



Phototactic behavior of native *Daphnia* in the presence of chemical cues from a non-native predator *Bythotrephes*

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Abstract

Chemical cues are used by many taxa to communicate within and among species. Behavioral defenses induced by predator cues are a mechanism by which prey species resist or avoid predator attack. This study examined the egg bank of native *Daphnia* species in a lake that has been invaded by *Bythotrephes longimanus*, an invertebrate zooplanktivore native to northern-central Europe and Asia (initial invasion 1994, population boom in 2009). *Daphnia* resting eggs from both pre- and post-*B. longimanus* invasion lake sediments were hatched and established as isofemale clonal lines. Phototactic behavior (a proxy for vertical migration behavior) was assessed in the presence and absence of *B. longimanus* cue. This was done to evaluate the hypothesis that the heavy predation imposed by *B. longimanus* would have been selected for *Daphnia* clones that are more negatively phototactic in the presence of *B. longimanus* cue, because *B. longimanus* is a visual predator. The behavior of the clones derived from pre-*B. longimanus* era resting eggs was not significantly different from the behavior of the clones from the post-*B. longimanus* era and exposure to predator cue did not affect the phototactic response of the clones. There was a significant difference in the phototactic behavior of the three *Daphnia* species tested (*Daphnia ambigua*, *Daphnia mendotae*, and *Daphnia pulicaria*). These results suggest that predation by *B. longimanus* is not the main factor that is influencing the phototactic behavior of *Daphnia* in the lake. Other factors such as fish predation may be playing a more significant role in this system.

Keywords Invasive species · Inducible defenses · Kairmones · Vertical migration · Zooplankton

Introduction

Chemical cues are present in ecosystems and serve as a method of communication within and between species. Chemical cues can be used in a variety of ways. For example, parasites can use chemical cues to locate a host (Runyon et al. 2006), social insects can use them to recognize nest-mates (Torres et al. 2007), and they can serve as an alarm signal that activates inducible defenses (von Frisch 1942;

Tollrian 1995). Inducible defenses are phenotypic changes triggered directly by cues associated with biotic agents, whereas constitutive defenses are always phenotypically present (Tollrian and Leese 2010). Constitutive defenses evolve under constant predation pressure, while inducible defenses are adaptations to fluctuating predation risk (Tollrian and Leese 2010).

Predation is a major selective force which affects the structure of biological communities as well as causes the evolution of inducible defenses in many prey species (Tollrian and Leese 2010). Among invertebrates, proximity to predators and competitors can induce defensive shifts in morphology, behavior, and life history (Dodson 1988; Pijanowska 1997; Pijanowska and Kowalczewski 1997a, b; Pijanowska et al. 2006). Predator-induced changes in prey can range from the production of spines and helmets (in *Daphnia*; Laforsch et al. 2006) to changes in shell thickness (in an intertidal snail; Trussell and Nicklin 2002), changes in activity (in a damselfly; Brodin and Johansson 2002), and changes in bet-hedging behavior (in birds; Fontaine and

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Martin 2006). The ability to measure these changes makes them a powerful tool for understanding evolutionary causes and ecological consequences of inducible responses.

The system: invasive predator and native prey

Bythotrephes longimanus is a non-native invasive zooplanktivore found in many north-temperate lakes of North America (Yan et al. 2011). A preferred prey of *B. longimanus* is the herbivore *Daphnia* (Boudreau and Yan 2003), which plays an important role both as a grazer of algae/bacteria and as a vital food source for recreationally and commercially important fish species (Lampert 2011). Thus, predation of *B. longimanus* on native *Daphnia* species has the potential to disrupt the functioning of many aquatic ecosystems (Boudreau and Yan 2003; Walsh et al. 2016a). For example, the vertical distribution of zooplankton in invaded lakes is changing; specifically, high densities of *B. longimanus* are correlated with deeper vertical distributions of native zooplankton species (Pangle et al. 2007; Bourdeau et al. 2011, 2015). Predation by *B. longimanus* is light dependent and *B. longimanus* is unable to feed on *Daphnia* by mechanoreception alone, making light a major factor determining the foraging impact of *B. longimanus* (Pangle and Peacor 2009; Jokela et al. 2013).

Light-induced behavior in *Daphnia* has been extensively studied (Ringelberg 1964; Zaret and Suffern 1976; De Meester 1989). It has been demonstrated that phototactic reaction to changes in light intensity can be altered by the presence of chemical cues from predators such as fish (Ringelberg 1991). Zooplankton diel vertical migration is a specific light-induced behavior which results in individuals being found in shallower water at night than during the day (Zaret and Suffern 1976). This behavior is commonly considered a predation-avoidance mechanism—by staying deeper in the water column during the day, zooplankton can reduce their risk of being detected by visually foraging predators such as fish (Zaret and Suffern 1976; Lampert 1989). Laboratory experiments have found that *Daphnia mendotae* from a lake invaded for approximately 20 years, migrate downward in response to cues specific to and produced directly by *B. longimanus* (Pangle and Peacor 2006; Bourdeau et al. 2013). It is often assumed that when native (i.e., naïve) prey encounter a novel predator, they are vulnerable due to the absence of a shared evolutionary history (Cox and Lima 2006). However, these studies demonstrate that at least one *Daphnia* species exhibits predator-induced defenses in response to the non-native predator. It is possible therefore, that native *Daphnia* populations can respond to the invasion of *B. longimanus* via a combination of plasticity and genetic adaptation, as has been demonstrated in other predator–prey systems (see Rabus et al. 2012; Stoks et al. 2015).

