PRIMARY RESEARCH PAPER



# Effects of chemical cues from two piscine predators, natal predator regime, and time since cue introduction, on the movements of aquatic isopods (Caecidotea communis)

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Abstract The ability to detect predators at a distance through chemical cues is often essential for prey, but spatial variation in predator presence and species may promote variability in the reactions of prey subpopulations. We collected isopods (Caecidotea communis) from three ponds: two with fish (sunfish in one, shiners in the other), and one without. We exposed individuals from these three subpopulations to kairomones and diet cues released by sunfish (Lepomis spp.) or golden shiners (Notemigonus crysoleucas). We used time-lapse photography to calculate the rate of movement and proportion of time isopods spent moving, both soon after cue introduction and twentyfive minutes later. Thus, we tested for reactions to cues, for rapid cue degradation or short-term habituation, and for effects of natal pond. The isopods from all subpopulations did not alter their rate of movement nor their percent of time moving when exposed to dechlorinated tap water or cues from sunfish. The isopods from the pond containing shiners significantly increased their proportion of time moving, and showed a not significant tendency to move more rapidly, when exposed to cues from shiners as opposed to dechlorinated tap water. Our results are the first to

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demonstrate that isopods may lack a behavioral response to chemicals indicating likely imminent danger from the same species of fish as occur in their natal pond.

Keywords Chemical cues - Crustacean - Predator– prey - Lentic

# Introduction

Avoiding predators, and thus serious injury or death, is one of the most essential daily tasks for prey (Lima & Dill, [1990\)](#page-10-0), often driving their adaptations to detect and respond appropriately (Brown, [2003\)](#page-9-0), even if the chemical information from predators does not constitute intentional signals (Wisenden & Chivers, [2006](#page-10-0)). Predation is reliably correlated with context-specific chemical cues that can guide behavioral decisionmaking in sympatric prey. Kairomones and disturbance cues cause prey to increase vigilance and prepare for evasive maneuvers, while alarm cues from conspecifics and dietary cues from predators invoke intense antipredator behavioral responses (Wisenden, [2015\)](#page-10-0). Typically, these inducible defenses are phenotypically plastic, with the degree to which they appear tailored to the magnitude of the threat as indicated by the biotic cues detected (Harvell, [1990\)](#page-9-0). Many inducible defenses have a memory component and are deployed more strongly in subsequent exposures (Harvell, [1990\)](#page-9-0). However, if these responses are energetically costly, and no danger manifests itself from the predator within a certain time window, individuals may reduce their response behaviors due to habituation (Lima & Dill, [1990](#page-10-0); Ferrari et al., [2010](#page-9-0)).

Freshwater isopods are benthic detritivores that serve an important function in the nutrient cycling of aquatic ecosystems (Wallace & Webster, [1996\)](#page-10-0) and can exist in particularly dense populations, especially in ponds without fish (Zhao et al., [2007](#page-11-0)). Visuallyoriented predators, such as many local fish, may exert significant selection pressures on these comparatively large, mobile but slow, members of the benthic macroinvertebrate fauna. Most fish species actively search for prey rather than employ passive ambush strategies, so actively moving prey typically suffer higher levels of predation than stationary prey (Wellborn et al., [1996\)](#page-10-0). Hence, decreased activity is commonly reported as an anti-predator response to fish (Abjornsson et al., [2004](#page-9-0)).

While prey can detect predators in numerous ways, olfactory cues (water-borne kairomones, conspecific alarm cues, diet cues, and disturbance cues; Chivers & Smith, [1998](#page-9-0); Kats & Dill, [1998](#page-10-0); Wisenden & Chivers, [2006\)](#page-10-0) typically predominate in ponds because rapid light attenuation hampers visual cues. Detection of species-specific kairomones can indicate proximity of the predator and trigger appropriate defense responses (Ferrari et al., [2010\)](#page-9-0). Exudates from predators can cause aquatic isopods to increase their respiration rate (Bengtsson, [1982\)](#page-9-0) or seek refuge (Hechtel et al., [1993\)](#page-9-0). Amphipods have been shown to reduce activity and show avoidance behaviors in response to chemical cues from fish and conspecific injury (Wudkevich et al., [1997](#page-10-0); Wisenden et al., [2001](#page-10-0); Smith & Webster, [2015\)](#page-10-0). Such a wide range of fish caused streamdwelling amphipods to settle on the benthos that Williams & Moore [\(1985](#page-10-0)) proposed the cue involved a basic fish secretion. Stream-dwelling isopods significantly reduce their activity in response to chemical cues in the skin mucus of five species of fish in three families (including non-predatory species), but particularly for centrarchids (Short & Holomuzki, [1992](#page-10-0)). In contrast, Spivey et al. ([2015\)](#page-10-0) found that while isopods from fish-less pools reduced their activity when presented with chemical alarm cues from macerated conspecifics, they did not react to chemical cues from predatory sunfish whether or not the fish had

