PREVENTING PREDATION: EVOLUTION AND ADAPTIVE PLASTICITY IN MORPHOLOGICAL DEFENSE OF AN INVASIVE SPECIES

By

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ABSTRACT

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Invasive species are one of the leading threats to global biodiversity, but until recently, little attention has been paid to how evolution contributes to invasive species effects despite important applications to conservation and our general understanding of evolution in the wild. Invasive species often experience strong selection upon introduction, which may result in adaptive evolution that could facilitate their successful integration into food webs and their effects on native species. Recent species invasions in North America offer a strong opportunity to address fundamental questions in evolutionary ecology as well as advance our understanding of invasive species effects. *Bythotrephes longimanus* (the spiny water flea) is a predatory zooplankton with a conspicuous tail spine that invaded the Great Lakes region during the 1980s and may be having large negative effects on fisheries. Previous field studies show the morphology and life history of *Bythotrephes* strongly vary spatially and temporally, but the cause is not known. Evolution by natural selection and phenotypic plasticity are potential sources of *Bythotrephes* trait variation, but heretofore, these sources of variation have not been investigated.

My dissertation research investigated ecological and evolutionary factors that influence morphological and life history variation in *Bythotrephes*. Using *Bythotrephes* collected from Lake Michigan, I found moderate-to-high genetic variation in distal spine and body length and maternal effects in both traits. Further, experiments revealed that spine length, body size, and clutch size respond plastically to temperature but not to fish predator cues, with higher

temperature inducing mothers to have smaller clutches of larger offspring (longer absolute distal spine and body length) that were better defended against predation. Although *Bythotrephes* use temperature as the proximate cue of plasticity, it is likely that the trait changes represent adaptations to varying fish predation risk which correlates with water temperature. I also found temporally and spatially variable selection on distal spine length consistent with seasonal changes in gape-limitation of fish predators and spatial heterogeneity of fish, respectively. Yet, despite net selection for increased distal spine length, I observed little evidence of an evolutionary response to selection based on comparisons of historic and contemporary wild-captured individuals and retrieved spines from sediment cores. In a companion study of Canadian Shield lakes, I identified gape-limited fish predators as agents of selection on *Bythotrephes* distal spine length. Specifically, I found selection for increased distal spine length in lakes dominated by a gape-limited fish predator and no significant selection in lakes dominated by a non-gape-limited fish predator. A large difference (20%) in average distal spine length between lakes of each predator type was consistent with the direction of selection, suggesting potential local adaptation of distal spine length to gape-limited fish predation.

The results of my dissertation indicate *Bythotrephes* respond to fish predation through multiple mechanisms, including phenotypic plasticity and evolutionary responses to selection. These responses to predation likely promote *Bythotrephes* success as an invasive species, and may also underlie negative effects on important Great Lakes fisheries through food web interactions. More generally, the results of my dissertation suggest the effects of invasive species may occur not just through their ecological interactions, but also through evolutionary and phenotypically plastic trait modifications. As invasive species continue to affect biodiversity worldwide, understanding the mechanisms behind invasive species effects is critical.

To my research assistants, for your curiosity, enthusiasm, and spirit

~ and ~

To my husband, for your strength, dedication, and unwavering support

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CHAPTER 1

A new beginning: How species invasions provide insight into evolutionary ecology

Andrea Lynn-Jaeger Miehls

Introduction

As an evolutionary ecologist, I view the natural world through a lens of questions: What are the determinants of trait phenotype? How do species traits affect interspecific interactions? How fast do traits evolve in the wild? What conditions are conducive to adaptive evolution? What conditions limit adaptive evolution? These questions have been the focus of the field of evolutionary ecology for decades. They are critical questions, and they are also difficult questions to address. For instance, consider predicting or measuring the rate of adaptive evolution. For many species, we often lack information regarding the duration of selective events, trait phenotype prior to selection, the identity of selective agents, and the amount of genetic variation in traits before selection (but note that there are exceptions, including long-term datasets for, e.g., collared flycatchers, *Ficedula albicollis*: Kruuk et al. 2001; Merilä et al. 2001; great tits, *Parus major*: Boyce and Perrins 1987; red deer, *Cervus elaphus*: Kruuk et al. 2000; 2002; red squirrels, *Tamiasciurus hudsonicus*: McAdam and Boutin 2003; 2004; Soay sheep, *Ovis aries*: Milner et al. 1999; 2000), all of which may hinder the prediction or measurement of evolutionary rates. Moreover, many studies lack temporal or spatial replication (Kingsolver et al. 2001), which may reduce confidence in measurements or hinder the ability to generalize results.

With the recognition of these challenges to the study of evolutionary ecology has also come the awareness that massive contemporary movements of species across the globe provide incredible opportunities for the study of evolution. The introduction of species to new areas (i.e., species invasions) is occurring at an unprecedented rate due to anthropogenic movement of species as well as anthropogenic alteration of the environment in ways that allow species to expand their historic distributions (Mack et al. 2000; Kolar and Lodge 2001). Although the ecology of species invasions has been well studied for decades (reviewed in, e.g., Elton 1958; Mack et al. 2000; Pimentel et al. 2000; Ehrenfeld 2010), it was not until recently that the value of species invasions for providing insight into evolutionary processes was widely appreciated (reviewed in, e.g., Levin 2003; Cox 2004; Sax et al. 2005; Callaway and Maron 2006; Strauss et al. 2006; Sax et al. 2007). However, it is interesting to note that, despite the recent merge of evolutionary ecology and invasion biology, parallels between species introductions and historic evolutionary events were recognized by Darwin (1859).

The primary value of the study of species invasions for our understanding of evolutionary ecology is the ability to observe evolution in "real time", free from many of the challenges that often plague evolutionary investigations. For example, species invasions are often replicated temporally and spatially, providing unplanned experiments (Grinnel 1919) for the study of evolutionary processes over scales that would otherwise be nearly impossible (Sax et al. 2005; 2007). Moreover, the characteristics of species invasions are often well known, e.g., information on the date of introduction and number of invading individuals is frequently available (Sax et al. 2005). Additionally, because of the interest in the ecology of species invasions, there are frequently data available on population growth rates post-invasion and possibly even specimens of invading individuals, often with their strong potential to answer evolutionary questions going unrecognized (A. Miehls, *pers. obs.*). Finally, if information on the native range is available, a wealth of evolutionary hypotheses can be generated based on differences in the ecology, and thereby differences in selective pressures, between the native and invaded range. Thus, the study of invasive species offers incredible opportunities to answer questions in evolutionary ecology that otherwise are extremely challenging to address.

Moreover, the study of invasive species also entails an important application due to the effects of invasive species on biodiversity and their economic cost. Species invasions are now considered one of the world's leading threats to biodiversity and ecosystem function (Mills et al. 1994; Vitousek et al. 1997; Pimentel et al. 2000; Kolar and Lodge 2001). Cost estimates for control and remediation from the early 2000s, which are certainly lower than current-day costs, indicate annual expenditures of over $141 billion in the United States and $248 billion worldwide (Pimentel et al. 2000; Cox 2004). These costs are in addition to other, more difficult to estimate, indirect costs to fisheries, navigation, and industry, and non-monetary costs to spiritual and aesthetic value of ecosystems. Given the severe effects of species invasions, invasive species are now considered part of a four-pronged ecological and evolutionary "revolution" (Cox 2004), in combination with habitat destruction and degradation, over-exploitation of plants and animals, and global climate change.

Evolutionary Ecology of Species Invasions

Invasive species experience many selective pressures in the invaded range, which they may respond to through phenotypic plasticity (i.e., the ability of a genotype to produce different phenotypes in response to differing environmental conditions; Baker 1965; Daehler 2003; Richards et al. 2006; Pichancourt and van Klinken 2012) and evolution (Mooney and Cleland 2001; Lee 2002; Cox 2004; Lambrinos 2004; Strayer et al. 2006). Phenotypic plasticity is considered advantageous for species invasions as plasticity enhances ecological niche breadth by allowing organisms to express advantageous phenotypes in a broad range of environments (Bradshaw 1965; Richards et al. 2006). When environmental conditions are similar between the native and invaded range, phenotypic plasticity may pre-adapt (Schlaepfer et al. 2009) invasive species, granting high fitness in the invaded range. However, invasive species will also experience numerous new selective pressures, wrought by novel predators, prey, competitors, and abiotic conditions in the invaded range. New selective pressures may result in evolution of invasive species traits (Lee 2002; Cox 2004; Lambrinos 2004; Bossdorf et al. 2005). There is growing evidence that evolution of invasive species traits may occur frequently, and that evolution is critically important to the establishment success of many invasive species (Cox 2004; Lambrinos 2004). Importantly, evolution of invasive species traits is not restricted to canalized traits (i.e., traits whose expression is unaffected by the developmental environment; Stearns 1989). Evolution of phenotypic plasticity (either through *de novo* evolution of plasticity or evolution of existing reaction norms) may also occur during species invasions (Sexton et al. 2002; Parker et al. 2003; Richards et al. 2006).

There is mounting evidence that phenotypic plasticity and evolution play a key role in the pronounced effects of invasive species on ecosystems. Regarding phenotypic plasticity, there are multiple examples that indicate phenotypic plasticity contributes to the competitive effects of invasive species on native species. Many plants are more vigorous in the invaded range due to phenotypic plasticity in resource acquisition traits (Chun et al. 2007; Zhao et al. 2010), which is thought to aid in the competitive displacement of native species (Blossey and Notzold 1995; Zhao et al. 2010). Behavioral plasticity in fish (e.g., American shad, *Alosa sapidissima*; Quinn and Adams 1996) and morphological plasticity in the European wild rabbit (*Oryctolagus* *cuniculus*; Williams and Moore 1989) may also increase their competitive ability and effects on native species.

There is also growing evidence that evolution of invasive species traits contributes to their ecological effects. For example, release of purple loosestrife (*Lythrum salicaria*) from specialized herbivores in the native European range, and subsequent evolution of phenotypic plasticity in North America, likely contributed to their competitive displacement of native species (Blossey and Notzold 1995; Chun et al. 2007). Further, evolution of longer legs by cane toads (*Bufo marinus*) has increased their range expansion rate in Australia from 10km yr^{-1} to over 50km yr^{-1} (Phillips et al. 2006), thus magnifying their already-severe ecological effects on native predators, prey, and competitors. Finally, Argentine ant (*Linepithema humile*) invasion in North America involved a reduction in genetic diversity that was adaptive in the invaded range due to decreased territoriality and intraspecific competition, which contributed to their competitive displacement of native ant species (Tsutsui et al. 2000; Holway and Suarez 2004).

Whether invasive species respond to selective pressures in the invaded range through phenotypic plasticity or evolution (including the evolution of plasticity) depends on the frequency and direction of environmental change. When environmental change is persistent and directional across multiple generations, evolutionary responses typically yield the highest fitness (Lee and Gelembiuk 2008; Svanbäck et al. 2009). Conversely, when environmental change involves short-term fluctuations (e.g., fluctuations within a generation, or between only a few generations), phenotypic plasticity typically yields the highest fitness (Lee and Gelembiuk 2008; Svanbäck et al. 2009). An important aspect of the fluctuating environments that select for phenotypic plasticity is that the cues for plasticity (i.e., the environmental conditions that trigger altered phenotype) must be consistently associated with selective environments (i.e., cues must reliably predict selective environments; Hairston 1987), and plasticity must yield higher fitness than constitutive trait expression across environments (Doughty and Reznick 2004). Thus, whether invasive species respond to novel selection pressures in the invaded range through phenotypic plasticity or evolution depends on temporal variation in selective pressures.

Focus of Dissertation

My dissertation focuses on evolutionary and phenotypically plastic morphological and life history responses of an invasive species to predation. I used an invasive species in this work because of the numerous advantages discussed above that species invasions provide toward addressing fundamental questions in evolutionary ecology, as well as the important application to conservation that the study of invasive species entails. My dissertation focuses on the following questions:

General: How do species respond to selection pressures in new environments? When are phenotypically plastic vs. evolved responses expected?

Chapter 2: How do genetic variation and maternal effects in traits under selection vary temporally?

Chapter 3: What environmental conditions are used as cues for phenotypic plasticity, and do cues need to be directly related to selective agents, or can proxy cues be used?

Chapter 4: How does natural selection vary temporally and spatially, and what factors constrain evolutionary responses to selection in the wild?

Chapter 5: Who are the agents of selection and how quickly do species traits evolve in the wild?

Study Organism

I used the recent invasion by *Bythotrephes longimanus* (the spiny water flea) in the Laurentian Great Lakes as a model system for addressing these questions. *Bythotrephes* are epilimnetic, planktivorous cladoceran zooplankton that were first found in Lake Ontario in 1982 (Barbiero and Tuchman 2004). Soon after their initial detection, *Bythotrephes* spread to Lakes Huron (1984), Erie (1985), Michigan (1986), and Superior (1987; Mills et al. 1993), as well as over 90 surrounding lakes (Strecker et al. 2006). *Bythotrephes* are an efficient predator of both large and small-bodied zooplankton (Schulz and Yurista 1999; Bunnell et al. 2011), and are also a relatively large and conspicuous prey item for many young and adult fish (Schneeberger 1991; Pothoven et al. 2007; 2012).

Effects of Bythotrephes *Invasion*

The initial effects of the *Bythotrephes* invasion were well documented in the Great Lakes, especially Lake Michigan (e.g., Lehman 1991; Lehman and Cáceres 1993; Schulz and Yurista 1999). The primary direct effect of *Bythotrephes* invasion was a shift in the size structure and abundance of zooplankton communities (Wahlström and Westman 1999; Bunnell et al. 2011), including the decline of four zooplankton species (*Leptodora kindtii*, *Daphnia mendotae*, *D. pulicaria*, and *D. retrocurva*; Barbiero and Tuchman 2004). Additionally, there is evidence (e.g., Mills et al. 1992; Lehman and Cáceres 1993; Dumitru et al. 2001; Yan et al. 2001) that *Bythotrephes* may be having indirect deleterious effects on juvenile and adult fish. Effects on juvenile fish may occur through competition, as suggested by declines associated with failed recruitment in yellow perch (*Perca flavescens*) in a small lake (Long Lake; Hoffman et al. 2001) and Lake Michigan (Vanderploeg et al. 2002; Dettmers et al. 2003) closely following *Bythotrephes* invasion. *Bythotrephes* can also affect fish by interfering with foraging ability, leading to slower fish growth and higher mortality (Barnhisel and Kerfoot 2004; Compton and Kerfoot 2004), and through direct physical injury following consumption, including stomach and digestive tract punctures in up to 30-40% of cases for small fish (Compton and Kerfoot 2004). Finally, recent precipitous declines in Lake Huron alewife (Johnson et al. 2005), and decreased condition of Pacific salmon (*Oncorhynchus* spp.), have been linked to *Bythotrephes* via food web interactions (Johnson et al. 2005; Eshenroder, R.L. pers. comm.).

Life History and Morphology of Bythotrephes

Bythotrephes reproduce by cyclic parthenogenesis and have a short generation time (10-15 day, depending on water temperature; Branstrator 2005) that may yield up to ten or more asexual generations during the growing season. The asexual generations are followed by sexual reproduction at the end of the season (Yurista 1992; Pothoven et al. 2001; Branstrator 2005). The *Bythotrephes* morphology features a large body size and long tail spine. The longest component of the tail spine is the distal spine, which is present at birth and does not change with instar development (i.e., the length of the distal segment does not grow; Burkhardt 1994; Sullivan and Lehman 1998). Instead, during the first two molts, total spine length increases through the addition of intercalary segments that each end with a paired barb (Branstrator 2005). The long spine can comprise over 10% of adult body mass (Sullivan and Lehman 1998; Branstrator 2005) and 80% of body length (Barnhisel and Harvey 1995). The length of the spine is considered a key trait influencing *Bythotrephes* food web dynamics, serving as an effective deterrent against predation by gape-limited fish (typically juvenile fish <100mm in length; Barnhisel 1991a; Barnhisel 1991b; Schneeberger 1991; Baker et al. 1992; Barnhisel and Harvey 1995).

Potential for Evolution and Phenotypic Plasticity in Bythotrephes *Traits*

The morphology and life history of *Bythotrephes* in the Great Lakes strongly vary temporally (Burkhardt 1994; Pothoven et al. 2001; 2003), but the cause is not known. In Lake Michigan, for example, *Bythotrephes* tail spine and body length are shortest at the start of the growing season (June-July), longest mid-growing season (August-September), and then decrease at the end of the growing season (October onward; Pothoven et al. 2001). Observed seasonal changes in *Bythotrephes* traits correlate with and have been theorized to be a defense against gape-limited fish predation, which increases throughout the growing season and typically comprises the dominant source of predation on *Bythotrephes* mid-to-late in the growing season (Branstrator 2005).