A resurrection ecology approach to study predator–prey interactions

The field of paleolimnology reconstructs past populations/communities and environmental changes in aquatic systems by comparing (sub)fossil remains of organisms (e.g., zooplankton, phytoplankton) with proxies of environmental changes archived in lake sediments (Kerfoot and Weider 2004). Living remains (such as zooplankton diapausing eggs) can be separated from sediments and used for laboratory ecological tests (Kerfoot and Weider 2004). Reviving diapausing eggs for laboratory tests (i.e., “resurrection ecology”) allows ecological and evolutionary hypotheses to be directly tested with ancestral individuals (Kerfoot et al. 1999; Kerfoot and Weider 2004). For parthenogenetically reproducing organisms such as *Daphnia*, hatchlings from resting eggs can be used to establish isofemale clonal lines. Individuals from these clonal lines can be used in experiments as replicates of the same genotype, to evaluate whether a population has evolved over time.

In this study, a resurrection ecology approach was used to recover and hatch *Daphnia* resting eggs from Lake Mendota (Madison, WI) sediments that were deposited before and after the *B. longimanus* invasion (lake invaded in 2009). The goal of this study was to determine whether the invasion of the non-native predator (*B. longimanus*) has caused a change (either constitutive or inducible) in the phototactic behavior of the native *Daphnia* species of lake Mendota. To address this question, 15-min phototactic assays (De Meester 1989) were performed to elucidate predator cue-induced depth selection behavior in post-invasion clones of three *Daphnia* species (*D. ambigua*, *D. mendotae*, and *D. pulicaria*) and pre-invasion clones of two *Daphnia* species (*D. ambigua* and *D. pulicaria*). We were unsuccessful in establishing pre-invasion clones of *D. mendotae*. We hypothesized that in the presence of *B. longimanus* chemical cues, post-invasion *Daphnia* would display negative phototactic behavior, while both the pre-invasion *Daphnia* and the *Daphnia* not exposed to chemical cues would display more positively phototactic behavior. Because *B. longimanus* are a visual predator, those *Daphnia* that have a shared history with *B. longimanus* would benefit from responding to the chemical cue by migrating down in the water column. Negative phototaxis should only be demonstrated in the presence of predator chemical cue because of potential trade-offs (i.e., reduced reproduction and/or growth in colder less productive deeper waters; Dawidowics and Loose 1992; Loose and Dawidowics 1994). We also hypothesized that larger-bodied *Daphnia* species would be more negatively phototactic, as their larger body size increases their visibility to visual predators such as *B. longimanus*. Predation of *B.*

longimanus on the focal *Daphnia* species is not restricted by gape limitation and therefore all species being studied would benefit from reduced visibility (Schulz and Yurista 1999).

Methods

Field sampling

Study site

Lake Mendota is a eutrophic, 39.6 km² lake in southeastern Wisconsin, USA (near the southern edge of *B. longimanus*' invasive range; Fig. 1S). The lake is dimictic and from mid-July to October, the bottom of the lake becomes anoxic (<0.5 mg/L) from 10 m below the surface to the bottom of the lake (25.3 m maximum depth and 12.7 m mean depth, North Temperate Lakes Long-Term Ecological Research, NSF 2001b; Walsh et al. 2016b). Lake Mendota is one of the North Temperate Lakes Long-Term Ecological Research (NTL-LTER) sites and has been consistently monitored since 1976. *Bythotrephes longimanus* was initially established in Lake Mendota in 1994 (detected via sediment cores), although it was not detected in NTL-LTER zooplankton samples until a 2009 population outbreak (Walsh et al. 2016b). Lake Mendota is an ideal lake to study the invasion of *B. longimanus* from the Great Lakes to smaller inland lakes, because of the long-term zooplankton database, previous sediment coring work, and importance of the lake to the surrounding community (Walsh et al. 2016a).

Core collection and sediment processing

To collect pre- and post-invasion resting eggs for hatching experiments, nine sediment cores were taken from the deep hole of Lake Mendota (43.10667° N, 89.42472° W, water depth = 25 m) in May 2017, using a gravity corer (0.5 m long, 63 mm inner diameter, 69 mm outer diameter). Cores were examined for the presence of layered sediments and absence of gas bubbles to ensure the integrity of the sediment layers. Cores that showed possible disruption (i.e., mixing of layers) were discarded. Polycarbonate tubes containing cores were transported to shore, where sediments were extruded and sliced at 2 cm intervals from 0 to 20 cm. Care was taken to avoid cross-contamination of different sediment layers via carefully extruding sections, slicing, and washing the extruder and slicer between samples. Samples were placed individually in 384 mL whirl-paks®, placed immediately in coolers containing ice packs, and then returned to the laboratory for further processing. Previous ²¹⁰Pb dating of Lake Mendota sediments was used to estimate the dates of the sediments collected and to determine

pre- and post-*B. longimanus* invasion sediments (Walsh et al. 2016b). In addition, *B. longimanus* tail spines preserve well in lake sediments (Beranek 2012) and thus the presence/absence of tail spines can be used as an indicator to assess the presence or absence of *B. longimanus* in the lake during each time period (Walsh et al. 2016b).

