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RESPONSE OF NATIVE PREY SPECIES TO AN EXOTIC INVASIVE PREDATOR: ECOLOGY AND EVOLUTION OF INDUCIBLE DEFENSES

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RESPONSE OF NATIVE PREY SPECIES TO AN EXOTIC INVASIVE PREDATOR: ECOLOGY AND EVOLUTION OF INDUCIBLE DEFENSES

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Abstract

One of the most common and devastating human impacts on the world's ecosystems is the introduction of non-native invasive species which can negatively impact agriculture, industry, human health, and the natural environment. The invasion of a non-native predator can have particularly damaging effects on native prey species. These impacts are generally attributed to a failure by the prey to recognize a novel enemy and respond appropriately due to lack of experience. However, the response of native prey species to non-native predators is not well understood. Knowledge of this relationship is important for informing policy on non-native species control and management strategies.

In my dissertation, I evaluated the response of three native North American zooplankton prey species (*Daphnia pulicaria*, *Daphnia mendotae*, and *Daphnia ambigua*) to a non-native zooplankton predator (the spiny water flea a.k.a. *Bythotrephes*) in Lake Mendota (WI). *Daphnia*, small invertebrate animals, are key members of lake ecosystems because they are primary algae grazers and serve as a major food source for commercially and recreationally important fish species. *Daphnia* are the preferred prey of *Bythotrephes*, a non-native invertebrate predator from Northern Eurasia. Predation of *Bythotrephes* on *Daphnia* species has the potential to disrupt the functioning of many lakes because when *Daphnia* are removed, there is less food for fish and less of a constraint on algae growth. For example, since the invasion of Lake Mendota, predation of *Bythotrephes* on *Daphnia* has led to a decrease in water clarity of nearly 1 meter, which represents an economic loss (i.e., water quality, recreational use) valued at $140 million per year. The goal of my dissertation is to better understand how native *Daphnia* species are responding to the invasion of spiny water fleas so that the impacts of current and future invasions can be better predicted, helping us to protect the health and stability of these ecosystems.

To explore how *Daphnia* are responding to this invasive predator, I isolated and hatched dormant (“resting”) *Daphnia* eggs (termed ephippia) that have been preserved in lake sediments. This innovative technique has allowed me to “time-travel” and compare the responses of *Daphnia* from before and after the spiny water flea invasion. I used this method to evaluate a variety of anti-predator defenses (Chapters 1-3) and then assessed how the development of these defenses impacted the population dynamics of *Daphnia* species at different levels of food availability (Chapter 4). I evaluated both pre-encounter defenses such as depth selection (phototactic) behavior (Chapter 1) and escape ability (Chapter 2), and post-encounter defenses such as changes in morphology (Chapter 3). My research aimed to fill knowledge gaps within the field of invasion biology by tracking the response of native prey species to a non-native predator through time.

The goal of my first chapter was to see if *Daphnia* from Lake Mendota alter their depth selection behavior when in the presence of *Bythotrephes* chemical cues. It has been noted that the vertical distribution of *Daphnia* in invaded lakes is changing. Specifically, high densities of *Bythotrephes* are correlated with deeper vertical distributions of *Daphnia*. *Bythotrephes* are visual predators that require light to forage, thus, *Daphnia* may be selecting deeper depths when *Bythotrephes* are present to avoid predation. I performed experiments assessing predator cue-induced depth selection behavior of *Daphnia* from both pre- and post- invasion time periods. Results from these experiments suggested that *Daphnia* in Lake Mendota were not altering their depth selection behavior as a strategy to avoid predation by *Bythotrephes*. This lack of a response appears to indicate a naïve prey behavior, which could explain the reduced *Daphnia* populations and corresponding decline in water clarity (due to increased algae growth) that has been documented in recent years.

For my second chapter, I conducted a series of laboratory experiments to assess the escape ability of pre- and post- invasion *Daphnia* in the presence and absence of *Bythotrephes* and fish chemical cues. It has been observed that those species, whose populations have been heavily impacted by the *Bythotrephes* invasion have slower swimming speeds, while those that have remained relatively unchanged have faster swimming speeds. Escape ability differed across *Daphnia* species and post-invasion *D. pulicaria* had overall enhanced escape ability in comparison to pre-invasion *D. pulicaria*, which may help them avoid attack by *Bythotrephes*. Differences in the escape ability of Lake Mendota *Daphnia* may have contributed to differences in vulnerability, which in turn, may have influenced the community changes observed after the establishment of *Bythotrephes*

The third chapter of my dissertation compared the morphology of *Daphnia* from pre- and post-invasion time periods in the presence and absence of *Bythotrephes* chemical cues. Chemical cues from predators can induce morphological changes in *Daphnia*, from the development of elongated heads and tail spines to enhanced or reduced eye diameter. These induced morphological changes are defensive agents, which make capture or ingestion by predators more difficult. By measuring previously unassessed traits, I found that native *Daphnia* are responding to *Bythotrephes* with species-specific morphological changes. Native *Daphnia* populations have tracked the introduction of the non-native predator *Bythotrephes* via a combination of inducible and constitutive morphological defenses.

For my final chapter (Chapter 4), I investigated how the population dynamics of two *Daphnia* species (*D. ambigua* and *D. pulicaria*) native to Lake Mendota are affected by the varying food availability (high versus low food availability) and the presence and absence of *Bythotrephes* chemical cues. Some *Daphnia* species develop antipredator defenses such as

changes in behavior, morphology, and life-histories in the presence of *Bythotrephes* chemical cues. Food availability impacts growth, reproduction, and development of antipredator defenses. Food availability, *Bythotrephes* predation risk, and *Daphnia* species identity all altered trends in *Daphnia* and ephippial densities, whereas *Daphnia* biomass was primarily driven by food availability and *Daphnia* species identity. My results from Chapter 4, highlight the importance of considering the indirect impacts that an invasion may have on the population dynamics of native prey species, as this may play a significant role in impacting ecosystem processes.

Chapter one—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 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 nestmates (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 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 km^2 lake in southeastern Wisconsin, USA (near the southern edge of *B. longimanus*' invasive range; Fig. S1). 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. *B. 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°, 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 ^{210}Pb 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 μmol 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 µm 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 stetting (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 µm 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 × 2 treatments × 4 replicates + 2 clones × 2 treatments × 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 S1). The response variable, average phototactic index, was checked for normality (shapiro.test function, Table S2) 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 S3–S8). 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 S3 and S6). 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 was determined to be from ~ 0 to 10 m and the hypolimnion was determined to be from ~ 12 to 24 m (Fig. S2). The hypolimnion (as is typical) was associated both with lower temperatures and lower concentrations of dissolved oxygen (Fig. S2). 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 S3; 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 S3; 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, $P = 2.8\text{E} - 07$; Table S3; Fig. S3). *Daphnia pulicaria* clones exhibited positive phototaxis, *D. mendotae* clones exhibited slight negative phototaxis, and *D. ambigua* clones exhibited negative phototaxis (Fig. S3). 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. ambigua* clones tested (Fig. 2). There were no significant interaction effects among treatment, history of coexistence, and species (Table S3).

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. S3). 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 zooplanktivourous fish are yellow perch (*Perca flavescens*) and white bass (*Morone chrysops*), while prior to 1987, cisco (*Coregonus artedi*) 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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Data availability statement

The data associated with this study is available from the corresponding author upon request.

References

Bates D, Kliegl R, Vasishth S, Baayen H (2015) Parsimonious mixed models. arXiv:1506.04967 **(stat.ME)**

Beranek A (2012) An assessment of the long-term phenology and impact of *Bythotrephes longimanus* in Island Lake Reservoir, Minnesota, using sediment records. Master thesis, Department of Integrated Biosciences, University of Minnesota, Minneapolis, Minnesota, USA. http://hdl.handle.net/11299/121007

Black AR, Dodson SI (2003) Ethanol: a better preservation technique for *Daphnia*. Limnol Oceanogr Methods 1:45–50. https://doi.org/10.4319/lom.2003.1.45

Bollens SM, Frost BW (1991) Ovigerity, selective predation, and variable diel vertical migration in *Euchaeta elongate* (Copepoda: calanoida). Oecologia 87:155–161. https://doi.org/10.1007/BF00325252

Boudreau SA, Yan ND (2003) The differing crustacean zooplankton communities of Canadian Shield lakes with and without the nonindigenous zooplanktivore *Bythotrephes longimanus*. Can J Fish Aquatic Sci 60:1307–1313. https://doi.org/10.1139/f03-111

Bourdeau PE, Pangle KL, Peacor SD (2011) The invasive predator *Bythotrephes* induces changes in the vertical distribution of native copepods in Lake Michigan. Biol Invasions 13:2533–2545. https://doi.org/10.1007/s10530-011-0073-0

Bourdeau PE, Pangle KL, Reed EM, Peacor SD (2013) Finely tuned response of native prey to an invasive predator in a freshwater system. Ecology 94:1449–1455. https://doi.org/10.1890/12-2116.1

Bourdeau PE, Pangle KL, Peacor SD (2015) Factors affecting the vertical distribution of the zooplankton assemblage in Lake Michigan: the role of the invasive predator *Bythotrephes longimanus*. J Great Lakes Res 41:115–124. https://doi.org/10.1016/j.jglr.2015.09.017

Brodin T, Johansson F (2002) Effects of predator-induced thinning and activity changes on life history in a damselfly. Oecologia 132:316–322. https://doi.org/10.1007/s00442-002-0938-0

Bungartz B, Branstrator DK (2003) Morphological changes in *Daphnia mendotae* in the chemical presence of *Bythotrephes longimanus*. Arch Hydrobiol 158: 97–108. https://doi.org/10.1127/0003-9136/2003/0158-0097

Burks RL, Jeppesen E, Lodge DM (2001) Littoral zone structure as *Daphnia* refugia against fish predators. Limnol Oceanogr 46:230–237. https://doi.org/10.4319/lo.2001.46.2.0230

Burks RL, Lodge DM, Jeppesen E (2002) Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. Freshw Biol 47:343–365. https://doi.org/10.1046/j.1365-2427.2002.00824.x

Cox JG, Lima SL (2006) Naiveté and an aquatic-terrestrial dichotomy in the effects of introduced predators. Trends Ecol Evol 21:674–680. https://doi.org/10.1016/j.tree.2006.07.011

Davies J (1985) Evidence for a diurnal horizontal migration in *Daphnia hyalina lacustris* Sars. Hydrobiologia 120:103–105. https://doi.org/10.1007/BF00032130

Dawidowics P, Loose CJ (1992) Metabolic costs during predator-induced diel vertical migration of *Daphnia*. Limnol Oceanogr 37:1589–1595. https://doi.org/10.4319/lo.1992.37.8.1589

De Meester L (1989) An estimation of the heritability of phototaxis in *Daphnia magna* Straus. Oecologia 78:142–144. https://doi.org/10.1007/BF00377210

De Meester L (1991) An analysis of the phototactic behavior of *Daphnia magna* clones and their sexual descendants. Hydrobiologia 225:217–227. https://doi.org/10.1007/978-94-017-0918-7_21

De Meester L (1993) Genotype, fish-mediated chemical, and phototactic behavior in *Daphnia magna*. Ecology 74:1467–1474. https://doi.org/10.2307/1940075

De Meester L (1996) Evolutionary potential and local genetic differentiation in a phenotypically plastic trait of a cyclical parthenogen, *Daphnia magna*. Evolution 50:1293–1298. https://doi.org/10.1111/j.1558-5646.1996.tb02369.x

Decaestecker E, De Meester L, Ebert D (2002) In deep trouble: habitat selection constrained by multiples enemies in zooplankton. Proc Natl Acad Sci USA 99:5481–5485. https://doi.org/10.1073/pnas.082543099

Dodson S (1988) The ecological role of chemical stimuli for the zooplankton: predator-avoidance behavior in *Daphnia*. Limnol Oceanogr 33:1431–1439. https://doi.org/10.4319/lo.1988.33.6part2.1431

Dumont HJ, Miron I, D'Allasta V, Decraemer W, Claus C, Somers D (1973) Limnological aspects of some Moroccan Atlas lakes, with reference to some physical and chemical variables, the nature and distribution of the phyto- and zoo-plankton, including a note on possibilities for the development of an inland fishery. Int Rev Hydrobiol 58:33–60. https://doi.org/10.1002/iroh.19730580104

Fontaine JJ, Martin TE (2006) Parent birds assess nest predation risk and adjust their reproductive strategies. Ecol Lett 9:428–434. https://doi.org/10.1111/j.1461-0248.2006.00892.x

Frisch D, Morton PK, Chowdhury PR, Culver BW, Colbourne JK, Weider LJ, Jeyasingh PD (2014) A millennialscale chronicle of evolutionary responses to cultural eutrophication in *Daphnia*. Ecol Lett 17:360–368. https://doi.org/10.1111/ele.12237

Fuchs B, Zimmermann B, Wabakken P, Bornstein S, Månsson J, Evans AL, Liberg O, Sand H, Kindberg J, Ågren EO, Arnemo JM (2016) Sarcoptic mange in the Scandinavian wolf *Canis lupus* population. BMC Vet Res 12:156–167. https://doi.org/10.1186/s12917-016-0780-y

Gillis MK, Walsh MR (2017) Rapid evolution mitigates the ecological consequences of an invasive species (*Bythotrephes longimanus*) in lakes in Wisconsin. Proc Biol Sci 284:20170814. https://doi.org/10.1098/rspb.2017.0814

Hembre LK, Peterson LA (2013) Evolution of predator avoidance in a *Daphnia* population: evidence from the egg bank. Hydrobiologia 700:245–255. https://doi.org/10.1007/s10750-012-1234-6

Huntley M, Brooks ER (1982) Effects of age and food availability on diel vertical migration of *Calanus pacificus*. Mar Biol 71:23–31. https://doi.org/10.1007/BF00396989

Jokela A, Arnott SE, Beisner BE (2013) Influence of light on the foraging impact of an introduced predatory cladoceran, *Bythotrephes longimanus*. Freshw Biol 58:1946–1957. https://doi.org/10.1111/fwb.12182

Kerfoot WC, Weider LJ (2004) Experimental paleoecology (resurrection ecology): chasing Van Valen’s Red Queen hypothesis. Limnol Oceanogr 49:1300–1316. https://doi.org/10.4319/lo.2004.49.4_part_2.1300

Kerfoot WC, Weider LJ (1999) A new approach to historical reconstruction: combining descriptive and experimental paleolimnology. Limnol Oceanogr 44:1232–1247. https://doi.org/10.4319/lo.1999.44.5.1232

Kilham SS, Kreeger DA, Lynn SG, Goulden CE, Herrera L (1998) COMBO: a defined freshwater culture medium for algae and zooplankton. Hydrobiologia 377:147–159. https://doi.org/10.1023/A:1003231628456

Kim N, Yan ND (2010) Methods for rearing the invasive zooplankter *Bythotrephes* in the laboratory. Limnol Oceanogr Methods 8:552–561. https://doi.org/10.4319/lom.2010.8.552

Kvam OV, Kleiven OT (1995) Diel horizontal migration and swarm formation in *Daphnia* in response to *Chaoborus*. Hydrobiologia 307:177–184. https://doi.org/10.1007/978-94-011-0021-2_20

Laforsch C, Beccara L, Tollrian R (2006) Inducible defenses: the relevance of chemical alarm cues in *Daphnia*. Limnol Oceanogr 51:1466–1472. https://doi.org/10.4319/lo.2006.51.3.1466

Lampert W (1989) The adaptive significance of diel vertical migration of zooplankton. Funct Ecol 3:21–27. https://doi.org/10.2307/2389671

Lampert W (2011) *Daphnia*: development of a model organism in ecology and evolution, excellence in ecology: book 21. In: Kinne O (eds) International ecology institute, Germany

Lauridsen TL, Buenk I (1996) Diel changes in the horizontal distribution of zooplankton in the littoral zone of two shallow eutrophic lakes. Arch Hydrobiol 137:161–176. https://doi.org/10.1007/s10750-007-9086-1

Leibold MA (1991) Trophic interactions and habitat segregation between competing *Daphnia* species. Oecologia 86:510–520. https://doi.org/10.1007/BF00318317

Leibold M, Tessier AJ (1991) Contrasting patterns of body size for *Daphnia* species that segregate by habitat. Oecologia 869:342–348. https://doi.org/10.1007/BF00317599

Loose CJ, Dawidowics P (1994) Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. Ecology 75:2255–2263. https://doi.org/10.2307/1940881

Michels H, Amsinck SL, Jeppesen E, De Meester L (2007) Interclonal variation in diel horizontal migration behavior of the water flea *Daphnia magna*—searching for a signature of adaptive evolution. Hydrobiologia 594:117–129. https://doi.org/10.1007/s10750-007-9086-1

Muirhead J, Sprules WG (2003) Reaction distance of *Bythotrephes longimanus,* encounter rate and index of prey risk for Harp Lake, Ontario. Freshw Biol 48:135–146. https://doi.org/10.1046/j.1365-2427.2003.00986.x

North Temperate Lakes Long-Term Ecological Research, NSF (2001a) North temperate lakes LTER: fish abundance 1981-current [Database]. https://lter.limnology.wisc.edu/data

North Temperate Lakes Long-Term Ecological Research, NSF (2001b) North temperate lakes LTER: physical limnology of primary study lakes 1981-current [Database]. https://lter.limnology.wisc.edu/data

North Temperate Lakes Long-Term Ecological Research, NSF (2001c) North temperate lakes LTER: zooplankton—Madison lakes area 1976-1994 1976–1994 [Database]. https://lter.limnology.wisc.edu/data

North Temperate Lakes Long-Term Ecological Research, NSF (2001d) North temperate lakes LTER: zooplankton—Madison lakes area 1997-current [Database]. https://lter.limnology.wisc.edu/data

Pangle KL, Peacor SD (2006) Non-lethal effect of the invasive predator *Bythotrephes longimanus* on *Daphnia mendotae*. Freshw Biol 51:1070–1078. https://doi.org/10.1111/j.1365-2427.2006.01555.x

Pangle KL, Peacor SD (2009) Light-dependent predation by the invertebrate planktivore *Bythotrephes longimanus*. Can J Fish Aquatic Sci 66:1748–1757. https://doi.org/10.18901139/06F09-0768 133

Pangle KL, Peacor SD, Johannsson OE (2007) Large nonlethal effects of an invasive invertebrate predator on zooplankton population growth rate. Ecology 88:402–412. https://doi.org/10.11391890/F0906-133 0768

Pasch B, Bolker BM, Phelps SM (2013) Interspecific dominance via vocal interactions mediates altitudinal zonation in neotropical singing mice. Am Nat 182:E161–E173. https://doi.org/10.1086/673263

Pijanowska J (1997) Alarm signals in *Daphnia*? Oecologia 112:12–16. https://doi.org/10.1007/s004420050277

Pijanowska J, Kowalczewski A (1997a) Cues from injured *Daphnia* and from cyclopoids feeding on *Daphnia* can modify life histories of conspecifics. Hydrobiologia 350:99–103. https://doi.org/10.1023/A:1003079614043

Pijanowska J, Kowalczewski A (1997b) Predators can induce swarming behavior and locomotory responses in *Daphnia*. Freshw Biol 37:649–656. https://doi.org/10.1046/j.1365-2427.1997.00192.x

Pijanowska J, Dawidowicz P, Weider LJ (2006) Predator-induced escape response in *Daphnia*. Arch Hydrobiol 167:77–87. https://doi.org/10.1127/0003-9136/2006/0167-0077

R Core Team (2019) R: a language and environment for statistical computing R foundation for statistical computing, Vienna. http://www.R-project.org

Rabus M, Waterkeyn A, Van Pottelbergh N, Brendonck L, Laforsch C (2012) Interclonal variation, effectiveness and long-term implications of *Triops*-induced morphological defences in *Daphnia magna* Strauss. J Plankton Res 34:152–160. https://doi.org/10.1093/plankt/fbr092

Ringelberg J (1964) The positively phototactic reaction of *Daphnia magna* Straus—a contribution to the understanding of diurnal migration. J Sea Res 2:319–406. https://doi.org/10.1016/0077-7579(64)90001-8

Ringelberg J (1991) Enhancement of the phototactic behavior in *Daphnia* by a chemical mediated by juvenile perch (*Perca fluviatilis*). J Plankton Res 12:17–25. https://doi.org/10.1093/plankt/13.1.17

Runyon JB, Mescher MC, De Moraes CM (2006) Volatile chemical cues guide host location and host selection by parasitic plants. Science 313:1964–1967. https://doi.org/10.1126/science.1131371

Schulz KL, Yurista PM (1999) Implications of an invertebrate predator's (*Bythotrephes cederstroemi*) atypical effects on a pelagic zooplankton community. Hydrobiologia 380:179–193. https://doi.org/10.1023/A:1003484813672

Stoks R, Govaert L, Pauwels K, Jansen B, De Meester L (2015) Resurrecting complexity: the interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea *Daphnia magna*. Ecol Lett 19:180–190. https://doi.org/10.1111/ele.12551

Strauss SY, Lau JA, Carroll SP (2006) Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? Ecol Lett 9:357–374. https://doi.org/10.1111/j.1461-0248.2005.00874.x

Timms RM, Moss B (1984) Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing in the presence of zooplanktivorous fish in a freshwater wetland ecosystem. Limnol Oceanogr 29:472–486. https://doi.org/10.4319/lo.1984.29.3.0472

Tollrian R (1995) Predator-induced morphological defenses: costs, life history shifts, and maternal effects in *Daphnia pulex*. Ecology 76:1691–1705. https://doi.org/10.2307/1940703

Tollrian R, Leese F (2010) Ecological genomics: steps towards unraveling genetic basis of inducible defenses in *Daphnia*. BMC Biol 8:51. https://doi.org/10.1186/1741-7007-8-51

Torres CW, Brandt M, Tsutsui ND (2007) The role of cuticular hydrocarbons as chemical cues for nestmate recognition in the invasive Argentine ant (*Linepithema humile*). Insectes Soc 54:336–373. https://doi.org/10.1007/s00040-007-0954-5

Trussell GC, Nicklin MO (2002) Cue sensitivity, inducible defense, and trade-offs in a marine snail. Ecology 83: 1635–1647. https://doi.org/10.1890/00129658(2002)083%5b1635:CSIDAT%5d2.0.CO;2

von Frisch K (1942) Über einen Schreckstoff der Fischhaut und seine biologische Bedeutung. Z Vgl Physiol 291:46–145. https://doi.org/10.1007/BF00304445

Vuorinen I, Rajasilta M, Salo J (1983) Selective predation and habitat shift in a copepod species—support for the predation hypothesis. Oecologia 59:62–64. https://doi.org/10.1007/BF00388073

Walsh JR, Carpenter S, Vander Zanden J (2016a) Invasive species triggers a massive loss of ecosystem services through a trophic cascade. Proc Natl Acad Sci USA 113:4081–4085. https://doi.org/10.1073/pnas.1600366113

Walsh JR, Munoz SE, Vander Zanden MJ (2016b) Outbreak of an undetected invasive species triggered by a climate anomaly. Ecosphere 7:e01628. https://doi.org/10.1002/ecs2.1628

Weider LJ (1984) Spatial heterogeneity of *Daphnia* genotypes: vertical migration and habitat partitioning. Limnol Oceanogr 29:225–235. https://doi.org/10.4319/lo.1984.29.2.0225

Wisconsin Department of Natural Resources (2018) Bureau of fisheries management. Fish stocking summary [Database]. https://dnr.wi.gov/topic/fishing/stocking/

Yan ND, Leung B, Lewis MA, Peacor SD (2011) The spread, establishment and impacts of the spiny water flea, *Bythotrephes longimanus*, in temperate North America: a synopsis of the special issue. Biol Invasions 13:2423–2432. https://doi.org/10.1007/s10530-011-0069-9

Zaret TM, Suffern JS (1976) Vertical migration of zooplankton as a predator avoidance mechanism. Limnol Oceanogr 21:804–813. https://doi.org/10.4319/lo.1976.21.6.0804

Figure captions

Figure 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.

Figure 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.

Figure 1.

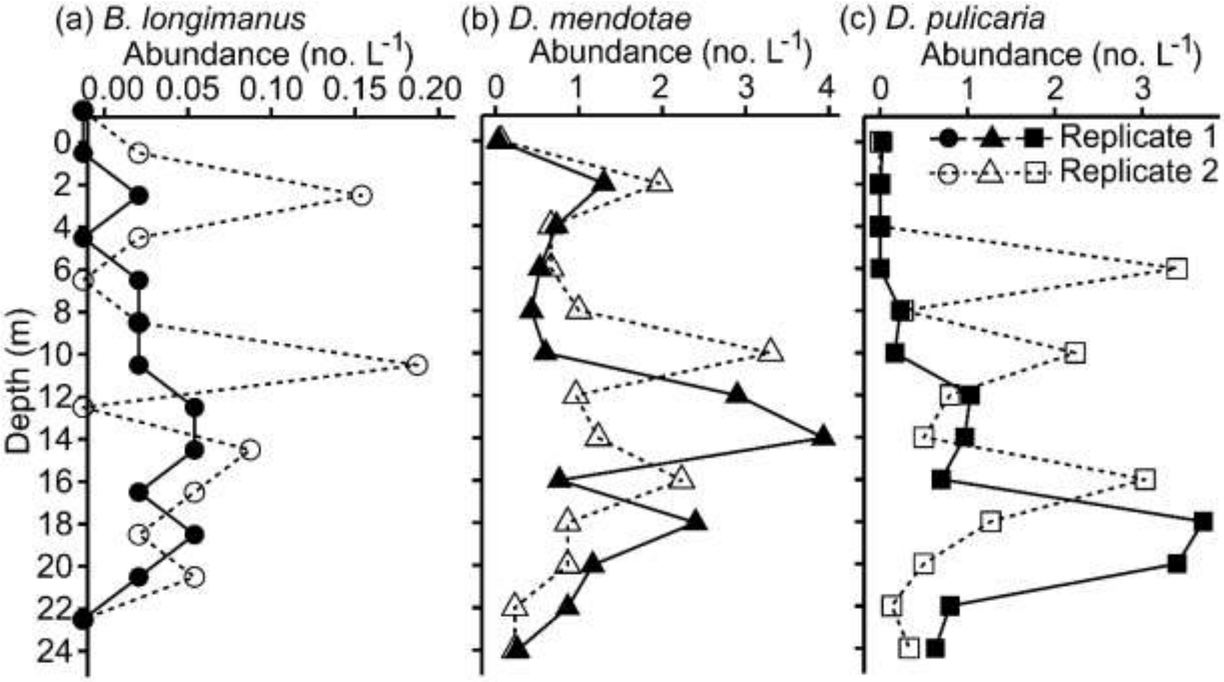
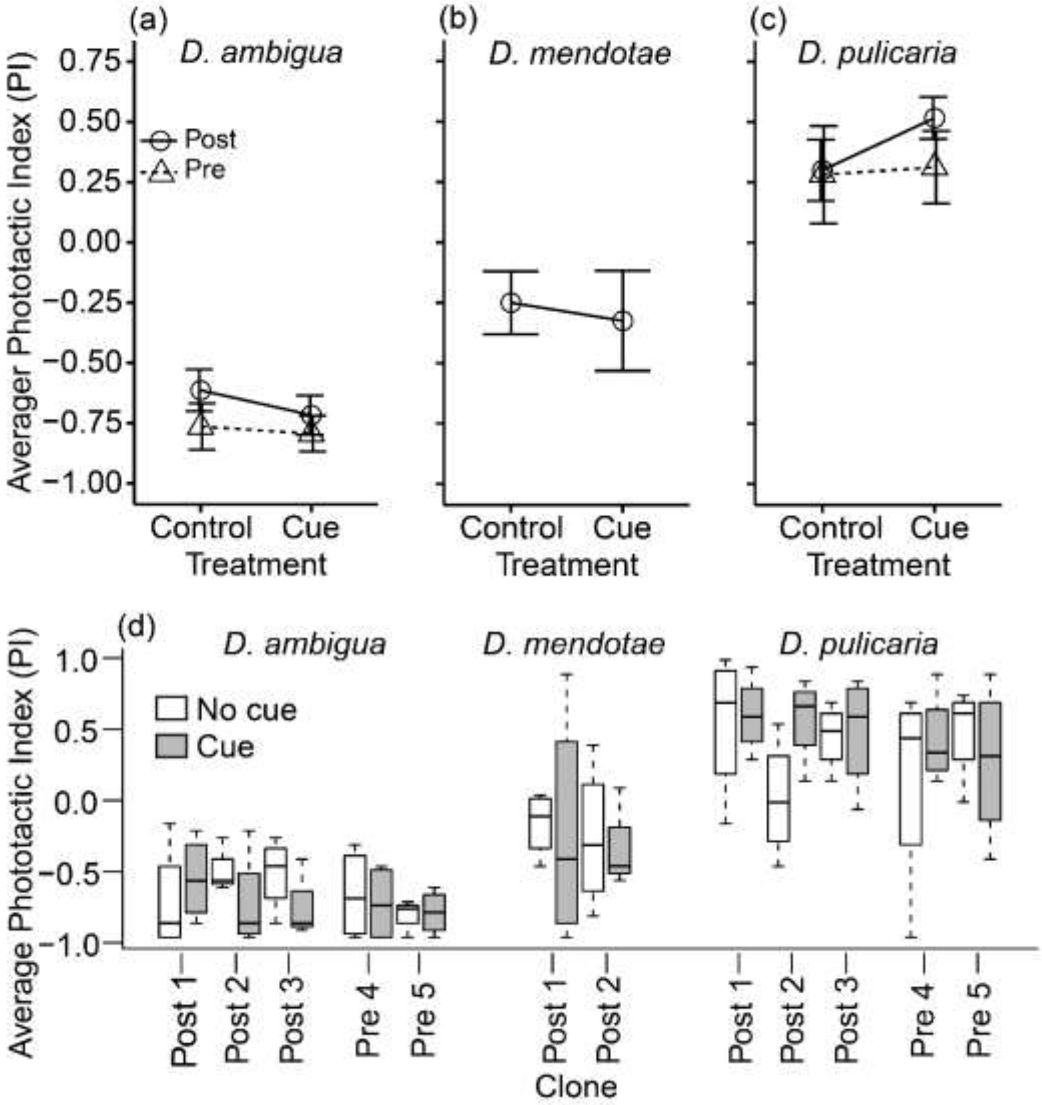

(a) *B. longimanus*
Abundance (no. L^{-1})
0.00 0.05 0.10 0.15 0.20
(b) *D. mendotae*
Abundance (no. L^{-1})
0 1 2 3 4
(c) *D. pulicaria*
Abundance (no. L^{-1})
0 1 2 3
Depth (m)
0 2 4 6 8 10 12 14 16 18 20 22 24
Replicate 1
Replicate 2

Figure 2.