recently consumed isopods. However, the authors themselves noted that the natal environment may explain the lack of response to predator kairomones and diet cues. Larval dragonflies from habitats with fish reduced foraging more in the presence of fish than did those from fish-less habitats (Pierce, [1988](#page-10-0)), and the aquatic isopod Asellus aquaticus (Linnaeus, 1758) displayed defensive behaviors only if it had prior exposure to the predatory species from which the cue was derived (Harris et al., [2013\)](#page-9-0). The reaction of prey to predatory cues may require previous exposure to those predators, and these threats may differ among disjunct habitats such as ponds (Ferrari et al., [2010](#page-9-0); Harris et al., [2013\)](#page-9-0).

Few studies have examined the relative performance of species with a limited ability to disperse from ponds with different predator regimes (Abjornsson et al., [2004](#page-9-0)). Freshwater isopods are capable of only passive dispersal among ponds (Wiggins et al., [1980\)](#page-10-0), limiting genetic communication in the absence of regular connections by flooding. Invertebrate prey community structure, genetic composition, and antipredatory behavior can differ greatly between fish-less and fish-containing habitats, even within clusters of close-set pools (Wellborn et al., [1996](#page-10-0); Abjornsson et al., [2004;](#page-9-0) De Meester et al., [2005;](#page-9-0) Levri et al., [2012\)](#page-10-0). Therefore, isopods likely also will demonstrate adaptations specific to the prevailing predator regime in their pond.

At Graver Arboretum (Bath, PA, USA), ponds differ dramatically in the population density of the isopod Caecidotea communis (Say, 1818) depending upon whether fish are present (unpublished data). In the present paper, we examined the reaction of isopods from different source ponds, two with and one without fish, when exposed to cues from sunfish (Lepomis spp.) and golden shiners [Notemigonus crysoleucas (Mitchell, 1814)], to determine whether individuals from the three subpopulations differed in olfactorymediated avoidance responses. We predicted that, despite geographic proximity, isopods from the ponds containing fish would reduce their movements in response to predatory cues while those from the pond without fish would not. We also examined whether the isopods further altered their behavior after time had elapsed since the initial cue detection, suggesting that either habituation in the prey or rapid degradation of the predatory cue occurred.

## **Methods**

#### Animal collection and cue preparation

All animals were collected from ponds at Graver Arboretum in Bath, PA  $(40^{\circ} 48' 00.24'' N, 75^{\circ} 21'$ 47.55" W). The isopods (Caecidotea communis) were collected from three ponds and maintained at  $4^{\circ}$ C in water and leaves from their specific pond. Two of these ponds are situated approximately 250 m apart within a woods comprised predominantly of tulip poplar (Liriodendron tulipifera L.), red maple (Acer rubrum L.), red oak (Quercus rubra L.), and beech trees (Fagus grandifolia Ehrh.): Scout Pond, a permanent pond that does not contain fish, and Virginia's Pond, a larger permanent pond with fish. Two sampling outings to Virginia's Pond conducted concurrent with these studies (one involving two snorkelers) collected and saw only golden shiners (Notemigonus crysoleucas). Golden shiners are one of the most common shiners in Pennsylvania (Steiner, [2002\)](#page-10-0), are common throughout the eastern United States (Page & Burr, [1991\)](#page-10-0), have an extremely dense population in Virginia's Pond, and will feed on isopods (personal observations). The third pond, Meadow Pond, is situated outside the forest about 500 m from Virginia's Pond and 250 m from Scout Pond. Meadow Pond has supported robust populations of sunfish for over a decade, including at least two species: bluegills (Lepomis macrochirus Rafinesque, 1819) and pumpkinseed [Lepomis gibbosus(Linnaeus, 1758)], both of which readily consume large numbers of isopods (Norton & Brainerd, [1993\)](#page-10-0). These species can interbreed and are difficult to distinguish until they develop adult coloration patterns, so our preparations of sunfish cues likely included both species. Both golden shiners and sunfish are gape-limited predators that hunt using visual cues (Breck & Gitter, [1983](#page-9-0); Reebs, [2002](#page-10-0)). The fish we used to create the chemical cue stimuli were large enough that they could consume the range of sizes of our experimental isopods (Long, unpublished data). The distance to our field site precluded daily gathering of experimental animals, but all isopods were tested within ten days of field collection.