Processing of resting eggs from the sediments and hatching protocols followed Frisch et al. (2014). *Daphnia* clones were established from resting egg hatchlings isolated from Lake Mendota sediment layers. A total of four pre- and eight post-*B. longimanus* invasion *Daphnia* clones were used in this experiment. Species tested included *D. pulicaria* (three post-invasion clones, two pre-invasion clones), *D. mendotae* (two post-invasion clones), and *D. ambigua* (three post-invasion clones, two pre-invasion clones). Post-invasion *Daphnia* included three *D. ambigua* and two *D. pulicaria* clones from approximately 2014–2017 (0–2 cm sediment layer) and one *D. pulicaria* and two *D. mendotae* clones from approximately 2008–2011 (4–6 cm sediment layer). Pre-invasion *Daphnia* included two *D. ambigua* and two *D. pulicaria* clones from approximately 1987–1990 (18–20 cm sediment layer). Low levels of hatching success from resting eggs from deeper sediments prevented the establishment of equal numbers of pre- and post-invasion clones. With many resurrection ecology studies of *Daphnia*, a major limitation is the number of viable hatchlings that can be established, especially from deeper sediment layers. We acknowledge that limitation in this present study, which may reduce the ability to make broader inferences in this system.

Stratified zooplankton samples

To examine the daytime vertical distribution of *Daphnia* and *B. longimanus* in Lake Mendota, a 30 L Schindler-Patalas Trap (292 × 292 × 413 mm) fitted with a 63 μm Nitex filter net (5.4 cm cod end and 31.1 cm long collecting into a 200 mL bucket with 61 μm stainless steel screen cloth) was used to collect stratified zooplankton samples at noon on 13 August 2018. We only sampled during the day because our primary interest was in how the *Daphnia* were distributed under daylight conditions. We acknowledge that the nighttime distributions of *Daphnia* in the lake are likely different from the daytime distributions, but daytime distributions are more directly comparable to our laboratory phototactic behavior experiments. Two trap samples were taken at 2 m intervals (0–24 m) at the Lake Mendota deep hole (43.10667° N, 89.42472° W, water depth = 25 m). Samples were poured through a 152 μm mesh sieve, shocked in 95% ethanol, and preserved in 70% ethanol following the methods of Black and Dodson (2003). All the *Daphnia* sp. and *B. longimanus* in the samples were identified to species and enumerated using a Leica MZ8 stereomicroscope on a Transmitted Light Stand HL with a NCL 150 light source.

The abundance of each species at each depth (i.e., number L^{-1}) for each of the two samples was graphed to demonstrate the vertical distribution of the species in the lake. Dissolved oxygen ($mg L^{-1}$) and temperature (degrees C) data from the weeks of 6 August 2018 and 22 August 2018 were obtained from the LTER database (North Temperate Lakes Long-Term Ecological Research, NSF 2001b) and were averaged to estimate the thermal stratification of the lake when zooplankton samples were collected.

Phototactic behavioral assays

The phototactic behavior of clones was quantified using a laboratory assay as described in De Meester (1989); this method has been used in a variety of published studies (e.g., De Meester 1991, 1996; Decaestecker et al. 2002; Hembre and Peterson 2013). The experimental setup consisted of clear polycarbonate cylinders (30.5 cm tall, 2.5 cm diameter) externally marked into three compartments: upper (U) 10 cm tall, middle (M) 12 cm tall, and lower (L) 3 cm tall. Tubes were placed in a three-sided black box in a darkened room and illuminated from above with a fiber optic light ($241.0 \mu mol m^{-2} s^{-1}$ at the water surface). To minimize light reflection, autoclaved black aquarium gravel was placed in the bottom of the tubes to a depth of 3 cm. To minimize variability due to daily endogenous rhythm, experiments were performed between 16.00 and 20.00 h on each experimental date. Cue treatments were prepared using a mortar and pestle to crush live-frozen *B. longimanus* (collected from several lakes in southern Ontario with varied invasion histories—EL Kiehnau, unpublished data) in a small amount of artificial lake medium (COMBO; Kilham et al. 1998). The crude homogenate was then placed on a $0.45 \mu m$ GF/F filter held in place on a vacuum filtration flask and COMBO was poured over the filter to disperse the chemical cues throughout the filtered medium to reach a final concentration of $2.7 B. longimanus L^{-1}$ of COMBO (following methods similar to Bungartz and Branstrator 2003). We chose to use chemical cue from frozen field-collected *B. longimanus* because of the difficulty of culturing *B. longimanus* in a lab setting (Kim and Yan 2010) and because previous research has demonstrated induction of defenses in *Daphnia* using chemical cues from *B. longimanus* frozen alive (Bungartz and Branstrator 2003). The compounds that comprise *B. longimanus* chemical cues are not known and thus artificial synthesis of chemical cue(s) was not an option. The no cue (control) treatments consisted solely of COMBO filtered through a separate $0.45 \mu m$ GF/F filter and vacuum apparatus.