(a)
D. ambigua
(b)
D. mendotae
(c)
D. pulicaria
Averager Phototactic Index (PI)
0.75
0.50
0.25
0.00
−0.25
−0.50
−0.75
−1.00
Post
Pre
Control
Cue
Treatment
(d)
D. ambigua
D. mendotae
D. pulicaria
Average Phototactic Index (PI)
1.0
0.5
0.0
−0.5
−1.0
No cue
Cue
Post 1
Post 2
Post 3
Pre 4
Pre 5
Post 1
Post 2
Post 1
Post 2
Post 3
Pre 4
Pre 5
Clone

Chapter one: Appendix S1. Supporting Information.

Supplement S1. Methods

We performed model selection/comparison using Akaike's Information Criterion (AIC_c) via the dredge function in the MuMIn package (Tables S4 and S7, Burnham and Anderson 2003, Barton 2016). In addition, to determine which driver variables most influenced phototactic behavior, we calculated relative importance values using the importance function in the MuMIn package (Tables S5 and S8, Burnham and Anderson 2003, Barton 2016). We checked the residuals for indications of non-linearity, normality and heteroscedasticity during the model evaluation process, and the final model passed all the standard assumptions for linear regression.

Supplement S1. References

Barton K (2016) MuMIn: model selection and model averaging based on information criteria (AICc and alike). R package version 1.15.1.

Burnham KP, Anderson DR (2003) Model selection and multimodel inference: a practical information-theoretic approach. Springer Science and Business Media, New York.

Liebig J, Benson A, Larson J, Makled TH, Fusaro A (2019) *Bythotrephes longimanus*: U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL, https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=162, Revision Date: 6/4/2013

Supplement S1. Tables

Table S1: Results of Bartlett test of homogeneity of variance among groups. Bold numbers indicate p values below the alpha level of 0.05. Tests are run on both the full dataset (*D. ambigua*, *D. mendotae*, and *D. pulicaria* data) and reduced dataset (from which *D. mendotae* data were excluded) because separate models were constructed using both the reduced and full dataset.

	Full dataset			**Reduced dataset**		
	Bartlet's K^2	**df**	**p value**	**Bartlet's K^2**	**df**	**p value**
Treatment	0.565	1	0.452	0.195	1	0.658
Species	10.445	2	**0.005**	8.258	1	**0.004**
History of coexistence	0.395	1	0.530	0.056	1	0.814

Table S2: Results of Shapiro-Wilk test of normality. Bold numbers indicate p values below the alpha level of 0.05. Tests are run on both the full dataset (*D. ambigua*, *D. mendotae,* and *D. pulicaria* data) and reduced dataset (from which *D. mendotae* data were excluded) because separate models were constructed using both the reduced and full dataset.

	W	**p value**
Full dataset	0.921	**2.91E-05**
Reduced dataset	0.907	**3.07E-05**

Table S3: Summary statistics for the linear model (using the full dataset) assessing the impact of treatment (T), history of coexistence (H), and species identity (S) on the average phototactic index of *Daphnia*. Bolded values indicate p values below the alpha level of 0.05. Not Available (NAs) are due to the absence of pre-invasion *D. mendotae* data. Treatment indicates the presence or absence of *Bythotrephes longimanus* chemical cue in the experimental column. History of coexistence indicates whether the focal *Daphnia* are from pre- or post-*B. longimanus* invasion time periods. Species identity indicates the species of the focal *Daphnia* (i.e., DP-*D. pulicaria*, DM-*D. mendotae*, or DA-*D. ambigua*). Phototactic index is a measure of movement toward or way from the light source. Sixty-one percent of the variance found in the response variable (average phototactic index) can be explained by the predictor variables. For the overall model, $F_{9,84} = 17.19$; P = 1.01E-15.

Coefficients	Estimate	Std. Error	t value	p value
Intercept	1.09837	0.02338	46.980	**< 2E-16**
T-Cue	-0.02544	0.03237	-0.786	0.434
H-Pre	-0.03670	0.03749	-0.979	0.330
S-DM	0.07942	0.03603	2.204	**0.030**
S-DP	0.18071	0.03237	5.583	**2.84E-07**
T-Cue: H-Pre	0.01874	0.05156	0.363	0.717
T-Cue: S-DM	0.00301	0.05051	0.060	0.953
T-Cue: S-DP	0.06341	0.04528	1.401	0.165
H-Pre: S-DM	NA	NA	NA	NA
H-Pre: S-DP	0.02825	0.05156	0.548	0.585
T-Cue: H-Pre: S-DM	NA	NA	NA	NA
T-Cue: H-Pre: S-DP	-0.04565	0.07186	-0.635	0.527

Table S4 Comparison of linear models assessing the impact of treatment (T), history of coexistence (H), and species identity (S) on the average phototactic index of *Daphnia*. Models used the full dataset. Treatment indicates the presence or absence of *Bythotrephes longimanus* chemical cue in the experimental column. History of coexistence indicates whether the focal *Daphnia* are from pre- or post-*B. longimanus* invasion time periods. Species identity indicates the species of the focal *Daphnia* (i.e., *D. pulicaria*, *D. mendotae*, or *D. ambigua*). Phototactic index is a measure of movement toward or away from the light source.

Model	**Model Variables**							**AIC_c**	**LogLiklihood**	**df**	**$\Delta\Delta\text{AIC}_c$**	**w_i**
	H	S	T	H:S	H:T	S:T	H:S:T					
Average Phototactic Index		X						-211.3	109.876	4	0.00	0.310
	X	X						-211.1	110.869	5	0.25	0.274
		X	X					-209.1	109.876	5	2.23	0.102
	X	X		X				-208.8	110.880	6	2.51	0.088
	X	X	X					-208.8	110.870	6	2.53	0.088
		X	X			X		-206.7	111.004	7	4.60	0.031
	X	X	X	X				-206.5	110.881	7	4.84	0.028
	X	X	X		X			-206.4	110.873	7	4.86	0.027
	X	X	X			X		-206.3	112.013	8	4.97	0.026
	X	X	X	X	X			-204.1	110.885	8	7.23	0.008
	X	X	X		X	X		-203.9	112.024	9	7.4	0.008
	X	X	X	X		X		-203.9	112.023	9	7.4	0.008
	X	X	X	X	X	X		-201.4	112.033	10	9.89	0.002
	X	X	X	X	X	X	X	-199.3	112.259	11	12.01	0.001
								-122.2	63.171	2	89.09	0.000
	X							-120.4	63.351	3	90.87	0.000
			X					-120.1	63.184	3	91.2	0.000
	X		X					-118.3	63.364	4	93.02	0.000
	X		X		X			-116.0	63.364	5	95.26	0.000

AIC statistics: *AICc* AIC statistic; *log likelihood*; *df* degrees of freedom; *ΔAICc* AICc minus top model AICc, *wi* model weight

Table S5 Relative importance values (RIVs) for driver variables of linear models (using the full dataset) assessing the impact of treatment (T), history of coexistence (H), and species identity (S) on the average phototactic index of *Daphnia*. RIVs are a summed and standardized indicator of predictor variable rank across all possible models. Treatment indicates the presence or absence of *Bythotrephes longimanus* chemical cue in the experimental column. History of coexistence indicates whether the focal *Daphnia* are from pre- or post-*B. longimanus* invasion time periods. Species identity indicates the species of the focal *Daphnia* (i.e., *D. pulicaria*, *D. mendotae*, or *D. ambigua*). Phototactic index is a measure of movement toward or away from the light source.

	S	**H**	**T**	**L:S**	**S:T**	**H:T**	**H:S:T**
Sum of weights	1	0.56	0.33	0.13	0.08	0.05	<0.01
N containing models	14	14	14	6	6	6	1

Table S6: Summary statistics for the linear model (using the reduced dataset) assessing the impact of treatment (T), history of coexistence (H), and species identity (S) on the average phototactic index of *Daphnia*. Bolded values indicate p values below the alpha level of 0.05. Treatment indicates the presence or absence of *Bythotrephes longimanus* chemical cue in the experimental column. History of coexistence indicates whether the focal *Daphnia* are from pre- or post-*B. longimanus* invasion time periods. Species identity indicates the species of the focal *Daphnia* (i.e., *D. pulicaria* or *D. ambigua*). Phototactic index is a measure of movement toward or away from the light source. Sixty-nine percent of the variance found in the response variable (average phototactic index) can be explained by the predictor variables. For the overall model, $F_{7,70} = 25.28$; the $P < 2.2E\text{-}16$.

Coefficients	**Estimate**	**Std. Error**	**t value**	**p value**
Intercept	1.16210	0.03808	30.521	**< 2E-16**
T-Cue	-0.04226	0.05271	-0.802	0.425
H-Pre	-0.06115	0.06106	-1.002	0.320
S-DP	0.31669	0.05271	6.008	**7.59E-08**
T-Cue: H-Pre	0.03090	0.08397	0.368	0.714
T-Cue: S-DP	0.11096	0.07373	1.505	0.137
H-Pre: S-DP	0.04859	0.08397	0.579	0.565
T-Cue: H-Pre: S-DP	-0.08251	0.11702	-0.705	0.483

Table S7 Comparison of linear models assessing the impact of treatment (T), history of coexistence (H), and species identity (S) on the average phototactic index of *Daphnia*. Models used the reduced dataset; *D. mendotae* data were excluded because no pre-invasion clones were included in the experiment. Treatment indicates the presence or absence of *Bythotrephes longimanus* chemical cue in the experimental column. History of coexistence indicates whether the focal *Daphnia* are from pre- or post-*B. longimanus* invasion time periods. Species identity indicates the species of the focal *Daphnia* (i.e., *D. pulicaria* or *D. ambigua*). Phototactic index is a measure of movement toward or away from the light source.

Model	**Model Variables**							**AIC$_c$**	**LogLiklihood**	**df**	**ΔΔAIC$_c$**	**w$_i$**
	H	S	T	H:S	H:T	S:T	H:S:T					
Average Phototactic Index		X						-98.6	52.478	3	0.00	0.251
	X	X						-98.6	53.556	4	0.07	0.243
		X	X					-96.5	52.539	4	2.10	0.088
	X	X	X					-96.4	53.622	5	2.22	0.083
	X	X		X				-96.3	53.563	5	2.34	0.078
		X	X			X		-96.2	53.540	5	2.38	0.076
	X	X	X			X		-96.1	54.639	6	2.54	0.071
	X	X	X		X			-94.1	53.642	6	4.53	0.026
	X	X	X	X				-94.1	53.630	6	4.55	0.026
	X	X	X		X	X		-93.7	54.662	7	4.91	0.022
	X	X	X	X		X		-93.7	54.646	7	4.94	0.021
	X	X	X	X	X			-91.7	53.649	7	6.93	0.008
	X	X	X	X	X	X		-91.2	54.668	8	7.38	0.006
	X	X	X	X	X	X	X	-89.2	54.944	9	9.39	0.002
								-7.4	5.779	2	91.23	0.000
	X							-5.8	6.070	3	92.81	0.000
			X					-5.2	5.779	3	93.40	0.000
	X		X					-3.6	6.070	4	95.04	0.000
	X		X		X			-1.3	6.082	5	97.30	0.000

AIC statistics: *AICc* AIC statistic; *log likelihood*; *df* degrees of freedom; *ΔAICc* AICc minus top model AICc, *wi* model weight

Table S8 Relative importance values (RIVs) for driver variables of linear models (using the reduced dataset) assessing the impact of treatment (T), history of coexistence (H), and species identity (S) on the average phototactic index of *Daphnia*. RIVs are a summed and standardized indicator of predictor variable rank across all possible models. Treatment indicates the presence or absence of *Bythotrephes longimanus* chemical cue in the experimental column. History of coexistence indicates whether the focal *Daphnia* are from pre- or post-*B. longimanus* invasion time periods. Species identity indicates the species of the focal *Daphnia* (i.e., *D. pulicaria* or *D. ambigua*). Phototactic index is a measure of movement toward or away from the light source.

	S	**H**	**T**	**H:S**	**S:T**	**H:T**	**H:S:T**
Sum of weights	1.00	0.59	0.43	0.20	0.14	0.06	<0.01
N containing models	14	14	14	6	6	6	1

Supplement S1. Figure captions

Figure S1: Invasion range of *Bythotrephes longimanus* in North America. Points indicate clustered specimen records (the larger the dot the more specimens collected in that area). The outset panel shows the Yahara chain of lakes: (a) Lake Mendota, (b) Lake Wingra, (c) Lake Monona, (d) Lake Waubesa, and (e) Lake Kegonsa. This map is a modification of a USGS nonindigenous aquatic species point map (Liebig et al. 2019).

Figure S2: Average **(a)** temperature and **(b)** dissolved oxygen of Lake Mendota water column. Data (including Secchi depth) are averages of samples taken on 6 August 2018 and 22 August 2018. Data were obtained from North Temperate Lakes Long-Term Ecological Research, NSF 2001b.

Figure S3: Phototactic index (PI) of the three *Daphnia* species. Boxplots show the third quartile, median, first quartile, and data outliers. Clones within species and treatments were pooled. 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.

Figure S1.

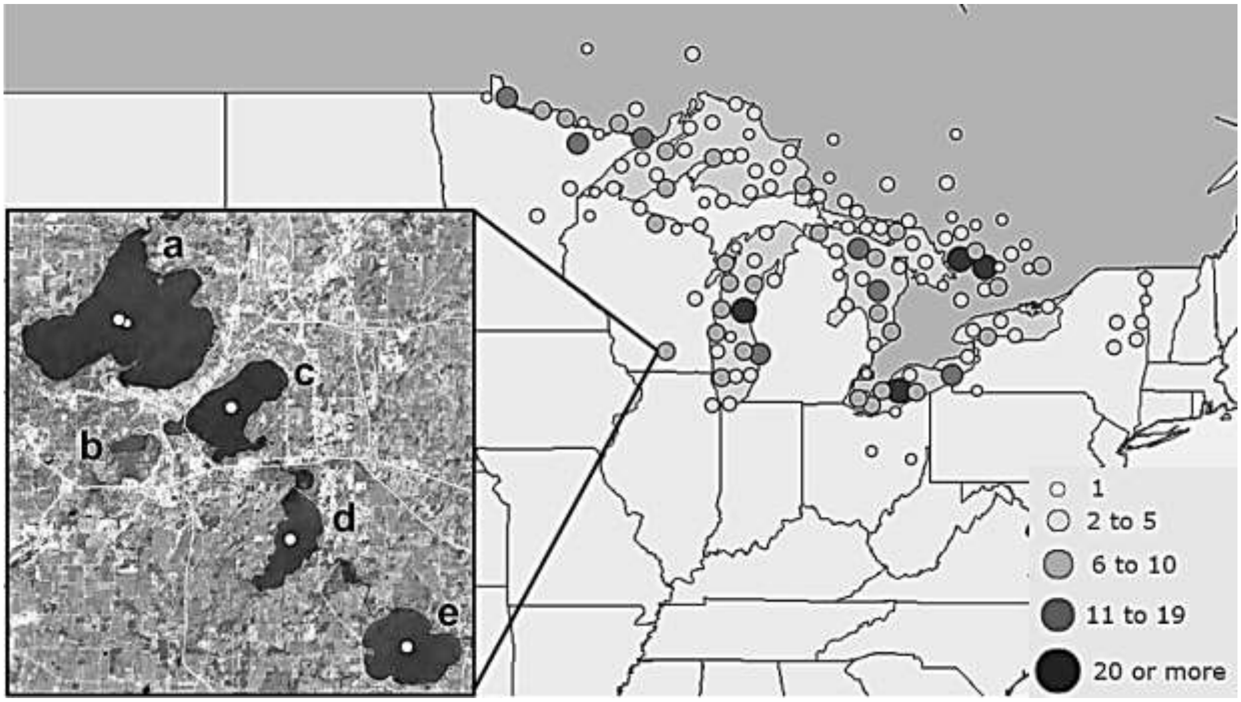


Figure S2.

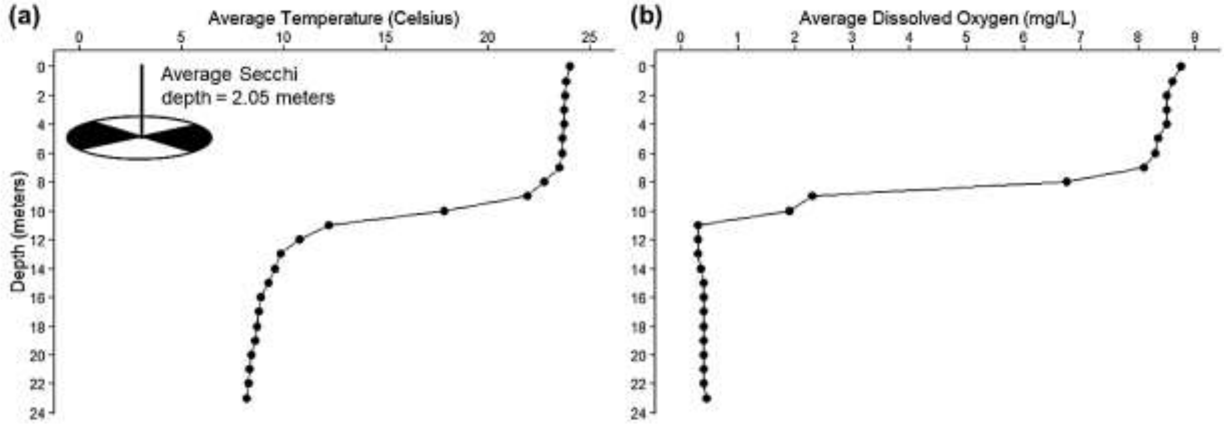
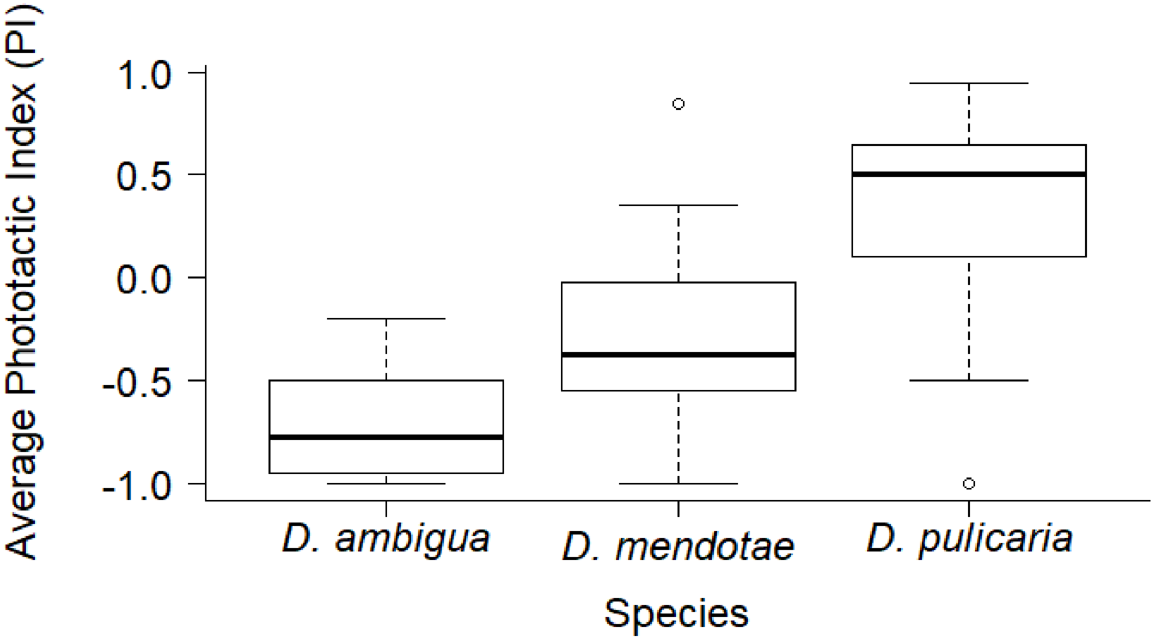

(a)
Average Temperature (Celsius)
0
5
10
15
20
25
Depth (meters)
0
2
4
6
8
10
12
14
16
18
20
22
24
Average Secchi
depth = 2.05 meters
(b)
Average Dissolved Oxygen (mg/L)
0
1
2
3
4
5
6
7
8
9
0
2
4
6
8
10
12
14
16
18
20
22
24

Figure S3.


Average Phototactic Index (PI)
1.0
0.5
0.0
-0.5
-1.0
D. ambigua
D. mendotae
D. pulicaria
Species

Chapter two—The great escape: Differential escape response of resurrected *Daphnia* to chemical cues from predators

Formatted for publication in *Hydrobiologia*

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Abstract

Non-native species introductions are becoming increasingly common, but long-term consequences of the introduction of non-native predators on native prey species remains poorly understood. *Bythotrephes cederströmii* is an invasive zooplanktivore with potential impacts on *Daphnia* communities across North America. We conducted a series of transfer experiments in the presence and absence of *Bythotrephes* and fish (*Poecilia reticulata*) chemical cues to evaluate the escape ability of three *Daphnia* species (*D. ambigua*, *D. mendotae*, and *D. pulicaria*) from both pre- and post-*Bythotrephes* invasion time periods. Comparison of *Daphnia* from pre- and post-invasion time periods revealed a constitutive increase in the escape ability of *D. pulicaria* which may help them avoid attack by *Bythotrephes*. These data contribute to a growing body of research that demonstrates that ecological invasions can prompt adaptive responses, altering the nature of interactions between invasive predators and native prey species.

Key words: evasiveness; kairomones; invasive species; anti-predator behavior; *Bythotrephes*

Introduction

One of the most devastating human impacts on the world's ecosystems is the introduction of non-native invasive species which can negatively impact agriculture, industry, human health, and the natural environment (U.S. Congress, 1993; Mooney & Cleland, 2001). Over the course of history, humans have served as either active or passive dispersal vectors for a wide diversity of species, often transporting species from their native ranges, and generating countless novel species combinations (Mooney & Cleland, 2001). Introductions of non-native species are increasingly common as global connectivity accelerates through human movement (Mooney & Cleland, 2001). The invasion of non-native predators can have particularly damaging effects on native prey species and the introduction of non-native predators is common in many systems

(Strauss et al., 2006). Native species can undergo rapid adaptation in response to directional selection imposed by non-native invaders, leading to changes in the morphology, physiology, and behavior of the native species (Strauss et al., 2006). It is necessary to explore what scenarios can lead to rapid adaptation to better understand how past interactions can shape contemporary communities, so that we can develop effective mitigation and conservation plans (Stockwell et al., 2003; Sax et al., 2007).

Here we examine the response of native *Daphnia* species to invasion by the predatory spiny water flea, *Bythotrephes cederströmii* (Bur et al., 1986; Korovchinsky & Arnott, 2019). *Bythotrephes* invaded the Laurentian Great Lakes in the 1980's and since then has spread to many other North American inland lakes (Schulz & Yurista, 1999). Since the invasion of *Bythotrephes*, changes in the diversity and community composition of zooplankton in invaded North American lakes has been noted (Lehman, 1991; Makarewicz et al., 1995; Yan et al., 2002). These changes are thought to be driven by *Bythotrephes* predation. *Bythotrephes* do not fit the standard invertebrate predator model because, while predaceous zooplankton typically prefer small-bodied zooplankton, *Bythotrephes* prefer large-bodied zooplankton, particularly *Daphnia* (Cladocera: Anomopoda) (Schulz & Yurista, 1999). Post-invasion shifts often involve persistence of *D. mendotae*, decreased abundance of other *Daphnia* species, and increased abundance of other smaller herbivorous zooplankton (Schulz & Yurista, 1999).

The impact of *Bythotrephes* on native *Daphnia* is of concern because in many lakes, *Daphnia* serve as primary algae grazers and are an important food source for commercially and recreationally important fish species (Lampert, 2011). Therefore, shifts in zooplankton community composition can have dramatic ecosystem wide impacts. For example, in 2009, a *Bythotrephes* population boom in Lake Mendota (WI) led to a trophic cascade, which resulted in a decline in water clarity of nearly 1 m due to predation of *Bythotrephes* on *Daphnia* (especially *D. pulicaria*) and a corresponding increase in algae growth (Walsh et al., 2016a). Ultimately, the zooplankton community shifted from dominance by *D. pulicaria,* a larger-bodied and more efficient algae grazer, to codominance by *D. pulicaria* and the smaller, less-efficient grazer *D. mendotae* (Walsh et al., 2016b). Furthermore, on several occasions, temperature-dependent *Bythotrephes* population booms have led to complete collapse of the Lake Mendota *Daphnia* community for as long as 250 days, causing ecosystem-wide impacts such as delay of the spring clearwater phase (Walsh et al., 2016b).

As demonstrated in Lake Mendota, invasion of a non-native predator can lead to decline or disappearance of some prey species and an increase in abundance of others. For native prey species facing a non-native predator, development of anti-predator defenses can be crucial for survival. In zooplankton such as *Daphnia*, defensive strategies can range from changes in morphology such as the development of defensive helmets to changes in behavior such as vertical migration (Diel et al., 2020). These defenses can either be constitutive—always present even when the predator is absent—or inducible—present only when the predator or chemical cues from the predator are detected (Diel et al., 2020).

Alertness is one potential anti-predator defense (Diel et al., 2020). *Daphnia* with increased alertness show faster evasion responses (Pijanowska et al., 2006) and increased swimming speed (Brewer et al., 1999). It has been well established that *Daphnia* species in the presence of fish chemical cues exhibit enhanced evasion efficiency (Brewer et al., 1999; Pijanowska et al., 2006; Pietrzak et al., 2017). Similarly, the evasion efficiency of *Daphnia* from Lake Michigan was enhanced when they were in the physical presence of *Bythotrephes* (Pichlová-Ptáčníková & Vanderploeg, 2011). Furthermore, populations of those species with the fastest documented swimming speeds (i.e., *D. mendotae* and diaptomid copepods) have remained relatively unchanged in Lake Michigan post- *Bythotrephes* invasion, while those species with slower documented swimming speeds (i.e., *D. pulicaria* and *D. retrocurva*) have been more heavily impacted (Pichlová -Ptáčníková & Vanderploeg, 2011). Altogether, these findings suggest that enhanced escape ability may be an important antipredator defense for *Daphnia* against *Bythotrephes*.

For this study, we conducted a series of transfer experiments in the presence and absence of *Bythotrephes* and fish (*Poecilia reticulata*) chemical cues to assess the escape ability of three *Daphnia* species (*D. ambigua*, *D. mendotae*, and *D. pulicaria*) from both pre- and post- *Bythotrephes* invasion time periods to determine whether *Daphnia* were exhibiting either induced or constitutive changes in escape ability. We set out to answer the following questions: 1) does escape ability of the prey species differ in the absence and presence of *Bythotrephes* chemical cues (induced change)?; 2) does the escape ability of pre- and post- invasion clones of the species differ regardless of the presence of predator chemical cues (constitutive change)?; and 3) do the three *Daphnia* species differ in their escape abilities regardless of the presence of predator chemical cues?

Methods

Origin of clones

The *Daphnia* clones used in this study were hatched from resting eggs isolated from the sediments of Lake Mendota, an inland lake located in southern Wisconsin (USA) initially invaded by *Bythotrephes* in 1994 (Walsh et al., 2016b). We were able to establish clonal lines of three *Daphnia* species (*D. ambigua*, *D. mendotae*, and *D. pulicaria*) from single hatchlings. Hatchlings (from resting eggs) were isolated from two different sediment depths: sediments deposited pre-*Bythotrephes* invasion (1987—1990) and sediments deposited post- *Bythotrephes* invasion (2009—2017). Previous ^{210}Pb dating of Lake Mendota sediments was used to estimate the dates of the sediments collected (Walsh et al., 2016b). Clonal lines were maintained under identical conditions for at least two generations before being used in experiments to reduce maternal effects. Clonal lines were grown separately in 5 L jars and were fed daily a 50:50 mixture of the green algae *Nannochloropsis* sp. (at a concentration of 34 million cells mL^{-1}; Nano 3600, Reed Mariculture, Campbell, CA) and *Scenedesmus acutus* (at a concentration of 1 mg C L^{-1}). For additional sediment core collection and hatching protocols see Kiehnau and Weider (2019).

General approach

To test escape ability, we conducted a series of transfer experiments. Transfer experiments were modeled after the methods outlined by Pietrzak et al. (2017) and consisted of blind sequential transferring of media from an initial jar, which contained a set volume of media and number of *Daphnia*, through a series of jars until the volume of media in each jar was equal (see Fig. 1 for a diagram of the experimental set-up). At the end of the experiments, the final distribution of animals in the jars was assessed to compare the expected versus observed distributions. It was expected that those individuals that were more evasive would not be easy to capture and would be found mainly in the starting containers. All transfer experiments were conducted by the same individual, so as not to confound a possible difference in experimenter pipetting technique.

Treatments included a control with no chemical cues present, a *Bythotrephes* cue treatment, and a fish cue treatment. Response to chemical cues from fish was tested in addition to the response to *Bythotrephes* cues because the procedure most closely imitates suction feeding predators such as fish (Ferry-Graham et al., 2003) rather than raptorial predators such as *Bythotrephes* (Muirhead & Sprules, 2003). Further, because *Daphnia* are known to respond to fish chemical cues with enhanced escape ability, the fish treatment served as a positive control (Pietrzak et al., 2017).

We tested the effect of previous exposure to *Bythotrephes* chemical cues on the escape ability by testing both pre- and post-invasion *D. ambigua*, *D. mendotae,* and *D. pulicaria*. Three post-invasion clones of each species were tested as well as two pre-invasion *D. ambigua* clones and three pre-invasion *D. pulicaria* clones (total of 14 clones). The uneven representation of *Daphnia* (i.e., lack of pre-invasion *D. mendotae* clones) is due to the low hatching success of ephippial eggs from deeper sediment layers and the difficulty of establishing *D. mendotae* hatchlings. Each treatment x clone combination was replicated 4 times (3 treatments x 14 clones x 4 replicates = 168 transfer experiments). Replicate experiments were carried out in random order in a well-lit room from June–November 2019 between 13.00-17.00 hrs.

Evasiveness experiments

A cohort of 100 pre-reproductive females of each clone were used in each experiment. Pre-reproductive animals were identified via size and the lack of a visible brood chamber. All experimental animals came from maternal lines raised under identical conditions (see above).

Animals receiving the cue treatments were incubated in 1 L of cue water for 24-48 hours prior to the test and control animals were incubated for 24-48 hours in control/no cue medium (i.e. COMBO). Both experimental and control media were supplemented with a food source (the 50:50 mixture of the green algae at the concentrations described above) at the time the incubation began and again at the 24-hour point.