Seasonal elongation of the tail spine and increased body size in *Bythotrephes* are consistent with two explanations: 1) adaptive phenotypic plasticity to reduce the risk of gape-limited fish predation; and 2) natural selection by gape-limited and non-gape-limited fish predators. Numerous studies of cladoceran zooplankton and other taxa (e.g., Dodson 1974; Agrawal 2001; Laforsch and Tollrian 2004; Kishida et al. 2006) have shown plasticity in prey morphology in response to gape-limited predators. These studies are suggestive that seasonal variation in *Bythotrephes* traits may represent plastic responses to gape-limited predation. Alternatively, seasonal variation in *Bythotrephes* traits may be due to temporally fluctuating selection by gape-limited and non-gape-limited fish predators. Many studies indicate gape-limited predation selects for increased morphological size (e.g., Paine 1976; Case 1978; Day et al. 2002; Zimmerman 2007; Urban 2008), whereas non-gape-limited predation selects for decreased morphological size if larger individuals are preferentially consumed (e.g., Hambright 1991; Urban 2007; 2008). Thus, natural selection by temporally varying predator gape-limitation may also explain seasonal variation in *Bythotrephes* traits. My dissertation explores these mechanisms as drivers of phenotypic change in *Bythotrephes* traits.

Chapter Outline

*Chapter 2: Genetic and maternal effects on tail spine and body length in the invasive spiny water flea (*Bythotrephes longimanus*)*

Chapter 2 provides a foundation for my entire dissertation by investigating genetic, maternal, and environmental sources of phenotypic variation in *Bythotrephes* distal spine and body length. I quantified these variance components during five time periods across three years using a laboratory clonal analysis of wild-captured *Bythotrephes* from Lake Michigan. I found that variance components for distal spine length were variable among but not within years. Spine length was always heritable but was not always influenced by maternal effects. In contrast, variance components for body length varied both within and among years, but likewise body length was always heritable and not always influenced by maternal effects. Results indicate *Bythotrephes* traits have heritable variation comparable to native species and other invasive species that would enable an evolutionary response to natural selection. This chapter was published in *Evolutionary Applications*:

Miehls, A.L.J., S.D. Peacor, and A.G. McAdam. 2012. Genetic and maternal effects on tail spine and body length in the invasive spiny water flea (*Bythotrephes longimanus*). *Evolutionary Applications* 5:306-316.

Chapter 3: Plastic response to a proxy cue of predation risk when direct cues are unreliable

Chapter 3 explores phenotypic plasticity in *Bythotrephes* morphological and life history traits in response to temperature and fish cues. Investigating phenotypically plastic response of

Bythotrephes traits to their environment is critical for understanding: 1) the basis of previously observed temporal trait variation; 2) possible adaptive plasticity in *Bythotrephes* traits that might facilitate their invasion in novel ecosystems; and 3) whether plasticity may serve as a constraint on an evolutionary response to selection. Using a laboratory experiment with *Bythotrephes* collected from Lake Michigan, I found no effect of fish (*Perca flavescens*) cues on *Bythotrephes* morphology or life history. In contrast, higher water temperature led to longer absolute spine and body length, increased investment in morphological defense of offspring (measured as the ratio of spine-to-body length), and decreased clutch size and age at reproduction. I argue that these plastic responses are unlikely to be adaptive to temperature *per se*, but rather my findings indicate that temperature serves as a proxy cue of predation risk.

Chapter 4: Evolutionary stasis despite selection on a heritable trait in an invasive zooplankton

Chapter 4 is the capstone chapter for the research on *Bythotrephes* evolutionary ecology in Lake Michigan. Chapter 4 presents an analysis of temporal and spatial variation in natural selection on *Bythotrephes* distal spine length in Lake Michigan and corresponding evolutionary response to selection. I found that selection varied temporally, with selection for decreased distal spine length early in the growing season, and strengthening selection for increased distal spine length later in the growing season, a trend consistent with seasonal changes in the relative strength of non-gape-limited and gape-limited fish predation. Selection also varied spatially, which is consistent with well-known heterogeneity of fish predators in large lakes. Yet, despite net selection for increased distal spine length (and known genetic basis of distal spine length; Chapter 2), I observed little evidence of an evolutionary response to selection.

Chapter 5: Gape-limited predators as agents of selection on the defensive morphology of an invasive species

Chapter 5 investigates the agents of selection on *Bythotrephes* distal spine length using a set of Canadian Shield lakes dominated by either a gape-limited or non-gape-limited fish predator. Chapter 5 is both a supporting chapter for Chapter 4 (by providing a test of selection by gape-limited and non-gape-limited fish predators) and also a stand-alone exploration of possible local adaptation of *Bythotrephes* distal spine length in response to different selective environments. I found that natural selection varied by gape-limitation, with strong selection for increased distal spine length in lakes dominated by gape-limited predation, and no significant selection in lakes dominated by non-gape-limited predation. Distal spine length was 20% longer in lakes dominated by gape-limited predation, suggesting local adaptation in response to selection for increased distal spine length. As all study lakes were invaded less than twenty years prior to my collections, my findings suggest rapid divergence in defensive phenotype in response to selection from predators.

Implications of Dissertation Research

My dissertation research provides multiple contributions to the study of invasive species and evolutionary ecology. Chapter 2 advances our current understanding of evolutionary ecology by investigating genetic variation in quantitative traits of an invasive species, for which the vast majority of previous work has focused on neutral genetic variation. Further, Chapter 2 quantifies maternal variation, an under-appreciated source of phenotypic variation, and tests the stability of genetic and maternal variation through time, a rarely completed analysis. Chapter 3 presents what I believe to be the first evidence for a plastic response of a prey to a correlate of predation risk, but no response to cues released from the predator. This work raises the possibility that organismal use of proxy cues in nature may be more common than previously realized, if proxy cues, due to their indirect and potentially surprising relationships to the adaptive responses they elicit, are less often investigated than more obvious potential direct cues of plastic responses. Chapter 5 demonstrates evolutionary stasis in an important trait governing interspecific interactions that is under significant, directional selection and has a strong genetic basis. This finding is based on one of the largest temporally and spatially replicated investigations of natural selection (47 total estimates) ever completed, and also uses multiple lines of evidence (wild captured individuals, paleoecology of retrieved spines) to show evolutionary stasis. This research indicates evolutionary stasis can occur, even when an evolutionary response is expected due to selection on genetically-based traits. Finally, Chapter 5 identifies gape-limited predators as agents of selection; prior evidence for natural selection in the wild by predators on traits of prey, especially invasive prey, is surprisingly limited. Moreover, the results of this chapter are suggestive that substantial differences in prey phenotype can evolve rapidly (less than twenty years) in response to predation.

Why a New Beginning?

I chose to precede the title of this chapter with “a new beginning”, a phrase not (entirely) intended as substanceless, emotive language (although, undoubtedly the title reflects some sentimentality as I conclude nine years of graduate study). Instead, this title is intended to echo an exciting moment in invasion biology and evolutionary ecology, as the current convergence of these fields brings about new discoveries in evolutionary ecology and new perspectives for invasion biology. I began this chapter with a discussion of the value of invasive species as model organisms for addressing major questions in evolutionary ecology. Ongoing work, including my own dissertation research, indicates the study of invasive species can provide numerous insights into evolutionary ecology that are otherwise incredibly challenging to attain. Thus, the study of invasive species is catalyzing many new discoveries in the field of evolutionary ecology. Further, what I mean by “new perspectives” is a re-conceptualization of species invasions as tremendous learning opportunities. Of course, invasive species have caused incredible damage to ecosystems, and I have no intention of glorifying or promoting these events. However, what I desire to emphasize is the importance of learning from invasions and using that knowledge toward a better end, whether it’s preventing future invasions or providing insight into processes governing the natural world. This is an optimist’s perspective, making the best out of a bad situation. Finally, on a more personal note, this dissertation defines a new beginning in my own life, as I conclude one professional endeavor and eagerly begin the next.

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CHAPTER 2

Genetic and maternal effects on tail spine and body length in the invasive spiny water flea (*Bythotrephes longimanus*)

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Abstract

Interest in the evolution of invasive species has grown in recent years, yet few studies have investigated sources of variation in invasive species traits experiencing natural selection. The spiny water flea, *Bythotrephes longimanus*, is an invasive zooplankton in the Great Lakes that exhibits seasonal changes in tail spine and body length consistent with natural selection. Evolution of *Bythotrephes* traits, however, depends on the presence and magnitude of quantitative genetic variation, which could change within or across years. Clonal analysis of wild-captured *Bythotrephes* indicated that variance components for distal spine length were variable among but not within years. Spine length was always heritable but was not always influenced by maternal effects. In contrast, variance components for body length varied both within and among years, but likewise body length was always heritable and not always influenced by maternal effects. Results indicate that important *Bythotrephes* traits have heritable variation comparable to native species and other invasive species that would enable an evolutionary response to natural selection. This evolutionary capacity could contribute to the widespread success and dramatic effects of *Bythotrephes* invasion in systems with diverse biotic and abiotic conditions.

Introduction

Invasive species are considered one of the leading threats to biodiversity and ecosystem function. Whereas the myriad and often severely negative ecological effects of species invasions have garnered considerable research attention for decades (reviewed in, e.g., Elton 1958; Mack et al. 2000; Pimentel et al. 2000; Ehrenfeld 2010), research on the evolution of invasive species has intensified only recently (reviewed in, e.g., Mooney and Cleland 2001; Lee 2002; Cox 2004; Lambrinos 2004; Strayer et al. 2006). This growing body of research suggests invasive species often evolve upon introduction into new environments due to novel selection pressures, population bottlenecks, founder effects, and hybridization with related species in the invaded range, as well as freedom from selection pressures in the native range (Lambrinos 2004; Bossdorf et al. 2005). Further, there is growing evidence that evolution of invasive species may play a key role in their pronounced ecological effects (Mooney and Cleland 2001; Lambrinos 2004; Strayer et al. 2006).

Although we are beginning to appreciate the importance of evolution to the ecological effects of invasive species, we still have limited knowledge of the rate of and constraints on invasive species evolution (but see Lavergne and Molofsky 2007; Dlugosch and Parker 2008; Colautti et al. 2010). In particular, we know little about genetic variation in invasive species traits despite its potential to constrain evolutionary rates (Lynch and Walsh 1998; Conner and Hartl 2004; but see Blows and Hoffman 2005). A wealth of studies of neutral (molecular) genetic variation in invasive species have been published (Dlugosch and Parker 2008), but there have been few studies of genetic variation in quantitative traits (reviewed in Table 2.1; see also Dlugosch and Parker 2008). Furthermore, the few studies that have addressed quantitative genetic variation suggest no consistent pattern in the amount of genetic variation in invasive species traits; quantitative genetic variation ranged from very low to exceptionally high (Table 2.1).

The amount of genetic variation for a given trait can also vary in space and time due to genotype-by-environment interactions (Pigliucci 2001) or the erosion of genetic variation by natural selection. For example, previous research with clonally reproducing zooplankton suggests that genetic variation, as well as the ratio of genetic variation to total phenotypic variation (i.e., heritability), can decline throughout the growing season due to clonal selection (Lynch 1984; Pfrender and Lynch 2000). Thus, to understand how genetic variation affects the evolution and persistence of invasive species, it is important that genetic variation be quantified across a range of environmental conditions across the growing season.

Recent species introductions into the North American Great Lakes offer a strong opportunity to investigate sources of variation in invasive species traits and their ramification for ecological interactions. One invasive species considered to have potent ecological effects in the Great Lakes is *Bythotrephes longimanus*, the spiny water flea (hereafter *Bythotrephes*; Figure 2.1), a predatory zooplankton native to Eurasia that was introduced to North America in the 1980s (Mills et al. 1993). The invasion of *Bythotrephes* has resulted in dramatic ecosystem changes due to their central position in the food web as a dominant predator of zooplankton (Bunnell et al. 2011) and common prey for fish (Pothoven et al. 2007). The primary ecological effects of *Bythotrephes* involve alteration of zooplankton community abundance and composition (Lehman 1991; Yan et al. 2002; Barbiero and Tuchman 2004), which may affect phytoplankton communities (Strecker and Arnott 2008) and larval fish (Hoffman et al. 2001) through trophic cascades.

In this study we used clonal analysis of wild-captured *Bythotrephes* raised across three generations in captivity to measure quantitative genetic variation in two *Bythotrephes* traits, namely their distal spine and body length, which affect their susceptibility to fish predators (see Study Species, below) and thus govern their food web interactions. We assessed whether genetic variation in *Bythotrephes* traits differed among five time periods across three years. Although the initial *Bythotrephes* invasion may have involved a genetic bottleneck (based on analysis of neutral genetic variation; Colautti et al. 2005), their rapid spread soon after invasion (Mills et al. 1993) as well as evidence for multiple introductions and gene flow among introduced populations (Colautti et al. 2005) all suggest genetic variation in the *Bythotrephes* population may be high. We, therefore, predicted *Bythotrephes* traits would contain significant genetic variation. Following previous studies of clonal selection in other cladoceran zooplankton (e.g., *Daphnia*; Lynch 1984; Pfrender and Lynch 2000), we further predicted that genetic variation would decline throughout the growing season.

Methods

Study Species

Bythotrephes reproduce by cyclic parthenogenesis and have a short generation time (10-15 day, depending on water temperature; Branstrator 2005). They produce multiple asexual generations during the growing season, which culminate in sexual reproduction at the end of the season (Yurista 1992; Pothoven et al. 2001; Branstrator 2005). A conspicuous trait of *Bythotrephes* is their long tail spine (Figure 2.1). The longest component of the tail spine is the distal spine (i.e., spine section from the tip of the spine to the first set of instar barbs), which is present at birth and does not change with instar development (i.e., the length of the distal segment does not grow). Instead, during the first two molts, total spine length increases through the addition of intercalary segments that each end with a paired barb (Branstrator 2005).

A large volume of research documenting fish predation on *Bythotrephes* indicates that fish are size-specific predators and suggests that natural selection on *Bythotrephes* traits may be occurring, although no study has explicitly measured natural selection. *Bythotrephes* are consumed by both gape-limited fish (typically juvenile fish less than 100mm length) and non-gape-limited fish (typically adult fish; Schneeberger 1991; Mills et al. 1992; Branstrator 2005; Pothoven et al. 2007), with gape-limited predation dominating later in the growing season (Branstrator 2005). Because gape-limited fish selectively choose prey based on prey size, late-season gape-limited fish predation may be an important source of selection on the size of *Bythotrephes* morphological traits. Field surveys showing a correlation between *Bythotrephes* spine and body size and the abundance of gape-limited fish (Straile and Hälbich 2000), diet analysis of wild-caught fish (Schneeberger 1991; Mills et al. 1992; Barnhisel and Harvey 1995), variable spine and body size of *Bythotrephes* among lakes with different fish predation regimes (Bilkovic and Lehman 1997; Sullivan and Lehman 1998), and evidence for selective feeding of fish on *Bythotrephes* based on size of their morphological traits (Barnhisel 1991a; 1991b) all suggest that fish may act as agents of natural selection on *Bythotrephes* morphological traits.

Field Collections

We collected *Bythotrephes* from Lake Michigan during 2007, 2008, and 2010 at either the 60m (2007, 2008; sampling coordinates: 43º 13.80' N 86º 29.58' W) or 45m (2010; sampling coordinates: 43º 12.40' N 86º 27.06' W) depth contours, approximately 12.5km and 9.5km, respectively, west of Muskegon, MI. There was a single collection of 60 *Bythotrephes* mid-growing season (September 27) in 2007, three collections of approximately 300 *Bythotrephes* each spanning the growing season (July 28, September 22, and November 3) during 2008, and a single collection of 25 *Bythotrephes* early in the growing season (July 28) during 2010. Because *Bythotrephes* resting eggs hatch only at the start of the growing season (Yurista 1992) and Lake Michigan is well mixed at our sampling locations (Beletsky et al. 2007), we assumed that our samples at different time periods represented repeated samples of the same population. We used a conical zooplankton net with a 1m diameter opening and 363μm mesh size towed vertically through the top 25m of the water column. On shipboard, *Bythotrephes* were placed into 60mL clear glass jars (one per jar) within 10min of collection. Jars contained approximately 50mL of Lake Michigan water previously passed through a 63μm sieve.

Laboratory Culturing

We reared *Bythotrephes* in biological incubators using methods derived from Kim and Yan (2010) and personal communications (K. Schulz, State University of New York – College of Environmental Science and Forestry, Syracuse, New York, USA). We maintained *Bythotrephes* at 15ºC with a 13:11 light:dark cycle in Lake Michigan water passed through GF/F Whatman filters. Water changes occurred every-other-day in 2007 and daily in 2008 and 2010. Each day we fed *Bythotrephes ad libitum* with approximately 150 *Artemia* nauplii that were less than 48hr old; in 2010 *Bythotrephes* were also given three *Daphnia pulicaria* daily.