Each experimental tube was filled with either predator chemical cue or no cue media, and then four pre-reproductive experimental animals per clone of each species were pipetted into the tubes. Pre-reproductive experimental animals were identified via size and the lack of a visible brood

chamber. All experimental animals came from maternal lines (stock cultures) raised under identical conditions for at least two generations (to reduce maternal effects; see Tollrian 1995 for an example of maternal effects in *Daphnia*). Stock cultures of clonal lineages were grown separately in several 3.79 L and 1 L jars (note: experimental animals for each clone were pooled from multiple jars into single jars prior to being haphazardly distributed into the experimental tubes). Stock jars were fed daily with the green algae *Nannochloropsis* sp. (Nano 3600™, Reed Mariculture, Campbell, CA). Nano 3600™ is a high-concentration (i.e., 68 billion cells/mL) “slurry” of dead algae that needs to be diluted before use. We diluted the algae (with COMBO) to a concentration of 34 million cells/mL and fed 1 mL of this diluted food per 200 mL liquid in the stock jars. No algae were added to the experimental tubes due to the short duration of the trials, the animals being well fed in the stock jars, and the potential impact of a food gradient on migration behavior. Trials took place on separate dates from January to March 2018, and each trial consisted of a 5-min dark acclimation period before a 10-min light treatment during which the number of individuals in each compartment (U, M, L) was recorded every minute. This was a double-blind experimental design with preparers of the tubes located in one room, while the observer (in all trials, ELK) was in a separate room. A third person randomized (using a random number generator) the order of tubes prior to the beginning of each trial and labeled the two treatments as either “A” or “B” with clones/species numerically coded. Thus, neither the preparers nor the observer knew the identity of either the treatments or the clones/species being tested to avoid possible observation bias.

The phototactic behavior of the animals was calculated using the following phototactic index (PI) developed by De Meester (1989): $PI = U - L / (U + M + L)$. U, M, and L are the numbers of animals observed in the upper, middle, and lower compartments of the column, respectively. PI values can range from -1 (all animals in the lower compartment) to 1 (all animals in the upper compartment). To minimize possible acclimation effects of switching from total darkness to light, only data (i.e., averaged) from the last 5 min of the light treatment were used. Three to four replicate assays of each clone-by-treatment combination were performed, depending on the number of pre-reproductive individuals that were available in the *Daphnia* cultures (i.e., a single pre-invasion *D. ambigua* clone and a single post-invasion *D. ambigua* clone have only three replicates due to limited availability of experimental animals).

Statistical analysis

All statistical analyses were conducted in R version 3.5.3 (R Core Team 2019). Zooplankton stratified abundance data

were binned into two categories, the epilimnion (0–10 m) and the hypolimnion (14–24 m) and were tested for normality using quantile–quantile plot and histogram visualizations as well as a Shapiro–Wilk test (shapiro.test function). The data were found to be non-normal ($W=0.70, P=7.4E-11$) and were transformed using Tukey’s ladder of powers (transformTukey function). After transformation, the data met all necessary assumptions and were analyzed using a two-way analysis of variance (ANOVA) with thermal stratification (epilimnion/hypolimnion) and species (*D. mendotae*/*D. pulicaria*/*B. longimanus*) as independent variables (note: *D. ambigua* was absent from our plankton samples—see below) and the Tukey-transformed abundance data as the dependent variable (anova function).

Linear models (lm function) were used to assess the importance of the predator chemical cue, history of coexistence, and species identity on the phototactic behavior of the *Daphnia* clones tested. Phototactic index data from 94 (10 clones \times 2 treatments \times 4 replicates + 2 clones \times 2 treatments \times 3 replicates) trials were analyzed using a Bartlett test to test homogeneity of variance among groups (bartlett.test function). Variance between groups was not equal (Table 1S). The response variable, average phototactic index, was checked for normality (shapiro.test function, Table 2S) and was transformed using Tukey’s ladder of powers, before running the analyses (transformTukey function). While in principle, clonal identity should be incorporated in the statistical model as a random effect, in practice its effect was negligible (the among-clone variance in average phototactic index was estimated to be zero in our data). It is reasonable to remove variance components that are not supported by the data and removing such terms does not mean the variance is truly zero, but that we lack evidence of it being different from zero (e.g., these results may change if a greater number of clones had been tested; Bates et al. 2015). Dropping

clone as a random effect allowed us to use a linear model approach, and the coefficients of the mixed model did not differ qualitatively from those of the linear model (species was always the only factor that had a significant effect on average phototactic index). This method of dropping random effects due to zero variance components has been utilized in other instances (e.g., Pasch et al. 2013; Fuchs et al. 2016), and is the approach we have taken.

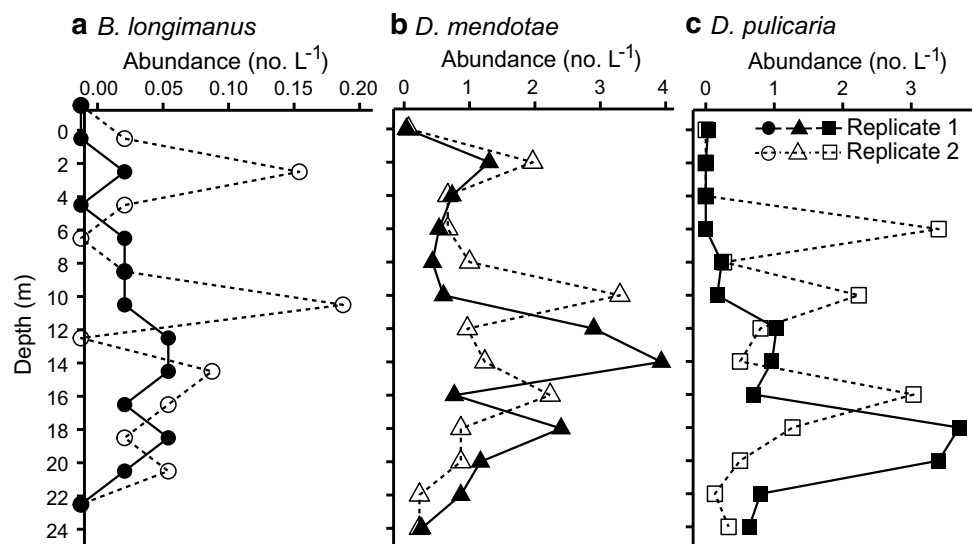
In the linear model, presence or absence of *B. longimanus* chemical cue (treatment), history of coexistence with *B. longimanus* (either pre- or post-invasion), species identity (*D. ambigua*, *D. mendotae*, or *D. pulicaria*), and the interaction among the three variables were treated as driver variables. Average phototactic index, a measurement of movement towards or away from the light, served as the response variable. Due to the unbalanced structure of the data (no pre-invasion *D. mendotae* were tested), models were constructed for both the full dataset and a reduced dataset which excluded the *D. mendotae* clones (Tables 3S–8S). Both models indicate that species is the only factor that has a significant effect on average phototactic index; therefore, the values from the model for the full dataset are used throughout the paper (Tables 3S and 6S). Details on model selection and testing of assumptions can be found in Appendix S1.