Bythotrephes chemical cue water was obtained by crushing live-frozen *Bythotrephes* (collected from Lake Mendota) in a small amount of COMBO, then pouring the *Bythotrephes* homogenate over a 0.45 μm GF/F filter held in place over a vacuum filtration device. *Bythotrephes* chemical cue water was prepared to a concentration of 2.7 *Bythotrephes* L^{-1} to simulate natural densities of *Bythotrephes* typically found in invaded lakes (Boudreau & Yan, 2004).

Fish cue water was obtained by incubating two approximately 5 cm long *Poecilia reticulata* in 3 L of COMBO for 24 hours, then filtering this media over a 0.45 μm GF/F filter. Fish were fed fish flakes in the aquarium before the incubation began but were not fed in the incubation container and were never fed *Daphnia* to avoid conflating kairomone, *Daphnia* alarm cues, and other chemical cues. Our design assumes that fish chemical cues are generalized across species. *P. reticulata* (commonly known as guppies) are not found in Lake Mendota, but we assume that the cues that they give off are comparable to those given off by native fish species. This assumption is based on the finding that kairomones from a variety of planktivorous or piscivorous fish displayed identical chemical characteristics and similar retention on high performance liquid chromatography (Brönmark & Hansson, 2012). This demonstrates that fish infochemicals are chemically similar, if not identical, and suggests that fish exude a generalized rather than species-specific cue which provides an evolutionary stable indicator of fish predation risk (Brönmark & Hansson, 2012). Furthermore, previous studies of *Daphnia magna* behavior demonstrate that predator avoidance behavior was induced by chemical cues from either starving planktivorous or piscivorous fish (Loose et al.,1993; Von Elert & Loose, 1996; Von Elert & Pohnert, 2000).

Before the start of the transfer experiment, all 100 animals were moved from the incubation jars to 400 mL beakers filled with 250 mL of either fresh *Bythotrephes* cue, fish cue, or control (no cue) media. A glass pipette with a 5 mm inner diameter and a 3 mL rubber bulb was used to blindly transfer animals through 10 subsequent 400 mL beakers (Fig. 1). The initial beaker contained 250 mL of experimental (control, fish cue, or *Bythotrephes* cue) media and 100 pre-reproductive *Daphnia*. Using a glass pipette to simulate a predator's attack, 225 mL of media (including *Daphnia*) was transferred from the first beaker to the second beaker. Media was similarly transferred from the second to the third beaker and so on 9 times. At each transfer, 25 mL of media was left in the source beaker (and 25 mL was left in all 10 beakers at the end of the experiment), so that the volume moved beaker-to-beaker was 225 mL during the first transfer and 25 mL at the final transfer (Fig. 1). To ensure random pipetting, the source transfer beaker was placed in an opaque sleeve and the pipette tip was placed randomly at different spots in the beaker at subsequent strokes. The volume to be reached was marked on each target beaker to ensure the proper amount of media was transferred.

At the end of each experiment, the number of animals in each beaker was recorded. Then, following the methods of Black and Dodson (2003), the experimental *Daphnia* were preserved by pooling the individuals from all beakers, shocking them in 95% ethanol, and then storing them in 70% ethanol.

Data analysis

All statistical analyses were performed in R ver. 3.6.3 (R Core Team 2020). The distribution of animals throughout the 10-beakers was compared across treatments to assess escape ability of *Daphnia*. An expected/observed metric was calculated to assess the evasiveness of daphnids as proposed by Pietzak et al. (2017). Linear mixed-effects models that were constructed using the lmer function in the lme4 package (Bates et al., 2015), were subsequently used to assess the importance of the predator chemical cues and history of coexistence (i.e., pre- versus post-invasion) on the escape ability of the *Daphnia* species tested. For all linear mixed-effects models, we performed model selection using Akaike's Information Criterion (AIC_c) via the dredge function in the MuMIn package (Burnham and Anderson 2003, Barton 2020; see tables in supplement). In addition, to determine which driver variables most influenced escape ability, we calculated relative importance values using the importance function in the MuMIn package (Barton 2020; see tables in supplement). Using the resid function in base R (R Core Team 2020), we checked the residuals for indications of non-linearity, normality, and heteroscedasticity during the model evaluation process, and the final model passed all the standard assumptions. Results of the final linear mixed effects models were further explored via Tukey adjusted post hoc analysis (using the emmeans function in the emmeans package; Lenth, 2020).

An expected/observed metric was calculated as the ratio of the number of daphnids expected to be left at each transfer under the assumption of no escape ability, proportional to the relative volume of water left to the observed numbers of daphnids that escaped at each transfer. The expected number of daphnids was calculated as $N_e = N_s * V_1/V_s$, where N_s is the number of individuals at the source container before the transfer, V_1 is the volume left in the source container after the transfer (25 mL each time in our experiments), and V_s is the initial volume of water at the source container before the transfer. Calculated expected/observed values were averaged so that there was a single metric for each clone, treatment, and replicate combination. Lower values of the expected/observed metric indicate higher escape ability, while higher values indicate lower escape ability.

The calculated ratios were transformed using Tukey's ladder of powers (transformTukey function in the rcompanion package; Mangiafico, 2020) before being analyzed with linear mixed-effects models. The full linear mixed-effects model for the complete data set included species identity (Species), history of coexistence with *Bythotrephes* (History), the presence of *Bythotrephes* chemical cues, fish chemical cues, or the absence of chemical cues (Treatment), and the interaction between species and treatment and history and treatment as fixed effects. The interactions between species and history and the three-way interaction were not included in the model because no pre-invasion *D. mendotae* were included in the experiment. Clonal identity (Clone) was included as a random effect. The full model was specified as follows: ExpObs ~ Species+ History +Treatment + Species:Treatment + Treatment:History + (1|Clone). After model selection (Table S1), the final model was specified as follows: ExpObs~Species + History + Treatment +(1|Clone).

After analysis of all three species together, data were analyzed with separate linear mixed-effects models for each species. The full linear mixed-effects models for *D. ambigua* and *D. pulicaria* included history, treatment, and their interaction as fixed effects, and clone as a random effect and were specified as follows: ExpObs ~ Treatment + History + Treatment:History + (1|Clone). After model selection, the final models were specified as follows for *D. ambigua* (Table S3): ExpObs ~ Treatment + (1|Clone) and for *D. pulicaria* (Table S7): ExpObs ~ Treatment + History + (1|Clone). As mentioned above, since we had no pre-invasion *D. mendotae* clones, the full (and final) model for this species included only treatment as a fixed effect, clone as a random effect and was specified as follows (Table S5): ExpObs ~ Treatment + (1|Clone).

Results

When the dataset including all three *Daphnia* species was assessed, species (*F*=9.1822, *df*=2,10, *P*=0.0055), treatment (*F*=6.3534, *df*=2,152, *P*=0.0022), and the random effect clone (*LRT*=6.1221, *df*=1, *P*=0.0134) were all found to have a significant effect on the probability of escaping (Table 1).

According to the expected/observed measure whereby lower values indicate higher escape ability, *D. ambigua* showed higher overall escape ability (1.06 ± 0.03, mean ± SE) than either *D. mendotae* (1.30 ± 0.06) or *D. pulicaria* (1.57 ± 0.06). The difference in escape ability between *D. ambigua* and *D. pulicaria* was significant (*z*=4.2750, *df*=10, *P*<0.0001), but the differences between *D. mendotae* and *D. ambigua* (*z*=1.969, *df*=10, *P*=0.0979), and *D. mendotae* and *D. pulicaria* (*z*=1.411, *df*=10, *P*=0.1584) were not.

While there were overall differences in escape ability amongst species, responses to treatments were relatively consistent across all *Daphnia* species (Fig. 2). Fish chemical cues elicited a significantly enhanced escape response of all *Daphnia* compared to the control and *Bythotrephes* cue treatments (*z*=-3.2390, *df*=152, *P*=0.0036 and *z*=-2.9090, *df*=152, *P*=0.0073 respectively); however, no significant difference in escape ability was detected between *Bythotrephes* chemical cue and control treatments (*z*=0.3300, *df*=152, *P*=0.7412). However, some clonal variation in response to cues was apparent. For example, *D. ambigua* post 1 clone exhibited a comparatively high escape across all cue treatments (Fig. 2).

History of coexistence with *Bythotrephes* did not have a significant effect across species (Table 1); however, when species data were analyzed separately (Table 2), we found that history of coexistence with *Bythotrephes* had a significant effect on the overall escape ability of *D. pulicaria* (*F*= 12.1594, *df*= 1, 4, *P*= 0.0252). Post-invasion clones of *D. pulicaria* had an overall enhanced escape ability (1.38 ± 0.07, mean ± SE) across treatments in comparison to pre-invasion *D. pulicaria* clones (1.76 ± 0.09; Fig. 2).

Discussion

Our study examined potential differences in prey escape ability by resurrecting clones of three different *Daphnia* species from pre- and post-*Bythotrephes* invasion time periods for our experiments and comparing their escape response in the presence and absence of *Bythotrephes* and fish (*P. reticulata*) chemical cues. Overall, we found that the presence of fish chemical cues induced an enhanced escape response, while the presence of *Bythotrephes* chemical cues did not (Table 1, Fig. 2). However, a significant constitutive increase in escape ability from pre- to post-invasion *D. pulicaria* was identified (Table 2, Fig. 2). We also found that there were general differences in escape ability across species. Escape ability ordered the species as follows: *D. ambigua* > *D. mendotae* > *D. pulicaria* (Fig. 2).

Previous research has demonstrated that species, such as *D. mendotae*, whose populations have remained relatively unchanged post-*Bythotrephes* invasion, have faster swimming speeds than species, such as *D. pulicaria*, whose populations have been heavily impacted by *Bythotrephes* invasion (Pichlová-Ptáčníková & Vanderploeg, 2011). While our analyses indicated that differences between *D. mendotae* and the other two species were not significant, we did find that of the three *Daphnia* species tested, *D. pulicaria*—especially pre-invasion clones—had the lowest average escape ability (Fig. 2). It is well documented that prey movement behavior affects the outcome of predator-prey interactions because prey movement serves both as a cue to predators and a determinant of encounter rate (Gerritsen & Strickler, 1977; Lima & Dill, 1990; Brewer & Coughlin, 1996; Dodson et al., 1997; Langkilde, 2009). Overall, this suggests that the variation in escape ability of members of the Lake Mendota

Daphnia assemblage may have contributed to the documented decline in *D. pulicaria*, which followed the population boom of *Bythotrephes* in 2009 (Walsh et al., 2016b).

Although the differences between *D. mendotae a*nd the other species were not found to be significant, *D. ambigua* was found to have a significantly higher escape ability than *D. pulicaria* (Table 1). This suggests that *D. ambigua* may also exhibit faster swimming speeds than *D. pulicaria*, although direct analysis of the swimming speeds of these daphnids would be needed to confirm this assertion. It is not clear how the invasion of *Bythotrephes* has impacted *D. ambigua* abundance in Lake Mendota, given that *D. ambigua* has only been identified in one Lake Mendota LTER zooplankton sample (on 21 July 1983) over the last ~40 years despite biweekly sampling of the pelagic zone (Lathrop, 2013; Magnuson et al., 2019) and being abundant in the resting egg bank for the last ~ 30 years (see Kiehnau & Weider, 2019 for further discussion).

Although an induced change in escape ability was not observed in response to *Bythotrephes* chemical cues, escape ability was significantly different between both control and fish and *Bythotrephes* and fish cue treatments (Table 1). This is in line with previous findings (Brewer et al., 1999; Pijanowska et al., 2006; Pietrzak et al., 2017) which demonstrate that evasion efficiency of *Daphnia* is enhanced in the presence of fish chemical cues. Additionally, although the difference was not significant, a somewhat similar pattern was shown for *Bythotrephes*, as escape response of several clones was enhanced in the presence of *Bythotrephes* chemical cues in comparison to controls (Fig. 2). This trend is supported by previous experimentation with *Daphnia* from Lake Michigan, which demonstrated that both *D. mendotae* and *D. pulicaria* exhibit enhanced swimming speeds when in the physical presence of *Bythotrephes* (Pichlová-Ptáčníková & Vanderploeg, 2011).

Although we did not find robust evidence of an induced change in escape response in any of the species we tested, we did find that *D. pulicaria* clones exhibited a constitutive increase in escape ability (Table 2). Post-invasion clones of *D. pulicaria* demonstrated greater overall escape ability in comparison to pre-invasion clones, regardless of the presence of predator cues (Fig. 2). In addition, there appears to be reduced clonal variation amongst post-invasion clones than amongst pre-invasion clones (Fig. 2). It is known that predator escape responses can be a target for selection (Pijanowska et al. 2006) and selection imposed by *Bythotrephes* on other anti-predator behavioral traits has been demonstrated (Landy et al., 2020). Together, our findings suggest that Lake Mendota *D. pulicaria* have developed an enhanced escape ability in response to the *Bythotrephes* invasion. However, other resurrection experiments have demonstrated that, despite clear evidence of directional selection by *Bythotrephes* on behavioral and life-history traits, Lake Mendota *Daphnia* have maintained extensive clonal variation in plasticity over time (Landy et al., 2020). Thus, further testing of additional pre- and post-invasion clones would be valuable to determine the robustness of our findings.

Variation in the swimming speeds of the *Daphnia* species found in Lake Mendota may have contributed to initial differences in vulnerability to *Bythotrephes,* akin to what may be occurring in Lake Michigan (Pichlová-Ptáčníková & Vanderploeg, 2011). This would help explain the drastic reduction of *D. pulicaria* that occurred following the population boom of *Bythotrephes* in 2009 (Walsh et al., 2016b). The partial recovery of *D. pulicaria* and resulting co-dominance of *D. pulicaria* and *D. mendotae* in Lake Mendota may be explained, in part, by the constitutive increase in escape ability found in post-invasion *D. pulicaria*. This, in combination with other anti-predator defenses such as the negative phototactic behavior noted by Landy et al. (2020), may have contributed to the persistence of *D. pulicaria* in Lake Mendota, despite its well-documented vulnerability to *Bythotrephes* in this and other systems (Schulz & Yurista, 1999; Walsh et al., 2016b).

In conclusion, we posit that differences in the escape ability of Lake Mendota *Daphnia* have contributed to differences in vulnerability, which in turn, have influenced the community changes observed after the establishment of *Bythotrephes* (Walsh et al., 2016b). Our findings contribute to a growing body of research which suggests that it is critical to recognize the evolutionary component of non-native predator/native prey species interactions in order to further our understanding of the long-term impacts of biological invasions on native communities (Mooney & Cleland, 2001; Strauss et al., 2006).

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Data availability statement

The data associated with this study are available in the Open Science Framework repository: https://osf.io/m68u9/?view_only=5c2eef4209b84b33906f94866b8ffb87.

Ethics approval

This work was approved by the University of Oklahoma Institutional Animal Care and Use Committee (protocol R18-006A).

References

Barton, K., 2020. MuMIn: Multi-Model Inference. R package version 1.43.17. https://CRAN.R-project.org/package=MuMIn

Black, A.R. & S.I. Dodson, 2003. Ethanol: A better preservation technique for *Daphnia*. Limnology and Oceanography: Methods 1: 45–50.

Boudreau, S.A. & N.D. Yan, 2004. Auditing the accuracy of a volunteer-based surveillance program for an aquatic invader *Bythotrephes*. Environmental Monitoring and Assessment 91: 17–26.

Brewer, M.C. & J.N. Coughlin, 1996. Virtual plankton—a novel approach to the investigation of aquatic predator-prey interactions. Marine and Freshwater Behaviour and Physiology 26: 91–100.

Brewer, M.C., P. Dawidowicz & S.I. Dodson, 1999. Interactive effects of fish kairomone and light on *Daphnia* escape behavior. Journal of Plankton Research 27: 1317–1335.

Brönmark, C. & L.-A. Hansson, 2012. Chemical Ecology in Aquatic Systems. Oxford University Press, Oxford.

Bur, M.T., D.M. Klarer & K.A. Krieger, 1986. First Records of a European Cladoceran, *Bythotrephes Cederstroemi*, in Lakes Erie and Huron. Journal of Great Lakes Research 12: 144–146.

Burnham, K.P. & D.R. Anderson, 2003. Model selection and multimodel inference: A practical information-theoretic approach. Springer Science and Business Media, New York.

Diel, P., M. Kiene, D. Martin-Creuzburg & C. Laforsch, 2020. Knowing the enemy: Inducible defenses in freshwater zooplankton. Diversity 12: 147.

Dodson, S.I., S. Ryan, R. Tollrian & W. Lampert, 1997. Individual swimming behavior of *Daphnia*—effects of food, light and container size in four clones. Journal of Plankton Research 19: 1537–1552.

Douglas, B., M. Maechler, B. Bolker & S. Walker, 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67: 1–48.

Ferry-Graham, L.A., P.C. Wainwright & G.V. Lauder, 2003. Quantification of flow during suction feeding in bluegill sunfish. Zoology 106: 159–168.

Gerritsen, J. & J.R. Strickler, 1977. Encounter probabilities and community structure in zooplankton – mathematical model. Journal of the Fisheries Research Board Canada 34: 73–82.

Kiehnau, E.L. & L.J. Weider, 2019. Phototactic behavior of native *Daphnia* in the presence of chemical cues from a non-native predator *Bythotrephes*. Oecologia 190: 799–809.

Kilham, S.S., D.A. Kreeger, S.G. Lynn, C.E. Goulden & L. Herrera, 1998. COMBO: a defined freshwater culture medium for algae and zooplankton. Hydrobiologia 377: 147–159.

Korovchinsky, N.M. & S.E. Arnott, 2019. Taxonomic resolution of the North American invasive species of the genus *Bythotrephes* Leydig, 1860 (Crustacea: Cladocera: Cercopagididae). Zootaxa 4691: 125–138.

Lampert, W., 2011. *Daphnia*: development of a model organism in ecology and evolution. In: Kinne, O. (eds) Excellence in ecology: book 21. International ecology institute, Oldendorf/Luhe, Germany.

Landy, J.A., A. Oschmann, S.B. Munch & M.R. Walsh, 2020. Ancestral genetic variation in phenotypic plasticity underlies rapid evolutionary changes in resurrected populations of waterfleas. Proceedings of the National Academy of Sciences, USA 117: 32565–32544.

Langkilde, T., 2009. Invasive fire ants alter behavior and morphology of native lizards. Ecology 90: 208–217.

Lathrop, R., 2013. Madison Wisconsin lakes zooplankton 1976 - 1994. Environmental Data Initiative. https://doi.org/10.6073/pasta/ec3d0186753985147d4f283252388e05. Accessed 2021-01-08.

Lehman, J.T., 1991. Causes and consequences of cladoceran dynamics in Lake Michigan: Implications of species invasion by *Bythotrephes*. Journal of Great Lakes Research 17: 437–445.

Lenth, R., 2020. emmeans: Estimated marginal means, aka least-squares means. R package version 1.4.7. https://CRAN.R-project.org/package=emmeans

Lima, S.L. & L.M. Dill, 1990. Behavioral decisions made under the risk of predation: A review and prospectus. Canadian Journal of Zoology 68: 619–640.

Loose, C.J., E. Von Elert & P. Dawidowicz, 1993. Chemically-induced diel vertical migration in *Daphnia*: A new bioassay for kairomones exuded by fish. Archiv fr Hydrobiologie 126: 329–337.

Magnuson, J., S. Carpenter & E. Stanley, 2019. North temperate lakes LTER: Zooplankton - Madison lakes area 1997 - current. Environmental Data Initiative. https://doi.org/10.6073/pasta/8b265c0300252c87805f26f41e174aa4.

Makarewicz, J.C., P. Bertram, T. Lewis & E.H. Brown, Jr., 1995. A decade of predatory control of zooplankton species composition of Lake Michigan. Journal of Great Lakes Research 21: 620–640.

Mangiafico, S., 2020. rcompanion: Functions to support extension education program evaluation. R package version 2.3.25. https://CRAN.R-project.org/package=rcompanion

Mooney, H.A. & E.E. Cleland, 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences 98: 5446–5451.

Muirhead, J. & W.G. Sprules, 2003. Reaction distance of *Bythotrephes longimanus*, encounter rate and index of prey risk for Harp Lake, Ontario. Freshwater Biology 48: 135–146.

Pichlová-Ptáčníková, R. & H.A. Vanderploeg, 2011. The quick and the dead: Might differences in escape rates explain the changes in the zooplankton community composition of Lake Michigan after invasion by *Bythotrephes*? Biological Invasions 13: 2595–2604.

Pietrzak, B., J. Pijanowska & P. Dawidowics, 2017. The effect of temperature and kairomone on *Daphnia* escape ability: A simple bioassay. Hydrobiologia 798: 15–23.

Pijanowska, J., P. Dawidowicz & L.J. Weider, 2006. Predator-induced escape response in *Daphnia*. Archiv fr Hydrobiologie 167: 77–87.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Sax, D.F., J.J. Stachowicz, J.H. Brown, J.F. Bruno, M.N. Dawson, S.D. Gaines, R.K. Grosberg, A. Hastings, R.D. Holt, M.M. Mayfield, M.I. O'Connor & W.R. Rice, 2007. Ecological and evolutionary insights from species invasions. Trends in Ecology and Evolution 22: 465–471.

Schulz, K.L. & P.M. Yurista, 1999. Implications of an invertebrate predator's (*Bythotrephes cederstroemi*) atypical effects on a pelagic zooplankton community. Hydrobiologia 380: 179–193.

Stockwell, C.A., A.P. Hendry & M.T. Kinnison, 2003. Contemporary evolution meets conservation biology. Trends in Ecology and Evolution 18: 94–101.

Strauss, S.Y., J.A. Lau & S.P. Carroll, 2006. Evolutionary responses of natives to introduced species: What do introductions tell us about natural communities? Ecology Letters 9: 354–371.

U.S. Congress, 1993. Office of technology Assessment, OTA-F-565. Washington, D.C., USA.

von Elert, E. & C.J. Loose, 1996. Predator-induced diel vertical migration in *Daphnia*: Enrichment and preliminary chemical characterization of a kairomone exuded by fish. Journal of Chemical Ecology 22: 885–895.

von Elert, E. & G. Pohnert, 2000. Predator specificity of kairomones in diel vertical migration of *Daphnia*: A chemical approach. Oikos 88: 119–128.

Walsh, J.R., S. Carpenter & J. Vander Zanden, 2016a. Invasive species triggers a massive loss of ecosystem services through a trophic cascade. Proceedings of the National Academy of Sciences of the United States of America 113: 4081–4085.

Walsh, J.R., S.E. Munoz & M.J. Vander Zanden, 2016b. Outbreak of an undetected invasive species triggered by a climate anomaly. Ecosphere 7: e01628.

Yan, N.D., R. Girard & S. Bourdreau, 2002. An introduced invertebrate predator (*Bythotrephes*) reduces zooplankton species richness. Ecology Letters 5: 481–485.

Tables

Table 1: **A.** Type II analysis of variable table with Kenward-Roger method from linear mixed effects models assessing the impact of *Daphnia* species identity (*D. ambigua*—DA, *D. mendotae*—DM, and *D. pulicaria*—DP), history of coexistence (daphnids from pre- or post-*Bythotrephes* invasion time periods), and treatment (the presence of *Bythotrephes*—Bytho, fish, or no—Control chemical cues) on average escape ability. Escape ability is expressed as a ratio of the expected to the observed number of daphnids left after a transfer. **B.** Results of Log-likelihood ratio test on random effects of linear mixed effects model described above. Single term deletions were used to compare the model with and without random effects. **C.** Post-hoc analysis of variables found to be significant in the final model. Multiple comparisons of means Tukey contrasts. Degrees of freedom method: Kenward-Roger. Bolded values indicate p values below the alpha level of 0.05.

A. Coefficient	**Sum Sq**	**Mean Sq**	**Num Df**	**Den Df**	**F value**	**P**
Species	0.3665	0.1833	2	10	9.1822	**0.0055**
History	0.0492	0.0492	1	10	2.4647	0.1475
Treatment	0.2536	0.1268	2	152	6.3534	**0.0022**

B. Random effect	**logLik**	**AIC**	**Df**	**LRT**	**P**
Clone	67.9910	-121.9800	1	6.1221	**0.0134**

C. Contrasts	**Estimate**	**SE**	**Df**	**z value**	**P**
DM-DA	0.1012	0.0514	10	1.9690	0.0979
DP-DA	0.1737	0.0406	10	4.2750	**5.75e-5**
DP-DM	0.0725	0.0514	10	1.4110	0.1584
Bytho-Control	0.0088	0.0267	152	0.3300	0.7412
Bytho-Fish	-0.0777	0.0267	152	-2.9090	**0.0073**
Control-Fish	-0.0865	0.0267	152	-3.2390	**0.0036**

Table 2: **A.** Type II analysis of variable tables with Kenward-Roger method from linear mixed effects models assessing the average escape ability of three *Daphnia* species expressed as a ratio of the expected to the observed number of daphnids left after a transfer. Impact of treatment (the presence of *Bythotrephes*, fish, or no chemical cues) on the escape ability of *D. ambigua*, *D. mendotae*, and *D. pulicaria* and history of coexistence (daphnids from pre- or post-*Bythotrephes* invasion time periods) on the escape ability of *D. pulicaria*. **B.** Results of Log-likelihood ratio test on random effects of linear mixed effects models described above. Single term deletions were used to compare the models with and without random effects. Bolded values indicate p values below the alpha level of 0.05.

A.	**Coefficient**	**Sum Sq**	**Mean Sq**	**Num df**	**Den df**	**F value**	**P**
D. ambigua	Treatment	0.0618	0.0309	2	53	1.7226	0.1885
D. mendotae	Treatment	0.1052	0.0526	2	31	1.7064	0.1981
D. pulicaria	Treatment	0. 1017	0.0509	2	64	2.9724	0.0583
	History	0. 2080	0. 2080	1	4	12.1594	**0.0252**

B.	**Random effect**	**logLik**	**AIC**	**df**	**LRT**	**P**
D. ambigua	Clone	23.5890	-39.1780	1	5.6013	**0.0180**
D. mendotae	Clone	6.05590	-4.1119	1	0.3994	0.5074
D. pulicaria	Clone	46.3490	-82.6970	1	-1.42e-14	1.0000

Figure captions

Fig. 1 Overview of the transfer experiment procedure. At the start of each experimental trial, the first beaker (of 10) contained 250 mL of either fresh *Bythotrephes cederströmii* (Bytho) chemical cues, *Poecilia reticulata* (Fish) chemical cues, or no cue (Control) media and 100 pre-reproductive *Daphnia*. Media was then blindly transferred with a pipette through subsequent beakers, so that at the end of the experiment, media would be equally distributed throughout the beakers (i.e., 25 mL in each). After the final transfer, the distribution of *Daphnia* throughout the beakers was compared across species and treatments to assess escape ability. Modified from Pietrzak et al. (2017).

Fig. 2 Escape ability of clones of three *Daphnia* species from pre- and post-*Bythotrephes* invasion time periods in the presence of no chemical cues (Control), *Poecilia reticulata* (Fish), and *Bythotrephes cederströmii* (Bytho) chemical cues. Escape ability is expressed as a ratio of the expected to the observed number of individuals left after a transfer (mean ± SE). Lower ratios indicate higher escape ability.

Fig. 1

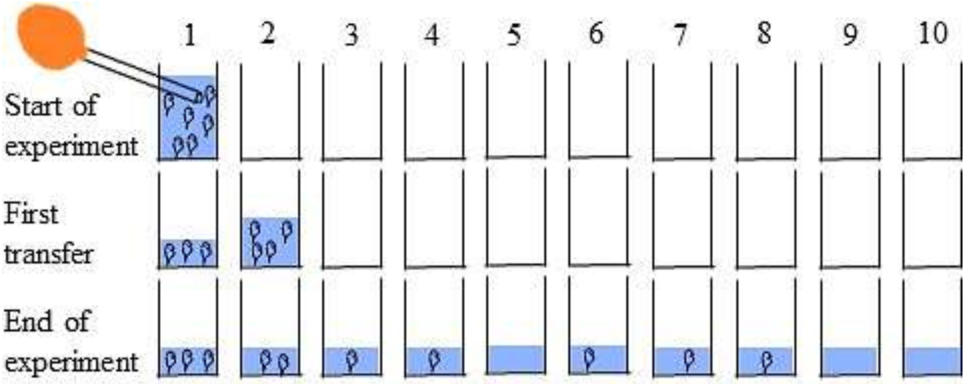
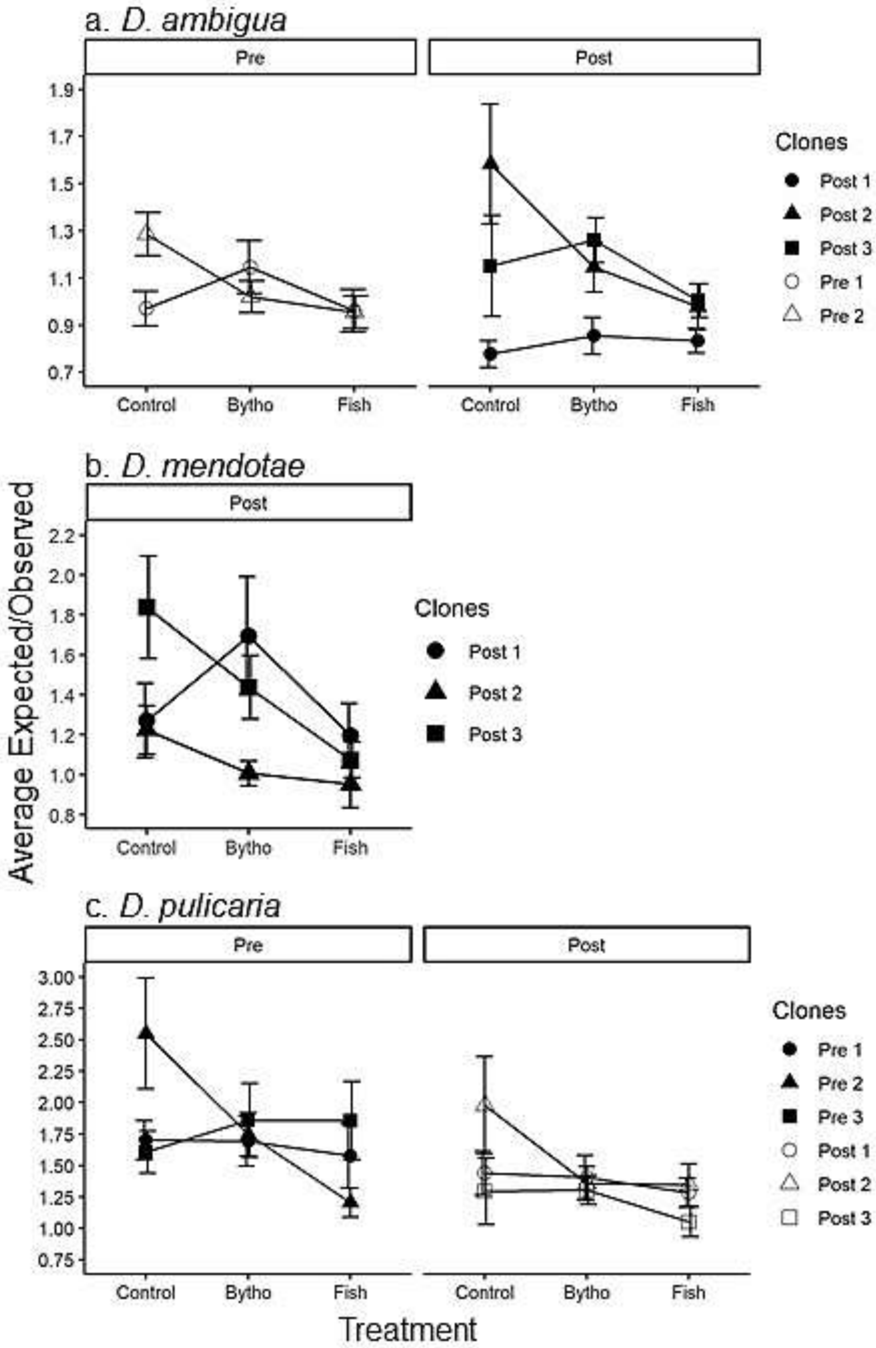

1
2
3
4
5
6
7
8
9
10
Start of experiment
First transfer
End of experiment

Fig. 2



a. D. ambigua
Pre
Post
1.9
1.7
1.5
1.3
1.1
0.9
0.7
Control
Bytho
Fish
Clones
Post 1
Post 2
Post 3
Pre 1
Pre 2
b. D. mendotae
Post
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
Clones
Post 1
Post 2
Post 3
Average Expected/Observed
c. D. pulicaria
Pre
Post
3.00
2.75
2.50
2.25
2.00
1.75
1.50
1.25
1.00
0.75
Clones
Pre 1
Pre 2
Pre 3
Post 1
Post 2
Post 3
Treatment

Chapter two: Appendix S1. Supporting Information.