We conducted experiments using sunfish kairomones and isopods from Scout Pond and Virginia's Pond in June and July 2019, and isopods from Meadow Pond in October and November 2020. Our experiments using shiner kairomones and isopods from Virginia's Pond were conducted in October and November 2019. Thus, none of our experiments were conducted during the isopod breeding season, which begins in winter (unpublished data). Fish were collected from the wild two to three weeks before the beginning of each series of trials. The fish were maintained in single-species community tanks in the laboratory that were filled with dechlorinated tap water, fish and their transport water (less than 25% of the tank water). During experiments, dechlorinated tap water was added to the tanks as needed to maintain water levels, but we performed no maintenance water changes. Aquarium filters without inserts were used to ensure adequate turbulence for oxygenation without removing chemical cues. To ensure that the fish would exude digested prey metabolites, sunfish were fed exclusively live isopods every other day from collection date through the duration of the experiment. Shiners were fed fish flakes and isopods for the duration of the experiment, but exclusively isopods within 24 h of any experimental trial. Therefore, the cue water likely contained isopod alarm cues and other disturbance cues in addition to predator kairomones (fish odor and diet cues). Water from the tanks was used immediately in experiments.

Multiple 10-gallon community tanks of fish were maintained (five tanks for the sunfish and four tanks for the shiners) to reduce issues of pseudo-replication, but it was impossible to use different fish in each experimental replicate. The sunfish were housed with at least 13 fish per tank, body lengths ranging from 2.5 to 10.0 cm (snout to tip of caudal fin), while there were approximately 45 golden shiners per tank, body lengths ranging from 4.20 to 9.75 cm. While our cue preparation likely produced a higher concentration of predator metabolites than is typically found in nature, the golden shiners in Virginia's Pond were observed to move in large, dense schools (two snorkelers using one hand-held seine net collected more than 200 fish in less than an hour) and we wanted our cue concentration at least as strong as an isopod in proximity to a shoal of feeding fish. While sunfish have greater body mass per unit of length than do shiners and tended toward the higher end of the size distribution, there was still more biomass of shiners than sunfish per liter. The sunfish concentration was constrained because this species was aggressive and attacked each other at higher densities, an issue that did not occur with the shiners.

<span id="page-3-0"></span>Sunfish do not travel in shoals as dense as the shiners, so the concentration of sunfish cues still exceeded that occurring in nature. Because stronger concentrations of predator odors have elicited stronger antipredator responses in prey (e.g., Ferrari et al., [2008](#page-9-0); Brown et al., [2009\)](#page-9-0), we preferred to use hyper-concentrated cue preparations.

Upon conclusion of each series of experiments, the animals were euthanized by freezing, except for some fish maintained afterwards in a display tank (that were not used in later experiments) and isopods that were fed to fish during experiments. Permission for sample collections was granted by Graver Arboretum and Muhlenberg College; Iyengar and Long each possessed PA state fishing licenses for animal collections.

## Study design

We collected isopods from Scout Pond, Virginia's Pond, and Meadow Pond for trials involving cues affiliated with sunfish, but only from Virginia's Pond for trials involving cues affiliated with shiners. Isopods ranged in size as indicated in Table 1. Isopods were placed individually in the center of a plastic arena (15 cm length, 11 cm width, 7 cm depth) containing a uniform sand benthos and room temperature (20  $^{\circ}$ C), dechlorinated tap water that either contained chemical cues (fish kairomones and alarm cues from conspecifics, ''cue'' treatment) or did not (''control'' treatment). Cue treatment involved only one type of fish (sunfish or shiners) at a time.