Clonal Lines

We used clonal lines (Lynch and Walsh 1998) to raise *Bythotrephes* through the F_2 generation (Figure 2.2). We initiated each clonal line with a wild-captured *Bythotrephes*; the young (F_1 generation) of the wild *Bythotrephes* were used to establish clonal sub-lines. We assumed all wild-captured *Bythotrephes* were genetically distinct, whereas we assumed all young born to a clonal line were genetically identical (Lynch and Walsh 1998). All young born in the lab were transferred to individual culturing jars within 24hr of birth. In 2007 and 2008, F_2 *Bythotrephes* were reared until they reproduced or died and preserved in 95% ethanol, whereas in 2010, F_2 *Bythotrephes* were preserved at birth (first instar). Consequently, in 2007 and 2008, F_2 *Bythotrephes* were preserved at various instar stages (1, 2, and 3). Whereas distal spine length does not change through instar development, body size increases with each instar (Burkhardt 1994), so we accounted for instar stage in the body length analysis. Two of our sampling periods, September 2007 and November 2008, lacked sufficient replication of instars among clonal and sub-clonal lines to estimate sources of phenotypic variation. Thus, only body length data in July and September 2008 and July 2010 were analyzed. However, clonal analyses of distal spine length were performed for all time periods (i.e., September 2007, July through November 2008, and July 2010).

Morphological Measurement

We photographed F_2 *Bythotrephes* using a digital camera attached to a dissecting microscope. *Bythotrephes* were oriented on their side such that the length of the body and tail spine was along a single plane of focus and the eye, thorax, and abdomen regions of the body were visible (Figure 2.1). Using ImageJ (Abramoff et al. 2004), we measured distal spine and body length to the nearest 0.001mm of F_2 *Bythotrephes* from the digital photographs. Whereas our measurement of distal spine length (measured from the tip of the spine to the anterior end of the first set of instar barbs; Figure 2.1) was standard for *Bythotrephes* research (e.g., Sullivan and Lehman 1998), our measurement of body length was not. We noted that some *Bythotrephes* had a bend at the junction of the thorax and abdomen (as in Figure 2.1) whereas others did not. Differences in body position could, therefore, inflate measurement variation of body length based on previously used measurement techniques (linear distance from the eye to the base of the tail spine, e.g., Sullivan and Lehman 1998). As a result, we measured body length from the anterior edge of the eye to the base of the tail spine along the midline of the body with segments spanning the head and eye region, thorax, and abdomen (Figure 2.1). Although some organisms are known to decrease in length after preservation in ethanol (e.g., larval fish; Moku et al. 2004), our test of *Bythotrephes* morphological shrinkage in 95% ethanol revealed no significant shrinkage of *Bythotrephes* distal spine or body length (Supplementary Material).

Heritability, Maternal Effects, and Statistical Analyses

Using our clonal breeding design, we quantified genetic, maternal, and environmental variance components for distal spine and body length from statistical models of among clonal-line, among sub-line, and within sub-line variation, respectively (Lynch 1985; Lynch and Walsh 1998; Figure 2.2). We fitted linear mixed effects models (LME) for each trait separately (i.e., distal spine or body length) using the nlme package (Pinheiro et al. 2009) in R version 2.10.1 (R Development Core Team 2009). In each case, the trait measured in F_2 offspring was modeled by random effects for clonal line and sub-line nested within clonal line. We assessed the significance of the random effects in two ways: 1) by obtaining 95% confidence intervals around the random effects through bootstrapping (Potvin and Roff 1993); and 2) through model comparisons using likelihood ratio tests (LRT). 95% confidence intervals were obtained by randomly resampling our dataset of distal spine and body lengths (1000 iterations, accounting for clonal and sub-line structuring), which created a distribution of variances around our random effects. For the LRT, we fitted two additional models for each trait, with each model containing successively fewer random effects. The first additional model for each trait contained the random effect for clonal line but not the random effect for sub-line. The second additional model for each trait was a linear model without either random effect. As *Bythotrephes* body size increases during instar development, we included instar as a fixed effect (categorical for instars 1, 2, and 3) in models of body length. The only fixed effect in models of distal spine length was the intercept.

We also tested for temporal differences in genetic and maternal variance components by assessing differences by month within 2008 (July, September, and November) and assessing differences across years. We compared a model containing time period (either month or year) as a fixed effect and all random effects described above to a model containing the same fixed and random effects, but which allowed clonal and sub-line variation to differ by time period using the varIdent function in the nlme package (Pinheiro and Bates 2000). We used a LRT to assess whether separate estimates of clonal and sub-line variation for each time period significantly improved the fit of the model. Note that this approach differs from simply assessing the significance of time period as a fixed effect, which would assess whether mean phenotypes (i.e., distal spine or body length) differ among time periods. In our analysis, we were testing whether time period affected the clonal line and sub-line random effects in the model.

Descendants of clonal organisms are effectively linkage groups for their entire genotype and, therefore, broad-sense heritability (H^2) is the appropriate measure of inheritance (Lynch and Walsh 1998; Conner and Hartl 2004). We estimated genetic (V_g), maternal (V_m), and environmental (V_e) variation from the variance among clonal lines, variance among clonal sub-lines, and variance within clonal sub-lines, respectively, from our mixed-effect model analysis of our clonal breeding design. Based on Houle (1992), we estimated the coefficient of genetic variation (CV_g) as:

$$CV_g = \frac{100 * \sqrt{V_g}}{mean},$$

accounting for clonal and sub-line structure in the trait mean. We used the variance components to calculate H^2 as:

$$H^2 = \frac{V_g}{V_g + V_m + V_e}.$$

Similar to the calculation of H^2, we calculated maternal effects (m^2) as the ratio of maternal variance (V_m) to total phenotypic variance ($V_g + V_m + V_e$).

Results

We obtained 13 clonal lines that produced F_2 *Bythotrephes* in September 2007, 18, 31, and 4 clonal lines for July, September, and November 2008, respectively, and 17 clonal lines for July 2010 (percent survival of clonal lines to the F_2 generation during the five time periods was 22, 6, 9, 2, and 68%, respectively).

F_2 *Bythotrephes* mean (± 1 SD; controlled for clonal and sub- line) distal spine length was 4.72 ± 0.73mm in September 2007, 5.11 ± 0.50mm in 2008 (all months combined), and 5.70 ± 0.34mm in July 2010. Distal spine length in 2010 was significantly greater than in 2007 ($p < 0.001$) and 2008 ($p < 0.001$); distal spine length in 2008 was also significantly greater than in 2007 ($p < 0.05$). Variance components for *Bythotrephes* distal spine length did not differ among months within 2008 (likelihood ratio test: $\chi^2_2 = 3.3$, $p > 0.05$), but differed across years (likelihood ratio test: $\chi^2_2 = 74.5$, $p < 0.001$). We found significant (based on 95% confidence intervals and the LRT) genetic variation in *Bythotrephes* distal spine length in 2007, 2008, and 2010 (Table 2.2; Figure 2.3), corresponding to CV_g estimates of 15.0, 8.1, and 4.1, respectively, and H^2 estimates of 0.76, 0.48, and 0.27, respectively (Table 2.2). We found significant maternal variation in distal spine length in 2008 and 2010, corresponding to m^2 estimates of 0.13 and 0.59, respectively, but not in 2007 (Table 2.2; Figure 2.3).

F_2 *Bythotrephes* mean body length for instars one, two, and three, was 1.53 ± 0.18mm, 1.79 ± 0.24mm, and 2.34 ± 0.15mm in July 2008 and 1.61 ± 0.29mm, 1.86 ± 0.24mm, and 2.21 ± 0.34mm in September 2008, respectively, and 1.58 ± 0.10mm for instar one in July 2010. Body length for instars one, two, and three did not differ by time period ($p > 0.05$ for all comparisons). In contrast to distal spine length, variance components for body length differed between months within 2008 (likelihood ratio test: $\chi^2_1 = 13.4$, $p < 0.001$) and also across years (likelihood ratio test: $\chi^2_2 = 72.5$, $p < 0.001$). We found significant genetic variation in *Bythotrephes* body length in July 2008, September 2008, and July 2010 (Table 2.2; Figure 2.4), corresponding to CV_g estimates of 7.8, 13.4, and 3.5, respectively, and H^2 estimates of 0.30, 0.48, and 0.13, respectively (Table 2.2). We found significant maternal variation in body length in July 2008 and July 2010, corresponding to m^2 estimates of 0.23 and 0.44, respectively, but not in September 2008 (Table 2.2; Figure 2.4).

Discussion

A growing body of research suggests that evolution in traits important to interspecific interactions of invasive species may play a key role in their effects on ecosystems (e.g., Mooney and Cleland 2001; Lambrinos 2004; Strayer et al. 2006). Our research quantified genetic variation in traits of the invasive zooplankton, *Bythotrephes longimanus*, a species that has caused severe and widespread ecological effects in the Great Lakes. We found that the two traits considered to have the greatest effect on food web interactions with fish, namely *Bythotrephes* distal spine and body length, both had significant genetic and maternal variation and exhibited moderate-to-high coefficients of genetic variation, broad-sense heritabilities, and maternal effects (Table 2.2). Moreover, genetic variation for *Bythotrephes* traits did not decline over the growing season, as is typical for cladoceran zooplankton, but instead remained similar for distal spine length and increased for body length (Table 2.2).

Coefficients of genetic variation and heritability estimates for *Bythotrephes* distal spine and body length are similar to other invasive species (Table 2.1) and heritability estimates fall within the range of previously published broad-sense heritabilities for native cladoceran zooplankton, suggesting that evolution of these important *Bythotrephes* traits may not be limited by low genetic variation. Our range in heritability estimates (Table 2.2) for *Bythotrephes* distal spine and body length is similar to the range of heritabilities (0.28 – 0.62; Pfrender and Lynch 2000) for body size of *Daphnia pulex*, a widespread native *Daphnia* species. The concordance of heritability estimates between the invasive *Bythotrephes* and native *Daphnia* is significant given that previous studies have suggested that low genetic variation relative to other sources of variation may prohibit evolution of invasive species. Multiple source populations have already been suggested for the *Bythotrephes* invasion based on evidence from neutral loci (Colautti et al.

2005) and may explain the presence of substantial genetic variation relative to other sources of variation in the traits we investigated.

Two potential sources of bias to our study must be considered. First, our heritability estimates were derived from a clonal analysis in the lab, which is typical for heritability estimates for cladocerans (e.g., Lynch 1984; Lynch and Walsh 1998; Pfrender and Lynch 2000), but which could introduce a bias due to experimentally controlled environmental conditions (Conner et al. 2003; Wolff 2003; Calisi and Bentley 2009; but see, Weigensberg & Roff 1996). Our estimates of environmental variation (Table 2.2) were consistently lower than genetic variation for *Bythotrephes* distal spine length, but similar to genetic variation for *Bythotrephes* body length and maternal variation for both traits. It is possible that low environmental variation in the laboratory inflated our estimates of heritability and maternal effects as compared to wild populations. Second, our analyses involved low sample sizes that resulted from the, at times, poor survival of clonal lines and logistical constraints of the labor-intensive culturing of *Bythotrephes* (Kim and Yan 2010), which necessitated lower sample sizes than studies of other organisms (e.g., *Daphnia*). It is possible that poor survival could have reduced our estimates of genetic and maternal variation if significant lab-based clonal selection occurred. However, there was no relationship between the magnitude of genetic or maternal variation and clonal line mortality; our two time periods with the lowest clonal line mortality, September 2007 and July 2010, represented the highest and lowest estimates of genetic variation in distal spine length. The lack of relationship between mortality and sources of variation indicates that mortality was random with respect to our clonal lines. Further, the absence of a significant difference in genetic variation by time period within 2008 for distal spine length was unlikely to be due to low statistical power, as these data were sufficient for detecting significant changes in genetic variation in distal spine length across years and changes in genetic variation for body length both within and across years. Thus, despite variable clonal line survival among time periods, we did not have any evidence for clonal selection associated with our culturing approaches and the remaining samples were sufficient to test our predictions.

Our findings did not support the prediction that genetic variation in *Bythotrephes* traits would decrease throughout the growing season, despite the similarity in life history between *Bythotrephes* and the other cladoceran zooplankton on which we based this prediction. Like most cladoceran species, *Bythotrephes* reproduce by cyclic parthenogenesis (i.e., multiple rounds of asexual reproduction culminating in sexual reproduction that produces resting eggs at the end of the growing season; Yurista 1992; Pothoven et al. 2001; Branstrator 2005). After sexual reproduction, genetic variation and broad-sense heritabilities for cladoceran traits are often high (with heritabilities falling within the range of 0.4 – 0.6) as each resting egg hatchling is genetically unique (Lynch 1984). During subsequent asexual reproduction, genetic variation typically is reduced and broad-sense heritabilities decline due to clonal selection, approaching zero at the end of the season (Lynch 1984). Contrary to the scenario of clonal selection eroding genetic variation over the growing season, genetic variation for *Bythotrephes* distal spine length did not differ during three sampling periods within one growing season (July – November 2008). Furthermore, genetic variation in *Bythotrephes* body length increased between July and September 2008. The increase of genetic variation in *Bythotrephes* body length was associated with a decrease in maternal variation that together resulted in increased heritability, despite a concomitant increase in environmental variation.

Multiple scenarios could explain why seasonal patterns in *Bythotrephes* genetic variation are dissimilar to other cladoceran zooplankton. Previous research documenting declines in genetic variation of *Daphnia* clones during the growing season (Lynch 1984) was associated with strong and consistent natural selection. Conversely, temporal fluctuation in the direction of selection across the growing season (Sasaki and Ellner 1997), such as from opposing selection pressures by gape-limited and non-gape-limited fish predators whose predation rate varies seasonally (Branstrator 2005), might maintain high levels of genetic variation in *Bythotrephes* traits both within and across seasons. Alternatively, phenotypically plastic responses to changing environmental conditions can prevent selection from eroding genetic variation (Ghalambor et al. 2007), and thus could explain the persistence of genetic variation in *Bythotrephes* traits. If clones differ in their plastic responses to the environment (i.e., if clones differ in their reaction norms), then certain clones may be favored in one set of environments and selected against in another set, thus maintaining genetic variation (Pigliucci 2001). Indeed, Burkhardt (1994) and Pothoven et al. (2003) suggested that *Bythotrephes* body size may be phenotypically plastic, potentially varying in response to their abiotic (e.g., temperature) and biotic (e.g., predator or prey presence) environment. Although our results for genetic variation in *Bythotrephes* distal spine and body length suggest a more complex scenario than unidirectional clonal selection through the growing season, to date, no study has estimated natural selection or quantified phenotypic plasticity experimentally in *Bythotrephes* traits. Thus, knowledge of selection and plasticity would be valuable for understanding why *Bythotrephes* distal spine and body length exhibit seasonal patterns in genetic variation that are atypical for a cyclic parthenogen.

Genetic variation in *Bythotrephes* distal spine and body length has implications for how *Bythotrephes* can respond to their food web interactions in the Great Lakes and could explain their invasive success in ecosystems with varying biotic conditions. Genetic variation allows for an evolutionary response to persistent environmental change across multiple generations (Lee and Gelembiuk 2008; Svanbäck et al. 2009). Our finding of moderate-to-high heritabilities throughout the growing season and across years suggests that *Bythotrephes* morphology could evolve in response to gape-limited fish predation, which increases throughout the growing season. Evolution of increased body size in response to gape-limited predation has previously been shown in *Ambystoma maculatum* (spotted salamander) larvae (Urban 2008) and *Daphnia* species (Spitze 1991). Moreover, *Bythotrephes* are abundant in a variety of lake environments with differing fish predator species and relative abundance of gape- versus non-gape limited predators (Bilkovic and Lehman 1997; Sullivan and Lehman 1998; Young and Yan 2008). Concordantly, previous research has shown that distal spine and body length are variable among lakes (Sullivan and Lehman 1998). Potentially, the variability in *Bythotrephes* spine and body length represents local adaptation to differing fish predation regimes, if *Bythotrephes* in other systems also maintain sufficient genetic variation to respond to selection from fish.