Supplement S1. Tables

Table S1. Comparison of linear mixed effects models assessing the impact of *Daphnia* species (S) identity (*D. ambigua, D. mendotae, D. pulicaria*), history (H) of coexistence (daphnids from pre- or post-Bythotrephes invasion time periods), and treatment (T, the presence of *Bythotrephes*, fish, or no chemical cues), on average escape ability. Expected/observed escape ability is expressed as a ratio of the expected to the observed number of daphnids left after a transfer. + indicate variables/interactions used in each model.

Model	**Model Variables**					**AIC_c**	**LogLik**	**df**	**ΔAIC_c**	**wi**
	Species (S)	**History (H)**	**Treatment (T)**	**S:T**	**H:T**					
Average expected/ observed	+	+	+			-156.8	86.864	8	0.00	0.548
	+		+			-155.9	85.321	7	0.88	0.353
	+	+	+		+	-152.4	86.896	10	4.43	0.060
	+	+				-148.8	80.681	6	7.98	0.010
	+	+	+	+		-148.5	87.249	12	8.34	0.008
	+					-147.9	79.139	5	8.91	0.006
	+		+	+		-147.7	85.707	11	9.10	0.006
			+			-146.8	78.598	5	10.00	0.004
		+	+			-146.6	79.566	6	10.21	0.003
	+	+	+	+	+	-143.8	87.252	14	13.06	0.001
		+	+		+	-142.3	79.599	8	14.53	0.000
						-138.7	72.416	3	18.14	0.000
		+				-138.5	73.384	4	18.30	0.000

AIC statistics: AIC_c AIC statistic; *LogLik* log likelihood; *df* degrees of freedom; ΔAIC_c AIC_c minus top model AIC_c, *wi* model weight

Table S2: Relative importance values (RIVs) for driver variable of linear mixed-effects models of assessing the impact of *Daphnia* species (S) identity (*D. ambigua*, *D. mendotae*, *D. pulicaria*), history (H) of coexistence (daphnids from pre- or post-Bythotrephes invasion time periods), and treatment (T, the presence of *Bythotrephes*, fish, or no chemical cues), on average escape ability. RIVs are a summed and standardized indicator of predictor variable rank across all possible models. Expected/ observed escape ability is expressed as a ratio of the expected to the observed number of daphnids left after a transfer.

	Species (S)	**History (H)**	**Treatment (T)**	**S:T**	**H:T**
Sum of weights	0.99	0.63	0.98	0.02	0.06
Number of containing models	8	8	9	3	3

Table S3. Comparison of linear mixed effects models assessing the impact of treatment (the presence of *Bythotrephes*, fish, or no chemical cues) and history of coexistence (daphnids from pre- or post-*Bythotrephes* invasion time periods) on the average escape ability of *D. ambigua*. Expected/observed escape ability is expressed as a ratio of the expected to the observed number of daphnids left after a transfer. + indicate variables/interactions used in each model.

Model	**Model Variables**			**AIC$_c$**	**LogLik**	**df**	**ΔAIC$_c$**	**wi**
	Treatment	**History**	**Treatment:History**					
Average expected/ observed				-57.2	31.795	3	0.00	0.491
	+			-55.9	33.527	5	1.22	0.267
		+		-54.9	31.805	4	2.28	0.157
	+	+		-53.5	33.537	6	3.67	0.078
	+	+	+	-48.3	33.554	8	8.88	0.006

AIC statistics: *AIC$_c$* AIC statistic; *LogLik* log likelihood; *df* degrees of freedom; *ΔAIC$_c$* AIC$_c$ minus top model AIC$_c$, *wi* model weight

Table S4: Relative importance values (RIVs) for driver variable of linear mixed-effects models of assessing the impact of treatment (the presence of *Bythotrephes*, fish, or no chemical cues) and history of coexistence (daphnids from pre- or post-*Bythotrephes* invasion time periods) on the average escape ability of *D. ambigua*. RIVs are a summed and standardized indicator of predictor variable rank across all possible models. Expected/ observed escape ability is expressed as a ratio of the expected to the observed number of daphnids left after a transfer.

	Treatment	**History**	**Treatment:History**
Sum of weights	0.35	0.24	<0.01
Number of containing models	3	3	1

Table S5. Comparison of linear mixed effects models assessing the impact of treatment (the presence of *Bythotrephes,* fish, or no chemical cues on the average escape ability of *D. mendotae*. Only post-invasion *D. mendotae* were assessed. Escape ability is expressed as a ratio of the expected to the observed number of daphnids left after a transfer.

Model	**Model Variable**	**AICc**	**LogLik**	**df**	**ΔAICc**	**wi**
	Treatment					
Average expected/ observed		-14.4	10.563	3	0.00	0.711
	+	-12.6	12.286	5	1.8	0.289

AIC statistics: *AIC_c* AIC statistic; *LogLik* log likelihood; *df* degrees of freedom; *ΔAIC_c* AIC_c minus top model AIC_c, *wi* model weight

Table S6: Relative importance values (RIVs) for driver variable of linear mixed-effects models of assessing the impact of treatment (the presence of *Bythotrephes*, fish, or no chemical cues) on the average escape ability of post-invasion *D. mendotae*. RIVs are a summed and standardized indicator of predictor variable rank across all possible models. Observed/expected escape ability is expressed as a ratio of the expected to the observed number of daphnids left after a transfer.

	Treatment
Sum of weights	0.289
Number of containing models	1

Table S7. Comparison of linear mixed effects models assessing the impact of treatment (the presence of *Bythotrephes*, fish, or no chemical cues) and history of coexistence (daphnids from pre- or post-*Bythotrephes* invasion time periods) on the average escape ability of *D. pulicaria*. Escape ability is expressed as a ratio of the expected to the observed number of daphnids left after a transfer. + indicate variables/interactions used in each model.

Model	**Model Variables**			**AICc**	**LogLik**	**df**	**ΔAICc**	**wi**
	Treatment	**History**	**Treatment:History**					
Average expected/ observed	+	+		-79.4	46.349	6	0.00	0.602
		+		-78.1	43.331	4	1.34	0.308
	+	+	+	74.4	46.350	8	4.99	0.050
	+			-73.1	42.017	5	6.28	0.026
				-71.9	39.129	3	7.50	0.014

AIC statistics: AIC_c AIC statistic; *LogLik* log likelihood; *df* degrees of freedom; ΔAIC_c AIC$_c$ minus top model AIC$_c$, *wi* model weight

Table S8: Relative importance values (RIVs) for driver variable of linear mixed-effects models of assessing the impact of treatment (the presence of *Bythotrephes*, fish, or no chemical cues) and history of coexistence (daphnids from pre- or post-*Bythotrephes* invasion time periods) on the average escape ability of *D. pulicaria*. RIVs are a summed and standardized indicator of predictor variable rank across all possible models. Observed/Expected escape ability is expressed as a ratio of the expected to the observed number of daphnids left after a transfer.

	Treatment	**History**	**Treatment:History**
Sum of weights	0.68	0.96	0.05
Number of containing models	3	3	1

Chapter three—The art of self-defense: morphological changes of *Daphnia* in response to the invasive predatory water flea, *Bythotrephes*

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Abstract

1. Non-native predators can have profound effects on native prey communities. Native prey species can respond to the invasion of non-native predators in a variety of ways—from no response to the development of inducible or constitutive defenses. Induced or constitutive morphological changes can function as defense mechanisms, making capture or ingestion by predators more difficult. We assess whether multiple *Daphnia* species native to Lake Mendota (U.S.A.) are responding to the invasion of the spiny water flea, *Bythotrephes*, by developing morphological defenses.

2. We used a novel resurrection ecology approach to recover and hatch *Daphnia* resting eggs from lake sediments that were deposited before and after the invasion of *Bythotrephes*. Established genotypes of two *Daphnia* species from pre- and post-invasion time periods were raised in the presence and absence of *Bythotrephes* chemical cues for four weeks. Multiple traits were measured and compared to test for inducible and constitutive defenses.

3. Daphnids displayed both constitutive and induced changes in key morphological traits. *Daphnia ambigua* expressed a constitutive reduction in eye diameter and adult body length while *Daphnia pulicaria* did not display any constitutive changes. Both *D. pulicaria* and *D. ambigua* exhibited inducible changes in morphology. For example, *D. ambigua* head length and *D. pulicaria* caudal spine length and adult body length were all altered by the presence of *Bythotrephes* chemical cues. By measuring previously unassessed traits, we found that native *Daphnia* are responding to *Bythotrephes* with species-specific morphological changes.

4. The design of our study allowed us to demonstrate that native prey populations can track the introduction of a non-native predator via a combination of inducible and constitutive defenses, but that despite this, native prey populations can still be heavily impacted by invasive predators.

Keywords: antipredator defense, *Daphnia pulicaria*, *Daphnia ambigua*, zooplankton, resurrection ecology, morphology, spiny water flea, novel predator

Introduction

The introduction of non-native predators is common in many systems. It is often assumed that native prey are particularly vulnerable to the invasion of non-native predators because of the lack of a shared evolutionary history (Cox & Lima, 2006; Sih et al., 2010). However, native prey species can respond to the introduction of non-native predators in several ways, ranging from extirpation to development of inducible or constitutive anti-predator defenses. Antipredator defenses may evolve when there is a reliable cue that both indicates the threat proximity and activates a defense in the prey species, when the prey defenses are effective against the predator, and when the cost incurred by developing the defense is offset by the benefit of the defense (Harvell & Tollrian, 1999). Inducible defenses are phenotypic changes that are only present when cues associated with biotic agents are present and thus are adaptations to strong but fluctuating predation risk (Tollrian & Leese, 2010). Constitutive defenses, on the other hand, are always phenotypically present and are more likely to evolve under constant predation pressure (Tollrian & Leese, 2010). Native prey populations can track environmental changes such as the introduction of a non-native predator via a combination of phenotypic plasticity and/or genetic adaptation (Stoks et al., 2015; Landy et al., 2020).

We assessed the impact of *Bythotrephes cederströmii* (Bur et al., 1986; Korovchinsky & Arnott, 2019), a visual raptorial zooplanktivore, on *Daphnia*, an herbivorous zooplankter. Both *Bythotrephes* and *Daphnia* are freshwater cladocerans. *Bythotrephes* is native to northern and central Europe and Asia and is thought to have been introduced to the Laurentian Great Lakes in the 1980s via ballast water in ships (Yan et al., 1992; 2011). Since their initial invasion, *Bythotrephes* have spread to many other inland lakes where they often reach extremely high densities (Yan et al., 2011). This has caused concern about the impacts of *Bythotrephes* on native zooplankton species, especially given the post-invasion shifts in community composition and diversity that have been noted (Kelley et al., 2013; Kerfoot et al., 2016). While some zooplankton species do not seem to recognize the presence of *Bythotrephes* (*Bosmina liederi*; Kerfoot et al., 2016), others seem to have developed anti-predator defenses including changes in life history (in *D. pulicaria*; Gillis & Walsh, 2017), vertical distribution (in copepods; Bourdeau et al., 2011), and morphology (in *D. mendotae*; Bungartz & Branstrator, 2003).

Daphnia species have been heavily impacted because they are the preferred prey of *Bythotrephes* (Yan et al., 2011). *Daphnia* serve both as an important food source for commercially and recreationally important fish species, and as a primary grazer of algae in lakes (Lampert, 2011). We wanted to better understand the impact of *Bythotrephes* on *Daphnia* native to Lake Mendota (Wisconsin, U.S.A.). Although Lake Mendota was initially invaded in 1995, a *Bythotrephes* population boom in 2009 led to a trophic cascade. Heavy predation of *Bythotrephes* on *Daphnia* led to low *Daphnia* densities, reduced grazing of algae, and ultimately decreased water clarity due to increased algal biomass (Walsh et al., 2016a).

We explored several non-exclusive hypotheses about how *Daphnia* morphology might respond to *Bythotrephes* chemical cues. The prey naivete hypothesis predicts that post-invasion daphnids will display morphological anti-predator defenses, while pre-invasion daphnids will not display such changes due to a respective presence and absence of a shared evolutionary history (H1; Table 1). As there are both large seasonal and annual fluctuations in *Bythotrephes* abundance (Walsh et al., 2016b), we postulated that *Daphnia* would develop a combination of constitutive and inducible defenses. We hypothesized that the anti-predator morphology of daphnids would either make the *Daphnia* less visible to predators (visibility hypothesis/H2; Table 1) or make them more difficult for *Bythotrephes* to handle and ingest (invertebrate predation hypothesis/H3; Table 1). In *Daphnia*, both reduced body lengths and smaller eye diameters decrease detection by visual predators such as *Bythotrephes* because pigmentation is concentrated mainly in the large black compound eye of these otherwise transparent animals (Zaret, 1972; Dodson, 1974; Boersma et al., 1998; Branstrator & Holl, 2000). On the other hand, defensive morphologies such as the development of elongated heads (helmets), caudal spines, and body lengths can reduce successful capture and ingestion of prey (Dodson, 1974; Pastorok, 1981; Balseiro & Vega, 1994). Therefore, there is a trade-off between daphnids either being smaller, more difficult to see, and easier to handle, or being larger, more difficult to handle, and easier to see. We used a novel resurrection ecology approach (Kerfoot et al., 1999) to directly reconstruct ancestral phenotypes and examine whether multiple *Daphnia* species showed induced and/or constitutive changes in morphology in response to the *Bythotrephes* invasion.

Methods

Origin of clones

Lake Mendota is a eutrophic lake located along the southern edge of *Bythotrephes* invasion range in southeastern Wisconsin (Walsh et al., 2016b). Analysis of sediment cores from Lake Mendota suggests the initial *Bythotrephes* invasion took place in 1994 (Walsh et al., 2016b). In addition to *Bythotrephes*, Lake Mendota contains a variety of visual and tactile zooplanktivores including *Chaoborus spp*., *Leptodora kindti*, and approximately 20 fish species (Magnuson et al., 2019; Magnuson et al., 2020, Spear et al., 2020). Aside from *Bythotrephes*, which experienced a population boom in 2009, these predators have maintained stable populations over the past ~30 years (Magnuson et al., 2019; Magnuson et al., 2020, Spear et al., 2020). Lake Mendota is a North Temperate Lakes Long-Term Ecological Research site (https://lter.limnology.wisc.edu) and thus, has been consistently monitored since 1976.

The *Daphnia* clones used in this study were hatched from resting eggs isolated from the sediments of Lake Mendota. *Daphnia* reproduce through cyclical parthenogenesis, meaning they have both clonal (apomictic) reproduction and can produce sexual (mictic) resting eggs (Lampert, 2011). Resting eggs settle in lake sediments, which layer over time, creating a “time-capsule” of *Daphnia* resting eggs. We were able to hatch three *Daphnia* species (*D. pulicaria*, *D. mendotae*, and *D. ambigua*) from resting eggs isolated from two different sediment depths: 1) pre-*Bythotrephes* invasion sediments (1987–1990) and post-*Bythotrephes* invasion sediments (2009–2017). Previous ^{210}Pb dating of Lake Mendota sediments was used to estimate the dates of the sediments collected (Walsh et al., 2016b). *Daphnia* clones from these different sediment layers were considered to belong to different subpopulations of one continuous population. Two clonal lineages were established per time period for each species. We describe results from *D. ambigua* and *D. pulicaria* clones; *D. mendotae* data were excluded due to high mortality. See Kiehnau and Weider (2019) for sediment core collection and hatching protocols and see supplement S1 for a description of stock culture care.

Experimental conditions

To address whether *Bythotrephes* chemical cues illicit morphological changes in *Daphnia* prey, *Daphnia* clones from pre- and post-invasion time periods were raised in *Bythotrephes* chemical cue and no cue media, then were preserved and key morphological traits were measured. The exposure experiment began in June 2018 and lasted four weeks. For each clonal line, four treatment and four control jars were established (2 species x 2 clones x 2 subpopulations x 2 treatments x 4 replicates = 64 total jars). Each jar was filled with 0.7 L artificial pond water (COMBO; Kilham et al., 1998) and was inoculated with 12 pre-reproductive individuals. Pre-reproductive individuals were identified via size and the lack of a visible brood chamber. For each clone, pre-reproductive individuals were pooled from multiple stock jars into a single jar prior to being haphazardly distributed into the experimental jars. All experimental animals came from maternal lines raised under identical conditions (see description in supplement S1). Experimental jars were placed in transparent plastic boxes (6 jars per box). Jar placement was randomly assigned at the start of the experiment and box placement was randomized every three days during the experiment. For the duration of the experiment, daphnids were grown at a constant temperature (~20°C) and received equal amounts of lighting 24 hours a day in a temperature-controlled room. Experimental jars were fed a 50:50 mixture of the green algae *Nannochloropsis* sp. (34 million cells mL^{-1}; Nano 3600™, Reed Mariculture, Campbell, CA) and live, chemostatically-cultured green algae *Scenedesmus acutus* (0.5 mg C L^{-1}).

Treatments consisted of *Bythotrephes* cue (treatment) and no cue (control) media. Treatment homogenate was made using a mortar and pestle to crush live-frozen *Bythotrephes* in a small amount of sterile autoclaved COMBO (1 *Bythotrephes* mL^{-1} COMBO). Live-frozen *Bythotrephes* were collected from several lakes in southern Ontario with varied invasion histories (EL Kiehnau, unpublished data). *Bythotrephes* cue (treatment) media was then made by adding 2 mL treatment homogenate to each treatment jar resulting in a final concentration of approximately 2.7 *Bythotrephes* L^{-1} (Bungartz & Branstrator, 2003). We used chemical cues from live-frozen, field collected *Bythotrephes* because previous research has demonstrated induction of morphological defenses in *Daphnia* using chemical cues from *Bythotrephes* frozen alive (Bungartz & Branstrator, 2003). Control jars were treated by adding 2 mL sterile autoclaved COMBO per jar. Fresh *Bythotrephes* cue or control inoculate were added to jars every three days during the experiment.

Response variables

In accordance with the methods of Bungartz & Branstrator (2003), the experiment was ended after four weeks and the contents of the jars were preserved. Jar contents were poured through a 64 µM Nitex (®) mesh sieve, shocked in 95% ethanol, and then transferred for long-term storage into 70% ethanol (Black & Dodson, 2003). All of the *Daphnia* in the jars at the end of the experiment were enumerated and categorized as belonging to one of the following life stages: pre-reproductive female, adult female without eggs, adult female with eggs, adult female with resting egg, or male. See supplementary methods, results and discussion for a detailed exploration of final abundance data.

Preserved animals were photographed using an iPhone 7 mounted on a dissecting microscope (Leica MZ8 stereomicroscope on a Transmitted Light Stand HL with an NCL 150 light source). Animals over the full size-range were selected randomly by inverting each sample bottle four times and using a wide-bore pipette to draw off animals. The first 10 individuals photographed per jar were classified as either pre- or post-reproductive and measured for the following traits: eye diameter (width of eye at widest location), head length (helmet crest to eye center), caudal spine length (caudal spine base to tip), and body length (caudal spine base to eye center) (Figure 1). Measurements were collected using ImageJ (Schneider et al., 2012). Traits were measured in pixels and subsequently converted into millimeters. Pixel length was determined from photographs of a stage-micrometer taken before *Daphnia* photos. After measurements were collected, relative ratios were calculated for eye diameter, head length, and caudal spine length so that comparisons could be made across individuals of varying size.

Relative ratios were calculated with body length as the denominator and the trait measurement as the numerator.

After measurements were collected from 10 individuals per jar, the total number of adults (post-reproductive individuals) measured per jar was assessed. If there were fewer than five adult *Daphnia* measured, measurements were collected from additional individuals until five adult *Daphnia* per jar (when possible) were measured. These additional adult measurements were only used in the analysis of body length, which only included data from adult individuals in order to assess potential changes in body length of adult *Daphnia*.

Statistical analysis

Statistical analyses were conducted using R version 3.6.3 (R Core Team 2020) or IBM SPSS (version 26; IBM 2020). Prior to analyses, body length, eye diameter ratio, head length ratio, and caudal spine ratio data were transformed in R using Tukey's ladder of powers (rcompanion package; Mangiafico, 2020) to meet multivariate analysis of covariance (MANCOVA) and analysis of covariance (ANCOVA) assumptions. Unless otherwise specified, the alpha level for all tests was set at 0.05.

Trait ratios were analyzed in SPSS using a MANCOVA (general linear model, multivariate option with type III sum of squares). We used separate MANCOVA tests to analyze *D. pulicaria* and *D. ambigua* data. Fixed factors included treatment (*Bythotrephes* chemical cue or no cue media), subpopulation (pre- or post-*Bythotrephes* invasion time periods), clone nested in subpopulation, subpopulation × treatment interaction, and clone nested in subpopulation × treatment interaction. Total abundance of daphnids at the end of the experiment (hereafter final abundance) was specified as a covariate because level of expression of defensive traits is influenced by prey density (Tollrian et al., 2015). Eye diameter ratio, head length ratio, and caudal spine length ratio were specified as dependent variables. Post-hoc ANCOVA tests were conducted for each dependent variable to further interpret the multivariate response. Because of inflated Type I error rate due to multiple testing (one test for each dependent variable, three in total), a Bonferroni-type adjustment was made and the alpha level for these tests was set at 0.017.

Adult body length data were analyzed in R using an ANCOVA with *D. pulicaria* and *D. ambigua* data analyzed using separate tests (R Core Team 2020). Similar to the MANCOVA tests, treatment, subpopulation, clone nested in subpopulation, subpopulation × treatment interaction, and clone nested in subpopulation × treatment interaction served as fixed factors, while final abundance was the covariate, and adult body length was the dependent variable.

Results

D. ambigua morphology

MANCOVA results (Table 2) indicated that predator cue treatments did not have an overall significant effect on the morphology of *D. ambigua* ($F_{3,21}$=0.967, p=0.427). Instead, the morphological response of *D. ambigua* clones to *Bythotrephes* chemical cues varied depending on whether they were from pre- or post-*Bythotrephes* invasion time periods ($F_{3,21}$=3.562, p=0.032). In addition, we found overall differences in the morphological traits of pre- and post-invasion clones, regardless of the presence of predator cues ($F_{3,21}$=9.753, p<0.001). Clonal differences within subpopulations were also apparent ($F_{6,42}$=10.885, p<0.001), indicating that although subpopulation identity influences morphological characteristics, clonal variability within subpopulations remains (Figure 2). In addition, final abundance of daphnids at the end of the experiment influenced *D. ambigua* morphology ($F_{3,21}$=6.767, p=0.002).

Additional univariate analyses indicated that caudal spine length ratio remained constant across time periods, treatments, and jars of varying final abundances, while significant changes in eye diameter ratio, head length ratio, and adult body length occurred (Table 2). Eye diameter ratio displayed significant clonal variation within subpopulations (Figure S1; $F_{2,23}$=20.477, p<0.001) although clones from post-invasion times had, on average, significantly smaller eye diameters than clones from pre-invasion times (Figure 2; $F_{1,23}$=31.821, p<0.001). Final abundance at the end of the experiment also influenced eye diameter, as *D. ambigua* from densely populated jars had smaller eye diameters than *D. ambigua* from lower density jars (Figure S1; $F_{1,23}$=15.269, p=0.001). The average head length ratio of pre- and post-invasion *D. ambigua* exposed to *Bythotrephes* chemical cues varied ($F_{1,23}$=7.962, p=0.010). Pre-invasion clones developed smaller head length ratios, while post-invasion clones developed larger head length ratios when exposed to predator cues (Figure 2). Significant clonal differences in head length ratio were also noted (Figure S2; $F_{2,23}$=12.540, p<0.001). For instance, the median head length ratio of clone pre 1 was 1.19-fold larger than clone pre 2 (Figure S2). The adult body length of post-invasion *D. ambigua* was significantly smaller than pre-invasion *D. ambigua* regardless of treatment or subpopulation identity (Figure 2; $F_{1,23}$=10.240, p=0.004). Overall, subpopulation identity, clonal identity, final abundance, and treatment × subpopulation interaction all impacted key morphological traits of *D. ambigua* (Table 2).

D. pulicaria morphology

The results of the MANCOVA (Table 3) indicated that predator cue treatment significantly affected *D. pulicaria* morphology regardless of subpopulation identity, indicating that *D. pulicaria* may respond to *Bythotrephes* with inducible changes in morphology ($F_{3,17}$=3.641, p=0.034). Although no overall subpopulation effects were observed ($F_{3,17}$=1.970, p=0.157), there were clonal differences within *D. pulicaria* subpopulations (Figure 3; $F_{6,34}$=3.973, p=0.004). Aside from clonal identity and exposure to predator cues, final abundance significantly affected the overall morphology of *D. pulicaria* clones ($F_{3,17}$=3.557, p=0.037).

Further univariate analysis revealed that eye diameter ratio remained constant across time periods, treatments, and jars of varying densities, while significant changes in head length ratio, caudal spine length ratio, and adult body length were observed (Table 3). The head length ratio of *D. pulicaria* clones varied significantly within subpopulations (Figure S3; $F_{2,19}$=7.209, p=0.005). For example, among *D. pulicaria* clones, median head length ratio of clone pre 1 was 1.2-fold larger than clone pre 2 (Figure S3), suggesting high variability in head length ratios within subpopulations. Treatment, on the other hand, significantly affected caudal spine length ratio regardless of subpopulation or clonal identity ($F_{1,19}$=10.409, p=0.004). The caudal spine length ratio of both pre- and post-invasion *D. pulicaria* clones decreased when daphnids were grown in the presence of *Bythotrephes* chemical cues (Figure 3). Finally, *D. pulicaria* adult body length was significantly affected by the treatment × subpopulation interaction ($F_{1,19}$=6.779, p=0.017). Body length of adult pre-invasion animals decreased in the presence of *Bythotrephes* chemical cues, while the body length of adult post-invasion animals increased in the presence of treatment (Figure 3). Altogether, the presence of predator cues, clonal identity, and treatment × subpopulation interaction all impacted the morphology of the *D. pulicaria* clones (Table 3).

Discussion

Daphnia native to Lake Mendota are responding to the invasion of *Bythotrephes* via a combination of constitutive and inducible changes in key morphological traits. We did not find evidence to support the prey naivete hypothesis (H1, Table 1) as both pre- and post-invasion daphnids can detect chemical cues from *Bythotrephes*. We found support for both the visibility hypothesis and the invertebrate predation hypothesis (H2 and H3, Table 1), as some daphnids displayed reduced eye diameters and body lengths, while others developed elongated body lengths. Different *Daphnia* species employed different antipredator defense strategies.

***D. ambigua* morphology**

Changes in *D. ambigua* morphology support the visibility hypotheses (H2, Table 1). We observed constitutive reductions in eye diameter ratio and adult body length. This may aid post-invasion *D. ambigua* in being less susceptible to *Bythotrephes* predation, as prey with smaller eye diameters and body lengths experience reduced detection by visual predators (Zaret, 1972; Dodson, 1974; Boersma et al., 1998; Branstrator & Holl, 2000; Beston et al., 2017). Although the morphological changes of *D. ambigua* support the visibility hypothesis in general, post-invasion *D. ambigua* clones exhibited increased head length ratios when raised in the presence of *Bythotrephes* chemical cues. This supports the invertebrate predation hypothesis (H3, Table 1) because elongated head length ratios are associated with the development of defensive “helmets” which make capture and ingestion by predators more difficult (Laforsch & Tollrian, 2004).

In accordance with previous findings, we found clone-specific variation in eye diameter (Brandon et al., 2015) and head length ratio (Bungartz & Branstrator, 2003). Because of the documented clonal variation in morphology, testing additional clones would verify the robustness of these observed morphological responses. However, the caudal spine length ratio of *D. ambigua* clones remained consistent across treatments and time periods. This lack of response is unsurprising, as elongated caudal spines are not a consistent antipredator response of *D. ambigua* (Dodson, 1989) and indicates that not all key morphological traits necessarily play a role in predator deterrence in all *Daphnia* species.

The level of expression of defensive traits decreases with increasing prey density (Tollrian et al., 2015). We observed several instances where final abundance influenced morphological changes in *D. ambigua*. For example, *D. ambigua* from jars with higher final abundances had smaller eye diameter ratios. Resources may have been limiting in jars with more individuals, and since *Daphnia* eye size is reduced by resource limitation (Brandon & Dudycha, 2014), it is likely that daphnids in jars with higher final abundances had fewer resources to divert to eye development. Similarly, pre-invasion *D. ambigua* developed reduced head length ratios when raised in the presence of *Bythotrephes* chemical cues. Cue jars tended to contain more *Daphnia* at the end of the experiment and those daphnids may have been experiencing food limitation. *Daphnia* experiencing food/nutrient limitation reduce their investment in defense, even when predator chemical cues are present (Jeyasingh & Weider, 2005). Thus, pre-invasion *D. ambigua* may have developed reduced head length ratios because they were experiencing food limitation.