While previous experiments with aquatic isopods have examined merely the proportion of time spent moving (e.g., Holomuzki & Hatchett, [1994](#page-10-0)) or frequency of gridlines crossed (e.g., Spivey et al., [2015\)](#page-10-0), we utilized camera-recorded tracked movements to calculate the more refined metric of movement rate (similar to Augusiak & Van den Brin, [2016](#page-9-0)). After a one-minute acclimation period, during which time the isopod was allowed to freely roam, an aerial photo of the container was taken (using an Olympus Touch TG-5 digital camera) every two seconds for a total of five minutes; this time frame was designated as the ''initial'' period. To test whether the cue degraded, or the isopods habituated to the environment, after twenty more minutes, there was again a one-minute acclimation period (to control for disturbance during the manual re-starting of the camera) followed by a photo every two seconds for five minutes; this second time frame was designated the ''subsequent'' treatment. For the trials utilizing isopods from Meadow Pond, we only photographed over the initial time period. McIntosh et al. ([1999\)](#page-10-0), studying the response of stream-dwelling larval mayflies to fish cues, found rapid responses: significant behavioral alterations within 5 min of addition of trout chemical cue. Therefore, we are confident that relevant responses, if they were going to occur, would have been visible in the time frames we examined.

Two cameras (or four cameras in 2020) were used simultaneously so we could conduct a "cue" and ''control'' replicate concurrently. We calculated the





Control is the dechlorinated tap water (no fish cue) treatment. N is the number of replicates that were conducted. Deletions were replicates in which the isopod never moved in either the initial or subsequent time periods, so the replicate was omitted from the data analysis (reducing  $N$ ) as it was assumed to be an aberrant, injured, or dead individual. Fish size is the total length of fish (tip of the snout to the tip of the longer lobe of the caudal fin) in the community tank used in creating experimental cue

rate of movement of each individual isopod over the five-minute series of experimental photographs (''initial'' and ''subsequent'' periods kept separate) using the manual tracking plug-in for the computer program ImageJ (Cordelières, [2005;](#page-9-0) Ferreira & Rasband, [2010–](#page-9-0)2012; Schindelin et al., [2012](#page-10-0)). We also calculated the proportion of time the isopod spent moving during trials by summing the number of photographs in which the animal moved at least a body's length since the previous photograph ("moved") and dividing that by the total number of photographs taken during the interval; "initial" was considered a separate data set from ''subsequent.''

There were at least fifteen trials conducted for each treatment. However, if an isopod never moved over the 25-min observation period it was dropped from the analysis to avoid including data from dead, injured or otherwise aberrant animals. This exclusion was rarely necessary (see Table [1](#page-3-0) for exact numbers, never more than two animals per treatment). No isopod was used in more than one trial. Between each trial, we changed the water and sand substratum and rinsed out the arena. To minimize the problem of pseudo-replication, we sequentially used chemical cues from each of the source tanks.

### Data analysis

Data from each type of kairomone cue (sunfish or shiner) were analyzed separately, but the same framework of statistical analyses were applied to all data sets, as follows. The dependent variable was either the rate of movement or the proportion of time moving (in separate analyses). For the data using isopods sourced from Scout Pond and Virginia's pond exposed to sunfish cues, we calculated two-way repeated measures ANOVAs using the natal pond of the isopods and cue/control as the two independent variables, with timeframe (initial/subsequent) as the repeated measure, an interaction term between cue and timeframe, and replicates nested within cue. The experiment examining cue from shiners used a oneway repeated measures ANOVA, without the independent variable ''natal pond.'' The experiment involving isopods sourced from Meadow Pond was evaluated separately using a one-way ANOVA with cue/control as the independent variable because behavior over time was not examined.

In all analyses, equal variance and normal distribution of residuals were assessed using the Levene's test and visual inspection of the normality curve of residuals, respectively, to confirm that the data met the assumptions inherent in parametric tests. All analyses, including those with proportion of time spent moving, met these assumptions without transformation except for the response variables in the experiment utilizing shiner cue. For those analyses alone, we  $ln(y)$  transformed the response variables of both treatments to meet assumptions before conducting the analysis. All statistical analyses were conducted using the computer program Data Desk (version 6.0; Data Description, Ithaca, New York). We used  $P < 0.05$  as the critical value in all pair-wise comparisons.