In addition to significant genetic variation, we also found significant maternal variation in *Bythotrephes* distal spine and body length that can affect phenotypic change between generations. The type of maternal effects we estimated are sometimes referred to as maternal identity effects (Sakwińska 2004) or intergenerational environmental effects (Schwaegerle et al. 2000). In a clonal analysis, maternal identity effects occur when sisters within a clonal line produce offspring that consistently differ despite being genetically identical and raised in a common environment. Maternal identity effects can be caused by maternal responses to small-scale environmental variation, which all experiments (even highly controlled laboratory experiments) inevitably involve (Schwaegerle et al. 2000), or by individual genetic differences caused by mutation. Because maternal identity effects have been shown to affect traits related to fitness (Sakwińska 2004), maternal identity effects may represent an important source of phenotypic variation in fitness-related traits of *Bythotrephes*.

In conclusion, our research has quantified genetic variation in two important ecological traits of an invasive species and has tested the stability of these sources of variation through time. Genetic variation in *Bythotrephes* distal spine and body length could contribute to the success of *Bythotrephes* at invading and dramatically altering ecosystems by permitting an evolutionary response to selection. This capacity for phenotypic change could explain, in part, the ability of *Bythotrephes* to invade lake ecosystems with variable biotic and abiotic conditions. As invasive species continue to threaten biodiversity worldwide, elucidating both the ecological and evolutionary foundations for their success will be critical.

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Tables

Table 2.1. Quantitative genetic variation in invasive species traits, including genetic and additive genetic variation (V_g and V_a, respectively), coefficients of additive genetic variation (CV_a), and broad- and narrow-sense heritabilities (H^2 and h^2, respectively).

Species	**Trait**	V_g	V_a	CV_a	H^2	h^2	**Reference**
Anolis cristatellus	Number of circum-trunk scales[1]					**0.27**	Eales et al. 2010
	Number of ventral scales[1]					**0.48**	Eales et al. 2010
Eurytemora affinis	Survival to metamorphosis: 15 PSU[2]				*0.79*		Lee et al. 2003
	Survival to metamorphosis: 0 PSU[2,3]				**1.40**		Lee et al. 2007
	Survival to metamorphosis: 5 PSU[2,3]				**0.54**		Lee et al. 2007
	Survival to metamorphosis: 25 PSU[2,3]				**0.46**		Lee et al. 2007
Leptinotarsa decemlineata	Development time: 17°C[4]			*5.50*	**0.55**		Boman et al. 2008
	Development time: 23°C[4]			*7.11*	**0.52**		Boman et al. 2008
	Female adult weight: 17°C			*5.60*	0.23		Boman et al. 2008
	Male adult weight: 17°C			*8.65*	**0.62**		Boman et al. 2008
	Female adult weight: 23°C			*14.64*	**0.55**		Boman et al. 2008
	Male adult weight: 23°C			*4.23*	0.07		Boman et al. 2008
	Emergence body mass[5]		$0.01x10^{-3}$			0.05	Piiroinen et al. 2011
	Overwintering body mass[5]		$0.05x10^{-3}$			0.12	Piiroinen et al. 2011
	Metabolic rate[5]		$0.05x10^{-3}$			0.06	Piiroinen et al. 2011
	Mass independent metabolic rate[5]		$0.05x10^{-3}$			0.08	Piiroinen et al. 2011
	Diapause behaviour[5]		$0.08x10^{-3}$			0.02	Piiroinen et al. 2011
	Overwintering survival[5]		$0.17x10^{-3}$			0.05	Piiroinen et al. 2011
	Days until entered diapause[5]		$1.69x10^{-3}$			0.16	Piiroinen et al. 2011

Table 2.1 (con't)

Species	**Trait**	V_g	V_a	CV_a	H^2	h^2	**Reference**
Mahonia genus	Seedling growth	0.01					Ross et al. 2009
Phalaris arundinacea	Relative growth rate[6]				**0.27**		Lavergne and Molofsky 2007
	Tillering rate[6]				**0.47**		Lavergne and Molofsky 2007
	Leaf number[6]				**0.27**		Lavergne and Molofsky 2007
	Stem height[6]				**0.49**		Lavergne and Molofsky 2007
	Root/shoot ratio[6]				**0.27**		Lavergne and Molofsky 2007
	Below ground biomass[6]				**0.26**		Lavergne and Molofsky 2007
	Above ground biomass[6]				**0.30**		Lavergne and Molofsky 2007
	Emergence time[6]				**0.08**		Lavergne and Molofsky 2007
Poecilia reticulata	Male body area			*11.79*		**0.87**	Brooks and Endler 2001a
	Male tail area			*13.89*		**0.41**	Brooks and Endler 2001a
	Male black area			*35.58*		**0.43**	Brooks and Endler 2001a
	Male fuzzy black area			*55.29*		**0.79**	Brooks and Endler 2001a
	Male iridescent area			*36.76*		**0.45**	Brooks and Endler 2001a
	Male orange area			*67.33*		**0.96**	Brooks and Endler 2001a
	Male orange chroma			*17.83*		0.15	Brooks and Endler 2001a
	Male orange brightness			*20.58*		**0.47**	Brooks and Endler 2001a
	Male total spot number			*23.67*		**0.79**	Brooks and Endler 2001a
	Male mean brightness			*4.53*		**0.58**	Brooks and Endler 2001a
	Male brightness contrast			*15.46*		**0.54**	Brooks and Endler 2001a
	Male mean chroma			*1.95*		**0.55**	Brooks and Endler 2001a
	Male color contrast			*9.53*		**0.38**	Brooks and Endler 2001a
	Female responsiveness to males					**0.27**	Brooks and Endler 2001b
	Female discrimination of males					0.11	Brooks and Endler 2001b
	Female preference for male traits[7]					0.02	Brooks and Endler 2001b
Potamopyrgus antipodarum	Size at first reproduction[8]	*0.74*			*0.09*		Dybdahl and Kane 2005
	Age at first reproduction[8]	*58.59*			*0.07*		Dybdahl and Kane 2005

Table 2.1 (con't)

Species	**Trait**	V_g	V_a	CV_a	H^2	h^2	**Reference**
	Total offspring[8]	*47.76*			*0.20*		Dybdahl and Kane 2005
	Reproduction rate[8]	*0.22*			*0.12*		Dybdahl and Kane 2005
Thymallus thymallus	Length at termination[9]					*0.07*	Koskinen et al. 2002
	Yolk-sac volume[9]					*0.04*	Koskinen et al. 2002
	Growth rate[9]					*0.21*	Koskinen et al. 2002
	Survival[9]					*0.12*	Koskinen et al. 2002
	Incubation time[9]					*0.37*	Koskinen et al. 2002
	Swim-up length[9]					*0.25*	Koskinen et al. 2002
	Hatching length[9]					*0.10*	Koskinen et al. 2002

Note: Statistically significant values are presented in bold; values reported without reference to statistical significance are presented in italics. We obtained values from a literature search using the ISI Web of Science® with the key words "invasive species" and "genetic variation". Only studies reporting univariate estimates of quantitative genetic trait variation of invasive species in their invaded range were included.

[1] Eales et al. (2010) derived h^2 from mother-offspring regression, and thus values may be inflated due to maternal effects.

[2] Only traits for invasive populations of *E. affinis* from Lee et al. (2003; 2007) were included.

[3] H^2 obtained by doubling intra-class correlations reported in Lee et al. (2007).

[4] CV_a and H^2 averaged across invasive populations in Boman et al. (2008).

[5] V_a and h^2 were calculated from V_{sire}, V_{dam}, V_{potato}, and V_{resid} reported in Piiroinen et al. (2011) following Falconer and Mackay (1996); only values for both males and females were included.

[6] H^2 determined from Figure 5 of Lavergne and Molofsky (2007) for invasive range.

[7] h^2 averaged across female preferences for the following male traits reported in Brooks and Endler (2001b): attractiveness, predicted attractiveness, body size, tail area, black area, fuzzy black area, orange area, orange chroma, orange brightness, iridescent area, spot number, mean brightness, brightness contrast, mean chroma, and color contrast.

[8] V_g and H^2 averaged across 12 and 15°C temperature treatments in Dybdahl and Kane (2005).

[9] h^2 averaged across populations in Koskinen et al. (2002).

Table 2.2. Genetic (V_g), maternal (V_m), and environmental (V_e) variance components, coefficient of genetic variation (CV_g), broad-sense heritability (H^2), and maternal effects (m^2) for distal spine and body length of *Bythotrephes longimanus*.

Time Period	V_g	**95% C.I.**		CV_g	V_m	**95% C.I.**		V_e	**95% C.I.**		H^2	m^2
		Lower	**Upper**			**Lower**	**Upper**		**Lower**	**Upper**		
Distal spine length												
2007 September	**0.498**	0.150	0.928	15.0	0.057	0.005	0.080	0.097	0.059	0.158	0.76	0.09
2008 Combined	**0.172**	0.085	0.260	8.1	**0.046**	0.011	0.122	0.138	0.109	0.176	0.48	0.13
2010 July	**0.055**	0.041	0.174	4.1	**0.118**	0.076	0.166	0.028	0.022	0.036	0.27	0.59
Body length												
2008 July	**0.016**	0.004	0.063	7.8	**0.012**	0.003	0.042	0.025	0.017	0.036	0.30	0.23
2008 September	**0.056**	0.028	0.109	13.4	<0.001	0	0.013	0.060	0.044	0.080	0.48	0
2010 July	**0.003**	0.001	0.013	3.5	**0.012**	0.005	0.022	0.012	0.009	0.015	0.13	0.44

Note: Variance components for distal spine length did not differ by month within 2008, but differed among years. Variance components for body length differed by month in 2008 and between years. Statistically significant ($p < 0.05$) genetic and maternal variance components based on likelihood ratio tests are presented in bold; 95% confidence intervals around the variance components are given.

Figures

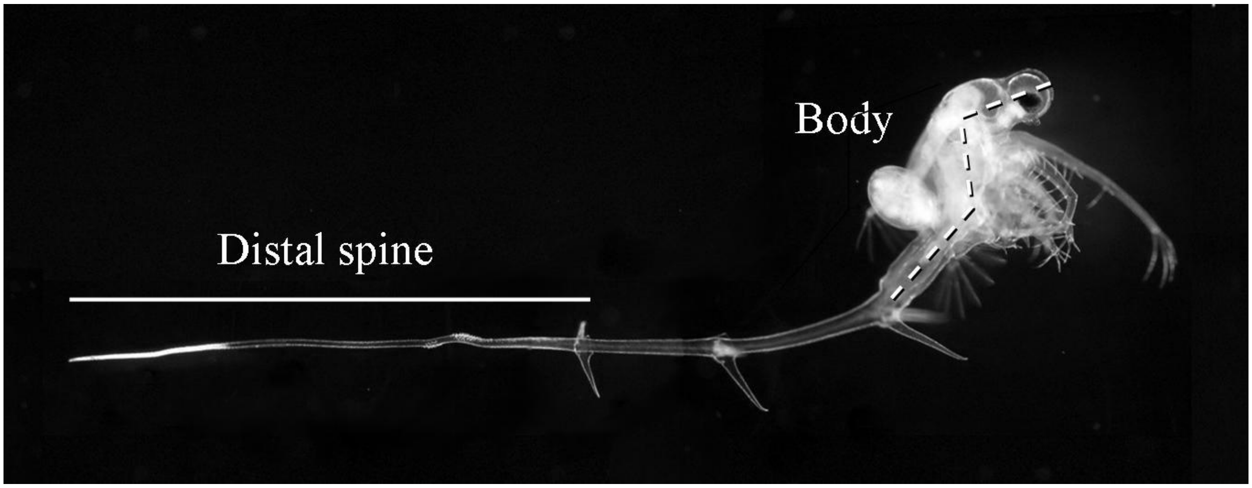

Figure 2.1. *Bythotrephes longimanus*, the spiny water flea. The *Bythotrephes* tail spine is comprised of the distal spine (measured from the tip of the spine to the anterior end of the first set of instar barbs; solid line) and up to two intercalary segments (for asexually produced *Bythotrephes*). Body length was measured from the anterior edge of the eye to the base of the tail spine along the midline of the body with segments spanning the head and eye region, thorax, and abdomen (dashed line). The *Bythotrephes* pictured is a third instar.

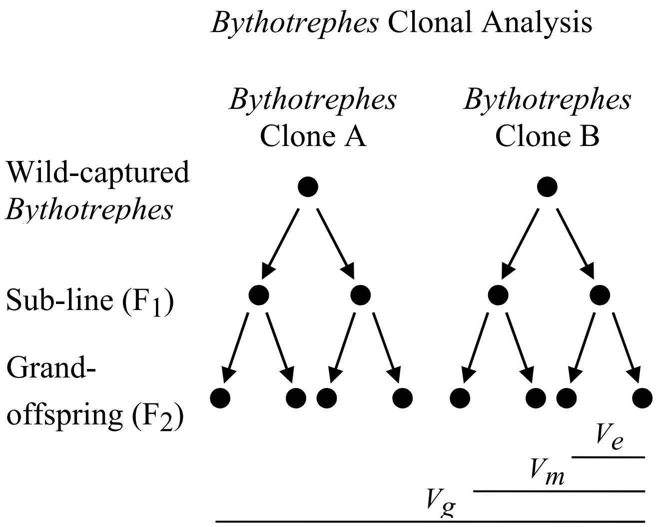

Figure 2.2. Clonal analysis design, with clonal sub-lines (F_1 generation) nested within clonal lines (wild-caught *Bythotrephes*); all analyses were completed using morphological data from the F_2 generation. V_g is the genetic variance, represented by the variance in morphology of F_2 individuals among clonal lines, V_m is maternal variance, represented by the variance among sub-lines within clones, and V_e is the residual environmental variance within sub-lines. Though two F_1 young per clonal line and two F_2 young per sub-line are pictured, all young born to clonal and sub-lines were reared. Figure modified from Lynch and Walsh (1998).

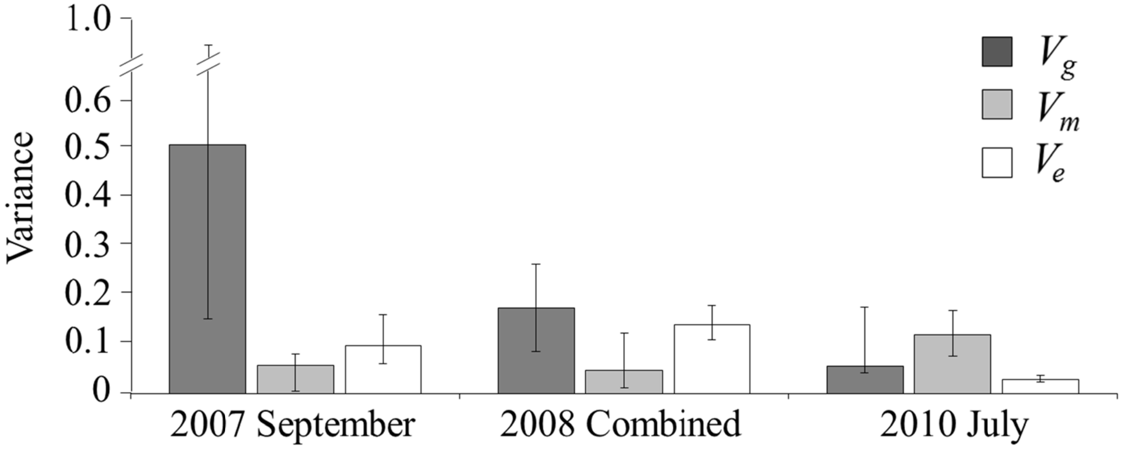

Figure 2.3. Genetic (V_g), maternal (V_m), and environmental (V_e) variance components for *Bythotrephes* distal spine length. Variance components did not differ by month within 2008, but differed among years. Bars are the 95% confidence intervals around the variance components.

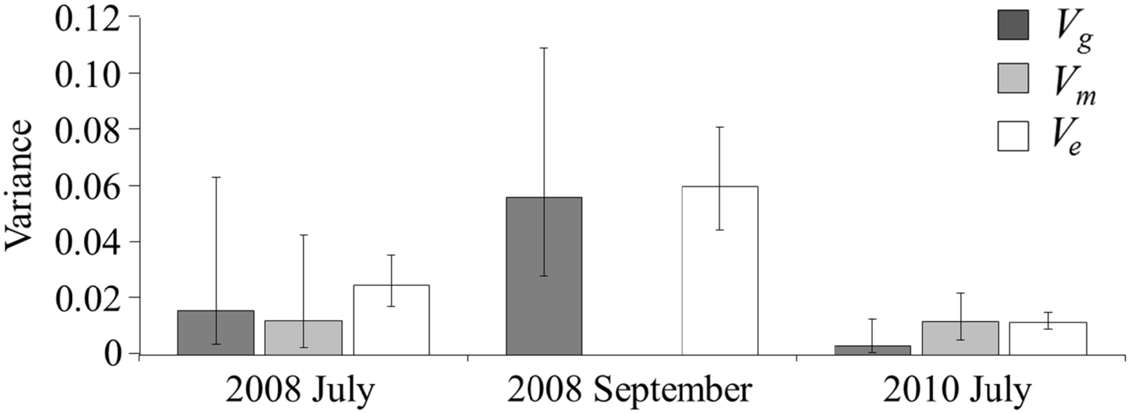

Figure 2.4. Genetic (V_g), maternal (V_m), and environmental (V_e) variance components for *Bythotrephes* body length. Variance components differed by month in 2008 and between years. Bars are the 95% confidence intervals around the variance components.