D. pulicaria morphology

The results of this study suggest that *D. pulicaria* are not using reduced visibility as a strategy to avoid *Bythotrephes*. For example, contrary to the predictions of the visibility hypothesis (H2; Table 1), neither post- nor pre-invasion *D. pulicaria* had significantly reduced eye diameter ratios when grown in the presence of predator chemical cues. In addition, when raised in the presence of *Bythotrephes* chemical cues, post-invasion clones had longer adult body lengths than pre-invasion clones. This increase in adult body size supports previous findings that noted a 21-32% increase in adult body size when pre- and post-invasion *D. pulicaria* from invaded lakes were compared (Gillis & Walsh, 2017). Increased body length was predicted by the invertebrate predation hypothesis (H3, Table 1) and is an antipredator defense typically triggered by invertebrate predators (Dodson, 1974). However, pre-invasion *D. pulicaria* clones, responded to the presence of *Bythotrephes* chemical cues like they would to fish chemical cues (H2, Table 1), and reduced their adult body lengths. Similar defense shifts—whereby *Daphnia* shift their reaction norms from before and after the introduction—have been observed in other systems which have experienced the introduction of a predator (Stoks et al., 2015). These shifts are thought to be fueled by the existence of ancestral plasticity and standing genetic variation for plasticity (Stoks et al., 2015) and previous research has revealed significant standing genetic variation in phenotypic plasticity of *Daphnia* from Lake Mendota for a variety of life history and behavioral traits (Landy et al., 2020).

Contrary to the invertebrate predation hypothesis (H3, Table 1), the caudal spine length ratio of both pre- and post-invasion *D. pulicaria* clones was smaller in the presence of *Bythotrephes* chemical cues. Elongated caudal spine length ratio is a typical anti-predator defense against invertebrate predators (Balseiro & Vega, 1994). Thus, it is surprising that *D. pulicaria* show the opposite trend. *D. pulicaria* clones may be developing reduced spine length ratios in the presence of *Bythotrephes* cues because even with elongated spines, *D. pulicaria* cannot get large enough to completely avoid capture and ingestion by *Bythotrephes* (Schulz & Yurista, 1999). Rather than devoting resources towards elongation, they may be focused on making changes to their 3-D body shape. For example, predator cues can induce “bulkiness” (Tollrian, 1995; Rabus & Laforsch, 2011) and body torsion (Herzog et al., 2016) as defensive mechanisms. Alternatively, *D. pulicaria* may be investing in non-morphological antipredator defenses such as altered life histories (Weider & Pijanowska, 1993) or enhanced alertness (Boersma et al., 1998). In fact, a recent study found that contemporary *D. pulicaria* clones from Lake Mendota matured earlier than clones from pre-invasion time periods, suggesting that *D.*

pulicaria have undergone shifts in life history traits following the establishment of *Bythotrephes* (Landy et al., 2020).

Neither the presence of *Bythotrephes* chemical cues nor a history of coexistence significantly affected *D. pulicaria* head length ratios. This is expected because *D. pulicaria* are not known to develop helmets in response to predators (Dodson, 1989). *D. pulicaria* clones did, however, display variability in head length ratios. This is unsurprising as morphological variation amongst *Daphnia* clones is common (Boersma et al., 1998).

Comparison of interspecific responses

Native *Daphnia* are responding to the invasion of *Bythotrephes* with both constitutive and induced changes in key morphological traits. Constitutive changes were expected due to strong *Bythotrephes* predation pressure (Walsh et al., 2016a). While *D. pulicaria* had no apparent constitutive defenses, *D. ambigua* expressed a constitutive reduction in eye diameter ratio and adult body length. Development of inducible defenses were also expected because of the large seasonal and annual fluctuations in *Bythotrephes* densities observed in Lake Mendota (Walsh et al., 2016a). Both *D. pulicaria* and *D. ambigua* exhibited inducible changes in morphology. *D. ambigua* head length ratio and *D. pulicaria* caudal spine length ratio and adult body length were all altered by the presence of *Bythotrephes* chemical cues.

Pre-invasion *Daphnia* detected and responded to *Bythotrephes* chemical cues despite the absence of a shared evolutionary history, rejecting the prey naivete hypothesis (H1; Table 1) and in contrast to previous predictions that many North American zooplankton species do not recognize the presence of *Bythotrephes* and do not exhibit behavioral or morphological defenses (Kerfoot et al., 2016). Pre-invasion *D. pulicaria* had reduced caudal spine length ratios and adult body lengths when raised in the presence of *Bythotrephes* chemical cues.

While the morphology of both *Daphnia* species changed in response to *Bythotrephes*, the key traits and the way the traits changed were species-specific. Changes in *D. ambigua* morphology generally followed the predictions of the visibility hypothesis (H2; Table 1), while *D. pulicaria* trait changes did not consistently follow the predictions of either hypothesis posed. For example, when exposed to *Bythotrephes* chemical cues, post-invasion *D. pulicaria* developed larger core body lengths (supporting H3; Table 1), but smaller caudal spine length ratios (opposing H3; Table 1). *D. pulicaria* may make capture and ingestion by *Bythotrephes* more difficult by focusing on 3-D body changes (for examples see Tollrian, 1995; Rabus & Laforsch, 2011; Herzog et al., 2016) rather than elongation as was predicted by the invertebrate predation hypothesis (H3, Table 1). Such proposed additional morphological responses warrant further examination.

Conclusion

Daphnia are responding to the invasion of *Bythotrephes* with species-specific changes in key morphological traits. It is likely, however, that they are combating *Bythotrephes* predation with a variety of antipredator defenses and thus, other changes such as life history and behavioral shifts should also be assessed. For example, both *D. mendotae* and *D. pulicaria* have exhibited life history shifts following the establishment of *Bythotrephes* (Landy et al., 2020). The differences in final abundance we observed, suggest that *D. ambigua* may have also experienced a shift towards production of more, smaller offspring (Figure S4); however, further verification via life history experiments is needed.

Although it is often assumed that native prey species are vulnerable to the invasion of non-native predators due to the lack of a shared evolutionary history (Cox & Lima, 2006; Sih et al., 2010), we have demonstrated that native prey populations can track the introduction of a non-native predator via a combination of inducible and constitutive defenses. These data contribute to growing evidence that native species, like invaders, can rapidly adapt to novel selective pressures (e.g. Langkilde, 2009; Stoks et al., 2015; Bible et al., 2017; Landy et al., 2020). Our findings suggest that despite the ability of prey to detect and respond to invasive predators with inducible and constitutive defenses, native prey populations can be heavily impacted by invasive predators (Walsh et al., 2016a). Although defenses often slow predation, they do not prevent predation entirely. Clearly, future research is warranted to decipher the ability of native prey species to use morphological, behavioral, and life history adaptations to serve as deterrents to novel predators.

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Data availability statement

The data associated with this study are available in the Open Science Framework repository: https://osf.io/86a4t/?view_only=bc5cd1d983a84616b3fc6ddeb1e568b2

References

Balseiro, E.G., & Vega, M. (1994). Vulnerability of *Daphnia middendorffiana* to *Parabroteas sarsi* predation: the role of the tail spine. *Journal of Plankton Research*, 16, 783–793. https://doi.org/10.1093/plankt/16.7.783

Beston, S.M., Wostl, E., & Walsh, M.R. (2017). The evolution of vertebrate eye size across an environmental gradient: phenotype does not predict genotype in a Trinidadian killifish. *Evolution*, 71, 2037–2049. https://doi.org/10.1111/evo.13283

Bible, J.M., Griffith, K.R., & Sanford, E. (2017). Inducible defenses in Olympia oysters in response to an invasive predator. *Oecologia*, 183, 809–819. https://doi.org/10.1007/s00442-017-3811-x

Black, A.R., & Dodson, S.I. (2003). Ethanol: a better preservation technique for *Daphnia*. *Limnology and Oceanography: Methods*, 1, 45–50. https://doi.org/10.4319/lom.2003.1.45

Boersma, M., Spaak, P., & De Meester, L. (1998). Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: the uncoupling of responses. *The American Naturalist*, 152, 237–248. https://doi.org/10.1086/286164

Bourdeau, P.E., Pangle, K.L., & Peacor, S.D. (2011). The invasive predator *Bythotrephes* induces changes in the vertical distribution of native copepods in Lake Michigan. *Biological Invasions*, 13, 2533–2545. https://doi.org/10.1007/s10530-011-0073-0

Brandon, C.S., & Dudycha, J.L. (2014). Ecological constraints on sensory systems: compound eye size in *Daphnia* is reduced by resource limitation. *Journal of Comparative Physiology A*, 200, 749–758. https://doi.org/10.1007/s00359-014-0918-y

Brandon, C.S., James, T., & Dudycha, J.L. (2015). Selection on incremental variation of eye size in a wild population of *Daphnia*. *Journal of Evolutionary Biology*, 28, 2112–2118. https://doi.org/10.1111/jeb.12711

Branstrator, D.K., & Holl, C.M. (2000). Planktivory by bluegill (*Lepomis macrochirus*) on *Leptodora kindti* in a small North American lake. *Hydrobiologia*, 437, 101–106. https://doi.org/10. 1023/A:1026530201948

Bungartz, B., & Branstrator, D.K. (2003). Morphological changes in *Daphnia mendotae* in the chemical presence of *Bythotrephes longimanus*. *Archiv fur Hydrobiologie*, 158, 97–108. https://doi.org/ 10.1127/0003-9136/2003/0158-0097

Bur, M.T., Klarer, D.M., Krieger, K.A. (1986). First Records of a European Cladoceran, *Bythotrephes Cederstroemi*, in Lakes Erie and Huron. *Journal of Great Lakes Research*, 12, 144–146. https://doi.org/10.1016/S0380-1330(86)71711-5

Cox, J., & Lima, S. (2006). Naiveté and an aquatic-terrestrial dichotomy in the effects of introduced predators. *Trends in Ecology and Evolution*, 21, 674–680. https://doi.org/10.1016/j.tree. 2006.07.011

Dodson, S.I. (1974). Adaptive change in plankton morphology in response to size-selective predation: a new hypothesis of cyclomorphosis. *Limnology and Oceanography*, 19, 721–729. https://doi.org/10.4319/lo.1974.19.5.0721

Dodson, S.I. (1989). The ecological role of chemical stimuli for the zooplankton: predator-induced morphology in *Daphnia*. *Oecologia*, 78, 361–367. https://doi.org/10.1007/BF00379110

Gillis, M.K., & Walsh, M.R. (2017). Rapid evolution mitigates the ecological consequences of an invasive species (*Bythotrephes longimanus*) in lakes in Wisconsin. *Proceedings of the Royal Society B*, 284, 20170814. http://dx.doi.org/10.1098/rspb.2017.0814

Harvell, C.D., & Tollrian, R. (1999). Why Inducible Defenses? Chapter 1. In: The ecology and evolution of inducible defenses (Eds: R. Tollrian & C.D. Harvell). Princeton University Press, Princeton, NJ.

Herzog, Q., Rabus, M., Ribeiro, B.W., Laforsch, C. (2016). Inducible defenses with a “twist”: *Daphnia barbata* abandons bilateral symmetry in response to an ancient predator. *PLoS ONE*, 11, e0148556. https://doi.org/10.1371/journal.pone.0148556

IBM. (2020). IBM SPSS statistics for Windows. Version 26.0. IBM, Armonk, New York, USA.

Jeyasingh, P.D., & Weider, L.J. (2005). Phosphorus availability mediates plasticity in life-history traits and predator-prey interactions in *Daphnia*. *Ecology Letters*, 8, 1021–1028. https://doi.org/10.1111/j.1461-0248.2005.00803.x

Kelley, N.E., Yan, N.D., Walseng, B., & Hessen, D.O. (2013). Differential short- and long-term effects of an invertebrate predator on zooplankton communities in invaded and native lakes. *Diversity and Distributions*, 19, 396–410. https://doi.org/10.1111/j.14724642.2012.00946.x

Kerfoot, W.C., Robbins, J.A., & Weider, L.J. (1999). A new approach to historical reconstruction: Combining descriptive and experimental limnology. *Limnology and Oceanography*, 44, 1232-1247. https://doi.org/10.4319/lo.1999.44.5.1232

Kiehnau, E.L., & Weider, L.J. (2019). Phototactic behavior of native *Daphnia* in the presence of chemical cues from a non-native predator *Bythotrephes*. *Oecologia*, 190, 799–809. https://doi.org/10.1007/s00442-019-04461-6

Kilham, S.S., Kreeger, D.A., Lynn, S.G., Goulden, C.E., & Herrera, L. (1998). COMBO: A defined freshwater culture medium for algae and zooplankton. *Hydrobiologia*, 377, 147–159. https://doi.org/10.1023/A:1003231628456

Korovchinsky, N.M., & Arnott, S.E. (2019). Taxonomic resolution of the North American invasive species of the genus *Bythotrephes* Leydig, 1860 (Crustacea: Cladocera: Cercopagididae). *Zootaxa*, 4691, 125–138. https://doi.org/10.11646/zootaxa.4691.2.2

Kerfoot, W.C., Hobmeier. M.M., Yousef, F., Moraska Lafrancois, B., Maki, R.P., & Hirsch, J.K. (2016). A plague of waterfleas (*Bythotrephes*): Impacts on microcrustacean community structure, seasonal biomass, and secondary production in a large inland-lake complex. *Biological Invasions*, 18, 1121–1145. https://doi.org/10.1007/s10530-015-1050-9

Laforsch, C., & Tollrian, R. (2004). Inducible defenses in multipredator environments: cyclomorphosis in *Daphnia cucullata*. *Ecology*, 85, 2302–2311. https://doi.org/10.1890/03-0286

Lampert, W. (2011). *Daphnia* as model organism. Chapter 1. In: *Daphnia*: Development of a model organism in ecology and evolution. Excellence in Ecology: Book 21. (Ed: O. Kinne). International Ecology Institute, Oldendorf/Luhe, Germany.

Landy, J.A., Oschmann, A., Munch, S.B., & Walsh, M.R. (2020). Ancestral genetic variation in phenotypic plasticity underlies rapid evolutionary changes in resurrected populations of waterfleas. *Proceedings of the National Academy of Sciences*, 117, 32535–32544. https://doi.org/10.1073/pnas.2006581117

Langkilde, T. (2009). Invasive fire ants alter behavior and morphology of native lizards. *Ecology*, 90, 208–217. https://doi.org/10.1890/08-0355.1

Magnuson, J., Carpenter, S., & Stanley, E. (2019). North Temperate Lakes LTER: Zooplankton - Madison lakes area 1997 - current. Environmental Data Initiative. https://doi.org/10.6073/pasta/8b265c0300252c87805f26f41e174aa4.

Magnuson, J., Carpenter, S., & Stanley, E. (2020). North Temperate Lakes LTER: Fish species richness 1981 - current. Environmental Data Initiative. https://doi.org/10.6073/pasta/dd290e862563c3f16edcb67552113d42.

Mangiafico, S. (2020). rcompanion: Functions to support extension education program evaluation. R package version 2.3.25. https://CRAN.R-project.org/package=rcompanion

Pastorok, R.A. (1981). Prey vulnerability and size selection by *Chaoborus* larvae. *Ecology*, 62, 1311–1324. https://doi.org/10.2307/1937295

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rabus, M., & Laforsch, C. (2011). Growing large and bulky in the presence of the enemy *Daphnia magna* gradually switches the mode of inducible morphological defenses. *Functional Ecology*, 25, 1137–1143. https://doi.org/10.1111/j.1365-2435.2011.01840.x

Schneider, C.A., Rasband, W.S., & Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature methods*, 9, 671–675. https://doi.org/10.1038/nmeth.2089

Schulz, K.L., & Yurista, P.M. (1999). Implications of an invertebrate predator's (*Bythotrephes cederstroemi*) atypical effects on a pelagic zooplankton community. *Hydrobiologia*, 380, 179–193. https://doi.org/10.1023/A:1003484813672

Sih, A., Bolnick, D.I., Luttbeg, B., Orrock, J.L., Peacor, S.D., Pintor, L.M., Preisser, E., Rehage, J.S., & Vonesh, J.R. (2010). Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos*, 119, 610–621. https://doi.org/10.1111/j.1600-0706.2009.18039.x

Spear, M.J., Wakker, P., Schmude, K.L., & Vander Zanden, J. (2020). Lake Mendota, Wisconsin, USA, (Non-Dreissenid) benthic macroinvertebrate abundance, biomass, and community composition 2016-2018. Environmental Data Initiative. https://doi.org/10.6073/pasta/5b74c51dfb8ff618aecda8b55d575b10.

Stoks, R., Govaert, L., Pauwels, K., Jansen, B., & De Meester, L. (2015). Resurrecting complexity: the interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea *Daphnia magna*. *Ecology Letters*, 19, 180–190. https://doi.org/10.1111/ele.12551

Tollrian, R. (1995). Predator-induced morphological defenses: costs, life history shifts, and maternal effects in *Daphnia pulex*. *Ecology*, 76, 1691–1705. https://doi.org/10.2307/1940703

Tollrian, R., & Leese, F. (2010). Ecological genomics: Steps towards unraveling genetic basis of inducible defenses in *Daphnia*. *BMC Biology*, 8, 51. https://doi.org/10.1186/1741-7007-8-51

Tollrian, R., Duggen, S., Weiss, L.C., Laforsch, C., & Kopp, M. (2015). Density dependent adjustment of inducible defenses. *Scientific Reports*, 5, 12736. https://doi.org/10.1038/srep12736

Walsh, J.R., Carpenter, S.R., & Vander Zanden, M.J. (2016a). Invasive species triggers a massive loss of ecosystem services through a trophic cascade. *Proceedings of the National Academy of Sciences*, 113, 4081–4085. https://doi.org/10.1073/pnas.1600366113

Walsh, J.R., Munoz, S.E., & Vander Zanden, M.J. (2016b). Outbreak of an undetected invasive species triggered by a climate anomaly. *Ecosphere*, 7, e01628. https://doi.org/10.1002/ecs2.1628

Weider, L.J., & Pijanowska, J. (1993). Plasticity of *Daphnia* life histories in response to chemical cues from predators. *Oikos*, 67, 385–392. https://www.jstor.org/stable/i283170

Yan, N.D., Dunlop, W.I., Pawson, T.W., & MacKay, L.E. (1992). *Bythotrephes cederstroemi* (Schoedler) in Muskoka Lakes: first records of the European invader in inland lakes in Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 49, 422–426. https://doi.org/10.1139 /f92-048

Yan, N.D., Leung, B., Lewis, M.A., & Peacor, S.D. (2011). The spread, establishment and impacts of the spiny water flea, *Bythotrephes longimanus*, in temperate North America: a synopsis of the special issue. *Biological Invasions*, 13, 2423–2432. https://doi.org/10.1007/s10530-011-0069-9

Zaret, T.M. (1972). Predators, invisible prey, & the nature of polymorphism in the Cladocera (class crustacea). *Limnology and Oceanography*, 17, 171–184. https://doi.org/10.4319/lo.1972.17.2.0171

Tables

Table 1: Proposed hypotheses regarding the development of morphological anti-predator defenses by native *Daphnia* in response to the invasive predator *Bythotrephes*.

No.	**Hypothesis name**	**Definition**
H1	Prey naiveté hypothesis[1,2]	If *Daphnia* lack a shared evolutionary history with *Bythotrephes* (i.e. pre-invasion daphnids) then they will not develop anti-predator defenses in response to *Bythotrephes* chemical cues.
H2	Visibility hypothesis[3]	If *Daphnia* are escaping *Bythotrephes* predation by reducing their visibility, then they will have smaller eye diameter ratios and reduced adult body lengths in response to *Bythotrephes* chemical cues.
H3	Invertebrate predation hypothesis[4]	If *Daphnia* are avoiding *Bythotrephes* predation by making handling and ingestion more difficult, then they will develop elongated head length ratios (i.e. helmets), caudal spine length ratios, and adult body lengths in response to *Bythotrephes* chemical cues.

Notes: References are given by numbers in the table: [1] Cox and Lima (2006), [2] Sih et al. (2010), [3] Zaret (1972), and [4] Dodson (1974).

Table 2: Effect of *Bythotrephes* chemical cue treatment (T), pre- or post-*Bythotrephes* invasion subpopulation identity (S), and clonal identity nested in subpopulation (C) on **A.** the morphology of *Daphnia ambigua* clones via MANCOVA, **B.** eye diameter ratio (EDR), head length ratio (HLR), and caudal spine length ratio (CSLR) via post-hoc univariate ANCOVAs, and **C**. adult body length via ANCOVA. A Bonferroni correction was applied to the alpha level for the univariate post-hoc tests due to multiple testing, alpha=0.0167. For other tests, alpha=0.05. P-values < alpha are shown in bold. *D. ambigua* were resurrected from Lake Mendota sediments deposited pre- and post-*Bythotrephes* invasion time periods.

	Treatment (T)		**Subpopulation (S)**		**Clone (C)**		**Final abundance**		**T x S**		**T x C**	
A.	$F_{3,21}$	*P*	$F_{3,21}$	*P*	$F_{6,42}$	*P*	$F_{3,21}$	*P*	$F_{3,21}$	*P*	$F_{6,42}$	*P*
All traits	0.967	0.427	9.753	**<0.001**	10.885	**<0.001**	6.767	**0.002**	3.562	**0.032**	2.043	0.081
B.	$F_{1,23}$	*P*	$F_{1,23}$	*P*	$F_{2,23}$	*P*	$F_{1,23}$	*P*	$F_{1,23}$	*P*	$F_{2,23}$	*P*
EDR	0.003	0.960	31.821	**<0.001**	20.477	**<0.001**	15.269	**0.001**	0.356	0.557	4.403	0.024
HLR	2.177	0.154	0.096	0.759	12.540	**<0.001**	2.032	0.167	7.962	**0.010**	0.696	0.509
CSLR	2.248	0.147	0.136	0.715	3.636	0.042	3.747	0.065	0.222	0.642	0.793	0.465
C.	$F_{1,23}$	*P*	$F_{1,23}$	*P*	$F_{2,23}$	*P*	$F_{1,23}$	*P*	$F_{1,23}$	*P*	$F_{2,23}$	*P*
BL	0.507	0.483	10.240	**0.004**	1.304	0.291	4.088	0.055	2.959	0.099	2.247	0.128

Table 3: Effect of *Bythotrephes* chemical cue treatment (T), pre- or post-*Bythotrephes* invasion subpopulation identity (S), and clonal identity nested in subpopulation (C) on **A.** the morphology of *Daphnia pulicaria* clones via MANCOVA, **B.** eye diameter ratio (EDR), head length ratio (HLR), and caudal spine length ratio (CSLR) via post-hoc univariate ANCOVAs, and **C**. adult body length via ANCOVA. A Bonferroni correction was applied to the alpha level for the univariate post-hoc tests due to multiple testing, alpha=0.0167. For other tests, alpha=0.05. P-values < alpha are shown in bold. *D. pulicaria* were resurrected from Lake Mendota sediments deposited pre- and post-*Bythotrephes* invasion time periods.

	Treatment (T)		**Subpopulation (S)**		**Clone (C)**		**Final abundance**		**T x S**		**T x C**	
A.	$F_{3,17}$	*P*	$F_{3,17}$	*P*	$F_{6,34}$	*P*	$F_{3,17}$	*P*	$F_{3,17}$	*P*	$F_{6,34}$	*P*
All traits	3.641	**0.034**	1.970	0.157	3.973	**0.004**	3.557	**0.037**	1.141	0.361	0.713	0.641
B.	$F_{1,19}$	*P*	$F_{1,19}$	*P*	$F_{2,19}$	*P*	$F_{1,19}$	*P*	$F_{1,19}$	*P*	$F_{2,19}$	*P*
EDR	1.805	0.195	5.783	0.027	3.859	0.039	5.275	0.033	0.783	0.387	2.026	0.159
HLR	2.145	0.159	0.247	0.625	7.209	**0.005**	0.772	0.391	0.661	0.426	0.483	0.625
CSLR	10.409	**0.004**	4.276	0.053	2.036	0.158	0.214	0.649	0.526	0.477	0.756	0.483
C.	$F_{1,19}$	*P*	$F_{1,19}$	*P*	$F_{2,19}$	*P*	$F_{1,19}$	*P*	$F_{1,19}$	*P*	$F_{2,19}$	*P*
BL	0.772	0.391	0.046	0.833	0.660	0.528	0.031	0.862	6.779	**0.017**	1.340	0.285

Figure captions

Figure 1: Specification of measurements (mm) collected from experimental *Daphnia*. HL = head length, BL = body length, CSL = caudal spine length, and ED = eye diameter.

Figure 2: Morphological response of *Daphnia ambigua* from pre- and post-*Bythotrephes* invasion time periods to *Bythotrephes* cues. Clones were raised in the presence (cue) and absence (control) of *Bythotrephes* chemical cues for four weeks. Clonal means (± 1 SE) are based on samples taken from four replicate jars, 10 individuals were measured per replicate jar. Clones were hatched from resting eggs extracted from Lake Mendota sediments. HL = head length, BL = body length, CSL = caudal spine length, and ED = eye diameter.

Figure 3: Morphological response of *Daphnia pulicaria* from pre- and post-*Bythotrephes* invasion time periods to *Bythotrephes* cues. Clones were raised in the presence (cue) and absence (control) of *Bythotrephes* chemical cues for four weeks. Clonal means (± 1 SE) are based on samples taken from four replicate jars, 10 individuals were measured per replicate jar when possible. Clones were hatched from resting eggs extracted from Lake Mendota sediments. HL = head length, BL = body length, CSL = caudal spine length, and ED = eye diameter.

Figure 1.

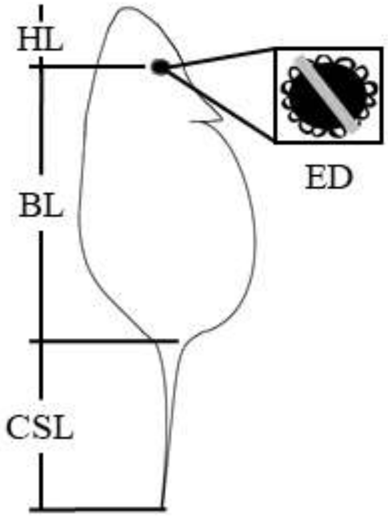
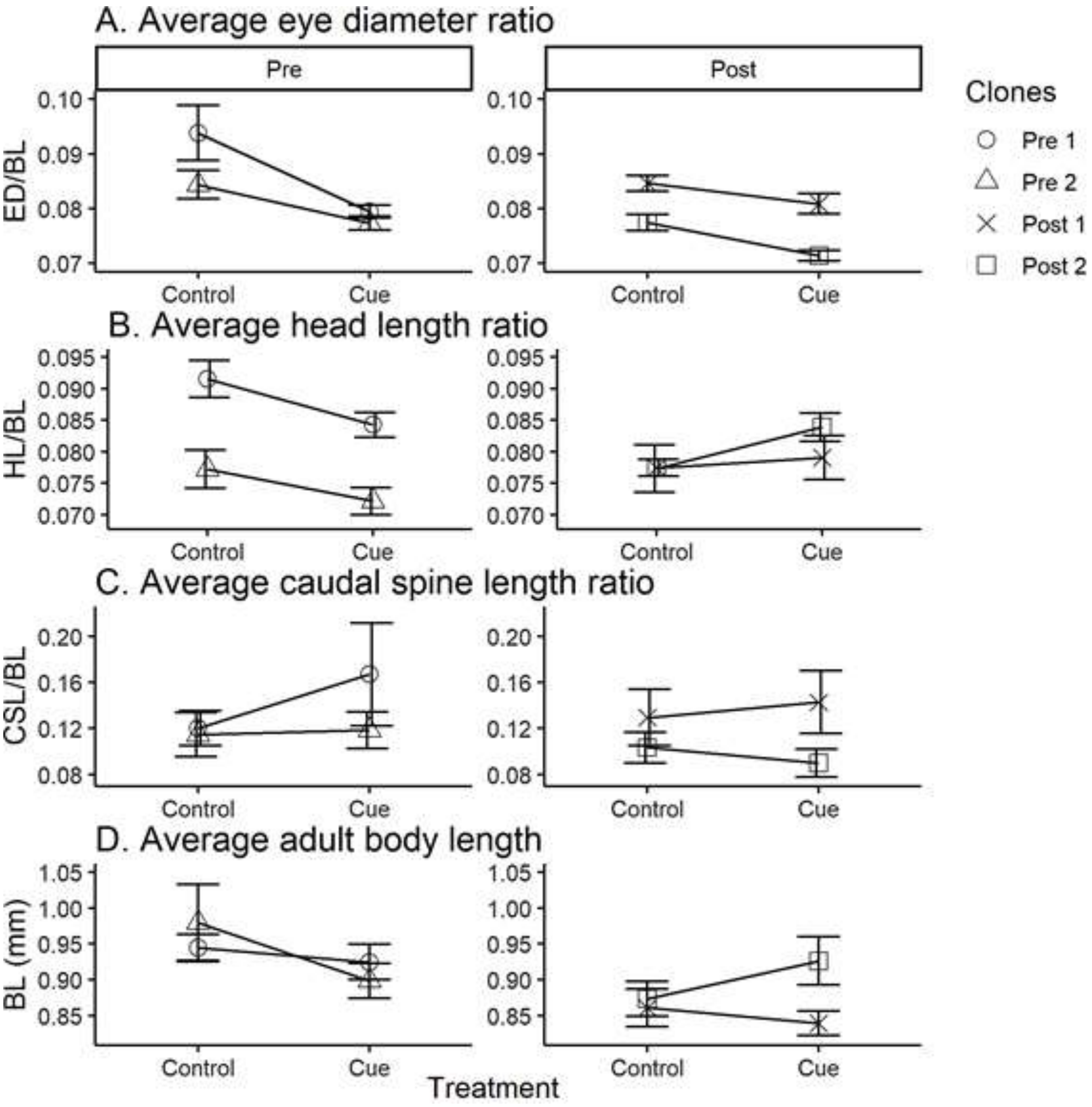
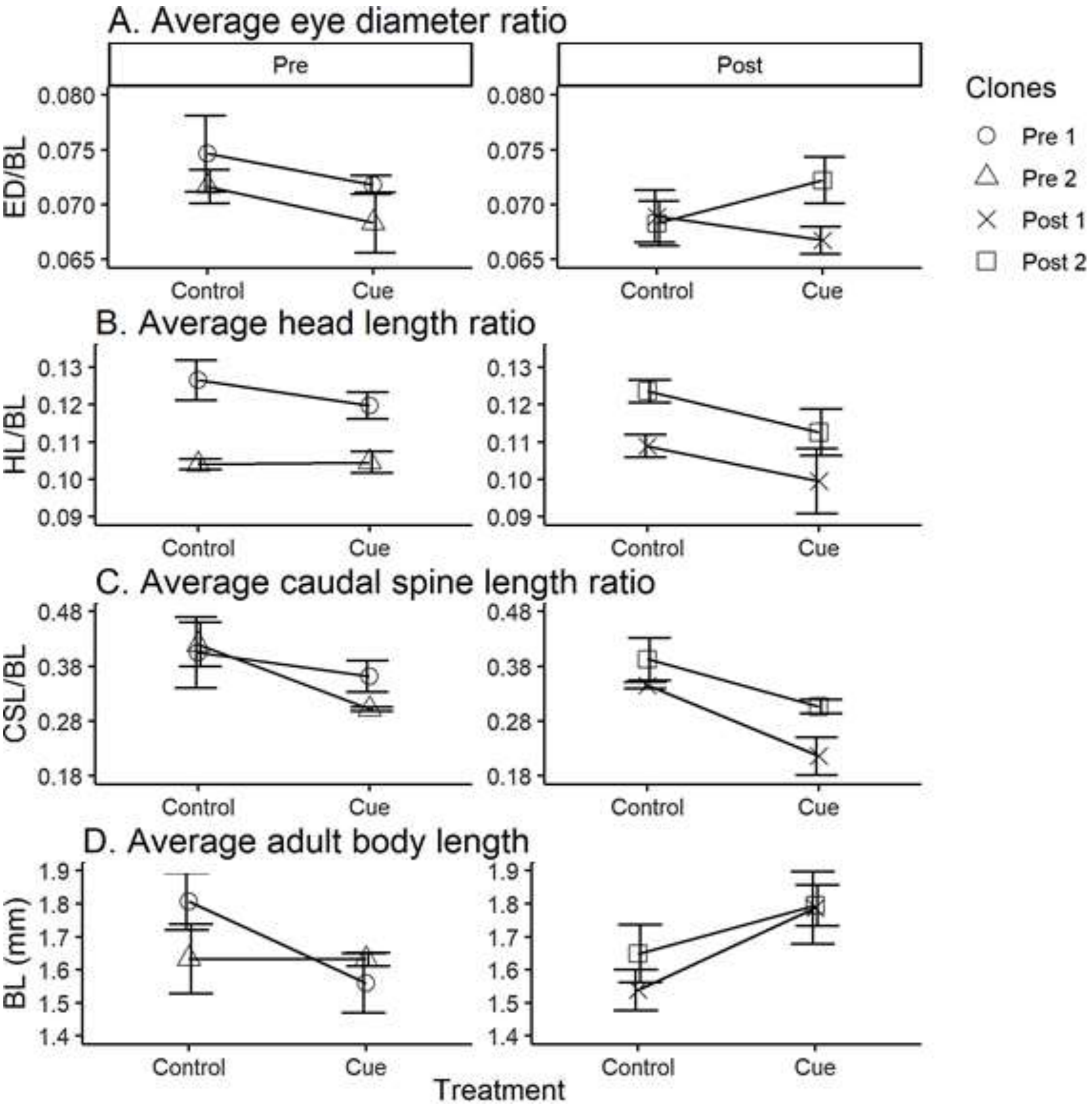


Figure 2.