#### Results

#### Cue detection and habituation effects analyses

In terms of the rate of movement when exposed to cues from tanks with feeding sunfish (Fig. [1](#page-5-0)A and 1B), the isopods from Scout Pond (the fish-less pond) and Virginia's Pond (the pond with shiners) did not show any significant response to cue  $(F_{(1,81)} = 1.07)$ ,  $P = 0.3084$ , significant change between timeframes  $(F_{(1,81)} = 3.30, P = 0.0731)$ , nor significant difference between ponds  $(F_{(1,81)} = 3.91, P = 0.0513)$ , and the cue\*timeframe interaction term was not significant  $(F_{(1,81)} = 0.79, P = 0.3760)$ . When examining the proportion of time spent moving in these same animals (Fig. [2](#page-5-0)A and B), the isopods did not show any significant response to cue  $(F_{(1,81)} = 0.002)$ ,  $P = 0.9616$ , nor difference between source ponds  $(F_{(1,81)} = 1.00, P = 0.3208)$ , and the cue\*timeframe interaction term was not significant  $(F_{(1,81)} = 0.64,$  $P = 0.34277$ , but they did display a significant reduction between the initial and subsequent timeframes  $(F_{(1,81)} = 7.38, P = 0.0081)$ . Similarly, isopods from Meadow Pond (the pond that sourced the sunfish for the experiments) did not show any significant response to cue from sunfish in terms of rate of movement (Fig. [1](#page-5-0)C;  $F_{(1,64)} = 0.12$ ,  $P = 0.7281$ , nor in terms of proportion of time spent moving (Fig. [2C](#page-5-0);  $F_{(1,64)} = 0.002$ ,  $P = 0.9645$ ).

In terms of rate of movement, isopods from Virginia's Pond did not show any significant response to cue from golden shiners (Fig. [3A](#page-6-0);  $F_{(1,27)} = 1.88$ ,

<span id="page-5-0"></span>

Fig. 1 Average  $(\pm 1.96SE)$  rate of movement of individual isopods from A Scout Pond (fish-less), B Virginia's Pond (containing golden shiners), and C Meadow Pond (containing sunfish). All fish cue treatments utilized sunfish (Lepomis spp.) that were fed isopods. The initial five-minute time frame started after a one-minute acclimation period, while the subsequent

five-minute period started 26 min after release. For the control and fish cue treatments, respectively,  $N = 15$  and 15 for isopods from Scout Pond,  $N = 15$  and 13 for isopods from Virginia's Pond, and  $N = 35$  and 31 for the isopods from Meadow Pond. The isopods from Meadow Pond were only observed during the initial time frame



**Fig. 2** Average  $(\pm 1.96SE)$  proportion of time spent moving of individual isopods from A Scout Pond (fish-less), B Virginia's Pond (containing golden shiners), and C Meadow Pond (containing sunfish) when exposed to cues from sunfish (Lepomis spp.). Times frames as described in Fig. 1. For the

 $P = 0.1813$  or significant change between timeframes  $(F_{(1,27)} = 0.75, P = 0.3927)$ , and the cue\*timeframe interaction term was not significant  $(F_{(1,27)} = 1.34,$  $P = 0.2575$ . However, these isopods did significantly increase the proportion of time spent moving in response to the cue from tanks with shiners compared with the control (Fig. [3B](#page-6-0);  $F_{(1,27)} = 11.07$ ,  $P = 0.0025$ , but there was no significant effect of timeframe  $(F_{(1,27)} = 1.29, P = 0.2669)$  and the

**Type of cue** control and fish cue treatments, respectively,  $N = 15$  and 15 for isopods from Scout Pond,  $N = 15$  and 13 for isopods from Virginia's Pond, and  $N = 35$  and 31 for the isopods from Meadow Pond

**0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1**

**Control Sunfish**

**Sunfish** 

**Type of cue** 

Control

cue\*timeframe interaction term was not significant  $(F_{(1,27)} = 0.67, P = 0.4219).$ 