APPENDIX

Appendix

Appendix A. Shrinkage of *Bythotrephes longimanus* distal spine length and body length in 95% ethanol.

Morphological shrinkage of organisms can occur in chemicals used for preservation (e.g., shrinkage of larval fish in 90% ethanol; Moku et al. 2004). Yan and Pawson (1998) found that the length of *Bythotrephes* morphological traits changed in various concentrations of sugar-formalin, a preservative derived from formaldehyde. However, no estimates of *Bythotrephes* morphological shrinkage in 95% ethanol have been published. We, therefore, completed an experiment to estimate shrinkage of *Bythotrephes* distal spine and body length in 95% ethanol.

We tested shrinkage of *Bythotrephes* distal spine and body length using *Bythotrephes* collected from Lake Michigan on July 31, 2008 and transported live to Michigan State University. *Bythotrephes* were photographed within five seconds of placement in ethanol using a camera mounted to a dissecting microscope. After photography, *Bythotrephes* were stored individually in ethanol for 126 days. *Bythotrephes* morphological traits were measured using ImageJ image analysis software (Abramoff et al. 2004). Distal spine length was measured from the tip of the spine to the anterior end of the first set of instar barbs and body length was measured from the anterior edge of the eye to the base of the tail spine along the midline of the body with segments spanning the head and eye region, thorax, and abdomen.

Although a percentage correction (final length / initial length * 100%) has previously been used to estimate morphological shrinkage in chemical preservatives, using a percentage correction assumes an isometric relationship between the numerator and the denominator. In the absence of isometry, analysis of the ratio (where variation in the numerator is of primary interest) can be confounded by variation in the denominator (Atchley et al. 1976; Albrecht et al. 1993;

Sokal and Rohlf 1995). To prevent the confounding of variation, we used ANCOVA-like models (Packard and Boardman 1988; Jackson et al. 1990; García-Berthou 2001) that accounted for allometric relationships, which we linearized through log-transformation to investigate shrinkage of *Bythotrephes* distal spine and body length.

We defined linear models of final length (day 126; log-transformed, continuous variable) predicted by initial length (day 1; log-transformed, continuous variable), instar (categorical for instars 1, 2, and 3), and an interaction between initial length and instar using R (version 2.10.1; R Development Core Team 2009). The interactions between initial length and instar were not significant for distal spine (general linear model: $F_{2, 42} = 0.01$; $p > 0.05$) or body length (general linear model: $F_{2, 54} = 0.67$; $p > 0.05$) and were, therefore, removed from both models. Non-significant interactions between traits and instar indicate that potential shrinkage of traits does not vary by instar. Upon removal of the interactions, instar was not significant for distal spine length (general linear model: $F_{2, 44} = 1.4$, $p > 0.05$), but was significant for body length (general linear model: $F_{2, 56} = 3.2$, $p = 0.05$). Therefore, instar was removed from the final model of distal spine length, but was retained in the final model of body length. The non-significant effect of instar on final distal spine length suggests that distal spine length of first, second, and third instars did not differ, whereas the significant effect of instar on final body length indicated significant differences of body length among instars, as would be expected given that *Bythotrephes* increase body length with each molt. Final length (log transformed) was significantly positively correlated with initial length for distal spine (log transformed; linear regression: $t_{46} = 21.2$; $p < 0.001$; intercept = 0.08 ± 0.08 mm; slope = 0.92 ± 0.04; adjusted r-squared = 0.91; values are reported as ± 1 SE) and body length (log transformed; linear regression: $t_{56} = 8.1$; $p < 0.001$; intercept = -0.09 ± 0.09 mm; slope = 0.81 ± 0.10; adjusted r-squared = 0.84).

In order to avoid bias in slope and intercept values associated with error in our predictor variable (i.e., log initial length), we used reduced major axis regression (RMA; Mitchell-Olds and Shaw 1987; Sokal and Rohlf 1995; McArdle 2003) to estimate slopes and intercepts for distal spine length and body length. The RMA slope for distal spine length was 0.97 (95% C.I. 0.94 to 1.12), whereas the slope for body length was 1.12 (95% C.I. 0.99 to 1.24); neither of the slopes differed from 1. Additionally, the RMA intercepts for distal spine length (intercept = 0.08 ± 0.08 mm) and body length (intercept = -0.09 ± 0.09 mm) were not significantly different from 0 ($p > 0.05$ for distal spine; $p > 0.05$ for body length). The slope equal to one and intercept equal to zero indicates that final and initial length scale isometrically for both distal spine and body length. As a result, there was no statistically significant shrinkage for *Bythotrephes* distal spine and body length in 95% ethanol.

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CHAPTER 3

Plastic response to a proxy cue of predation risk when direct cues are unreliable

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Abstract

Phenotypic plasticity in response to proximate cues that directly affect fitness or cues directly released by selective agents has been well documented. For example, prey respond to light level when light level affects predation risk, and prey respond to cues released by predators to reduce predation risk. Less well understood is the potential for organisms to use proximate cues that are indirectly related to the selective agent (e.g., prey response to a cue that correlates with, but does not directly affect, predation risk). Previous field studies indicate that body size and spine length of an invasive cladoceran in Lake Michigan, *Bythotrephes longimanus* (the spiny water flea), increase throughout the growing season, coincident with a decrease in clutch size. Although the cause of the seasonal trait changes is not known, the changes are associated with warmer water temperature and an increased risk of gape-limited fish predation. Using a laboratory experiment, we found no effect of fish (*Perca flavescens*) chemical cues (kairomones) on *Bythotrephes* morphology or life history. In contrast, higher water temperature led to longer absolute spine and body length, increased investment in morphological defense of offspring (measured as the ratio of spine-to-body length), and decreased clutch size and age at reproduction. These plastic responses are unlikely to be adaptive to temperature *per se*, but rather our findings indicate that temperature serves as a proxy cue of predation risk. Temperature

correlates with risk of gape-limited juvenile fish predation, which is the dominant source of predation on *Bythotrephes* later in the growing season. We argue that for *Bythotrephes*, temperature is a more reliable cue of gape-limited predation risk than fish kairomones, as kairomones from non-gape-limited adult fish (to which a plastic response would have little effect) are present throughout the season. Organisms may, therefore, not only respond to changes in abiotic factors because those factors directly affect risk, but also when the abiotic cues serve as proxies signaling changes in predation risk.

Introduction

Organisms respond to many biotic and abiotic environmental conditions through phenotypic plasticity (i.e., the ability of a genotype to produce different phenotypes in response to differing environmental conditions; Pigliucci 2001), but the environmental conditions to which organisms respond, as well as the trait modifications that result, are highly variable among species and even among populations within a species. Consequently, investigating which aspects of the environment are used as cues for phenotypic plasticity and how plastic responses affect fitness have been the focus of a large body of research. Biotic and abiotic environmental conditions used as proximate cues for phenotypic plasticity typically either directly affect fitness or are directly released by selective agents. For example, prey may use light level as a proximate cue for anti-predator responses when light level directly affects predation risk (e.g., Orrock et al. 2004). Similarly, prey may use chemicals released by predators (termed kairomones) as proximate cues for anti-predator responses (Tollrian and Dodson 1999; Laforsch and Tollrian 2004).

However, proximate cues do not necessarily have to directly affect fitness or be directly released by selective agents, but may instead be factors that are correlated in time or space (Hairston 1987). Theory (Hairston 1987) suggests prey may evolve to use correlated environmental cues as proxies for predation risk when the correlated cues are reliable predictors of predation risk. For example, an abiotic variable such as temperature may correlate with predation risk, without directly affecting predation risk, and thus serve as a “proxy cue”. There is general support for the use of proxy cues as reliable indicators of changing environmental conditions (e.g., beginning or end of growing season), such as the use of photoperiod for breaking or inducing diapause (Stross 1966), but experimental support for proxy cues eliciting a response in an organism to other organisms, such as predators, is lacking.

Bythotrephes longimanus (the spiny water flea; hereafter *Bythotrephes*; Figure 3.1a) are cladoceran zooplankton native to Eurasia that were introduced to the Laurentian Great Lakes in the 1980s (Mills et al. 1993). *Bythotrephes* are a dominant predator of zooplankton (Bunnell et al. 2011) and common prey for fish (Pothoven et al. 2007). The morphology and life history of *Bythotrephes* in the Great Lakes strongly vary temporally (Burkhardt 1994; Pothoven et al. 2001; 2003), but the cause is not known. In Lake Michigan, for example, *Bythotrephes* tail spine and body length are shortest at the start of the growing season (June-July), longest mid-growing season (August-September), and then decrease at the end of the growing season (October onward; Pothoven et al. 2001).

Seasonal changes in *Bythotrephes* traits may be due to phenotypically plastic responses to their biotic and/or abiotic environment, but heretofore cues for plasticity in *Bythotrephes* traits have not been investigated. Observed seasonal changes in *Bythotrephes* traits correlate with and have been theorized to be a defense against gape-limited fish predation (typically juvenile fish <100mm length), which increases throughout the growing season and typically comprises the dominant source of predation on *Bythotrephes* mid-to-late in the growing season (Branstrator 2005). Seasonal elongation of the tail spine and increased body size in *Bythotrephes* may thus represent plastic responses to reduce the risk of gape-limited fish predation. Consequently, a likely proximate cue for predation risk is kairomones released by gape-limited fish predators. However, *Bythotrephes* are also consumed by non-gape-limited (adult) fish (Pothoven et al. 2007) throughout the growing season, which also release kairomones, and to which morphological plastic responses would likely not be adaptive because predation by non-gape-limited predators is not affected by the morphology of *Bythotrephes*. The effectiveness of induced defenses by *Bythotrephes* is thus contingent on the particular fish present, and therefore fish kairomones may not be expected to be a reliable signal of predation risk. Alternatively, water temperature correlates with both observed seasonal changes in *Bythotrephes* traits (Burkhardt 1994) as well as risk of gape-limited fish predation, and thus, water temperature could serve as a reliable proxy signal of predation risk from gape-limited fish. We, therefore, conducted a study that tested fish predator kairomones and temperature as proximate cues of *Bythotrephes* morphological and life history plasticity.

Methods

Methodological Overview

Using a laboratory experiment, we investigated the effect of fish predator kairomones and temperature on *Bythotrephes* morphological and life history traits. Our experimental design entailed raising *Bythotrephes* clonal lines in the presence or absence of fish kairomones (by using water obtained from five fish and five control aquaria) at 9°C and 15°C (one incubator per temperature). We raised eight *Bythotrephes* clonal lines per replicate aquarium per temperature treatment, for a total of 40 clonal lines in each of the four temperature-fish kairomone treatments (total of 160 clonal lines).

Bythotrephes *Morphology and Life History*

The longest section of *Bythotrephes*' prominent tail spine is the distal spine, which extends from the tip of the spine to the first pair of instar barbs (Figure 3.1a). The distal spine is present at birth and does not change with instar development, although other traits, such as body size, increase with each instar molt (Burkhardt 1994). Instead, during the first two molts, total spine length increases through the addition of intercalary segments separated by paired barbs (Branstrator 2005). *Bythotrephes* reproduce by cyclic parthenogenesis, with multiple asexual generations during the growing season culminating in sexual reproduction at the end of the season (Yurista 1992; Pothoven et al. 2001).

Fish Kairomone Collection

We obtained predator fish kairomones from yellow perch (*Perca flavescens*; 50-120mm total length), an important predator of *Bythotrephes* in the Great Lakes (Schneeberger 1991; Baker et al. 1992). Perch were held in five replicate aquaria and fed *Bythotrephes* (see Appendix A for detailed methods). Water was collected from the five aquaria containing fish and from five identically treated control aquaria (that lacked only the perch) and frozen in 100mL aliquots (frozen kairomone concentration of 1 fish: 2L water) for later experiments with *Bythotrephes*. The effectiveness of our kairomones was examined by measuring the behavioral response of *Daphnia mendotae* to preserved fish kairomones (see Appendix B for detailed methods).

Field Collection of Experimental Bythotrephes

We collected 160 *Bythotrephes* from Lake Michigan on July 28, 2010, a time period representing the middle of the *Bythotrephes* growing season. The lake was stratified, with average water temperatures in the epilimnion (0-12m deep) and metalimnion (13-25m deep) of 24ºC and 14ºC, respectively. *Bythotrephes* collection occurred at the 45m depth contour (sampling coordinates: 43º 12.40' N 86º 27.06' W), approximately 9.5km west of Muskegon, MI. We used a conical zooplankton net with a 1m diameter opening and 363μm mesh size towed vertically through the epilimnion and metalimnion. On board the ship, *Bythotrephes* were placed into 60mL glass jars (one per jar) within 10min of collection. We retained only first and second instar *Bythotrephes* with very small brood pouches (used to assess reproductive state). Jars contained approximately 50mL of Lake Michigan water passed through a 63µm sieve.

Experimental Design

Wild-captured *Bythotrephes* were split equally between 9°C and 15°C temperature treatments maintained in biological incubators (one incubator per temperature). The colder temperature represented Lake Michigan water temperature at the beginning and end of the *Bythotrephes* growing season, whereas the warmer temperature represented Lake Michigan water temperature mid-growing season (Pothoven et al. 2001). Although water temperatures are often greater than 15°C during the growing season (Pothoven et al. 2001), our previous experience as well as others (Garton et al. 1990) indicate raising *Bythotrephes* at higher temperature (20°C and greater) increases mortality dramatically, and thus we used a conservatively low upper temperature limit.

Within each temperature treatment (i.e., incubator), half of the *Bythotrephes* were raised in water containing fish kairomones and half in control water, for a total of eight wild-captured *Bythotrephes* per replicate aquaria per temperature treatment. We completed daily water changes to counter breakdown of kairomones. The frozen control and kairomone water was thawed each day of the experiment; each 100mL aliquot was diluted with an additional 200mL of Lake Michigan water passed through GF/F Whatman filters to a final kairomone concentration of 1 fish: 6L water. *Bythotrephes* were maintained with a 13:11 light:dark cycle and fed *ad libitum* daily with approximately 150 *Artemia* nauplii less than 24hr old and three juvenile *Daphnia pulicaria* (Kim and Yan 2010; pers. comm. K. Schulz, State University of New York – College of Environmental Science and Forestry, Syracuse, New York, USA).

Clonal Lines

We raised *Bythotrephes* in clonal lines (Lynch and Walsh 1998) through the F_2 generation. Previous research has shown that cladoceran zooplankton exhibit plastic responses within and among generations, often with the strongest responses occurring through maternal effects (Tollrian 1995; Yurista 2000). Each clonal line was initiated with one wild-captured *Bythotrephes* whose daughters (F_1 generation) were used to establish sub-lines. All *Bythotrephes* from a common clonal line were exposed to the same temperature and fish kairomone treatments. Wild-captured and F_1 *Bythotrephes* were preserved in 95% ethanol the day they reproduced or died; all F_2 *Bythotrephes* were preserved the day they were born. Approximately one-third of wild-captured *Bythotrephes* in each temperature treatment, as well as one-quarter of F_1 *Bythotrephes* in the 15°C treatment and one-third in the 9°C treatment were culled randomly due to a limited supply of kairomone water (a greater proportion of F_1 *Bythotrephes* raised at 9°C were culled to reduce kairomone consumption due to their longer generation time; see results).

Morphological and Life History Traits

We measured the following *Bythotrephes* morphological and life history traits: distal spine length, S2 length (present for second and third instar *Bythotrephes*), S3 length (present for third instar *Bythotrephes*), body length (Figure 3.1a), the ratio of distal spine-to-body length, clutch size (i.e., number of offspring), days to first reproduction, and instar at first reproduction (*Bythotrephes* can reproduce in either the second or third instar stage; Pothoven et al. 2001; 2003). To ensure that we analyzed individuals of differing life history strategies separately, we only considered second instar measurements for those individuals that first reproduced at their second instar and only measured third instar individuals that first reproduced at their third instar.