A. Average eye diameter ratio
Pre
Post
ED/BL
0.10
0.09
0.08
0.07
Control
Cue
Clones
○ Pre 1
△ Pre 2
× Post 1
□ Post 2
B. Average head length ratio
HL/BL
0.095
0.090
0.085
0.080
0.075
0.070
C. Average caudal spine length ratio
CSL/BL
0.20
0.16
0.12
0.08
D. Average adult body length
BL (mm)
1.05
1.00
0.95
0.90
0.85
Treatment

Figure 3.


A. Average eye diameter ratio
Pre
Post
ED/BL
0.080
0.075
0.070
0.065
Control
Cue
Clones
○ Pre 1
△ Pre 2
× Post 1
□ Post 2
B. Average head length ratio
HL/BL
0.13
0.12
0.11
0.10
0.09
C. Average caudal spine length ratio
CSL/BL
0.48
0.38
0.28
0.18
D. Average adult body length
BL (mm)
1.9
1.8
1.7
1.6
1.5
1.4
Treatment

Chapter three: Appendix S1. Supporting Information.

Supplement S1. Methods

Care of stock cultures

Clonal cultures were established from single parthenogenetic female hatchlings kept in laboratory conditions prior to experimentation. Stock cultures of clonal lineages were grown separately in several jars filled with 4 L artificial pond water (COMBO; Kilham et al. 1998). Stock jars were fed daily with a 50:50 mixture of the green algae *Nannochloropsis* sp. (34 million cells/mL; Nano 3600™, Reed Mariculture, Campbell, CA) and live, chemostatically-cultured green algae *Scenedesmus acutus* (0.5 mg C^{L-1}). Stock cultures were grown at a constant temperature (~20°C) and received equal amounts of incidental ambient lighting 24 hours a day.

Statistical analysis of final abundance data

Final daphnid abundance data were analyzed in R version 3.6.3 (R Core Team 2020) using generalized linear mixed-effects models with negative binomial error distributions (glmmTMB package, Brooks et al. 2017). We constructed separate models for the *D. pulicaria* and *D. ambigua* data sets. In both models, final abundance was the dependent variable, while treatment, subpopulation, and subpopulation × treatment interaction served as fixed effects, and clone (nested in subpopulation) served as a random effect. All model assumptions were tested using residual diagnostics (DHARMa package, Hartig 2020). We assessed associations between the treatment applied to a jar and reproductive individuals observed in the jar at the end of the experiment—specifically the presence of females carrying resting eggs, gravid females, or males—using contingency tables and Fisher's Exact Tests. Fisher's Exact Tests determined whether certain treatments and reproductive types were found together more often than would be expected by chance. No males were observed in *D. ambigua* jars so a total of five Fisher's Exact

Tests were run. Due to multiple tests, a Bonferroni-type adjustment was made, and the alpha level was set at 0.01.

Supplement S1. Results

D. ambigua abundance

The final abundance of both pre- and post-invasion *D. ambigua* clones was higher in jars that contained *Bythotrephes* chemical cues (Figure S4; z=6.333, p=2.4x10^{-10}). Subpopulation identity did not significantly affect final abundance (z=0.483, p=0.629). Despite this difference in final abundance between treatments, there was no significant relationship between treatment and the number of females carrying resting eggs or gravid females in the jars (Table S1). No males were found in any *D. ambigua* microcosms at the end of the experiment.

D. pulicaria abundance

The final abundance within *D. pulicaria* microcosms was lower in treatment jars than in control jars (z=-2.190, p=0.0285), regardless of whether clones were from pre- or post-invasion time periods (Figure S4; z=0.405, p=0.685). In addition, a significant relationship between treatment and gravid females was observed (Fisher's exact test, p=0.003). Gravid females were more likely to be found in control jars than in jars which contained *Bythotrephes* chemical cues (Figure S5). Other life stages such as females carrying resting eggs and males, were approximately equivalent across treatments and subpopulations (Table S1).

Supplement S1. Discussion

D. ambigua abundance

At the end of the experiment, *D. ambigua* were more abundant in jars with *Bythotrephes* chemical cues (Figure S4). *D. ambigua* may be exhibiting life history shifts in response to the predator cues. Individual life-history features are responsive to different types of predators. For example, if *D. ambigua* are responding with a typical prey response to a visual predator, we would expect daphnids to mature more rapidly at smaller sizes and to produce more, but smaller, offspring. In contrast, a characteristic prey response to an invertebrate predator would be to delay reproduction and shunt energy into growth to reach a larger and presumably less-vulnerable size as quickly as possible (Weider and Pijanowska 1993). Since *D. ambigua* are more abundant in cue jars, it seems they may be displaying a typical prey response to a visual predator, whereby they produce more, smaller individuals. This supposition is further supported by the fact that other *D. ambigua* morphological changes such as reduced eye diameters and adult body lengths conform to the visibility hypothesis.

Alternatively, *D. ambigua* may be denser in jars containing predator cues because of a fertilizing effect of the cue addition. The extra material added to cue jars (ground frozen *Bythotrephes* suspended in COMBO) may have served as an additional carbon source which stimulated bacterial growth. Zooplankton bacterivory is well-known (Brendelberger 1991) and thus an increase in bacterial abundance would ultimately serve as an additional food source. A similar “fertilization” effect, however, was not observed for *D. pulicaria* (Figure S4). This may be because *Daphnia* species with coarser filtering meshes show lower bacteria retention efficiencies (Brendelberger 1991) and some small daphnid species similar in size to *D. ambigua* (e.g., *D. cucullata*), have finer filtering mesh than larger species like *D. pulicaria* (Brendelberger and Geller 1985). Thus, *D. ambigua* may be more efficient at consuming bacteria than *D. pulicaria*.

D. pulicaria abundance

D. pulicaria were more abundant in jars without *Bythotrephes* chemical cues (Figure S4). This is contrary to *D. ambigua,* which were more abundant in jars containing *Bythotrephes* chemical cues. One potential explanation for this, is that *D. pulicaria* may exhibit a general stress response to *Bythotrephes* chemical cues. For example, when exposed to fish chemical cues, *D. magna* exhibit a stress response whereby they produce high levels of heat-shock protein 60 (Pauwels and De Meester 2005). This functions as a temporary coping mechanism to deal with novel predator stress; however, because protein induction is energetically costly, daphnids cannot maintain a constant high level of heat-shock proteins (Pauwels and De Meester 2005). If *D. pulicaria* respond to *Bythotrephes* chemical cues with a stress response, this may explain why this species seems to be particularly vulnerable to *Bythotrephes* predation.

On the other hand, reduced final abundance of *D. pulicaria* clones in cue jars may indicate a life-history response to *Bythotrephes* chemical cues. A typical invertebrate predator life-history response involves the production of fewer, but larger offspring (Weider and Pijanowska 1993). This could explain why fewer daphnids were observed in cue jars. This type of life-history shift, however, contradicts previous research which found that *D. pulicaria* from invaded lakes produced both more offspring and offspring that were larger at maturation than *D. pulicaria* from uninvaded lakes, both in the presence and absence of fish chemical cues (Gillis and Walsh 2017). Additional life-history experiments would further elucidate whether *D. pulicaria* exhibit a life-history shift when exposed to *Bythotrephes* chemical cues.

Supplement S1. References

Brendelberger, H. (1991). Filter mesh size of cladocerans predicts retention efficiency for bacteria. *Limnology and Oceanography*, 36, 884–894.

Brendelberger, H., & Geller, W. (1985). Variability of filter structures in eight *Daphnia* species: mesh sizes and filtering areas. *Journal of Plankton Research*, 7, 473–486.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., & Bolker, B.M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9, 378–400.

Gillis, M.K., & Walsh, M.R. (2017). Rapid evolution mitigates the ecological consequences of an invasive species (*Bythotrephes longimanus*) in lakes in Wisconsin. *Proceedings of the Royal Society B*, 284, 20170814.

Hartig, F. (2020). DHARMa: Residual diagnostics for hierarchical (multi-level / mixed) regression models. R package version 0.3.1.

Kilham, S.S., Kreeger, D.A., Lynn, S.G., Goulden, C.E., & Herrera, L. (1998). COMBO: A defined freshwater culture medium for algae and zooplankton. *Hydrobiologia*, 377, 147–159.

Pauwels, K., Stoks, R., & De Meester, L. (2005). Coping with predator stress: Interclonal differences in induction of heat-shock proteins in the water flea *Daphnia magna*. *Journal of Evolutionary Biology*, 18, 867–872.

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Weider, L.J., & Pijanowska, J. (1993). Plasticity of *Daphnia* life histories in response to chemical cues from predators. *Oikos*, 67, 385–392.

Supplement S1. Tables

Table S1: Fisher's Exact tests of independence between treatment applied (presence and absence of *Bythotrephes* chemical cues) and reproductive individuals (the presence of gravid females, females carrying resting eggs, or males) observed at the end of a four-week microcosm exposure experiment. No males were found in *Daphnia ambigua* jars so that test was not performed. Bonferroni-adjusted alpha =0.01, significant p-values are in bold.

		P-values	
	Gravid females	**Females carrying resting eggs**	**Males**
D. ambigua	1	0.654	N/A
D. pulicaria	**0.003**	0.024	0.197

Supplement S1. Figure captions

Figure S1: Effect of **A.** final abundance (individuals L^{-1}) of animals remaining at the conclusion of the four-week exposure experiment; and **B.** clonal identity on the eye diameter ratio of *Daphnia ambigua*. Eye diameter ratio was calculated by dividing eye diameter by body length. Both final abundance and clonal identity had a significant effect on eye diameter ratio regardless of whether the individuals were grown in the presence (cue) or absence (control) of *Bythotrephes* chemical cues. Box plots show the third quartile, median, first quartile, and data outliers.

Figure S2: Effect of clonal identity on the head length ratio of *Daphnia ambigua*. Head length ratio was calculated by dividing head length by body length. Clonal identity had a significant effect on head length ratio regardless of whether the individuals were grown in the presence (cue) or absence (control) of *Bythotrephes* chemical cues. Box plots show the third quartile, median, first quartile, and data outliers.

Figure S3: Effect of clonal identity on the head length ratio of *Daphnia pulicaria*. Head length ratio was calculated by dividing head length by body length. Clonal identity had a significant effect on head length ratio regardless of whether the individuals were grown in the presence (cue) or absence (control) of *Bythotrephes* chemical cues. Box plots show the third quartile, median, first quartile, and data outliers.

Figure S4: Final abundance (individuals L^{-1}) of **A.** *Daphnia ambigua* and **B**. *D. pulicaria* after a four-week microcosm exposure experiment. *Daphnia* from pre- and post-*Bythotrephes* invasion time periods were raised in the presence (cue) and absence (control) of *Bythotrephes* chemical cues. Box plots show the third quartile, median, first quartile, and data outliers.

Figure S5: Proportion of *D. ambigua* (DA) and *D. pulicaria* (DP) microcosm jars containing adult females bearing eggs (AdEgg), adult females bearing resting eggs (AdEph), and males

(Male) after a four-week exposure experiment. Daphnids were raised in the presence (cue) and absence (control) of *Bythotrephes* chemical cues. Clones from pre- and post-*Bythotrephes* invasion time periods were pooled. No males were found in *D. ambigua* jars.

Figure S6: Morphological response of *Daphnia ambigua* from pre- and post-*Bythotrephes* invasion time periods to *Bythotrephes* cues. Clones were raised in the presence (cue) and absence (control) of *Bythotrephes* chemical cues for four weeks. Clonal means (± 1 SE) are based on samples taken from four replicate jars, 10 individuals were measured per replicate jar. Clones were hatched from resting eggs extracted from Lake Mendota sediments. HL = head length, BL = body length, CSL = caudal spine length, and ED = eye diameter.

Figure S7: Morphological response of *Daphnia ambigua* from pre- and post-*Bythotrephes* invasion time periods to *Bythotrephes* cues. Clones were raised in the presence (cue) and absence (control) of *Bythotrephes* chemical cues for four weeks. Clonal means (± 1 SE) are based on samples taken from four replicate jars, 10 individuals were measured per replicate jar. Clones were hatched from resting eggs extracted from Lake Mendota sediments. HL = head length, BL = body length, CSL = caudal spine length, and ED = eye diameter.

Figure S1.

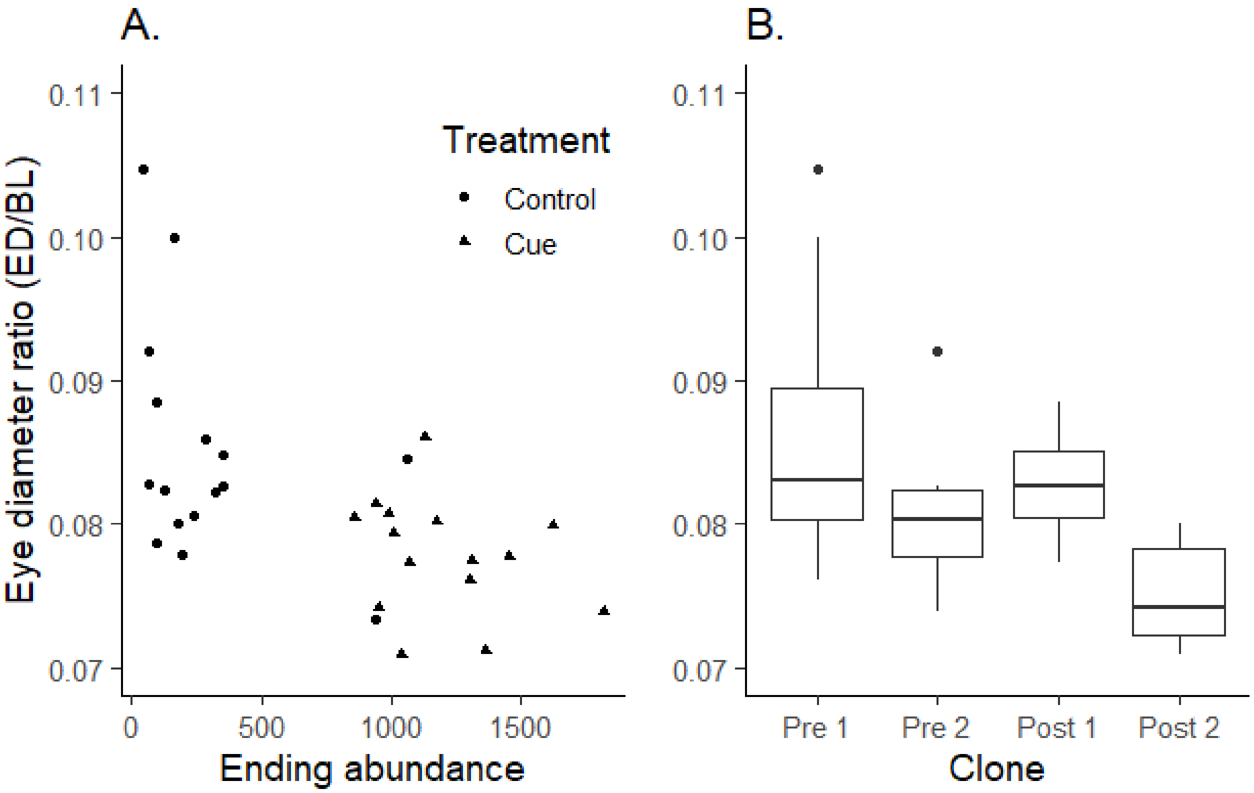

A.
B.
Eye diameter ratio (ED/BL)
0.11
0.10
0.09
0.08
0.07
Treatment
Control
Cue
0
500
1000
1500
Ending abundance
0.11
0.10
0.09
0.08
0.07
Pre 1
Pre 2
Post 1
Post 2
Clone

Figure S2.

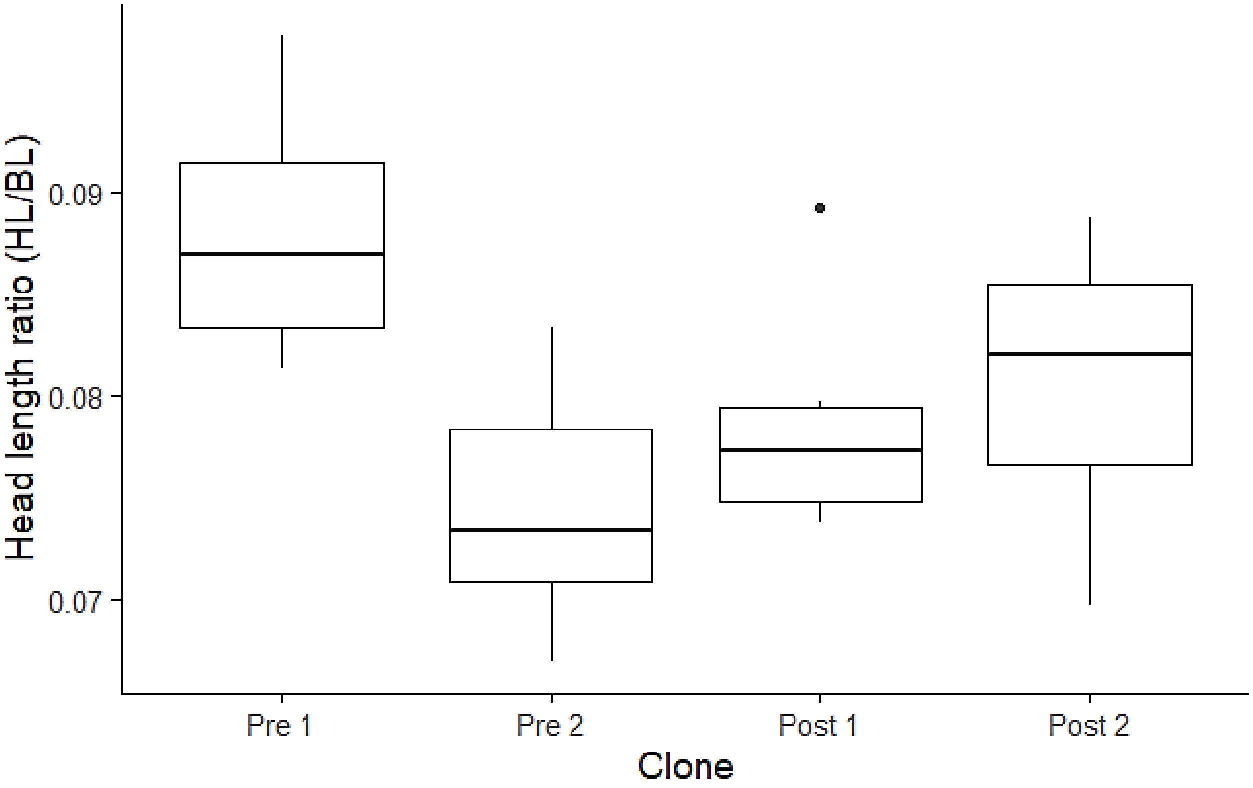

Head length ratio (HL/BL)
0.09
0.08
0.07
Pre 1
Pre 2
Post 1
Post 2
Clone

Figure S3.

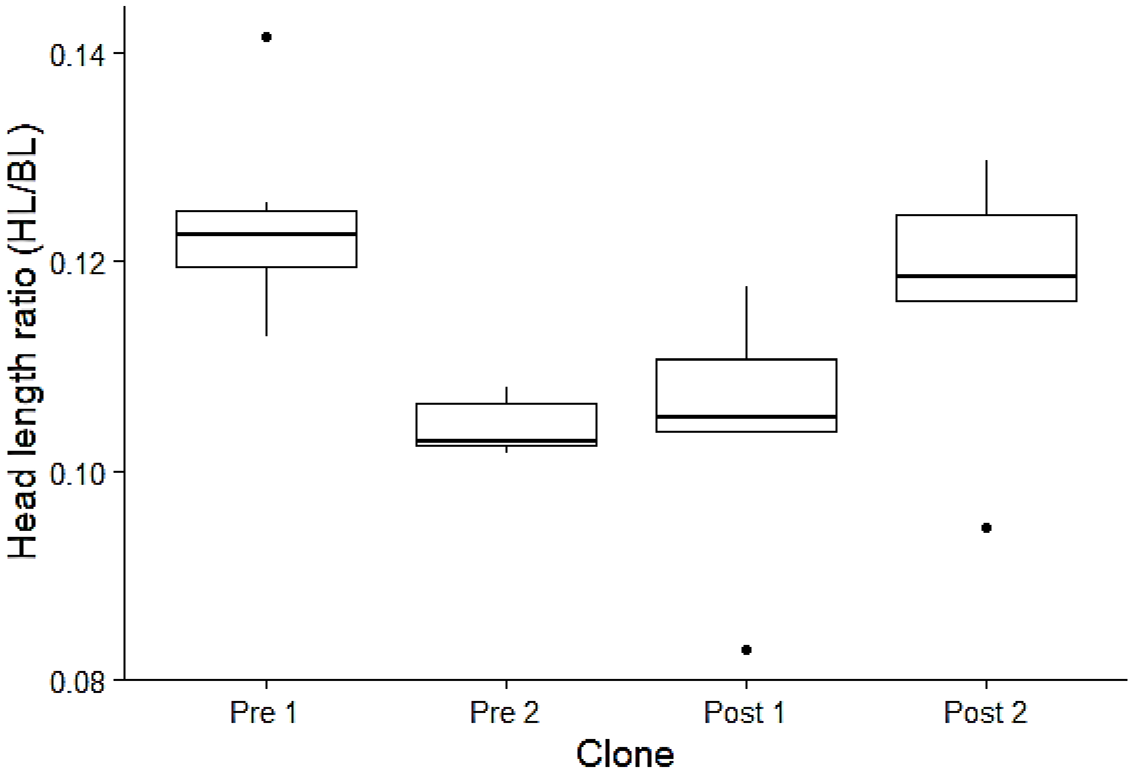

0.14
0.12
0.10
0.08
Head length ratio (HL/BL)
Pre 1
Pre 2
Post 1
Post 2
Clone

Figure S4.

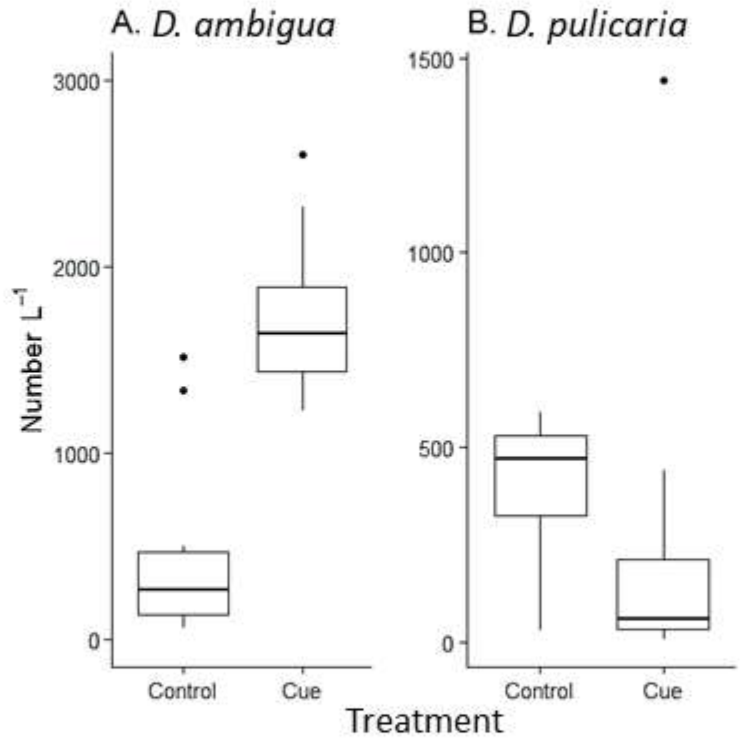

A. *D. ambigua*
B. *D. pulicaria*
Number L^{-1}
3000
2000
1000
0
1500
1000
500
0
Control
Cue
Control
Cue
Treatment

Figure S5.