Results

Vertical distributions in Lake Mendota

Species differences were observed in the daytime vertical distribution of *Daphnia* and *B. longimanus* in Lake Mendota (Fig. 1; interaction effect of species and stratification layer, $F_2=4.73, P=0.01$). As noted above, no *D. ambigua* were found in any of the water column samples. The epilimnion

Fig. 1 Abundance (number L^{-1}) of **a** *Bythotrephes longimanus*, **b** *Daphnia mendotae*, and **c** *Daphnia pulicaria* throughout the water column of Lake Mendota at noon on 13 August 2018. The abundance data were collected from two 30 L Schindler-Patalas trap samples, each replicate is plotted separately



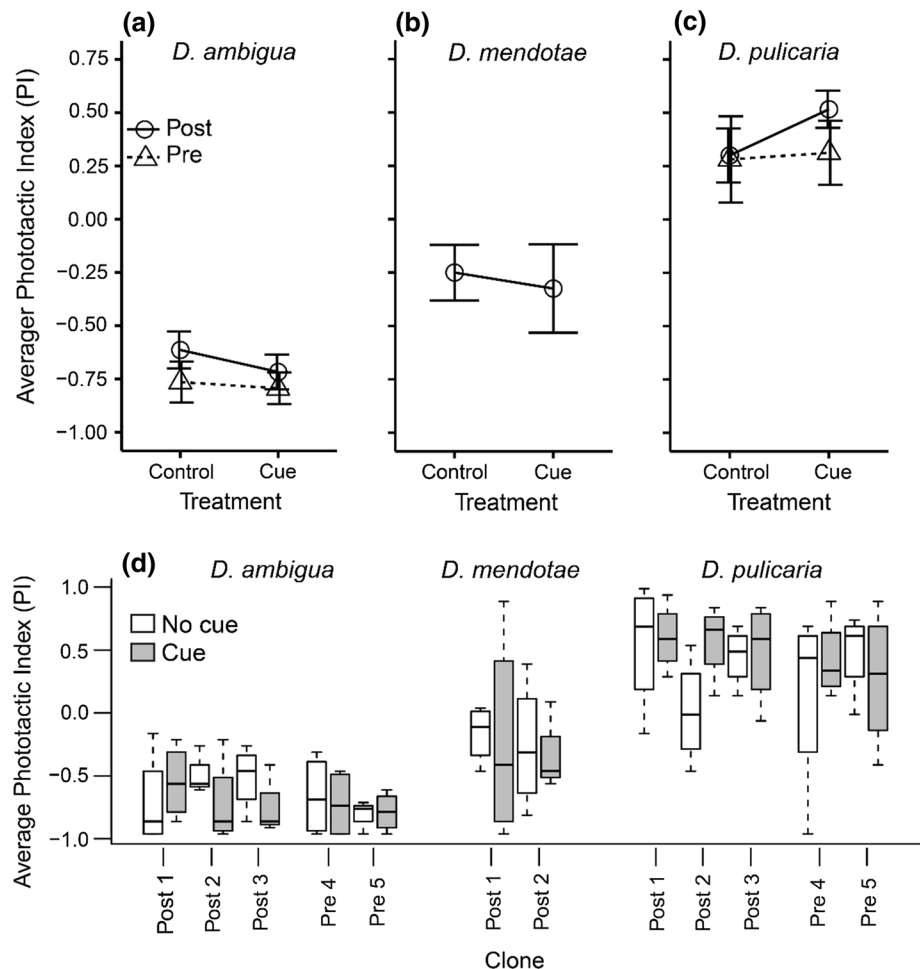
was determined to be from ~0 to 10 m and the hypolimnion was determined to be from ~12 to 24 m (Fig. 2S). The hypolimnion (as is typical) was associated both with lower temperatures and lower concentrations of dissolved oxygen (Fig. 2S). Thermal stratification layer (epilimnion vs hypolimnion) was found to have a significant effect on the overall abundance of *Daphnia* and *B. longimanus* ($F_1 = 12.04$, $P = 9.2E - 04$). *Daphnia mendotae* and *D. pulicaria* dominated the *Daphnia* community and were most abundant in the hypolimnion. *Daphnia mendotae* densities peaked at 10–14 m, higher in the water column than *D. pulicaria*, which exhibited a peak at 16–18 m (Fig. 1). Smaller peaks were seen in the epilimnion at 2 m (*D. mendotae*) and 6 m (*D. pulicaria*) (Fig. 1). *Bythotrephes longimanus* was found at low densities throughout the water column, with slight peaks at 4 m and 12 m (Fig. 1). When comparing abundance of *D. mendotae*, *D. pulicaria*, and *B. longimanus* across depths, *D. pulicaria* was found deeper in the water column than *D. mendotae* and the distribution of *B. longimanus* appeared to overlap more with *D. mendotae* than *D. pulicaria* (Fig. 1). While species distributions overlapped, the depths at which they were most abundant differed for