## **Discussion**

Our experimental sunfish species are native to the eastern United States, dominate many warm water habitats, and are diurnal feeders that are "sight-

<span id="page-6-0"></span>

Fig. 3 Average ( $\pm$  1.96SE) A rate of movement and B proportion of time spent moving by individual isopods from Virginia's Pond (containing golden shiners). The fish cue treatment utilized

feeders'' (Steiner, [2002](#page-10-0), p. 131) so adaptive behavioral responses that would reduce the visibility of prey might be expected. Sunfish have a wide, overlapping biogeographic distribution with Caecidotea communis (Natureserve, [2019](#page-10-0)), suggesting that historical selection pressures should have driven predator-recognition in the isopods. Previous laboratory experiments have demonstrated that riparian isopods reduce their movements in response to fish cues from a range of species (Holomuzki & Short, [1988;](#page-10-0) Huang & Sih, [1991;](#page-10-0) Short & Holomuzki [1992\)](#page-10-0). We predicted that even isopods from fish-less ponds would react to fish cues due to neophobia (elevated avoidance response in reaction to novel cues; Brown et al., [2013\)](#page-9-0) or past selection pressures in the isopod lineage at an evolutionary timescale. Surprisingly, none of the isopods in our experiments responded significantly to sunfish cues, regardless of their natal pond. Additionally, while the isopods from Virginia's Pond reacted significantly to cue from sympatric golden shiners, they increased, rather than decreased, their proportion of time spent moving, and tended to move more rapidly (although this latter response was not significant and may have been driven by the greater proportional time spent moving).

While some prey only react to predators that are actively consuming conspecifics (Alexander & Covich, [1991\)](#page-9-0), we used cues from fish that had been fed



golden shiners (N. crysoleucas) that were fed isopods. Time-frames as described in Fig. [1.](#page-5-0)  $N = 14$  for the control treatment and 15 for the fish cue treatment in  $(A)$  and  $(B)$ 

isopods ad libitum within the past 24 h, so digested conspecific metabolites should have been present in our cue water. Our cue preparation involved a much denser population of predators than that of Holomuzki & Hatchett's [\(1994](#page-10-0)) laboratory experiments, which used one fish in 20 1iters of dechlorinated tap water. Our predator densities were higher than those typically found in nature. Our minimum distance requirement for the proportion of time moving response was larger than that used by Augusiak &Van den Brin ([2016](#page-9-0)), making ours a conservative response that was more likely to agree with previous authors. However, we did not observe the expected reduction in the proportion of time spent moving. Additionally, if isopods respond only to the alarm cues (Chivers et al., [1996](#page-9-0)) of dying conspecifics (as described in Spivey et. al., [2015](#page-10-0) for Caecidotea intermedia (Forbes, 1876), but not found when Sehr  $&$  Gall,  $2016$  studied the same species), we likely should have detected a reaction, as we fed live isopods to the fish within 24-h of trials.

While Spivey et al [\(2015](#page-10-0)) also reported lack of response in a congeneric isopod to dietary cues from sunfish consuming conspecifics, the isopods they examined came from a fish-less pond. The authors themselves noted the natal environment may explain their results, as Harris et al.  $(2013)$  $(2013)$  noted (for a different species of isopod) that prior experience with relevant cues was needed to develop anti-predatory behaviors; innate responses were not enough. In comparison, our studies involved a consistent lack of response to sunfish cues across isopods from three source ponds: one without fish, one with shiners, and one with sunfish. Therefore, not only is this lack of response widespread across ponds, lack of previous experience with this specific predator cannot be the explanatory factor. As Spivey et al. ([2015\)](#page-10-0) noted, their study was the second-known to demonstrate a failure by prey to respond to a predator's dietary alarm cues (the first involved tailed-frog tadpoles; Feminella & Hawkins, [1994](#page-9-0)). Our study is now only the third to show this result.