Digital photographs of *Bythotrephes* (oriented as in Figure 3.1a) taken with a dissecting microscope were analyzed with ImageJ (Abramoff et al. 2004) to measure morphological traits to the nearest 0.001mm. Distal spine length was measured from the tip of the spine to the anterior end of the first pair of instar barbs; S2 (and, similarly, S3) length was measured from the anterior end of the first (second) pair of instar barbs to the anterior end of the second (third) pair of instar barbs (Figure 3.1a; Sullivan and Lehman 1998). We measured body length from the anterior edge of the eye to the base of the tail spine along the midline of the body with segments spanning the head and eye region, thorax, and abdomen (Figure 3.1a; Miehls et al. 2012).

Statistical Analyses

The effect of temperature and fish kairomones on *Bythotrephes* morphology and life history was tested statistically using linear mixed effects models (LME) fitted for each trait separately using the nlme package (Pinheiro et al. 2009) in R version 2.12.2 (R Development Core Team 2011). We first analyzed the effect of fish kairomone treatment within each temperature treatment. When no significant effect of fish kairomone treatment was found, we pooled the fish kairomone treatments and tested for a significant effect of temperature.

Models for traits of wild-captured *Bythotrephes* included temperature or fish kairomone treatment as fixed effects and aquaria (i.e., aquaria of fish kairomone or control water origin) as a random effect. Models for traits of F_1 *Bythotrephes* included temperature or fish kairomone treatment as fixed effects, and clonal line nested within aquaria as random effects. For F_2 *Bythotrephes*, sub-line nested within clonal line was included as an additional random effect. We assessed the significance of each random effect through model comparisons using likelihood ratio tests (LRT). For the LRT, we fitted up to three additional models for each trait, with each model containing successively fewer random effects to compare the fit of more complicated models to the data versus the fit of a simpler model that excluded the random effect of interest.

Because *Bythotrephes*' distal spine is present at birth (and does not change length with instar development), distal spine length of wild-captured *Bythotrephes* should not have been affected by our experimental treatments and any difference in distal spine length by experimental treatment for F_1 and F_2 *Bythotrephes* represents maternal effects. Differences in first instar body length by experimental treatment for F_1 and F_2 *Bythotrephes* also represent maternal effects. Because no F_1 *Bythotrephes* reproduced at their second instar in the colder temperature treatment (see results), we do not present data for body length or the ratio of distal spine-to-body length for second instar, F_1 *Bythotrephes* in the colder temperature.

Results

Survival of Clonal Lines to F_2 Generation

Lab-based mortality in our clonal rearing resulted in 21 and 17 clonal lines in our fish kairomone and control treatments, respectively, that survived to produce F_2 *Bythotrephes* at 15°C (75% and 68% survival, respectively), and 4 and 7 clonal lines in our fish kairomone and control treatments, respectively, at 9°C (15% and 26% survival, respectively). Although fewer clonal lines survived to the F_2 generation in the colder temperature, the mortality rate (per day) was identical between the temperature treatments, with approximately 1% and 2% of wild-captured and F_1 *Bythotrephes*, respectively, dying per day in both temperature treatments.

Response to Predator Kairomones

Fish kairomones did not affect any of the *Bythotrephes* traits we measured (Figs. 2-3; Appendix C: Table 3.1; Appendix D: Figure 3.1). The lack of response was consistent across temperature treatments (15°C and 9°C), generations (wild-captured, F_1, and F_2 generations), and instars (one, two, and three).

Response to Temperature

In contrast to fish kairomones, temperature had a strong effect on *Bythotrephes* morphology and life history (Figs. 1-3; Appendix C: Table 3.2). Below, we report trait responses to temperature for each generation (wild-captured, F_1, and F_2), as well as instar within each generation for traits known to vary with instar development (i.e., body length and the ratio of distal spine-to-body length).

Body length of second instar, wild-captured *Bythotrephes* (measured after producing offspring; see methods) was greater in the higher temperature (Figure 3.2c); no other morphological traits of reproductively mature (i.e., second or third instar), wild-captured *Bythotrephes* showed a response to temperature (Figure 3.2; Appendix D: Figure 3.1). Temperature also did not affect clutch size of wild-captured *Bythotrephes* (Figure 3.3a). Wild-captured *Bythotrephes* produced offspring with longer distal spines (F_1: Figure 3.2a) that also exhibited greater investment in morphological defense (ratio of distal spine-to-body length; F_1, Instar 1: Figure 3.2d) in the higher temperature.

Body length of third instar, F_1 *Bythotrephes* was greater at the higher temperature (Figure 3.2c), but no other morphological traits of reproductively mature F_1 *Bythotrephes* showed a response to temperature (Figure 3.2; Appendix D: Figure 3.1). Clutch size of F_1 *Bythotrephes* was smaller at the higher temperature (Figure 3.3a); the F_2 offspring produced at the higher temperature had longer distal spine (F_2: Figure 3.2a) and body length (F_2, Instar 1: Figure 3.2c), and also exhibited greater investment in morphological defense (F_2, Instar 1: Figure 3.2d). F_1 *Bythotrephes* reproduced twice as quickly at the higher temperature (Figure 3.3b) and reproduction occurred at both the second and third instar stages, whereas all F_1 *Bythotrephes* reproduced in their third instar at the colder temperature. Of note, greater distal spine-to-body length of F_2 offspring at the higher temperature was not a product of changes resulting from growth. At both temperatures, body length increased proportionally more than distal spine length with increased size (Figure 3.2b). Yet, F_2 offspring at the higher temperature (which were larger in absolute size) still showed greater ratios of distal spine-to-body length and also had markedly longer distal spines than offspring of the same body size at the lower temperature (Figure 3.2b).

Discussion

Our experiment clearly showed a strong plastic response of a prey to a correlate of predation risk, but no response to cues emanating directly from the predator. Response to a correlate of predation risk (that does not directly affect predation risk), without a concomitant response to cues directly released from the predator, has rarely, if ever been documented. Support for the use of proxy cues of predation risk is provided by theory (Hairston 1987), which suggests prey may evolve to use a correlated environmental cue as a proxy for predation risk when the correlated cue is a reliable predictor of predation risk. For *Bythotrephes* in Lake Michigan, temperature positively correlates with predation risk by gape-limited juvenile fish, which are the dominant predators of *Bythotrephes* later in the growing season; thus, temperature serves as a reliable predictor of size-dependent predation risk. Conversely, the presence of fish kairomones might be an indicator of overall predation risk, but fish kairomone presence is not a reliable predictor of size-dependent predation risk, to which plastically induced defenses would be effective. Plastic induction of defenses in response to kairomones from non-gape-limited adult fish, which are present throughout the growing season, would have little benefit. Here we found striking response of *Bythotrephes* morphology and life history to temperature (Figure 3.1b), but no response to kairomones from an important fish predator.

Field studies have documented temporal changes in *Bythotrephes* traits that correspond to the trait plasticity we observed in response to temperature, thus suggesting plastic responses to temperature might underlie observed seasonal variation in the wild. For example, field surveys in multiple lakes have shown an increase in body size and distal spine length that positively correlates with water temperature (Lake Constance: Straile and Hälbich 2000; Lake Michigan: Burkhardt 1994; Pothoven et al. 2001; 2003). Further, multiple studies also documented decreased clutch size and/or reproduction at younger instar stages that was associated with warmer water temperature (Straile and Hälbich 2000; Pothoven et al. 2001; 2003), strongly paralleling the trait changes we observed between our temperature treatments.

The lack of *Bythotrephes* response to fish kairomones may be surprising given the numerous examples of cladoceran zooplankton (Tollrian and Dodson 1999) that plastically respond to predator fish kairomones as well as the known importance of *Bythotrephes* traits (e.g., distal spine length) for deterring gape-limited fish predation (Barnhisel 1991a; 1991b). However, we can rule out multiple potential methodological concerns. We are confident that our representative fish predator, yellow perch (*Perca flavescens*), was an appropriate species to test as yellow perch are an important predator of *Bythotrephes* in the Great Lakes (Schneeberger 1991; Baker et al. 1992) and also have a closely related species (European perch, *Perca fluviatilis*) that preys upon *Bythotrephes* in their native range (Thorpe 1977; Straile and Hälbich 2000). We also confirmed that our freezing methodology effectively preserved fish kairomones by demonstrating a behaviorally plastic response of *D. mendotae* (Appendix B) and observing that the water containing fish kairomones (but not the control water) noticeably smelled of fish (personal observation). Further, we used a fish kairomone concentration (1 fish: 6L water) that previous studies (Reede 1995; Rose et al. 2001) using similarly sized fish (40-100mm length) have shown to elicit strong responses in cladoceran zooplankton. The fish also were fed (and ate as confirmed through diet analysis) *Bythotrephes* in order to increase the probability that fish scent would represent a threat (Stabell et al. 2003; Bourdeau 2010). Finally, the trait magnitudes of our laboratory-raised *Bythotrephes* fell within natural field variation of *Bythotrephes* traits, suggesting that our *Bythotrephes* were not constrained by a laboratory condition that prevented natural growth. Thus, we are confident that the lack of a *Bythotrephes* plastic response to fish kairomones is a real, biologically meaningful result.

We argue that temperature is a proxy cue for predation risk that elicits adaptive phenotypic responses based on three lines of reasoning: 1) the morphological and life history responses we observed, which involved an altered reproductive strategy with temperature, are not consistent with adaptive response to temperature *per se*; 2) the responses we observed are, instead, consistent with adaptive response to risk of gape-limited fish predation, which positively correlates with temperature; and 3) the use of the most common cue of fish predation risk, kairomones, as a proximate cue for predation risk would not be adaptive. We discuss each of these reasons below.

The observed plastic responses of *Bythotrephes* to temperature are unlikely to be adaptive to temperature *per se* given four reasons. First, one of the most well-supported rules of plasticity in response to temperature for ectotherms is the temperature-size rule, which states that individuals (within a species) raised at higher temperatures grow quickly, but reach a smaller final size than individuals raised at colder temperatures (Atkinson 1994; 1995); the temperature-size rule has been supported for over 80% of ectotherms investigated (Atkinson 1994). Smaller body size at higher temperature is physiologically adaptive for many ectotherms, as higher temperature causes increased metabolic rate, and consequently results in a higher energetic demand, which creates a cost to larger body size (Kozłowski et al. 2004). Indeed, many cladoceran zooplankton (Gillooly and Dodson 2000; Hart and Bychek 2011) follow the temperature-size rule. However, in contrast to the temperature-size rule, *Bythotrephes* had larger body size and longer distal spine length at the higher temperature, suggesting plasticity in *Bythotrephes* traits may not be physiologically adaptive to temperature *per se*. Second, *Bythotrephes* also produced offspring with longer distal spines relative to body length (i.e., larger ratio of distal spine-to-body length) at the higher temperature. Greater investment in distal spine length (relative to body size) likely has no adaptive value to higher temperature *per se*, and may even carry both a physiological cost (due to increased structural investment in a trait that has little energetic return, i.e., a trait that does not contribute to resource acquisition) and physical cost (due to increased weight which may promote sinking in warmer water, which is less viscous than colder water; Atkinson 1994). Third, *Bythotrephes* clutch size decreased with temperature (despite a concomitant increase in maternal body size), a finding that would have little adaptive value to temperature *per se* and which is also opposite to another general rule, the size-fecundity relationship (which states that larger individuals have greater fecundity; Stearns 1992; Roff

2002). Fourth, some F_1 *Bythotrephes* at the higher temperature reproduced at their second instar stage, whereas all F_1 *Bythotrephes* at the colder temperature delayed reproduction to their third instar stage. Although we might expect temperature to affect growth rate (such as the number of days between instar molts; Branstrator 2005), it is unlikely that temperature should alter development such that reproduction occurs at different stages.

The differences in *Bythotrephes* phenotype at different temperatures are consistent with the use of temperature as a proxy for adaptive response to predation risk. Given the increase in *Bythotrephes* distal spine length and the ratio of distal spine-to-body length with temperature, *Bythotrephes* exhibit their greatest investment in morphological defense later in the growing season, when water temperature is warmest. Predation by gape-limited juvenile fish is also greatest later in the growing season (due to growth of juvenile fish from completely gape-limited size classes early in the growing season into partially gape-limited classes mid-to-late in the season), and can even be the dominant source of predation on *Bythotrephes* (Branstrator 2005). Thus, temperature correlates with gape-limited predation, which allows temperature to serve as a proxy for predation risk. Previous research has demonstrated that longer spines of *Bythotrephes* (Barnhisel 1991a; 1991b) as well as other cladoceran zooplankton (*Daphnia lumholtzi*; Swaffar and O'Brien 1996) provide protection against gape-limited fish predation. Correspondingly, our observed plastic response of *Bythotrephes* traits to temperature would likely have adaptive value for reducing the risk from gape-limited fish predation.

Critical to our argument, temperature can serve as a reliable cue of predation risk in this predator-prey system, but fish kairomones are not reliable cues. Because adult fish are present throughout the growing season (and, therefore, fish kairomones are always present), but predation risk increases throughout the growing season due to increased gape-limited fish predation, there is a poor association between fish kairomones and predation risk. Further, there is also a poor association between fish kairomones and predation risk to which plastic responses would be beneficial. Large size and morphological defenses would have little adaptive value against adult fish predators early in the growing season as adult fish are a source of non-gape-limited predation. Thus, plastic morphological response to kairomones from fish would entail no fitness advantage, and so kairomones would not serve as a useful cue for reducing predation risk.

We believe our study is among the first to show a plastic response of a prey to a correlate of predation risk, but no response to cues released from the predator. Although previous studies have suggested (e.g., *Bosmina*: Kappes and Sinsch 2002) or are consistent with (e.g., *D. lumholtzi*; Yurista 2000) the use of temperature as a proxy for predation risk, the organisms studied also showed a concordant plastic response to predator kairomones (Kappes and Sinsch 2002; Dzialowski et al. 2003). There is general support for the sole use of proxy variables as reliable cues of correlated environmental conditions from the well-known use of photoperiod for breaking or inducing diapause (Stross 1966) and the use of temperature for adaptive sex determination in many ectotherms (e.g., Conover 1984; Warner and Shine 2008).

Notably, the use of temperature as an indirect, proxy cue for plasticity by *Bythotrephes* is mechanistically different from other studies that have suggested the use of “indirect” cues for plasticity. For example, Orrock et al. (2004) found that field mice (*Peromyscus polionotus*) use moonlight and precipitation (which they termed indirect cues), but not cues directly released by the predator (i.e., predator urine), as proximate cues for behaviorally plastic responses to reduce predation risk. Although moonlight and precipitation are not directly associated with the predator (as, comparatively, predator urine would be), moonlight and precipitation may still directly affect predation risk through altered prey visibility to predators. Thus, the use of abiotic cues that affect predation risk by field mice contrasts with the use of temperature by *Bythotrephes* as temperature has no direct effect on predation risk of *Bythotrephes*.

Three lines of evidence suggest there are costs associated with large morphological size early in the growing season, and support why *Bythotrephes* exhibit a plastic response to fish predation risk (via temperature as a proxy cue) rather than constitutive (fixed) expression of large size and enhanced morphological defense. First, large size increases visibility of prey to fish predators (Tollrian 1995), thus expression of large size and enhanced morphological defense by *Bythotrephes* early in the growing season during dominant non-gape-limited predation likely would entail a cost to *Bythotrephes*. Second, we found that the production of larger offspring was associated with reduced clutch size for *Bythotrephes*, suggesting a size-number trade-off in offspring investment (Smith and Fretwell 1974), which would entail a cost early in the growing season when higher fecundity would be advantageous due to low conspecific abundance and abundant prey resources (Burkhardt 1994; Pothoven et al. 2001; 2003). Third, large size early in the growing season, when water is colder and therefore more viscous (Atkinson 1994), could entail an energetic cost due to increased drag during swimming. Indeed, costs associated with drag could be an alternative explanation for *Bythotrephes* morphological plasticity. However, it is unlikely that the plastic responses we observed are adaptive for reducing drag, particularly responses involving distal spine length which is unlikely to be a significant source of drag in comparison to body length or brood pouch size (pers. comm. C. Laforsch and Q. Herzog, Ludwig-Maximilians-University, Munich, Germany).