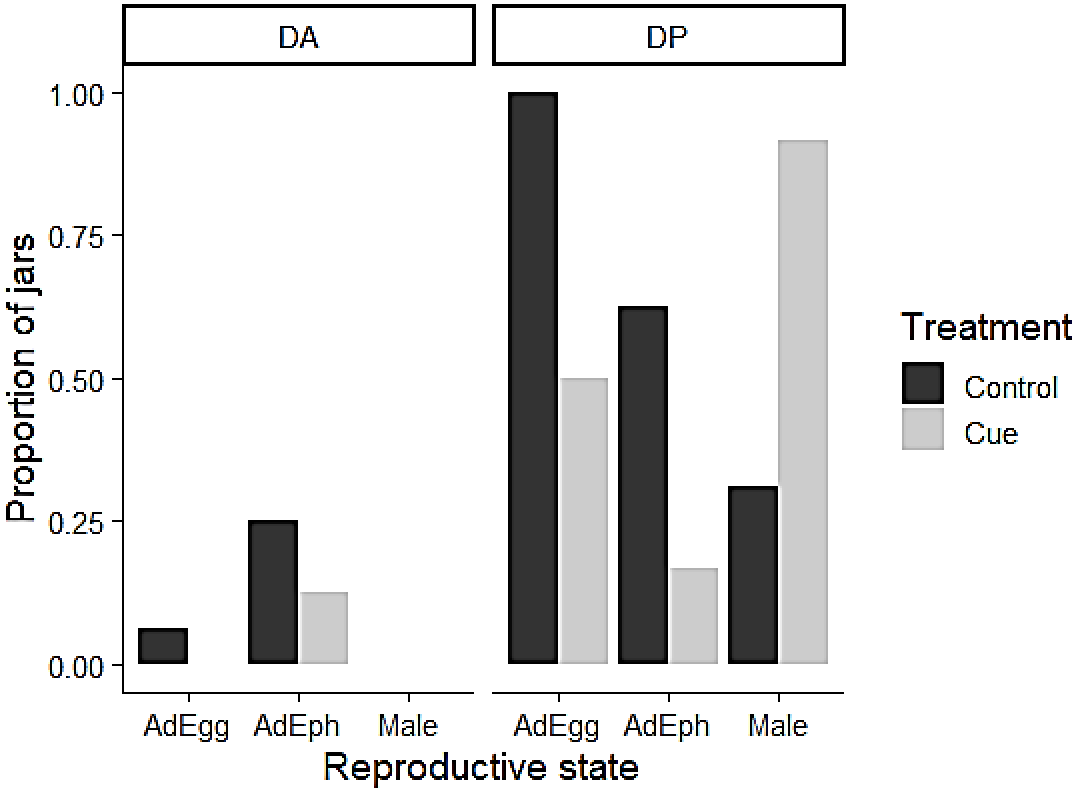
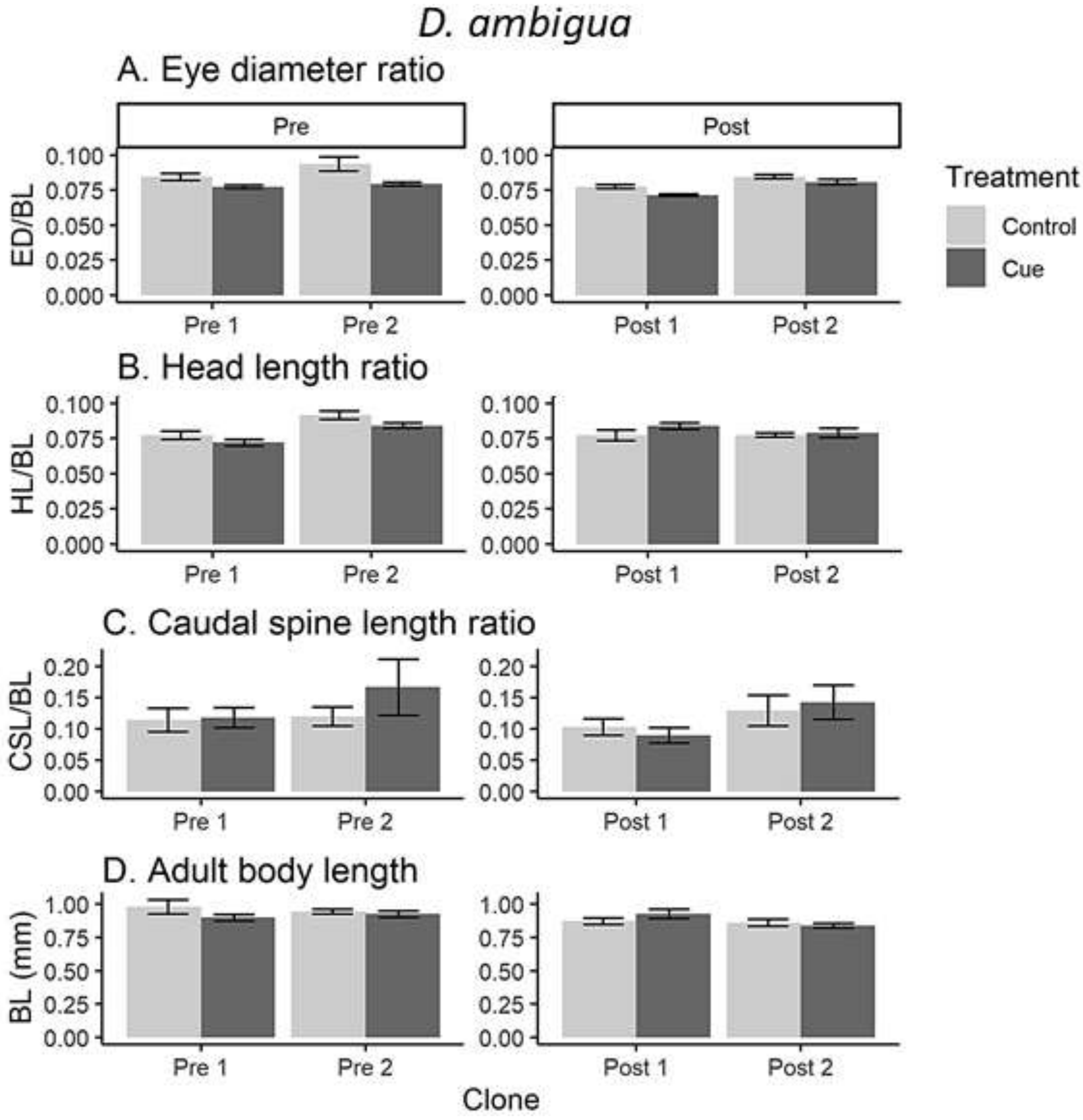
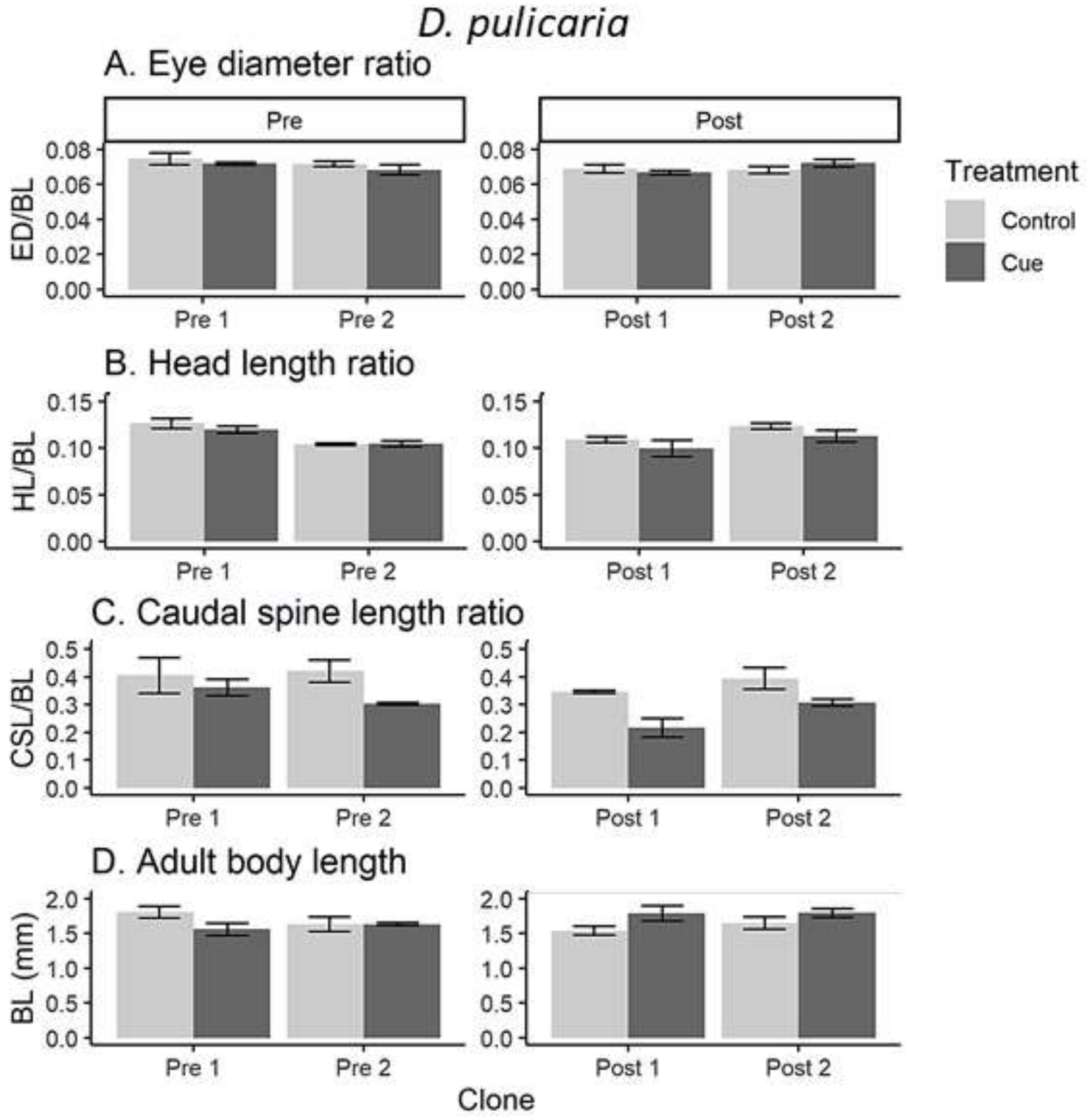

DA
DP
1.00
0.75
0.50
0.25
0.00
Proportion of jars
AdEgg
AdEph
Male
AdEgg
AdEph
Male
Reproductive state
Treatment
Control
Cue

Figure S6.



D. ambigua
A. Eye diameter ratio
Pre
Post
ED/BL
0.100
0.075
0.050
0.025
0.000
Pre 1
Pre 2
Post 1
Post 2
Treatment
Control
Cue
B. Head length ratio
HL/BL
C. Caudal spine length ratio
CSL/BL
0.20
0.15
0.10
0.05
0.00
D. Adult body length
BL (mm)
1.00
0.75
0.50
0.25
0.00
Clone

Figure S7.



D. pulicaria
A. Eye diameter ratio
Pre
Post
ED/BL
0.08
0.06
0.04
0.02
0.00
Pre 1
Pre 2
Post 1
Post 2
Treatment
Control
Cue
B. Head length ratio
HL/BL
0.15
0.10
0.05
0.00
C. Caudal spine length ratio
CSL/BL
0.5
0.4
0.3
0.2
0.1
0.0
D. Adult body length
BL (mm)
2.0
1.5
1.0
0.5
0.0
Clone

Chapter four—Interactions of food quantity and predator chemical cues on *Daphnia* population dynamics

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Abstract

1. Invasive predators can have large impacts on the dynamics of native prey species both through consumption and non-lethal impacts. The development of antipredator defenses—such as shifts in life history, behavior, or morphology—is well documented in many different systems. However, the impact of non-lethal effects on prey population dynamics is poorly understood despite its important role in shaping ecosystems. In addition, individuals are often experiencing multiple stressors and environmental cues at the same time, which may either strengthen or weaken their antipredator responses.
2. Despite its important role in shaping ecosystems, the combined impact of predator cues and food availability on prey population dynamics is poorly understood. We aimed to establish whether food availability and predation risk from an invasive predator alters the population dynamics of native prey species.
3. We investigated how the population dynamics of two *Daphnia* species (*D. ambigua* and *D. pulicaria*) native to Lake Mendota are affected by low and high food availability conditions, in the presence and absence of chemical cues from the invasive zooplanktivorous cladoceran, *Bythotrephes*.
4. Our data showed that food quantity, prey species identity, and the presence of *Bythotrephes* chemical cues were the chief drivers of changes in both *Daphnia* density and ephippial production, while *Daphnia* biomass was primarily influenced by food quantity and prey species identity. In addition, algal biomass trends were driven by food quantity and the presence of *Bythotrephes* chemical cues.
5. Understanding the impact of invasive predators on native prey is one of the most pressing challenges facing biologists today This study contributes to increasing our understanding

of the combined effects of signals of predation risk and food availability on population-level dynamics of native prey species.

Keywords: *Bythotrephes*, eco-evolutionary dynamics, invasive predator, kairmones, native prey, population dynamics, spiny waterflea, zooplankton

Introduction

Predators can have significant impacts on prey dynamics via direct consumption of prey (Murdoch et al., 2003). However, direct predation is only one effect that a predator has on its prey. Non-lethal effects such as the development of antipredator defenses in response to predator cues is a common occurrence in both aquatic and terrestrial systems (Harvell, 1990; Tollrian & Harvell, 1999; Nelson et al., 2004). Antipredator defenses can range from changes in individual morphological characteristics or life history traits to changes in behavior (Harvell, 1990; Tollrian & Harvell 1999, Nelson et al., 2004). These traits constitute a type of adaptive prey response that decreases predation success (Harvell, 1990; Tollrian & Harvell, 1999). However, this benefit comes at a cost. Development of defensive traits can lead to decreased growth and reproduction (Abrams, 1984; Black & Dodson, 1989; Riessen & Sprules, 1990; Nelson et al., 2004; Kopp & Gabriel, 2006; Dambacher & Ramos-Jiliberto, 2007). In addition, shifts in life history features such as changes in fecundity, body size, or age of maturation may serve as a predator defense (Tollrian, 1995; Goitom et al., 2018; Gu et al., 2020). Together, predator induced life history shifts and the demographic costs of defenses can alter population dynamics and thus, play a significant role in impacting ecosystem-level processes (Goitom et al., 2018; Gu et al., 2020).

Understanding the impact of invasive predators on native prey is one of the most important challenges facing biologists today (Berthon, 2015). Invasive predators have been shown to have non-lethal effects on native prey through both induced and constitutive phenotypic changes in a variety of systems (Berthon, 2015). Here we examine non-lethal effects of an invasive predator, *Bythotrephes cederstroemi*, on native *Daphnia* prey in Lake Mendota (WI). *Bythotrephes* is an invasive predatory cladoceran that has impacted the ecosystem of the Laurentian Great Lakes and many other North American inland lakes (Yan et al., 2011). For example, the arrival of *Bythotrephes* in Lake Mendota coincided with loss and reduction in density of several *Daphnia* species and a corresponding decrease in water clarity due to increased algae growth (Walsh, Carpenter, et al., 2016; Walsh, Munoz, et al., 2016). Further, laboratory experiments have shown that some *Daphnia* species modify their behavior, morphology, and life histories in the presence of *Bythotrephes* chemical cues (Bungartz & Branstrator, 2003; Landy et al., 2020). Development of these antipredator defenses could, in turn, be causing indirect effects on *Daphnia* population dynamics. It is important to consider the indirect impacts that an invasive predator may have on the population dynamics of native prey species.

Growth, reproduction, and development of antipredator defenses are all impacted by food availability. It is known that organisms reduce their investment in defense when they are experiencing food limitation (Jeyasingh & Weider, 2005; Tollrian et al., 2015). In Lake Mendota, there are seasonal shifts in algae quantity (Walsh et al., 2018), with algal biomass being inversely correlated with *Bythotrephes* density (Walsh et al., 2018). Thus, this observation begs for the exploration of the population dynamics of *Daphnia* in both high and low food quantity conditions. Seasonal changes in food availability could not only affect the ability of daphnids to express defenses but also impact their population growth and survivorship (Vanni & Lampert, 1992).

The combined role of predator cues and food availability on prey population dynamics is poorly understood despite its important role in shaping ecosystems (Gu et al., 2020). We used an experimental approach to investigate how the population dynamics of two *Daphnia* species (*D. ambigua* and *D. pulicaria*) native to Lake Mendota are affected by the varying food availability (high versus low food availability) and the presence and absence of *Bythotrephes* chemical cues. Our goals were to establish whether food availability and *Bythotrephes* predation risk alter the population dynamics of native *Daphnia* species. Overall, we hypothesized that there would be a greater density and biomass of individuals under no cue conditions than cue conditions because the daphnids will not be devoting resources towards the development of antipredator defenses (Abrams, 1984; Kopp & Gabriel, 2006; Dambacher & Ramos-Jiliberto, 2007). However, under low food conditions, we predicted that the densities and biomass of the cue and no cue treatments would be more similar because food limitation would reduce investment in defenses (Jeyasingh & Weider, 2005; Tollrian et al., 2015).

For interspecific comparisons, it was expected that in the absence of predator cue, differential abilities in resource use will dictate prey species density and biomass (Leibold, 1996). Since *D. pulicaria* is larger than *D. ambigua* and larger body sizes are related to higher filtering rates, which allow for uptake of more food, that in turn enables greater growth and reproduction (Burns, 1969), it was anticipated that in the absence of predator cues *D. pulicaria* would reach a higher biomass than *D. ambigua*. *D. ambigua*, on the other hand, were expected to reach higher densities because their smaller size and lower energy requirements allow more individuals to be supported at lower (algal) food levels (Lynch et al., 1986). As a result, we postulated that overall, there would be equal amounts of algae biomass in the *D. ambigua* and *D. pulicaria* jars and that food quantity treatments would drive algae biomass trends.

In addition to changes in biomass and density, we assessed fluctuations in resting egg (ephippial) production. *Daphnia* are cyclical parthenogens, and thus, they have two distinct modes of reproduction: asexual parthenogenetic reproduction for rapidly reproducing many offspring in favorable environments and sexual reproduction for producing resting eggs (ephippia) as an egg bank to survive in harsh environments (Pijanowska & Stolpe, 1996). We predicted that ephippia would be most abundant in the high food and *Bythotrephes* chemical cue treatments because diapause response to predator chemical cues (Pijanowska & Stolpe, 1996; Slusarczyk et al., 2013) and fluctuating food availability (Deng, 1996) are common phenomena. Ephippia production in the presence of a predator can be adaptive because, although there is an immediate cost to putting resources into dormant stages that will not immediately contribute to population growth, higher fitness can be achieved through survival in the diapausing state (Pijanowska & Stolpe, 1996; Slusarczyk et al., 2013). Thus, increased ephippia production would also be expected under high food conditions because high food availability will lead to higher *Daphnia* reproduction and eventually crowding conditions. Crowding cues are known to induce ephippia formation (Vanni & Lampert, 1992; LeBlanc & Medlock, 2015).

Methods

Study site

Lake Mendota is a dimictic, eutrophic lake (39.6 km^2, 25.3 m maximum depth, and 12.7 m mean depth) located in southeastern Wisconsin, USA (Magnuson et al., 2021; Walsh, Munoz, et al., 2016). As one of the North Temperate Lakes Long-Term Ecological Research (NTL-LTER) sites, Lake Mendota has been consistently monitored since 1976. Lake Mendota was initially invaded by *Bythotrephes* in 1994 (detected via sediment cores) and is located along the southern edge of the invasion range (Walsh, Munoz, et al., 2016). Three species of *Daphnia* are abundant in the egg bank of Lake Mendota: *D. ambigua*, *D. pulicaria*, and *D. mendotae* (EL Kiehnau, unpublished data). The seasonal peaks of these *Daphnia* species overlap (Lathrop, 2013; Magnuson et al., 2019). However, from 1976-2015, *D. ambigua* has been found in the water column only once, on 21 June 1983 (Lathrop, 2013; Magnuson et al., 2019). Since the *Bythotrephes* invasion, the zooplankton community composition of Lake Mendota has shifted from dominance of *D. pulicaria* to co-dominance of *D. pulicaria* and *D. mendotae* (Walsh et al., 2017). There are seasonal shifts in algal quantity in Lake Mendota with algal biomass being lowest in the fall, when *Bythotrephes* density is the highest (Walsh et al., 2018). Because of the long-term zooplankton database, previous sediment coring work, and importance of the lake to the surrounding community, Lake Mendota is an ideal lake to study the impact of *Bythotrephes* invasion on native *Daphnia* species (Walsh, Carpenter, et al., 2016).

Sediment core collection and processing

Post-invasion resting eggs for hatching experiments were taken from 9 sediment cores collected from the deep hole of Lake Mendota (43.10667° N, 89.42472°, 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 ^{210}Pb dating of Lake Mendota sediments was used to estimate the dates of the sediments collected and to determine pre- and post-*Bythotrephes* invasion sediments (Walsh, Munoz, et al., 2016). In addition, *Bythotrephes* 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 *Bythotrephes* in the lake during each time period (Walsh, Munoz, et al., 2016).

Species used in this experiment included three post-invasion clones each from both *D. pulicaria* and *D. ambigua*. *D. mendotae* was excluded from this study due to the difficulty in maintaining adequate laboratory stocks. Clones were established from resting egg hatchlings isolated from Lake Mendota sediments. Processing of resting eggs from the sediments and hatching protocols followed Frisch et al. (2014). The three *D. ambigua* clones, and one *D. pulicaria* clone were hatched from resting eggs laid down in approximately 2014–2017 (0–2 cm sediment layer) and the two remaining *D. pulicaria* clones were established from resting eggs dating to approximately 2008–2011 (4–6 cm sediment layer).

Clone maintenance

Clonal cultures were initiated from single parthenogenetic females and kept under laboratory conditions prior to experiments. Stock cultures of clonal lineages were grown separately in several jars filled with 5 L of artificial pond water (COMBO; Kilham et al., 1998). Stock jars were fed daily with a 50:50 mixture of the green algae *Nannochloropsis* sp. (at a concentration of 34 million cells mL^{-1}; Nano 3600™, Reed Mariculture, Campbell, CA) and *Scenedesmus acutus* (at a concentration of 0.5 mg C L^{-1}). Stock cultures were grown in a temperature-controlled room (~20°C) and received equal amounts of lighting on a 12:12 L:D light cycle.

Experimental design

A two-factor factorial design was used to test the effects of *Bythotrephes* chemical cue and food quantity on the population dynamics of *Daphnia* species. The three post-*Bythotrephes* invasion clones of each *Daphnia* species (*D. ambigua* and *D. pulicaria*) were used to create single-species monoculture microcosms. The experimental design consisted of two predator cue treatments (cue/no cue) × two food quantities (high food/low food) × two *Daphnia* species (*D. ambigua*/*D. pulicaria*) × four replicates, resulting in a total of 32 experimental (microcosm) units.

Microcosms were created in 900 mL jars filled with 700 mL of either cue or no cue media and a small amount of cetyl alcohol, which acted as a surfactant to prevent animals from being trapped at the air-water interface. Every microcosm was inoculated with 27 pre-reproductive *Daphnia* (i.e., 9 per clone) at the start of the experiment. Experimental animals came from maternal lines (stock cultures) raised under identical conditions (see above) for at least two generations to reduce maternal effects (see Tollrian, 1995 for an example of maternal effects in *Daphnia*). Pre-reproductive experimental animals were identified via size and the lack of a visible brood chamber. Experimental animals for each clone were pooled from multiple jars into a single jar (of a single clone) from which individuals were selected and haphazardly distributed among the experimental jars.

Because of differences in body size, equal species densities resulted in initial differences in total biomass among monocultures. Initial biomass estimates in μg dry mass L^{-1} were: *D. ambigua*= 74.153 and *D. pulicaria*= 385.279. Biomass of pre-reproductive individuals was estimated by measuring the body length of 25 pre-reproductive individuals of each clone (75 individuals per species), calculating an average body length for each species, and then estimating average biomass using established length-weight regressions (Lynch et al., 1986). It is likely that different starting biomasses did not influence the overall outcome of the experiment (Steiner et al., 2005; Dzialowski, 2010). For example, the biomass of each species increased in monocultures following the start of the experiment and all species attained comparable peak biomass levels (see below).

Treatments

Treatments included high and low food quantities as well as the presence and absence of *Bythotrephes* chemical cues. Food availability was altered to include low (17 million cells mL^{-1} *Nannochloropsis sp*. and 0.25 mg C L^{-1} *S. acutus*) and high (34 million cells mL^{-1} *Nannochloropsis sp*. and 0.5 mg C L^{-1} *S. acutus*) food quantity conditions. Experimental jars were inoculated with food at the start of the experiment and then every three days for the first 10 days of the experiment. Then, once population densities were sufficient, feeding was increased to every other day for the duration of the experiment (i.e., 52 days).

Cue media was made to a concentration of 2.7 *Bythotrephes* L^{-1}. Cue treatments were made by adding 1 mL autoclaved COMBO for every live-frozen *Bythotrephes*, homogenizing using a pestle, and then pouring the crude homogenate over a 42.5 mm Whatman GF/F filter held in place on a vacuum filtration flask. After the media was filtered, 1.875 mL of the cue media was added to each "cue treatment" jar, bringing the final concentration of the jar to ~2.7 *Bythotrephes* L^{-1}. *Bythotrephes* used to make cue treatments were live-frozen and were collected from several lakes with varied *Bythotrephes* invasion histories (EL Kiehnau, unpublished data). We chose to use chemical cue from frozen field-collected *Bythotrephes* because of the difficulty of culturing *Bythotrephes* in a lab stetting (Kim & Yan, 2010) and because previous research has demonstrated induction of defenses in *Daphnia* using chemical cues from *Bythotrephes* frozen alive (Bungartz & Branstrator, 2003). The compounds that comprise *Bythotrephes* chemical cues are not known and thus artificial synthesis of chemical cue(s) was not an option. A concentration of approximately 2.7 *Bythotrephes* L^{-1} was chosen to simulate natural densities of *Bythotrephes* typically found in invaded lakes (Boudreau & Yan, 2003). The no cue (control) treatments consisted solely of autoclaved COMBO filtered through a separate 42.5 mm Whatman GF/F filter and vacuum apparatus. Cue/no cue treatments were refreshed, and placement of jars was randomized every three days throughout the experiment.

Sampling and data collection

The experiment ran for a total of 52 days. *Daphnia* were sampled and media for chlorophyll-a analysis was collected every 13 days for the duration of the experiment (i.e., four sampling events). We collected samples by gently mixing each jar (inverting three times) and decanting off 100 mL of media. The sample was then filtered through 80 μm Nitex® mesh to separate the *Daphnia* from the media. The *Daphnia* samples were immediately fixed in 95% ethanol and then preserved in 70% ethanol (Black & Dodson, 2003). Then, 50 mL of 100 mL of media collected was filtered onto a 25 mm Whatman GF/F filter. These filters were then wrapped in aluminum foil and frozen until the end of the experiment when chlorophyll-a analysis was conducted. Fresh COMBO was added to jars following each sampling event to account for sampling and evaporative losses and to allow for the maintenance of a constant volume of 700 mL in each jar.

In vitro chlorophyll-a (chl-a) was extracted and measured using an acetone extraction (Arar and Collins 1997). Filters were submerged in 90% acetone for 12–15 hours, then the filter/acetone was spun down using an Eppendorf centrifuge (Model 5804; 1500 rpm for five minutes), and chlorophyll-a was measured (after calibration from a random subsample) using the chl NA module in a Turner model TD 700 bench top fluorometer (Turner Designs, Sunnyvale, California, USA). Chlorophyll-a concentrations were used as a bulk measure of algal biomass (Arar & Collins, 1997).

Daphnia and ephippia from preserved subsamples were counted and core body length (mm) of *Daphnia* was measured. Biomass of *Daphnia* was then calculated using established length-weight regressions (Lynch et al., 1986). Biomass was calculated to allow for comparison across species since *D. ambigua* and *D. pulicaria* vary in size.

Statistical analysis

We preformed all analyses in R version 3.6.3 (R Core Team, 2020). To test our hypothesis that varying predator threat and food availability would lead to changes in *Daphnia* and algal biomass (µg L^{-1}), we ran linear mixed effects models using the lmer function in the lme4 package (Bates et al., 2015). Before running the analyses, we checked the response variables for normality and ultimately transformed both average *Daphnia* biomass and algal biomass using the transformTukey function in the rcompanion package (Mangiafico, 2020). Driver variables were specified as species identity, cue treatment, food quantity, and their interactions. To account for repeated sampling, we included jar number and sampling day as random factors in our models. We performed model selection using Akaike's information criterion (AIC_c; Burnham & Anderson, 2003) to determine which driver variables most influenced *Daphnia* and algal biomass and used the MuMIn package (Barton, 2020) to perform model comparisons. If models had a $\Delta AIC_c < 2$, they were considered equally parsimonious (Burnham & Anderson, 2003). Residuals of the top model were plotted using quantile-quantile plots to check for homoscedasticity. All models were compared using relative importance values (RIVs), a summed and standardized indicator of predictor variable rank across all possible models. Relative importance values are the sum of Akaike weights (w_i) of the predictor variables species identity, cue treatment, food quantity and their interactions for each of the biomass responses we examined (Burnham & Anderson, 2003). We calculated the R^2 of the optimal models using the r.squaredGLMM function in the MuMIn package (Barton, 2020). We report both the marginal coefficient of determination—R_m^2, which takes into consideration only variance explained by the fixed factors, and the conditional coefficient of determination—R_c^2, which includes the effect of the random factors.

We analyzed *Daphnia* and ephippial density (no. L^{-1}) with generalized linear mixed effect models (GLME) using the glmer and glmer.nb functions in the lme4 R package (Bates et al., 2015). As the *Daphnia* density data conformed with a Poisson distribution, GLME analysis was performed with Poisson error distribution and log link function. The ephippia density data conformed with a negative binomial distribution so GLME analysis was performed with negative binomial error distribution and log link function. In both models, the driver variables included species identity, cue treatment, food quantity, and their interactions. We included sampling day and jar number as random effects to account for repeated sampling. We used the same model selection based on AIC criterion and R^2 calculations as was described for the biomass analysis.

Results

Daphnia biomass

Relative importance values indicated that species identity, food quantity, their interaction, and the presence/absence of *Bythotrephes* chemical cues were all important drivers of *Daphnia* biomass (Table 1). On average, *D. pulicaria* jars had higher total biomass than *D. ambigua* jars (Figure 1). For both species, the jars receiving high food treatments had higher total *Daphnia* biomass than the jars receiving low food treatments (Figure 1). However, this trend was more exaggerated in *D. ambigua* than in *D. pulicaria* (Figure 1). Of the *D. ambigua* jars receiving low food treatments, the jars that also received *Bythotrephes* chemical cues had higher average biomass, but of the jars receiving high food treatments, the jars also receiving *Bythotrephes* chemical cues had lower average biomass (Figure 1). Similar to the *D. ambigua* trends, *D. pulicaria* jars receiving both low food and cue treatments had higher biomass than those receiving low food and no cue treatments (Figure 1). However, under high food conditions, the cue treatment jars on average had higher biomass than no cue jars from day 13-day 26, but lower biomass than the no cue treatment jars from day 39-day 52 (Figure 1).

Daphnia density

Food quantity and species identity were the major drivers of *Daphnia* density (Table 2). On average, *D. ambigua* maintained higher densities than *D. pulicaria* under all treatment combinations (Figure 1). For both species, high food jars were on average more densely populated than low food jars (Figure 1). Other factors such as the presence of *Bythotrephes* chemical cues, interactions among food quantity × cue, and food quantity × species identity also impacted *Daphnia* density (Table 2). For example, for both species the highest average densities were found in the high food × no cue condition while the lowest average densities were found in the low food × no cue condition (Figure 1). Also, under low food conditions, *D. ambigua* and *D. pulicaria* (to a lesser extent) no cue jars were less dense than cue jars, but under high food conditions, no cue jars were denser than cue jars (Figure 1).

Ephippia density

Relative importance values indicated that species and food quantity were the most important drivers of ephippial density (Table 2). *D. pulicaria* on average produced more ephippia than *D. ambigua* (Figure 1). Ephippia production was higher on average in the high food jars, and for both species, the highest average ephippial density was found in the high food

× no cue condition (Figure 1). While *D. pulicaria* ephippia production seemed to increase over time, this trend was not apparent in *D. ambigua* (Figure 1).

Algal biomass

Food quantity was consistently a strong predictor for algal biomass (Table 1). Algal biomass was on average higher in the high food treatments than in the low food treatments (Figure 1). In all treatments, algal biomass decreased from day 13-day 26, then for most treatments' biomass increased from day 26-52. However, for the *D. ambigua* high food × no cue treatment and the *D. pulicaria* high food × cue and no cue treatments, algal biomass increased from day 26-day 39, then decreased from day 39-day 52 (Figure 1). There was no notable difference in algal biomass between *D. ambigua* and *D. pulicaria* jars. Beyond the effects of food quantity, the addition of *Bythotrephes* chemical cues also influenced algal biomass (Table 1). This pattern is likely driven by the notable increase in algal biomass observed on days 39 and 52 in the *D. ambigua* low food × no cue jars (Figure 1).

Discussion

We used an experimental approach to investigate how the population dynamics of two *Daphnia* species (*D. ambigua* and *D. pulicaria*) native to Lake Mendota are affected by the varying food availability (high versus low food availability) and the presence and absence of *Bythotrephes* chemical cues. Food availability, *Bythotrephes* predation risk, and *Daphnia* species identity all altered trends in *Daphnia* and ephippial densities, whereas *Daphnia* biomass was primarily driven by food availability and *Daphnia* species identity. It is important to consider the indirect impacts that an invasion may have on the population dynamics of native prey species as this may play a significant role in impacting ecosystem processes.

Daphnia biomass and density

As expected, we found that *D. ambigua* reached higher densities than *D. pulicaria*, while *D. pulicaria* reached higher total biomass than *D. ambigua*. This was expected because *D. pulicaria* has higher filtering rates which allows for greater growth and reproduction (Burns, 1969), while *D. ambigua* are smaller and have lower energy requirements, thus more individuals can be supported on less algae (Lynch et al., 1986). Biomass and density of both species was higher in the high food treatments than the low food treatments as expected due to increased population growth rates (Vanni & Lampert, 1992).