The increased movement of isopods in response to cues from shiners, but not in response to native sunfish cues, is surprising, as both fish species are common throughout their widespread biogeographic range. Furthermore, while golden shiners are reported to feed on zooplankton, insect larvae, and algae and we have observed them consuming isopods in our tanks, bluegill sunfish consume aquatic insects, crustaceans, and minnows, while pumpkinseed feed on snails and benthic aquatic insects (Steiner, [2002](#page-10-0)). Both species of sunfish voraciously attack and decimate isopod populations in our tanks (personal observations), so we had predicted a stronger defensive response would occur when exposed to the sunfish. Perhaps more continuously moving isopods present a target that is more difficult to track for the shiners that typically forage in the plankton. Additionally, if ponds with sunfish typically mirror the habitat parameters of Meadow Pond, isopods may have a spatial refuge from these predators. In Meadow Pond, the dearth of deciduous leaves may constrain foraging isopods to a thin belt of the very shallow weedy littoral zone which may preclude larger deep-bodied sunfish. In contrast, in Virginia's Pond, the streamlined shiners are better able to enter the relatively weed-free shallows more often and the continuous dense benthic cover of leaves likely facilitates isopod movements into deeper waters. This possibility of a spatial refuge deserves further attention as it is supported by the occurrence of isolated hot spots of high isopod density in extremely shallow, leaf-inundated areas under isolated bankside trees at Meadow Pond. However, the low general population density of isopods in this pond and high numbers of small sunfish suggest isopods must reside in proximity to sunfish.

The previous work reporting isopod responses to sunfish cues have used riparian species from other genera (Holomuzki & Short, [1988](#page-10-0), [1990](#page-10-0); Short & Holomuzki, [1992](#page-10-0); Holomuzki & Hatchett, [1994\)](#page-10-0), but other researchers studying pond Caecidotea sp. reported a lack of a response (Spivey et al., [2015](#page-10-0)), similar to our present findings. The difference may be habitat-specific, rather than genus-specific. The rapid, unidirectional flow and turbulence may render cue recognition more adaptive in lotic systems. Highly concentrated cues in streams likely represent imminent threats, while in pond waters the cues may linger after the predators have moved on. However, lake isopods respond to cues from predatory fish (Harris et al., [2013\)](#page-9-0), so some lentic systems demonstrate these defensive behaviors. Pond isopods might require the confluence of multiple evidences of imminent threat before reacting (as in Spivey et al., [2015;](#page-10-0) Zaguri et al., [2018\)](#page-10-0). In addition, the isopods might rely on detecting pressure waves from approaching predators rather than chemical cues, use the dilution or confusion effect as behavioral defensive strategies (Alcock, [2009\)](#page-9-0), or merely remain hidden under leaves rather than slowing down a comparatively lethargic pace. While our experimental design could not test for these possibilities, such investigations would be of interest in future studies.

Our results on pond isopods apparently conflict with those of Short & Holomuzki ([1992\)](#page-10-0), who reported that isopods reduced their movements in response to chemical cues from a large range of fish species, including sunfish, such as we used in our current experiments. However, not only did those researchers utilize riparian isopods and the two species of sunfish differed from the two we utilized (although they also noted decreased movements in response to cues from fish of two non-Centrarchidae families; Short & Holomuzki, [1992](#page-10-0)), their preparation of cues differed from that of most studies on this topic. Short & Holomuzki ([1992\)](#page-10-0) scraped mucus from the sides of fish and placed it on the bottom of the experimental arena, rather than using only water-soluble cues. If the mucus remained intact as strands on the benthos, the physical presence of the mucus may have contributed to the slower movements of the isopods, perhaps entangling their appendages or increasing the time spent grooming.

Our studies are not the first to note a lack of response: Spivey et al. ([2015\)](#page-10-0) reported similar results for a congeneric species, although that species reduced movements in response to alarm cues from con-specifics. Harris et al. [\(2013](#page-9-0)), working on another congener, found that the isopods only responded to cues from predatory fish if they were collected from a habitat containing this predator. While Vollmer & Gall  $(2014)$  $(2014)$  noted that isopods with prior experience with predatory crayfish did not respond to crayfish kairomones, their study did not use diet cues. Spivey et al. ([2015\)](#page-10-0) noted that few studies have reported a failure by prey to respond to dietary alarm cues from a predator (theirs was only the second-known study to do so), so our findings add to this small collection. However, while Spivey et al.'s [\(2015](#page-10-0)) isopods came from a pond without fish, our findings were consistent whether the isopods came from ponds without fish, or from ponds that contained either invasive or native predatory fish. Thus, ours is the first set of experiments to demonstrate a lack of behavioral response to predatory cues by isopods from ponds with predators.