Whereas we have not tested the adaptive value of *Bythotrephes* trait plasticity *per se*, we suggest that the plastic response of *Bythotrephes* traits to temperature is adaptive based on the framework of Doughty and Reznick (2004) who present an argument for adaptation based on six indirect lines of evidence (criteria), as follows. Adaptive plasticity must involve production of different phenotypes in different developmental environments (criterion 1) which must be present in nature (i.e., environmental heterogeneity; criterion 2). We meet these criteria by demonstrating the production of different *Bythotrephes* phenotypes at different temperatures (criterion 1) that represent natural seasonal temperature variation in Lake Michigan (criterion 2; Pothoven et al. 2001). Further, alternative phenotypes should differ in fitness between the different environments (criterion 3) and the developmental environments must be reliable cues for plasticity (criterion 4). We presented three logical arguments above for why we expect the alternative *Bythotrephes* phenotypes to differ in fitness between the different temperature ranges due to their correspondence to differing predation risk (criterion 3), although we have not explicitly measured selection on spine length here. We have also presented an argument for why temperature is a reliable predictor of fish predation risk (criterion 4). Finally, genetic variation for plasticity should exist in the responding traits (criterion 5) and comparative evidence should be present from other systems (criterion 6). Studies documenting genetic variation and providing comparative evidence for *Bythotrephes* trait plasticity are, unfortunately, lacking (i.e., no data are available to address criteria 5 and 6). However, it is reasonable to assume genetic variation for plasticity (criterion 5) exists in our system, given that gene-by-environment interactions are common across a wide variety of organisms (Pigliucci 2005). Overall, we are able to meet four of the six criteria discussed by Doughty and Reznick (2004), with a reasonable assumption of the presence of a fifth criterion, and consequently conclude that *Bythotrephes* plasticity in response to temperature is likely adaptive.

Plasticity in response to an abiotic, proxy cue of predation risk has implications for our understanding of the evolution of environmentally cued plasticity, as well as the success of invasive species, such as *Bythotrephes*, specifically. Our research provides experimental evidence that proxy cues can induce plastic responses in prey to reduce predation risk, when direct cues from the predator are unreliable. Organismal use of proxy cues in nature may be more common than previously realized, if proxy cues, due to their indirect and potentially surprising relationships to the adaptive responses they elicit, are less often investigated than more obvious potential direct cues of plastic responses. Moreover, the use of abiotic, proxy cues for reducing predation risk may be important to species invasions, particularly the *Bythotrephes* invasion. Interactions with predators have long been recognized as strong drivers of the success or failure of species invasions (Elton 1958). Indeed, lack of natural enemies (Keane and Crawley 2002) is often cited as evidence for the successful establishment of invasive species in nonnative ecosystems. Conversely, when potential predators are present, defenses may promote persistence through reducing predation risk. It is possible that *Bythotrephes*' ability to plastically adjust morphology and life history to defend against predation has contributed to their ability to establish in nonnative ecosystems. It is important to note, however, that the use of a correlated, proxy cue may potentially restrict which ecosystems *Bythotrephes* are capable of invading, if temperature regimes must correlate with predation risk. As invasive species may be more likely to invade ecosystems with similar abiotic conditions (e.g., temperature regime) to their native range (Kolar and Lodge 2001), our research suggests that the success of *Bythotrephes* specifically, and invasive species in general, may be due to abiotic-matching of cues for adaptive plasticity.

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Figures

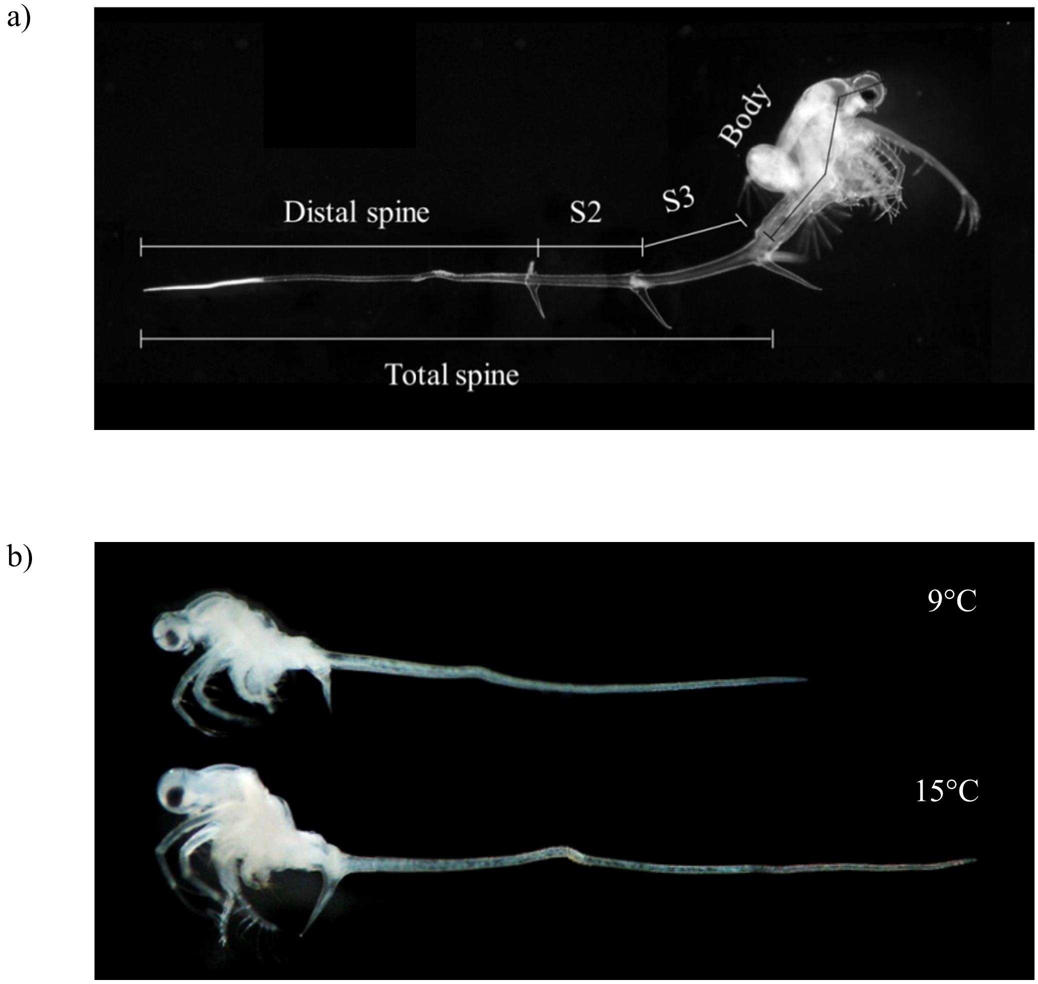
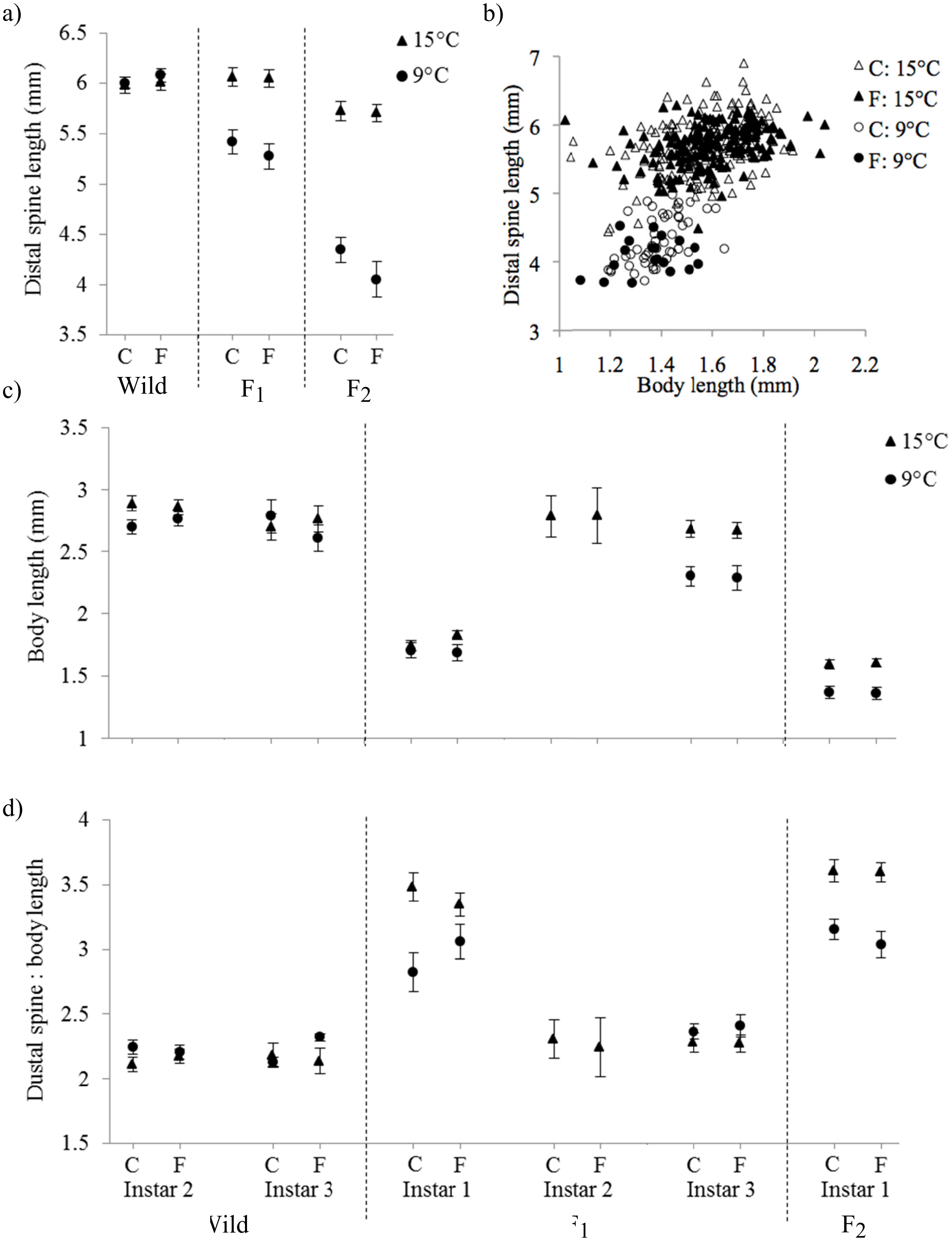


Figure 3.1. *Bythotrephes longimanus*, the spiny water flea. a) *Bythotrephes* morphological traits (the individual pictured is a third instar); b) F_2 offspring representing the average morphology of individuals born to clonal lines raised at 9°C and 15°C.

Figure 3.2. Predator and temperature effects on *Bythotrephes*: a) distal spine length; b) distal spine length versus body length of F_2 offspring; c) body length; and d) the ratio of distal spine to body length. Error bars are standard errors. C: control treatment; F: fish treatment.

Figure 3.2.


a)
Distal spine length (mm)
6.5
6
5.5
5
4.5
4
3.5
▲ 15°C
● 9°C
C
F
C
F
C
F
Wild
F_1
F_2
b)
Distal spine length (mm)
7
6
5
4
3
1
1.2
1.4
1.6
1.8
2
2.2
Body length (mm)
△ C: 15°C
▲ F: 15°C
○ C: 9°C
● F: 9°C
c)
Body length (mm)
3.5
3
2.5
2
1.5
1
▲ 15°C
● 9°C
d)
Dustal spine : body length
4
3.5
3
2.5
2
1.5
C
F
Instar 2
C
F
Instar 3
C
F
Instar 1
C
F
Instar 2
C
F
Instar 3
C
F
Instar 1
Vild
F_1
F_2

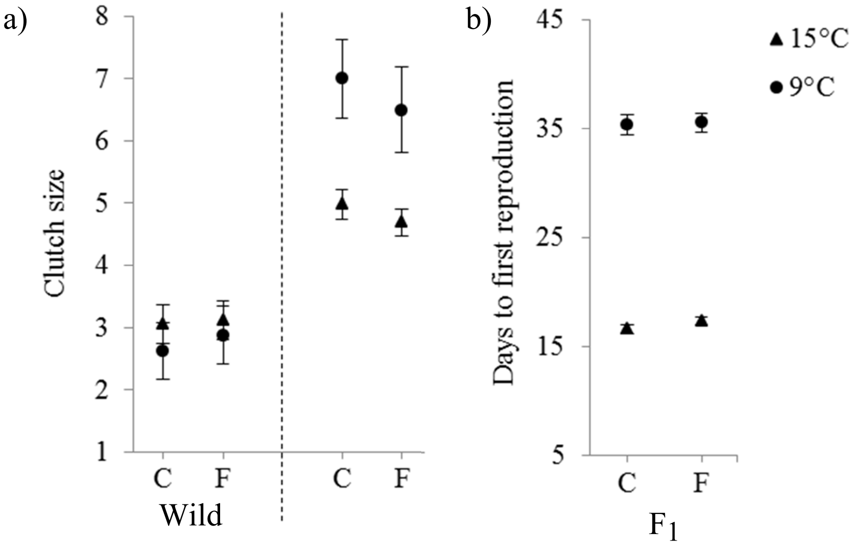


Figure 3.3. Predator and temperature effects on *Bythotrephes*: a) clutch size; and b) days to first reproduction. Error bars are standard errors. C: control treatment; F: fish treatment.

APPENDICES

Appendices

Appendix A: Fish Kairomone Collection

We obtained predator fish kairomones from yellow perch (*Perca flavescens*), one of the primary fish predators of *Bythotrephes* in Lake Michigan (Schneeberger 1991; Baker et al. 1992). Twenty yellow perch (50-100mm total length) were collected on July 14, 2010 from Lake Lansing (Ingham County, MI) using a 25ft beach seine (DNRE Scientific Collector's Permit No. 0536). Fish were placed in five 8L aquaria in the laboratory within 1hr of collection; each aquarium received one 50-70mm, two 80-100mm, and one 100-120mm fish (four fish total per aquarium). Five control aquaria were also set up and treated identically except for the addition of fish. Aquaria contained oxygenated, room temperature (21°C) Lake Michigan water passed through a 63µm sieve and were kept under low light with a 13:11 light:dark cycle. Fish were allowed a three day acclimation period during which all aquaria (fish and control) were inoculated with a natural assemblage of zooplankton. On the fourth day, all aquaria were inoculated with 200 *Bythotrephes* collected from Lake Michigan; previous research (e.g., Stabell et al. 2003; Bourdeau 2010) has shown that the strongest plastic responses of prey to predators were elicited when the predator had consumed prey conspecifics. Subsequent gut content analysis confirmed that perch in every aquarium consumed *Bythotrephes.* Twenty-four hours after addition of *Bythotrephes*, fish were removed from their acclimation aquaria, and all aquaria were emptied, rinsed, and re-filled with Lake Michigan water passed through GF/F Whatman filters; fish were then returned to their original aquaria. After 24hr, all water from the fish and control aquaria was collected and frozen in 100mL aliquots (frozen kairomone concentration of 1 fish: 2L water); previous research has shown that freezing is an effective method to preserve predator kairomones (Bourdeau, P., unpublished; Pangle, K. unpublished).

Appendix B: *Daphnia* Behavioral Bioassay

We conducted a *Daphnia mendotae* behavioral bioassay using a laboratory column experimental design (Loose and Dawidowicz 1994; Pangle and Peacor 2006) to assess whether our procedure to produce and preserve fish kairomones was effective. Previous laboratory research has shown that *D. mendotae* in Lake Michigan vertically migrate to colder, deeper water in response to predator kairomones (*Bythotrephes*: Pangle and Peacor 2006; yellow perch: Bourdeau, P., unpublished; Pangle, K., unpublished), and thus we predicted that *D. mendotae* would vertically migrate when exposed to our fish kairomone water.

We collected *D. mendotae* from Lake Michigan on October 18, 2010 at the 110m depth contour (sampling coordinates: 43º 12.11' N 86º 34.28' W), approximately 19km west of Muskegon, MI. In the laboratory, we filled twenty 1.5cm diameter, 60cm length transparent acrylic tubes (columns) with either fish kairomone or control water (both previously frozen). There were two replicate columns for each of the five fish and five control aquaria (20 columns total). Each 100mL aliquot of frozen control and kairomone water was diluted with an additional 200mL of Lake Michigan water passed through GF/F Whatman filters. Columns were illuminated from above and immersed in a water bath that maintained a temperature gradient of 19°C at the surface to 14°C at the bottom. Three *D. mendotae* were placed in each column and allowed to acclimate to the experimental conditions for 1hr, during which they were fed *Ankistrodesmus ad libitum*. Vertical position of all *D. mendotae* was recorded hourly for 4hr. Differences in *D. mendotae* mean depth (averaged across individuals in the same column) between the fish kairomone and control water were tested statistically with linear mixed effects models (LME) that included fish kairomone treatment as a fixed effect and aquaria nested within observation period as random effects using the nlme package (Pinheiro et al. 2009) in R version 2.12.2 (R Development Core Team 2011).

D. mendotae exposed to fish kairomones migrated deeper in the columns (average depth ± SE: 27.7 ± 3.3cm) than *D. mendotae* exposed to control water (average depth: 20.6 ± 3.1cm; $t_{35} = -2.14$; $p < 0.05$), confirming that our procedure to produce and preserve fish kairomones was effective. The LRT indicated no significant effect of observation period ($p > 0.05$) or aquaria ($p > 0.05$) on *Daphnia* mean depth.