each species; species identity had a significant effect on abundance (Fig. 1; $F_2 = 27.70$, $P = 1.9E - 09$).

Phototactic behavioral assays

There were no significant differences between cue and no-cue treatments in the average phototactic index of the *Daphnia* tested (t value = -0.79 , $P = 0.43$; Table 3S; Fig. 2). Further, pre- and post-invasion (proxy for coevolutionary history) *Daphnia* did not display significantly different phototactic behaviors (t value = -0.98 , $P = 0.33$; Table 3S; Fig. 2). However, there was a species-level effect, whereby the phototactic behavior of each species was significantly different (DA–DM t value = 2.20 , $P = 0.03$; DA–DP t value = 5.58 , $P = 2.8E - 07$; Table 3S; Fig. 3S). *Daphnia pulicaria* clones exhibited positive phototaxis, *D. mendotae* clones exhibited slight negative phototaxis, and *D. ambigua* clones exhibited negative phototaxis (Fig. 3S). Interspecific differences in phototactic behavior were greater than intraspecific differences, but there were some (albeit negligible) differences in reaction norms (i.e., plasticity) among clones within species, especially for the *D. pulicaria* and *D.*

Fig. 2 Average (± 1 SE) phototactic index (PI) of pre- and post-invasion *Daphnia* in the presence and absence of chemical cues from *Bythotrephes longimanus* are plotted in panels (a–c). Average PI of *Daphnia* clones in the presence and absence of chemical cues from *B. longimanus* are plotted in panel (d). Boxplots show the third quartile, median, first quartile, and data outliers. All *Daphnia* were resurrected from Lake Mendota sediments and were categorized as either pre- or post-*B. longimanus* invasion (triangles and circles, respectively, in panels (a–c)). PI values can range from -1 to 1 , positive values indicate movement toward the light source, while negative values indicate movement away from the light source



ambigua clones tested (Fig. 2). There were no significant interaction effects among treatment, history of coexistence, and species (Table 3S).

Discussion

The goal of this study was to determine whether the invasion of *B. longimanus* has caused changes (either constitutive or inducible) in the phototactic behavior of the *Daphnia* species of Lake Mendota. In the behavior assays, each *Daphnia* species displayed a distinct phototactic behavior, which was not affected by treatment or exposure history (pre- or post-invasion). The natural daytime distribution of *Daphnia* and *B. longimanus* in Lake Mendota was as expected (i.e., larger *Daphnia* species found deeper in the water column and *B. longimanus* found closer to the surface). However, these distributions taken on a single sampling date did not match the results of the phototactic assays. It is not surprising that the species-specific phototactic behaviors found in the laboratory experiments did not match the natural daytime distributions of *Daphnia* in the lake. The laboratory experiments were conducted in a controlled setting, assessing only the impact of *B. longimanus* chemical cues on phototactic behavior, while the field observations were subject to additional environmental factors such as temperature, turbulence, food patches, and presence of other predators. It is likely that multiple environmental factors are influencing the natural daytime distributions of *Daphnia* in the lake. Unfortunately, no other *Daphnia* vertical distribution data are available for Lake Mendota to determine the robustness of these findings.

Treatment effects

The *Daphnia* tested were either not able to detect the *B. longimanus* chemical cue or did not respond to the presence of the cue by altering their phototactic behavior. Treatment did not have a significant effect on the phototactic behavior of the *Daphnia* tested (Fig. 2). *Daphnia* are known to respond to *B. longimanus* chemical cues (Bourdeau et al. 2011). However, it is possible that a longer exposure period to *B. longimanus* chemical cue may be necessary to induce a change in phototactic behavior. Previous *Daphnia*–*Bythotrephes* vertical migration experiments have involved exposure lengths ranging from 4 h to 4 days (Pangle and Peacor 2006; Bourdeau et al. 2013), while the *Daphnia* in our study were exposed to chemical cues for the duration of 15-min experimental trials. Another possibility for the lack of response is that *B. longimanus* may not have been present at high enough densities in Lake Mendota for long enough periods of time to impose a strong selection pressure for the *Daphnia* to evolve a response to their chemical cue. *Bythotrephes longimanus* experience seasonal booms and crashes in

population growth correlated to surface water temperatures (Walsh et al. 2016b). The sporadic nature of *B. longimanus*' temperature-dependent population dynamics likely impacts the ability of *Daphnia* to develop anti-predator defenses (Walsh et al. 2016b).

