky et al. 2008). Such effects may be particularly important for organisms with complex life cycles because the effects of predators and contaminants vary across life stages (Touchon et al. 2013). For example, we know that the presence of various pesticides as well as chemical cues of predator presence can affect oviposition site choice behavior and larval development in a wide variety of organisms, including vertebrates and invertebrates (reviewed in Relyea and Hoverman 2006, Vonesh and Kraus 2009). However, this study is the first to examine whether contaminant mixtures alter the way in which predator cues affect oviposition behavior, larval abundance, and ultimately adult emergence. Here, we showed that PPCPs and predator chemical cues do affect mosquito egg deposition, larval abundance, and adult emergence.

Although we acknowledge that *Culex* mosquitoes release a pheromone during oviposition that attracts other females to oviposit in the same pools (Laurence and Pricekett 1985, Clements 1999), we found that, in the absence of predator cues, egg rafts accumulated slower in the PPCP treatments relative to the control. However, when predator cues were present, egg rafts accumulated faster in DEET compared to the control (Fig. 2a), suggesting that mosquitoes preferentially oviposit in DEET-contaminated systems with predators (or predator cues). Indeed, this suggests that the oviposition site selection of early colonists could compound the effects of PPCPs by attracting subsequent oviposition events (Laurence and Pricekett 1985, Clements 1999). Although PPCPs did not strongly influence *Culex* larval abundance solely by inducing direct mortality, the results were consistent with *Culex* egg raft deposition patterns in which there were fewer *Culex* larvae in the predator cue treatments (Fig. 2b). Interestingly, mosquitoes exposed to no predator cues had a lower probability of adult emergence than mosquitoes that developed with predator cues across PPCP treatments (Fig. 2c), and as expected, there were lower numbers of adults emerging from PPCP treatments with predator cues (Fig. 2d). The presence of predator chemical cues, therefore, may have negative effects on larval growth and development which either delays or inhibits metamorphosis. Such negative effects of predation risk have been observed in other taxa as well (McCollum and Leimberger 1997, Benard 2004, Peckarsky et al. 2008).

Emergence of adult mosquitoes was not substantially influenced by the presence of PPCPs (Fig. 2c, d). Adult abundance largely mirrored *Culex* egg raft deposition and larval abundance but with two exceptions. First, in the absence of predator cues, mosquitoes emerged in higher numbers from DEET treatments than the controls (Fig. 2d). Second, there was no adult emergence for mosquitoes exposed to caffeine with or without the presence of predator cues (Fig. 2c, d). This is consistent with other studies showing that caffeine can adversely affect insect larval development (Laranja et al. 2003, 2006). However, pools that contained a mixture of all three PPCPs, including caffeine, had a higher probability of adult emergence than DEET and triclosan alone (Fig. 2c). This observation reaffirms the need to consider how PPCPs in isolation can have different outcomes than when combined with other PPCPs as mixtures (Backhaus 2014).

Of the PPCPs examined here, caffeine and DEET produced the most surprising results. *Culex* egg raft deposition (Fig. 2a) and larval abundance (Fig. 2b) in the caffeine treatments were not vastly different than the other PPCP treatments; yet, there were no mosquitoes that emerged as adults in pools that contained caffeine. Previous research has shown that caffeine can influence larval development in *Aedes aegypti* Linnaeus mosquitoes at concentrations not much higher than the concentrations used in this study (Laranja et al. 2003, 2006). This study further suggests that caffeine at low, environmentally realistic concentrations may affect larval

development in multiple mosquito species. However, the mixture treatment, which contained caffeine, did not appear to impede development of mosquito larvae (Fig. 2c, d). This suggests that the effects of caffeine may be reduced or eliminated by the presence of other PPCPs, which may be, for example, due to an upregulation of detoxifying enzymes induced by other chemicals. Recent findings have revealed that mosquito fish exposed to reclaimed water from wastewater treatment facilities accumulated caffeine in their tissues (Wang and Gardinali 2012, 2013). Considering that we found that caffeine alone and mixed with other PPCPs affected mosquito fish predation rates (Fig. 1a) and that larvae survival was not affected by the mixture treatments containing caffeine, caffeine may be having important effects on mosquito population sizes and thus community dynamics.

The effects of DEET were also not easily predicted from its toxicological effects. *Culex* mosquitoes oviposited more in pools that contained DEET with predator cues than without predator cues (Fig. 2a). Considering that DEET did not have a large effect on *G. holbrooki* predation of mosquito larvae, DEET may be acting as an ecological trap (Battin 2004, Vonesh and Kraus 2009, Hale and Swearer 2016) by reducing predator avoidance behavior of ovipositing mosquitoes. This implies that adult mosquitoes are laying their eggs in habitats that may ultimately be lethal to their offspring as a result of predator presence. While there were more egg rafts laid in pools with DEET and predator cues, we found more individuals that reached adulthood when they were exposed to DEET without predator cues as opposed to DEET with predator cues (Fig. 2c, d). Studies have shown that DEET is nontoxic to freshwater insects (Campos et al. 2016); however, when combined with predator cues, we found that DEET influenced the survival of mosquito larvae. This finding argues for a better understanding of the complex interactions between biologically active PPCPs and biological cues on community-level processes, like predator–prey interactions.

Conclusion

This research demonstrates the need to consider how synthetic chemicals can alter predator–prey interaction strengths of species found in contaminated aquatic ecosystems. The chemicals used in this study illustrate that PPCPs can influence predator consumption of prey and the behavior of their prey. Both predator consumptive and nonconsumptive effects can influence population dynamics and cascade through food webs, ultimately influencing ecosystem function. As shown here, abiotic (i.e., PPCPs) and biotic (i.e., predator cues) factors may interact in a way that cannot be predicted by their individual effects. Future research should consider how abiotic and biotic factors interact with one another to evaluate how contaminants affect communities.

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