For both species (albeit weaker for *D. pulicaria*), the trend was that no cue treatment jars had higher densities under high food conditions, but cue treatment jars had higher densities under low food conditions. This difference can likely be explained by the fact that in the high food jars, daphnids are diverting resources toward the development of antipredator defenses, thereby taking away from their reproductive output (Abrams, 1984; Black & Dodson, 1989; Riessen & Sprules, 1990; Nelson et al., 2004; Kopp & Gabriel, 2006, Dambacher & Ramos-Jiliberto, 2007). Under low food conditions, however, daphnids may not be able to develop antipredator defenses due to low food availability and thus their density is similar to that of the no cue jars (Jeyasingh & Weider, 2005; Tollrian et al., 2015).

Trends in *D. ambigua* biomass mirrors the density trends and thus can be explained by similar justification. *D. pulicaria* biomass does not mirror density trends at the beginning of the experiment. Jars that received both cue and high food treatments, had higher biomass values than expected from day 13–26. This is likely because the jars contained a greater proportion of large adult individuals. Previous research supports the idea that the presence of predators can lead to changes in demographic interactions of *Daphnia* (Goitom et al., 2018). In addition, at the start of the experiment, the jars receiving both cue and high food treatments had the highest ephippial production. Thus, the increased biomass during this period may be due to the presence of large ephippial females.

Previous studies have found variation in the life history response of different *Daphnia* species to the *Bythotrephes* invasion (Gillis & Walsh, 2017; Landy et al., 2020). For example, *D. pulicaria* from invaded lakes were found to be larger at maturation and to have greater investment in reproduction than *D. pulicaria* from uninvaded lakes (Gillis & Walsh, 2017). In addition, contemporary *D. pulicaria* matured 2-3% earlier than *D. pulicaria* from pre-invasion time periods (Landy et al., 2020). Together it is thought that these changes may boost population growth rates of *D. pulicaria* in *Bythotrephes* invaded lakes. We did not observe a notable increase in density or biomass of *D. pulicaria* in jars receiving cue treatments; however, all of the clones used in our experiments were from post-invasion time periods. There is variation in how different *Daphnia* species respond to the *Bythotrephes* invasion. For example, contemporary post-invasion *D. mendotae* populations, showed a 37% reduction in reproductive investment and matured at a size that was ~7% smaller than pre-invasion *D. mendotae* (Landy et al., 2020). These results highlight the microevolutionary potential and plasticity of *Daphnia* clones that are subjected to an invasive predator like *Bythotrephes*, which can further impact population and ecosystem dynamics.

Ephippia density

D. pulicaria produced more ephippia on average than *D. ambigua* throughout the experiment. Overall, trends in ephippial density closely tracked trends in *Daphnia* biomass and production of ephippia tended to increase over time in all treatments. Previous research has demonstrated that dormant eggs tend to be produced after populations reach saturation because the cost of sexual reproduction is diminished when populations approach maximum density

(Gerber et al., 2018). Environmental signals responsible for initiating sexual reproduction vary in type and number, as well as among species and even among clones within a species (LeBlanc & Medlock, 2015). It does not appear as though *Bythotrephes* chemical cues are a signal for sexual reproduction in *D. ambigua,* but it may be a signal for *D. pulicaria*. At the start of the experiment (day 13–26), all *D. pulicaria* jars receiving chemical cues (regardless of food level) produced the highest numbers of ephippia.

Algal biomass

As we predicted, the high food jars did, on average, have higher algal biomass than low food jars, indicating that our different food availability treatments were effective. We did not observe any consistent patterns in algal biomass in response to the species identity of the *Daphnia* or the presence of *Bythotrephes* chemical cues. However, the algal biomass values on days 39 and 52 (i.e., conclusion of the experiment) for the *D. ambigua* jars that received no cue and high food were dramatically and consistently higher than any other algal biomass values. This result may be explained by variation in *D. ambigua* clonal grazing efficiency and clonal selection (Tessier et al., 2000). For example, it is possible that treatment jars may have diverged in clonal composition where by one of the three *D. ambigua* clones used to start the microcosms may have either a low grazing efficiency or high nutrient-recycling rate (i.e., serving as a fertilization effect) and may have been selected for in the jars receiving the low food and no cue treatments. If this clone dominated the microcosms at the end of the experiment that may help to explain the dramatic spike in algal biomass in these jars at the end of the experiment. It is well-known that clonal differences in both grazing efficiency and nutrient recycling/retention efficiencies exist in natural *Daphnia* populations (Tessier et al., 2000; Frisch et al., 2014).

Unfortunately, tracking shifts in clonal composition/dominance was beyond the scope of this study, and thus, this conjecture cannot be tested.

Conclusion

To test the interactions of food quantity and predator chemical cues on *Daphnia* population dynamics, we administered two food availability (high versus low food availability) and two cue treatment (no cue versus *Bythotrephes* chemical cues) conditions to laboratory microcosms of *Daphnia* and recorded population parameters. We found that food quantity, species identity, and the presence of *Bythotrphes* cues influenced *Daphnia* and ephippial density, while food quantity and species identity were the primary drivers of shifts in *Daphnia* biomass. Food quantity and the presence of *Bythotrephes* cues were the most important predicters of algal biomass. By excluding the direct predation from *Bythotrephes*, the present study provided a creative way to explore non-lethal effects of an invasive predator on native prey species and provides insight into the combined effects of signals of predation risk and food availability on population-level dynamics of native prey species.

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Data availability statement

The data associated with this study are available in the Open Science Framework repository: https://osf.io/bt76d/?view_only=271d4b14748449d881e6af6320e2e3ed

References

Abrams, P. A. (1984). Foraging time optimization and interactions in food web. *The American Naturalist*, *124*, 80–96. https://doi.org/10.1086/284253

Arar, E. J., & Collins, G. B. (1997). Method 445.0 *In vitro* determination of chlorophyll *a* and pheophytin *a* in marine and freshwater algae by fluorescence. U.S. Environmental Protection Agency, Washington, DC.

Barton, K. (2020). MuMIn: Multi-model inference. R package version 1.43.17. https://CRAN.R-project.org/package=MuMIn

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

Beranek, A. (2012). An assessment of the long-term phenology and impact of *Bythotrephes longimanus* in Island Lake Reservoir, Minnesota, using sediment records. Master thesis, Department of Integrated Biosciences, University of Minnesota, Minneapolis, Minnesota, USA. http://hdl.handle.net/11299/121007

Berthon, K. (2015). How do native species respond to invaders? Mechanistic and trait-based perspectives. *Biological Invasions*, *17*, 2199–2211. https://doi.org/10.1007/s10530-015-0874-7

Black, A. R., & Dodson, S. I. (1989). Demographic costs of *Chaoborus*-induced phenotypic plasticity in *Daphnia pulex*. *Oecologia*, *83*, 117–122. https://doi.org/10.1007/BF00324642

Black, A. R., & Dodson, S. I. (2003). Ethanol: a better preservation technique for *Daphnia*. *Limnology and Oceanography: Methods*, *1*, 45–50. https://doi.org/10.4319/lom.2003.1.45

Boudreau, S. A., & Yan, N. D. (2003). The differing crustacean zooplankton communities of Canadian Shield lakes with and without the nonindigenous zooplanktivore *Bythotrephes longimanus*. *Canadian Journal of Fisheries and Aquatic Sciences*, *60*, 1307–1313. https://doi.org/10.1139/f03-111

Bungartz, B., & Branstrator, D. K. (2003). Morphological changes in *Daphnia mendotae* in the chemical presence of *Bythotrephes longimanus*. *Archiv fur Hydrobiologie*, *158*, 97–108. https://doi.org/10.1127/0003-9136/2003/0158-0097

Burnham, K. P., & Anderson, D. R. (2003). Model selection and multimodel inference: A practical information-theoretic approach (2nd ed.). Springer-Verlag.

Burns, C. W. (1969). Relation between filtering rale, temperature, and body size in four species of *Daphnia*. *Limnology and Oceanography*, *14*, 693-700. https://doi.org/10.4319/lo.1969.14.5.0693

Dambacher, J. M., & Ramos-Jiliberto, R. (2007). Understanding and predicting effects of modified interactions through a qualitative analysis of community structure. *The Quarterly Review of Biology*, *82*, 227–250. https://doi.org/10.1086/519966

Deng, H. (1996). Environmental and genetic control of sexual reproduction in *Daphnia*. *Heredity*, *76*, 449–458. https://doi.org/10.1038/hdy.1996.67

Dzialowski, A. R. (2010). Experimental effect of consumer identity on the invasion success of a non-native cladoceran. *Hydrobiologia*, *652*, 139–148. https://doi.org/10.1007/s10750-010-0326-4

Frisch, D., Morton, P. K., Chowdhury, P. R., Culver, B. W., Colbourne, J. K., Weider, L. J., & Jeyasingh, P. D. (2014). A millennial-scale chronicle of evolutionary responses to cultural eutrophication in *Daphnia*. *Ecology Letters*, *17*, 360–368. https://doi.org/10.1111/ele.12237

Gerber, N., Kokko, H., Ebert, D., & Booksmythe, I. (2018). *Daphnia* invest in sexual reproduction when its relative costs are reduced. *Proceeding of the Royal Society B: Biological Sciences*, *285*, 20172176. https://doi.org/10.1098/rspb.2017.2176

Gillis, M. K., & Walsh, M. R. (2017). Rapid evolution mitigates the ecological consequences of an invasive species (*Bythotrephes longimanus*) in lakes in Wisconsin. *Proceeding of the Royal Society B: Biological Sciences*, *284*, 20170814. https://doi.org/10.1098/rspb.2017.0814

Goitom, E., Kilsdonk, L. J., Brans, K., Jansen, M., Lemmens, P., & De Meester, L. (2018). Rapid evolution leads to differential population dynamics and top-down control in resurrected *Daphnia* populations. *Evolutionary Applications*, *11*, 96–111. https://doi.org/10.1111/eva.12567

Gu, L., Qin, S., Lu, N., Zhao, Y., Zhou, Q., Zhang, L., Sun, Y., Huang, Y., Lyu, K., & Yang, Z. (2020). *Daphnia mitsukuri* traits responding to predation cues alter its population dynamics. *Ecological Indicators*, *117*, 106587. https://doi.org/10.1016/j.ecolind.2020.106587

Harvell, C.D. (1990). The ecology and evolution of inducible defenses. *The Quarterly Review of Biology*, *65*, 323–340. https://doi.org/10.1086/416841

Jeyasingh, P. D., & Weider, L. J. (2005). Phosphorus availability mediates plasticity in life-history traits and predator-prey interactions in *Daphnia*. *Ecology Letters*, *8*, 1021-1028. https://doi.org/10.1111/j.1461-0248.2005.00803.x

Kilham, S. S., Kreeger, D. A., Lynn, S. G., Goulden, C. E., & Herrera, L. (1998). COMBO: a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia*, 377, 147–159. https://doi.org/10.1023/A:1003231628456

Kim, N., & Yan, N. D. (2010). Methods for rearing the invasive zooplankter *Bythotrephes* in the laboratory. *Limnology and Oceanography: Methods*, *8*, 552–561. https://doi.org/10.4319/lom.2010.8.552

Kopp, M., & Gabriel, W. (2006), The dynamic effects of an inducible defense in the Nicholson–Bailey model. *Theoretical Population Biology*, *70*, 43–55. https://doi.org/10.1016/j.tpb.2005.11.002

Landy, J. A., Oschmann, A., Munch, S. B., & Walsh, M. R. (2020). Ancestral genetic variation in phenotypic plasticity underlies rapid evolutionary changes in resurrected populations of waterfleas. *Proceeding of the National Academy of Sciences of the United States of America*, 117, 32535–32544. https://doi.org/10.1073/pnas.2006581117

Lathrop, R. (2013). Madison Wisconsin lakes zooplankton 1976 - 1994. Environmental Data Initiative. https://doi.org/10.6073/pasta/ec3d0186753985147d4f283252388e05

LeBlanc, G. A., & Medlock, E. K. (2015). Males on demand: The environmental-neuro-endocrine control of male sex determination in daphnids. *FEBS Journal*, *282*, 4080–4093. https://doi.org/10.1111/febs.13393

Leibold, M. A. (1996). A graphical model of keystone predators in food webs: trophic regulation of abundance, incidence, and diversity patterns in communities. *The American Naturalist*, 147, 784–812. https://doi.org/10.1086/285879

Lynch, M., Weider, L. J., & Lampert, W. (1986). Measurement of the carbon balance in *Daphnia*. *Limnology and Oceanography*, *31*, 17–33. https://doi.org/10.4319/lo.1986.31.1.0017

Mangiafico, S. (2020). rcompanion: Functions to support extension education program evaluation. R package version 2.3.25. https://CRAN.R-project.org/package=rcompanion

Magnuson, J., Carpenter, S., & Stanley, E. (2019). North temperate lakes LTER: Zooplankton - Madison lakes area 1997 - current. Environmental Data Initiative. https://doi.org/10.6073/pasta/8b265c0300252c87805f26f41e174aa4.

Magnuson, J., Carpenter, S., & Stanley, E. (2021). North Temperate Lakes LTER: Physical Limnology of Primary Study Lakes 1981 - current. Environmental Data Initiative. https://doi.org/10.6073/pasta/d4b9574043d6378391da731be81b6455.

Murdoch, W., Briggs, C., & Nisbet, R. (2003). Consumer–resource dynamics. Princeton University Press.

Nelson, E. H., Matthews, C. E., & Rosenheim, J. A. (2004). Predators reduce prey population growth by inducing changes in prey behavior. *Ecology*, *85*, 1853–1858. https://doi.org/10.1890/03-3109

Pijanowska, J., & Stolpe, G. (1996). Summer diapause in *Daphnia* as a reaction to the presence of fish. *Journal of Plankton Research*, 18, 1407–1412. https://doi.org/10.1093/plankt/18.8.1407

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Riessen, H. P., & Sprules, W. G. (1990). Demographic costs of antipredator defenses in *Daphnia pulex*. *Ecology*, *71*, 1536–1546. https://doi.org/10.2307/1938290

Slusarczyk, M., Ochocka, A., & Biecek, P. (2013). Prevalence of kairmone-induced diapause in Daphnia magna from habitats with and without fish. *Hydrobiologia*, *715*, 225–232. https://doi.org/10.1007/s10750-013-1552-3

Steiner, C. F., Darcy-Hall, T. L., Dorn, N. J., Garcia, E. A., Mittelbach, G. G., & Wojdak, J. M. (2005). The influence of consumer diversity and indirect facilitation on trophic level biomass and stability. *Oikos*, *110*, 556–566. https://doi.org/10.1111/j.0030-1299.2005.13665.x

Tessier, A. J., Leibold, M. A., & Tsao, J. (2000). A fundamental trade-off in resource exploitation by *Daphnia* and consequences to plankton communities. *Ecology*, *81*, 826–841. https://doi.org/10.1890/0012-9658(2000)081[0826:AFTOIR]2.0.CO;2

Tollrian, R. (1995). Predator-induced morphological defenses: costs, life history shifts, and maternal effects in *Daphnia pulex*. *Ecology*, *76*, 1691–1705. https://doi.org/10.2307/1940703

Tollrian, R., Duggen, S., Weiss, L. C., Laforsch, C., & Kopp, M. (2015). Density-dependent adjustment of inducible defenses. *Scientific Reports*, *5*, 12736. https://doi.org/10.1038/srep12736

Tollrian, R., & Harvell, C. D. (Eds.), (1999). The Ecology and Evolution of Inducible Defenses. Princeton University Press.

Vanni, M. J., & Lampert, W. (1992). Food quality effects on life history traits and fitness in the generalist herbivore *Daphnia*. *Oecologia*, *92*, 48–57. https://doi.org/10.1007/BF00317261

Walsh, J. R., Carpenter, S., & Vander Zanden, J. (2016). Invasive species triggers a massive loss of ecosystem services through a trophic cascade. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, 4081–4085. https://doi.org/10.1073/pnas.1600366113

Walsh, J. R., Munoz, S. E., & Vander Zanden, M. J. (2016). Outbreak of an undetected invasive species triggered by a climate anomaly. *Ecosphere*, *7*, e01628. https://doi.org/10.1002/ecs2.1628

Tables

Table 1 Top models for relationships between variation in predator threat, food availability, and *Daphnia* biomass, and algal biomass in experimental microcosms. AIC statistics include: *AIC_c* AIC statistic; *LL* log likelihood; *df* degrees freedom; R_m^2 marginal coefficient of determination; R_c^2 conditional coefficient of determination; ΔAIC_c AIC_c minus top model AIC_c; and *wi* model weight. RIVs for each variable are presented in (**bold**) next to the model variable the first time it appears (i.e., Food quantity (**1**), Species (**1**)).

Model	Model Variables	AIC_c	LL	Df	R_m^2	R_c^2	ΔAIC_c	wi
***Daphnia* biomass**	Food quantity (**1**), Species (**1**), Food quantity × Species (**0.79**)	216.5	-100.793	7	0.277	0.670	0.00	0.285
	Food quantity, Species, Cue (**0.63**), Food quantity × Species	217.9	-100.370	8	0.279	0.671	1.43	0.140
	Food quantity, Species, Cue, Food quantity × Species, Food quantity × Cue (**0.32**)	218.1	-99.278	9	0.285	0.672	1.56	0.131
Algae biomass	Cue (**0.81**), Food quantity (**0.74**),	-395.9	204.293	6	0.033	0.390	0.00	0.186
	Cue, Food quantity, Cue × Food quantity (**0.30**)	-395.7	205.317	7	0.042	0.398	0.19	0.169
	Cue	-395.1	202.804	5	0.019	0.377	0.78	0.126
	Food quantity	-394.2	202.353	5	0.014	0.372	1.68	0.080

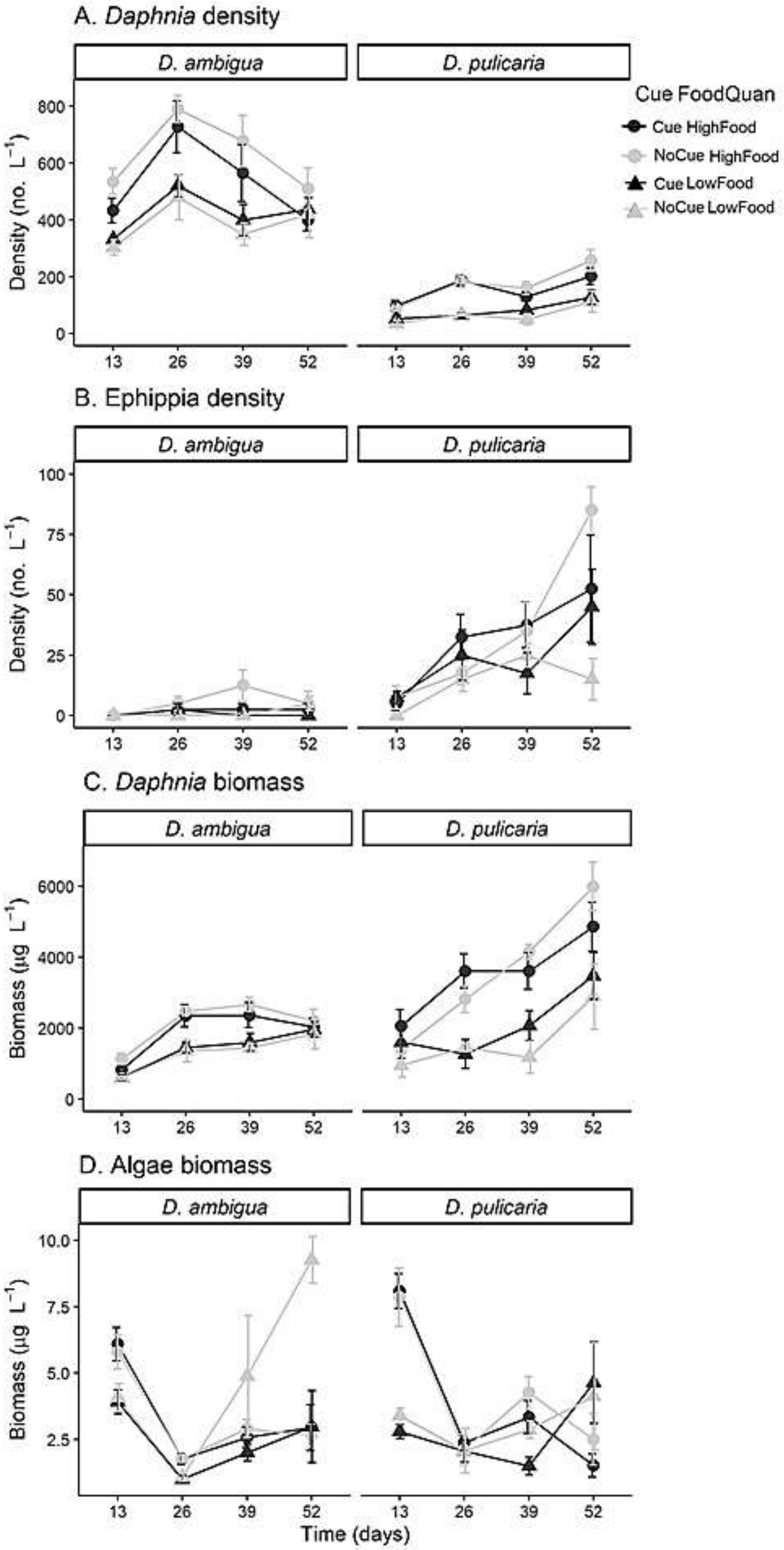
Table 2 Top models for relationships between variation in predator threat, food availability, and *Daphnia* and ephippial densities in experimental microcosms. AIC statistics include: AIC_c AIC statistic; *LL* log likelihood; *df* degrees freedom; R_m^2 marginal coefficient of determination; R_c^2 conditional coefficient of determination; ΔAIC_c AIC$_c$ minus top model AIC$_c$; and *wi* model weight. RIVs for each variable are presented in (**bold**) next to the model variable the first time it appears (i.e., Food quantity (**1**), Species (**1**)).

Model	**Model Variables**	**AIC$_c$**	**LL**	**Df**	**R_m^2**	**R_c^2**	**ΔAIC$_c$**	**wi**
***Daphnia* density**	Food quantity (**1**), Species (**1**), Food quantity × Species (**0.98**), Cue (**0.73**), Food quantity × Cue (**0.60**)	3208.3	-1595.567	8	0.909	0.994	0.00	0.363
	Food quantity, Species, Food Quantity × Species	3209.0	-1598.160	6	0.900	0.994	0.67	0.260
	Food quantity, Species, Cue, Food quantity × Cue, Food quantity × Species, Species × Cue (**0.27**)	3209.9	-1595.171	9	0.910	0.994	1.52	0.170
Ephippia density	Species (**1**), Food quantity (**0.99**), Cue (**0.74**), Species × Cue (**0.50**), Food quantity × Cue (**0.42**)	729.4	-356.084	8	0.291	0.417	0.00	0.152
	Species, Food quantity, Cue, Species × Cue	729.6	-357.310	7	0.282	0.395	0.17	0.139
	Food quantity, Species, Food quantity × Species (**0.44**)	729.7	-358.507	6	0.264	0.371	0.33	0.129
	Species, Food quantity	729.8	-359.643	5	0.240	0.344	0.40	0.125
	Species, Food quantity, Cue, Food quantity × Cue	730.4	-357.712	7	0.265	0.383	0.98	0.093
	Species, Food quantity, Cue, Species × Cue, Food quantity × Species	730.4	-356.616	8	0.303	0.415	1.06	0.089
	Species, Food quantity, Cue, Species × Cue, Food quantity × Species, Food quantity × Cue	730.4	-355.458	9	0.309	0.434	1.06	0.089
	Species, Food quantity, Cue, Food quantity × Species, Food quantity × Cue	731.4	-357.079	8	0.280	0.399	1.99	0.056

Figure captions

Figure 1 Impact of varying food quantity and predator threat on A.) *Daphnia* density (no. L^{-1}), B.) ephippial density (no. L^{-1}), C.) *Daphnia* biomass (μg L^{-1}), and D.) algal biomass (μg L^{-1}) in microcosm jars during the course of the experiment. Cue/no cue indicates the presence/absence of *Bythotrephes* chemical cues; high food jars received twice as much food as low food jars. (See methods for details).

Figure 1.



A. Daphnia density
D. ambigua
D. pulicaria
Density (no. L^{-1})
0
200
400
600
800
13
26
39
52
Cue FoodQuan
Cue HighFood
NoCue HighFood
Cue LowFood
NoCue LowFood
B. Ephippia density
Density (no. L^{-1})
0
25
50
75
100
C. Daphnia biomass
Biomass (µg L^{-1})
0
2000
4000
6000
D. Algae biomass
Biomass (µg L^{-1})
2.5
5.0
7.5
10.0
Time (days)

Synthesis

Predation of *Bythotrephes* on *Daphnia* has the potential to disrupt the ecosystem functioning in many lakes because when *Daphnia* are removed from these aquatic systems, there is less food for fish and lower grazing pressure to constrain algae growth. Understanding the response of *Daphnia* to *Bythotrephes* is important not only because of the wide-scale negative impacts of this invader, but also because it provides a baseline for studies of similar non-native predator-native prey relationships. The introduction of non-native species is happening at an unprecedented rate, which is likely only to increase in the future (Mooney & Cleland, 2001). My dissertation combined several lines of enquiry to investigate the ecology and evolution of antipredator defenses of native *Daphnia* species in response to the exotic invasive predator *Bythotrephes*. Both pre- (Chapter 1 - phototactic behavior and Chapter 2 - escape response) and post- (Chapter 3 - morphology) encounter antipredator defenses were explored, as well as the potential combined impacts of varying *Bythotrephes* threat and food availability on *Daphnia* population dynamics (Chapter 4 - population experiment).

Although it is often assumed that native prey species are vulnerable to the invasion of non-native predators due to the lack of a shared evolutionary history (Cox & Lima, 2006; Sih et al., 2010), I have demonstrated that native prey populations can track the introduction of a non-native predator via a combination of inducible and constitutive defenses. 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 my first chapter demonstrated that although each *Daphnia* species displayed a distinct phototactic behavior, this behavior was not affected by the presence of *Bythotrephes* chemical cues or exposure history (i.e., whether the clone was from pre- or post-invasion time periods). In my second chapter, I posited that differences in the escape ability of Lake Mendota *Daphnia* have contributed to differences in vulnerability, which in turn, have influenced the community changes observed after the establishment of *Bythotrephes* (Walsh et al., 2016b). In my third chapter, I found that *Daphnia* are responding to the invasion of *Bythotrephes* with species-specific changes in key morphological traits. Finally, the results of my fourth chapter demonstrated that food quantity, species identity, and the presence of *Bythotrephes* cues influenced *Daphnia* and ephippial density, while food quantity and species identity were the primary drivers of shifts in *Daphnia* biomass.

Despite the documented ability of native prey to detect and respond to invasive predators with inducible and constitutive defenses, native *Daphnia* populations have still been heavily impacted by the invasive predator *Bythotrephes* (Walsh et al., 2016a). Although antipredator defenses often slow predation, they do not prevent predation entirely. Clearly, future research is warranted to decipher the ability of native *Daphnia* prey species to use morphological, behavioral, and life-history adaptations to serve as deterrents to *Bythotrephes*. In particular experiments with live *Bythotrephes* are needed to determine how development of these defenses affects predation rates/success. The inherent difficulty of maintaining viable laboratory cultures of *Bythotrephes* (Kim & Yan, 2010) precluded the use of live predators in my experiments.

My findings contribute to a growing body of research, which suggests that it is critical to recognize the evolutionary component of non-native predator/native prey species interactions in order to further our understanding of the long-term impacts of biological invasions on native communities (Mooney & Cleland, 2001; Strauss et al., 2006). It is clear that native species, like invaders, can rapidly adapt to novel selective pressures (e.g. Langkilde, 2009; Stoks et al., 2015;

Bible et al., 2017; Landy et al., 2020). Research such as mine, which focused on understanding how interactions between native and non-native species change over time, will become increasingly important, as we work to understand the dynamics of such perturbations and the impacts on future ecosystems. These aspects become especially critical in the face of continued movement/dispersal of non-native species due to human activities on a global scale.

References

Bible, J.M., Griffith, K.R., & Sanford, E. (2017). Inducible defenses in Olympia oysters in response to an invasive predator. *Oecologia*, 183, 809–819. https://doi.org/10.1007/s00442-017-3811-x

Cox, J., & Lima, S. (2006). Naiveté and an aquatic-terrestrial dichotomy in the effects of introduced predators. *Trends in Ecology and Evolution*, 21, 674–680. https://doi.org/10.1016/j.tree. 2006.07.011

Kim, N., & Yan, N.D. (2010). Methods for rearing the invasive zooplankter *Bythotrephes* in the laboratory. *Limnology and Oceanography: Methods*, 8, 552–561. https://doi.org/10:4319/lom.2010.8.552

Landy, J.A., Oschmann, A., Munch, S.B., & Walsh, M.R. (2020). Ancestral genetic variation in phenotypic plasticity underlies rapid evolutionary changes in resurrected populations of waterfleas. *Proceedings of the National Academy of Sciences*, 117, 32535–32544. https://doi.org/10.1073/pnas.2006581117

Langkilde, T. (2009). Invasive fire ants alter behavior and morphology of native lizards. *Ecology*, 90, 208–217. https://doi.org/10.1890/08-0355.1

Mooney, H.A., & Cleland, E.E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences*, 98, 5446–5451. https://doi.org/10.1073/pnas.091093398

Sih, A., Bolnick, D.I., Luttbeg, B., Orrock, J.L., Peacor, S.D., Pintor, L.M., Preisser, E., Rehage, J.S., & Vonesh, J.R. (2010). Predator–prey naïveté, antipredator behavior, and the ecology of predator invasions. *Oikos*, 119, 610–621. https://doi.org/10.1111/j.1600-0706.2009.18039.x

Stoks, R., Govaert, L., Pauwels, K., Jansen, B., & De Meester, L. (2015). Resurrecting complexity: the interplay of plasticity and rapid evolution in the multiple trait response to strong changes in predation pressure in the water flea *Daphnia magna*. *Ecology Letters*, 19, 180–190. https://doi.org/10.1111/ele.12551

Strauss, S.Y., Lau, J.A., & Carroll, S.P. (2006). Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters*, 9, 357–374. https://doi.org/10.1111/j.1461-0248.2005.00874.x

Walsh, J.R., Carpenter, S.R., & Vander Zanden, M.J. (2016a). Invasive species triggers a massive loss of ecosystem services through a trophic cascade. *Proceedings of the National Academy of Sciences*, 113, 4081–4085. https://doi.org/10.1073/pnas.1600366113

Walsh, J.R., Munoz, S.E., & Vander Zanden, M.J. (2016b). Outbreak of an undetected invasive species triggered by a climate anomaly. *Ecosphere*, 7, e01628. https://doi.org/10.1002/ecs2.1628