History of coexistence effect

History of coexistence did not change phototactic behavior as pre- and post-invasion *Daphnia* did not display distinctly different phototactic behaviors (Fig. 2). This may be due to the inconstant predation pressure imposed by *B. longimanus* (as mentioned above). It could also be that altered phototactic behavior is not the most effective or efficient anti-predator response against *B. longimanus*. *Daphnia* are known to respond to predator chemical cues with a variety of morphological and behavioral defenses (for examples, see Pijanowska 1997; Pijanowska and Kowalczewski 1997a, b; Laforsch et al. 2006; Pijanowska et al. 2006). In addition, *B. longimanus* may not be the strongest factor influencing the vertical distribution of the *Daphnia* of Lake Mendota. Other invertebrate (e.g., *Chaoborus* and *Notonecta*) and fish (e.g., *Lepomis*) predators are known to alter the vertical migration behavior of *Daphnia* (Dodson 1988). Alternatively, the lack of a difference in phototactic behavior between pre- and post-invasion *Daphnia* may be due to the limited number of clones tested (four pre-invasion clones and eight post-invasion clones), or the absence of pre-invasion *D. mendotae* clones (due to low hatching success and unsuccessful attempts to establish the few hatchlings). *Daphnia mendotae* are known to be relatively unaffected by *B. longimanus* invasions, thus are likely to have a successful anti-predator defense (Yan et al. 2011).

Species differences

Predation of *B. longimanus* on the focal *Daphnia* species is not restricted by gape limitation (Schulz and Yurista 1999) and thus contrary to our initial predictions, we found that larger-bodied *Daphnia* species were less negatively phototactic, despite being more visible to visual predators (Fig. 2, Fig. 3S). We found that *D. pulicaria*, the largest species, showed the greatest positive phototactic response and was found in the top of the experimental column, *D. mendotae*, the medium-sized species, dominated the middle of the column, while *D. ambigua*, the smallest species, showed negative phototaxis and was found in the bottom of the column. The results of this study are contrary to the common findings that larger zooplankton migrate further down in the water column, because they are the most visible (and presumably vulnerable) to visual predators (such as *B. longimanus* and fish; Dodson 1988; Muirhead and Sprules 2003; Pangle et al. 2007).

The distinct phototactic behaviors of the *Daphnia* species in this study may be related to vertical partitioning of the water column in the absence of cues from predators other than *B. longimanus*. Vertical partitioning of the water column is known to reduce interspecific competition among *Daphnia* species (Leibold 1991). For example, Leibold and Tessier (1991) found that *Daphnia* demonstrate greater habitat segregation in lakes with high levels of predation. Specifically, these authors observed that *D. pulicaria* occupied the epilimnion at low levels of fish predation but were restricted to the hypolimnion under conditions of high fish predation. In contrast, the smaller-bodied *D. mendotae* always utilized the epilimnion (Leibold and Tessier 1991). Therefore, the phototactic behavior of the *Daphnia* in the experimental tubes may be explained by the lack of fish chemical cues rather than the presence of *B. longimanus* chemical cues.

In contrast to our lab results, the natural daytime distribution of *Daphnia* in Lake Mendota appears to follow the trend predicted by high levels of fish predation (i.e., larger species restricted to the hypolimnion and the smaller species able to utilize the epilimnion; Fig. 1). Since 1985, Lake Mendota has been stocked every 1–2 years with young walleye (*Sander vitreus*) and northern pike (*Esox lucius*) and there are approximately 20 fish species in the lake (Wisconsin Department of Natural Resources 2018; North Temperate Lakes Long-Term Ecological Research, NSF 2001a). All of the fish are zooplanktivores at either one life stage (as larval or young fish) or throughout their entire life (North Temperate Lakes Long-Term Ecological Research, NSF 2001a). The dominant zooplanktivorous fish are yellow perch (*Perca flavescens*) and white bass (*Morone chrysops*), while prior to 1987, cisco (*Coregonus artedii*) dominated (North Temperate Lakes Long-Term Ecological Research, NSF 2001a). These data support the idea that Lake Mendota is a high predation environment for *Daphnia* (consistent with the distribution of *Daphnia* in the lake). However, counter to our original prediction, in the phototactic assays, the largest species (*D. pulicaria*) was positively phototactic and the smallest species (*D. ambigua*) was negatively phototactic regardless of the presence of *B. longimanus* chemical cue. Therefore, our results suggest that chemical cues from the visual predator *B. longimanus* may not be the primary factor influencing the phototactic behavior and/or vertical distribution of *Daphnia* in Lake Mendota. Rather, other visual predators (i.e., fish) may be playing a more substantial role in determining the vertical distribution and migration of *Daphnia*, as has been shown extensively in other systems (Lampert 2011).

Clonal differences

Our study was unable to detect a significant within-species clonal effect, and therefore “clone” as a factor was removed from our analyses. Despite intraspecific (i.e., clonal)

differences being negligible, there does appear to be some suggestion of differences in the reaction norms within species (i.e., *D. pulicaria* and *D. ambigua*; Fig. 2). For example, two post-invasion *D. ambigua* clones (DA2-post and DA3-post) showed the predicted response pattern (i.e., going from more positive phototaxis in the no cue treatment to more negative phototaxis in the cue treatment), while the third post-invasion *D. ambigua* clone (DA1-post) showed the opposite response (i.e., going from more negative phototaxis in the no cue treatment to more positive phototaxis in the cue treatment). These patterns suggest some genetic variability in response to the chemical cue. When the post-invasion *D. ambigua* clone data were analyzed separately, however, it was found that treatment (i.e., cue versus no cue), clone, and the interaction of treatment and clone did not have a significant effect on average phototactic index (treatment t value = 1.05, $P = 0.31$; clone 1–2 t value = 1.40, $P = 0.18$; clone 1–3 t value = 1.26, $P = 0.23$; treatment and clone 1–2 interaction t value = -1.74, $P = 0.10$; treatment and clone 1–3 interaction t value = -1.66, $P = 0.12$). Clone-specific differences in phototactic and/or vertical migration behaviors are common (Weider 1984; De Meester 1993), and therefore it is not surprising that the clones within a species displayed variation (albeit nonsignificant) in their phototactic behavior. As previously noted, the lack of notable intraspecific differences in our study may be related to the limited number of clones tested and limited number of trials conducted.