The only significant comparison for isopods exposed to sunfish cues from Scout and Virginia's Pond was a slowing of movement over the course of the experiment. This pattern likely was part of a prolonged, continuous reaction to the adverse experience of whole-body perturbation that occurred when the animals were moved into the experimental arena. Likely it had nothing to do with time-deterioration of warning cues, as even the initial timeframe had no significant effect of cue.

While previous studies reported only the percent of time spent moving (e.g., Holomuzki & Hatchett, [1994\)](#page-10-0), our experiments examined the response at a finer level of detail, calculating the actual rate of movement as well as proportion of time moving. In our artificial arena environment, possibly the lack of response by animals when exposed to fish cues compared with control treatments was because they were searching for shelter. However, if that was the case, we would expect faster movement in the fish cue treatment, and that did not occur. The non-significant trend of isopods to move more rapidly when exposed to cues from golden shiners is interesting but does not seem likely to indicate increased searching for shelter because a similar trend did not manifest in response to predatory sunfish cues. Our qualitative comparisons of the tracks of the isopods did not appear to show more exploratory patterns with increased numbers of sharp turns when exposed to fish cue, whether that cue was

from shiners or sunfish, as may be expected from animals searching unsuccessfully for cover.

While the size of the isopods in our summer experiments was substantially smaller than in the fall, we do not think that the tendency to increase movement when exposed to cues from shiners (as shown in Fig. [3\)](#page-6-0), is due to an ontogenetic shift in response. The size difference across these studies was due to the life cycle of this species: eggs are produced during a single discrete time period in winter and summer populations do not contain multiple generations. The larger animals in fall experiments not only likely cannot move more rapidly than a foraging shiner, they failed to significantly alter their movement rates in response to shiner cues, only altering the proportion of time moving. Large isopods were also found in Meadow pond in the fall (Table [1](#page-3-0)) and those did not respond to the cue in sunfish treatments. Therefore, we conclude it likely that all developmental stages of this species of isopods do not respond to shiner cues.

Similarly, we do not believe our results reflect sex differences among the isopods in our studies. We used similarly sized animals in both treatments (Table [1\)](#page-3-0) that were likely all females or pre-reproductive males, as adult males are approximately  $1.5 \times$  to  $2 \times$  longer than females (Keogh and Sparkes, [2003](#page-10-0)). We did not conduct our experiments during the mating season, and there were no notable coloration differences among individuals (versus sex-associated or parasitized-associated colors found by Merilaita & Jormalainen, [1997;](#page-10-0) Jormalainen et al., [2001;](#page-10-0) Vesakoskia et al., [2008](#page-10-0); Park & Sparkes, [2017](#page-10-0)). Harris et al. [\(2013\)](#page-9-0) posited that mate-searching males of a different isopod species might respond more strongly than females to predator cues due to larger ranges or elevated sizerelated risk. However, Jormalainen et al. ([1995](#page-10-0)) found no size-preference by predators for isopods (unless animals were aerially dropped into the tank). Our results did not show a wide variance in isopod responses and the variance was similar across treatments, further disputing sex-related response differences. We used a size range that encompassed a large proportion of the population. However, future experiments might profitably examine responses of sexually mature males to predators during the mating season.

Our experiments indicated that these pond-dwelling isopods do not demonstrate inducible defenses that manifest themselves as reduced movement, nor dramatic acceleration, upon detection of chemical cues from predatory fish, but it is not clear from our experiments whether the isopods cannot detect the cues that predators are near, do not respond, or respond in ways other than altering movement rates or frequency. Especially in small pond habitats with fish, foregoing the evolution of cue detection but instead constantly attempting to remain in a protected environment might be the sensible strategy for slowmoving prey that are largely restricted to a twodimensional habitat. Our experiments utilized single isopods, in containers with a homogenous array of cue. Whether animals in groups respond differently to the presence of predators, or to pressure waves from predators, and whether a cue gradient (such as could be provided in a flume) would cause isopods to alter their movement trajectories, would all be of future interest.

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#### Declarations

Conflict of interest Elizabeth C. Long and Erika V. Iyengar declare that there are no known potential sources of conflict of interest.

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