Impact of *Bythotrephes* on Lake Mendota *Daphnia* community

By assaying “resurrected” *Daphnia* clones from resting eggs for an ecologically relevant trait such as phototactic behavior, we have shown that the level of predation by *B. longimanus* on *Daphnia* in eutrophic Lake Mendota has not caused the *Daphnia* community to alter their phototactic behavior. However, different life stages may exhibit different phototactic behaviors, as has been previously demonstrated (e.g., Dumont et al. 1973); there can be ontogenetic shifts in day depth/diel vertical migration behavior. Adults tend to reside deeper in the water column during the day and migrate over a larger amplitude than juveniles (Dumont et al. 1973; Huntley and Brooks 1982) and egg-bearing animals migrate over larger distances than animals without eggs (Vuorinen et al. 1983; Bollens and Frost 1991). We tested only pre-reproductive individuals in our study. So, future work should examine potential differences in phototaxis among different ontogenetic stages (i.e., adults, egg-carrying adults) of various clones.

As mentioned above, the most negatively phototactic and smallest species, *D. ambigua*, was not found in the stratified water column samples from Lake Mendota on the single

(August 2018) sampling date. In addition, this species has only been identified in a Lake Mendota LTER zooplankton sample once (21 June 1983) over the last ~40 years despite biweekly sampling of the pelagic zone (1976–2017; North Temperate Lakes Long-Term Ecological Research, NSF 2001c, d), and being abundant in the resting egg bank for the last ~30 years (E. Kiehnau, personal observation). It is possible that *D. ambigua* exhibit horizontal migration, residing in the near-shore littoral zone of the lake during the day to avoid predation by fish and invertebrate predators (as routine monitoring/sampling is done at the deepest pelagic site in the lake). Previous studies have demonstrated that *Daphnia* move towards macrophytes in the littoral zone during the day to avoid fish and invertebrate predation (Davies 1985; Kvam and Kleiven 1995; Lauridsen and Buenk 1996; Burks et al. 2001, 2002; Michels et al. 2007). This is because macrophytes represent a refuge and the foraging efficiency of fish is lowered in such structurally complex microhabitats (Timms and Moss 1984; Lauridsen and Buenk 1996). However, this trend is more often observed in shallow lakes, where diel vertical migration is not possible. Additional field sampling and laboratory experiments are warranted to assess whether horizontal migration of species like *D. ambigua* is taking place in Lake Mendota.

The results from our experiments suggest that *Daphnia* from Lake Mendota do not respond to chemical cues from *B. longimanus* via inducible or constitutive changes in phototactic behavior. This lack of a response appears to be indicative of a naïve prey behavior, which matches with recent *Daphnia* community data. In recent years, temperature-dependent *B. longimanus* population booms have led to total collapse of the Lake Mendota *Daphnia* community (with the longest crash to date lasting 250 days; Walsh et al. 2016b). These collapses have dramatic ecosystem-wide effects such as delay of the spring clearwater phase (Walsh et al. 2016b).

Although *Daphnia* from Lake Mendota are not responding to *B. longimanus* by altering their phototactic behavior, they may be responding in other ways. For example, it has been demonstrated that invasion of *B. longimanus* in Lake Mendota is associated with rapid increase in body size of *Daphnia* (in comparison to *Daphnia* from a non-invaded lake) and that these changes have a genetic component (Gillis and Walsh 2017). Future works should focus on investigating whether there have been inducible or constitutive changes in other anti-predator defenses (i.e., morphology, life histories).

Conclusions

Predation by *B. longimanus* on *Daphnia* has the potential to disrupt lake ecosystem functioning because when *Daphnia* are removed from these systems, there is less food for fish

and less of a constraint on algae growth. These impacts are being observed. For example, in Lake Mendota (WI), predation of *B. longimanus* on *Daphnia* has led to a decrease in water clarity of nearly 1 m (when comparing pre-*Bythotrephes* 1995–2007 and post-*Bythotrephes* 2009–2014 times), which represents an economic loss (i.e., water quality, recreational use) valued at \$140 million per year (Walsh et al. 2016a). Thus, the Lake Mendota system illustrates how non-native species can impact the ecological functioning and evolutionary trajectories of native species by altering trophic interactions, along with other important traits (e.g., anti-predator defenses, changing habitat and resource use). The ability of native species to respond evolutionarily to an invasive non-native species is dependent on the genetic structure and variability of native populations, the strength of the impact of the invader, and the invasion and evolutionary history of the species (Strauss et al. 2006). The work presented in this study highlights the potential utility of resurrection ecology in studying how native prey species respond to the invasion of a non-native predator.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal rights statement All applicable institutional and national guidelines for the care and use of animals were followed.

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