Appendix C: Statistical Tables

Table 3.1. Effect of fish kairomones (presence / absence) on the morphology and life history of *Bythotrephes longimanus* raised at 9°C and 15°C. The generation and instar of *Bythotrephes* used in each analysis is noted. Significance of random effects for aquaria (all generations), clonal line (F_1 and F_2 generations), and clonal sub-line (F_2 generation only) was assessed using likelihood ratio tests. Gen: generation; Aq: aquaria; CL: clonal line; SL: sub-line; N/A: not applicable.

			9°C temperature treatment					**15°C temperature treatment**				
Trait	**Gen**	**Instar**	**Kairomone treat**		**Aq**	**CL**	**SL**	**Kairomone treat**		**Aq**	**CL**	**SL**
			F (df)	***p***	***p***	***p***	***p***	**F (df)**	***p***	***p***	***p***	***p***
Body length	Wild	2	0.64 (1,8)	N.S.	N.S.	N/A	N/A	0.12 (1,8)	N.S.	N.S.	N/A	N/A
	Wild	3	1.07 (1,3)	N.S.	N.S.	N/A	N/A	0.21 (1,7)	N.S.	N.S.	N/A	N/A
	F_1	1	0.04 (1,4)	N.S.	N.S.	N.S.	N/A	2.66 (1,7)	N.S.	N.S.	N.S.	N/A
	F_1	2	N/A	N/A	N/A	N/A	N/A	0.001 (1,4)	N.S.	N.S.	N.S.	N/A
	F_1	3	0.02 (1,8)	N.S.	N.S.	N.S.	N/A	0.02 (1,8)	N.S.	N.S.	*	N/A
	F_2	1	0.02 (1,5)	N.S.	***	N.S.	*	0.08 (1,8)	N.S.	***	***	***
Distal spine : body length	Wild	2	0.28 (1,8)	N.S.	N.S.	N/A	N/A	0.73 (1,8)	N.S.	N.S.	N/A	N/A
	Wild	3	18.39 (1,1)	N.S.	N.S.	N/A	N/A	0.12 (1,7)	N.S.	N.S.	N/A	N/A
	F_1	1	1.45 (1,4)	N.S.	N.S.	N.S.	N/A	0.91 (1,7)	N.S.	N.S.	N.S.	N/A
	F_1	2	N/A	N/A	N/A	N/A	N/A	0.06 (1,4)	N.S.	N.S.	N.S.	N/A
	F_1	3	0.21 (1,7)	N.S.	N.S.	N.S.	N/A	0.005 (1,8)	N.S.	N.S.	**	N/A
	F_2	1	0.79 (1,5)	N.S.	N.S.	***	N.S.	0.01 (1,8)	N.S.	***	***	***
Distal spine length	Wild	All	0.78 (1,8)	N.S.	N.S.	N/A	N/A	0.07 (1,8)	N.S.	N.S.	N/A	N/A
	F_1	All	0.71 (1,8)	N.S.	*	*	N/A	0.02 (1,8)	N.S.	N.S.	***	N/A
	F_2	1	1.87 (1,5)	N.S.	**	***	***	0.02 (1,8)	N.S.	***	***	***

Table 3.1 (con't)

Trait	Gen	Instar	9°C temperature treatment					9°C temperature treatment				
			Kairomone treat		Aq	CL	SL	Kairomone treat		Aq	CL	SL
			F (df)	*p*	*p*			F (df)	*p*	*p*		
S2 length	Wild	2 and 3	0.02 (1,8)	N.S.	N.S.	N/A	N/A	1.29 (1,8)	N.S.	N.S.	N/A	N/A
	F_1	2 and 3	0.85 (1,8)	N.S.	N.S.	***	N/A	0.09 (1,8)	N.S.	*	***	N/A
S3 length	Wild	3	0.001 (1,4)	N.S.	N.S.	N/A	N/A	0.17 (1,6)	N.S.	N.S.	N/A	N/A
	F_1	3	2.86 (1,7)	N.S.	N.S.	N.S.	N/A	0.08 (1,8)	N.S.	N.S.	***	N/A
Clutch size	Wild	3	0.16 (1,8)	N.S.	N.S.	N/A	N/A	0.02 (1,8)	N.S.	*	N/A	N/A
	F_1	3	0.31 (1,5)	N.S.	N.S.	***	N/A	0.75 (1,8)	N.S.	N.S.	N.S.	N/A
Age at first reproduction	F_1	3	0.01 (1,5)	N.S.	*	*	N/A	2.15 (1,8)	N.S.	N.S.	*	N/A

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; N.S.: non-significant (significance of random effects based on likelihood ratio tests).

Table 3.2. Effect of temperature (9°C versus 15°C) on the morphology and life history of *Bythotrephes longimanus*. The generation and instar of *Bythotrephes* used in each analysis is noted. Significance of random effects for aquaria (all generations), clonal line (F_1 and F_2 generations), and clonal sub-line (F_2 generation only) was assessed using likelihood ratio tests. Gen: generation; Aq: aquaria; CL: clonal line; SL: sub-line; N/A: not applicable.

Trait	**Gen**	**Instar**	**Temperature treatment**		**Aq**	**CL**	**SL**
			F (df)	***p***	***p***	***p***	***p***
Body length	Wild	2	6.40 (1,107)	**	N.S.	N/A	N/A
	Wild	3	0.50 (1,16)	N.S.	N.S.	N/A	N/A
	F_1	1	3.69 (1,23)	N.S.	N.S.	N.S.	N/A
	F_1	3	28.27 (1,44)	***	N.S.	*	N/A
	F_2	1	34.55 (1,38)	***	***	***	***
Distal spine : body length	Wild	2	2.92 (1,89)	N.S.	N.S.	N/A	N/A
	Wild	3	0.29 (1,12)	N.S.	N.S.	N/A	N/A
	F_1	1	17.12 (1,20)	***	N.S.	N.S.	N/A
	F_1	3	1.65 (1,42)	N.S.	N.S.	**	N/A
	F_2	1	24.54 (1,38)	***	***	***	***
Distal spine length	Wild	All	0.45 (1,121)	N.S.	N.S.	N/A	N/A
	F_1	All	56.78 (1,69)	***	N.S.	***	N/A
	F_2	1	175.77 (1,38)	***	***	***	***
S2 length	Wild	2 and 3	1.71 (1,140)	N.S.	N.S.	N/A	N/A
	F_1	2 and 3	3.03 (1,45)	N.S.	N.S.	***	N/A
S3 length	Wild	3	0.88 (1,13)	N.S.	N.S.	N/A	N/A
	F_1	3	1.24 (1,44)	N.S.	N.S.	***	N/A

Table 3.2 (con't)

Trait	**Gen**	**Instar**	**Temperature treatment**		**Aq**	**CL**	**SL**
			F (df)	***p***	***p***	***p***	***p***
Clutch size	Wild	3	2.19 (1,64)	N.S.	N.S.	N/A	N/A
	F_1	3	25.15 (1,36)	***	N.S.	N.S.	N/A
Age at first reproduction	F_1	3	900.84 (1,36)	***	*	*	N/A

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; N.S.: non-significant (significance of random effects based on likelihood ratio tests).

Appendix D: Supplementary Figures

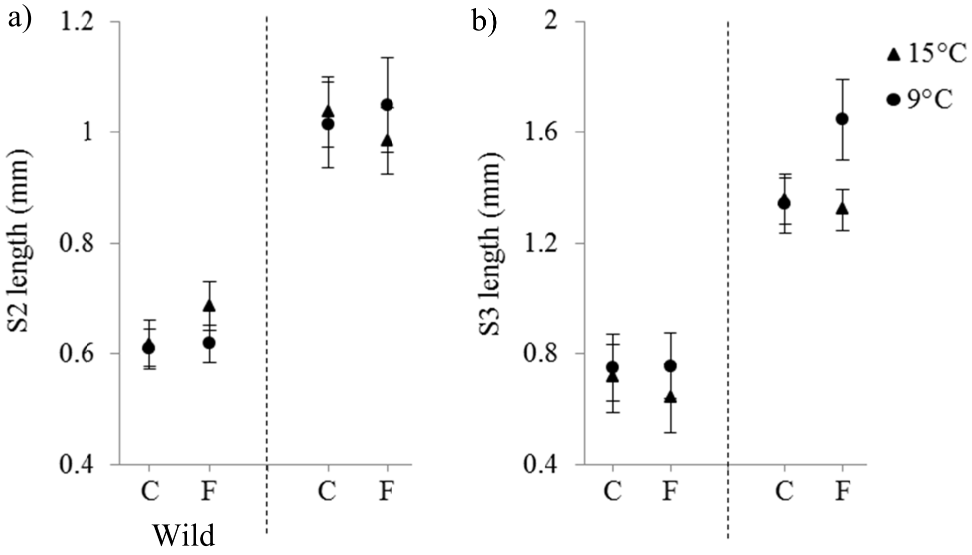


Figure 3.4. Predator and temperature effects on *Bythotrephes*: a) S2 length; and b) S3 length. Error bars are standard errors. C: control treatment; F: fish treatment.

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CHAPTER 4

Evolutionary stasis despite selection on a heritable trait in an invasive zooplankton

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Abstract

Natural selection is fundamental to evolutionary ecology, yet few studies rigorously assess spatial and temporal variation in selection, despite evidence that selection can vary widely in both dimensions. Accurately characterizing spatio-temporal variation in selection is important, as it may constrain evolutionary responses to selection. Here we quantified the strength of natural selection on the defensive morphology (distal spine) of an invasive zooplankton, *Bythotrephes longimanus*, in Lake Michigan across multiple months during three growing seasons and between four and six spatial replicates within the lake. We also used multiple lines of evidence (historic and contemporary wild-captured individuals, paleoecology of retrieved spines) to assess phenotypic change in distal spine length since invasion. We found that selection varied temporally, with selection for decreased distal spine length early in the growing season, and strengthening selection for increased distal spine length later in the growing season. This seasonal trend in natural selection is consistent with seasonal changes in the relative strength of non-gape-limited and gape-limited fish predation. Selection also varied spatially, which is consistent with well-known heterogeneity of fish predators in large lakes. Yet, despite net selection for increased distal spine length (and known genetic basis of distal spine length), we observed little evidence of an evolutionary response to selection. We discuss multiple factors that

likely limited an evolutionary response to selection, including genetic correlations, trade-offs between different components of fitness, and phenotypic plasticity.

Introduction

Evolution by natural selection is considered to be common in nature and numerous studies have documented trait changes over time consistent with adaptive evolution (reviewed in Hendry and Kinnison 1999; Kinnison and Hendry 2001; Reznick and Ghalambor 2001). Mechanistically, the evolutionary response to selection can be predicted from univariate models by the product of heritability and natural selection on the trait of interest (Falconer and Mackay 1996). These models of evolutionary change are well supported (Falconer and Mackay 1996; Lynch and Walsh 1998; but see Morrissey et al. 2010), yet there are a surprising number of examples where no evolutionary response is observed in the wild despite selection acting on traits with a known genetic basis (discussed in Merilä et al. 2001). Thus, one of the critical contemporary questions for evolutionary ecology is: what limits adaptation in the wild?

Many recent studies have addressed this question and have identified multiple potential limits to adaptive evolution. First, genetic correlations may reduce evolutionary responses to selection, even given substantial genetic variation in individual traits (Walsh and Blows 2009; Kingsolver and Diamond 2011). Second, trade-offs between different components of fitness (e.g., survival vs. fecundity) can constrain evolutionary responses to selection, if total selection on a trait across all fitness components is less than selection from a single fitness component (Roff 2002). Similarly, if the association of a trait with fitness changes with ontogeny, total selection measured across all life stages may be less than selection measured at a particular life stage, thus constraining evolution (Schluter et al. 1991; Kingsolver et al. 2012). Third, phenotypic plasticity (i.e., the ability of a genotype to produce different phenotypes in response to differing environmental conditions; Pigliucci 2001) in traits can constrain evolutionary responses if there is a genetic correlation between the trait and its degree of plasticity

(Schlichting 2004; Pfennig et al. 2010), or through altered trait heritability in different environments (Wilson et al. 2006). Finally, temporal and/or spatial fluctuations in the magnitude and/or direction of selection can yield weaker net selection than single selective events, thus constraining evolution (Siepielski et al. 2009; Bell 2010; Kingsolver and Diamond 2011). For example, strong directional selection may be followed by periods of no selection (thus lowering net selection through time), or periods of opposing directional selection (which could further lower or even negate the effects of prior selection, yielding no net selection; Bell 2010; Kingsolver and Diamond 2011). Thus, there is a need for temporal replication to fully characterize selection; similarly, there may also be a need for spatial replication, if selection is spatially heterogeneous. Yet, despite these needs, many studies lack temporal and/or spatial replication (temporal replicates reviewed by Kingsolver et al. 2001: range = 1–10; median = 1; spatial replicates: range = 1–12; median = 1; Kingsolver et al. 2001).

Invasive species are valuable study systems for investigating temporal and spatial patterns of natural selection, and how variation in selection and the other constraints affect the evolutionary response to selection (Sax et al. 2005; 2007). Selection on traits of invasive species is often strong, and numerous studies have shown evolution in invasive species traits (reviewed in, e.g., Mooney and Cleland 2001; Lee 2002; Lambrinos 2004). *Bythotrephes longimanus* (the spiny water flea; hereafter *Bythotrephes*; Figure 4.1) are invasive, cladoceran zooplankton native to Eurasia that were first found in the Laurentian Great Lakes in the early 1980s (Mills et al. 1993) and Lake Michigan, specifically, in 1986 (Evans 1988). *Bythotrephes* are a dominant predator of zooplankton (Bunnell et al. 2011) and common prey for fish (Pothoven et al. 2007), and are having widespread negative effects on food webs through trophic cascades (Hoffman et al. 2001; Strecker and Arnott 2008). One of the key traits of *Bythotrephes* governing their interspecific interactions is their long defensive tail spine, the longest component of which is the distal spine (Figure 4.1), which is considered to function as a defense against fish predation (Branstrator 2005).

Previous research (e.g., Burkhardt 1994; Sullivan and Lehman 1998; Pothoven et al. 2001; 2003) suggests *Bythotrephes* distal spine experiences natural selection, but heretofore, no study has explicitly measured natural selection nor has any study investigated a potential evolutionary response of distal spine length to selection. The most likely agents of selection on *Bythotrephes* distal spine length are their fish predators (Sullivan and Lehman 1998). *Bythotrephes* are consumed by both gape-limited fish (i.e., fish predators constrained by mouth size from consuming larger size classes; typically juvenile fish) and non-gape-limited fish (i.e., fish predators unconstrained by mouth size; typically adult fish; Schneeberger 1991; Mills et al. 1992; Pothoven et al. 2007; 2012). Research on other taxa indicates gape-limited predation selects for increased morphological size (e.g., Paine 1976, Case 1978, Day et al. 2002; Zimmerman 2007; Urban 2008), whereas non-gape-limited predation selects for decreased morphological size if larger individuals are preferentially consumed (e.g., Hambright 1991; Urban 2007; 2008). Thus, *Bythotrephes* likely experience opposing selection from their gape-limited and non-gape-limited fish predators.

Research on Lake Michigan (Pothoven et al. 2012) and other lakes (Straile and Hälbich 2000; Branstrator 2005) indicates predation on *Bythotrephes* by gape-limited and non-gape-limited predators varies seasonally; therefore, *Bythotrephes* distal spine length could experience temporal variation in selection. Gape-limited predation increases throughout the growing season due to growth of juvenile fish into sizes capable of consuming *Bythotrephes*; by the end of the growing season, gape-limited predation is typically the dominant source of predation on

Bythotrephes due to the abundance as well as high energetic demand of juvenile fish relative to adult fish (Straile and Hälbich 2000; Branstrator 2005; Pothoven et al. 2012). Given this temporal variation in predator gape-limitation, we would expect selection for decreased distal spine length early in the growing season (due to non-gape-limited adult fish), and selection for increased distal spine length later in the growing season (due to gape-limited juvenile fish). This temporal variation in selection may limit an evolutionary response in distal spine length, depending on the relative strength of selection from these two sources (i.e., net selection). Moreover, the abundance of adult and juvenile fish predators is known to be spatially variable in Lake Michigan and other large lakes (O'Gorman et al. 1991; Dettmers et al. 2005; Miehls and Dettmers 2011), thus selection may also vary spatially.

We conducted a study investigating temporal and spatial patterns of natural selection on *Bythotrephes* distal spine length and the potential for an evolutionary response to selection. We collected *Bythotrephes* from Lake Michigan during multiple months in three growing seasons (July-September: 2007; July-October: 2008-2009) and multiple sites (six sites: 2007; four sites: 2008-2009) along an 8.0km transect. For each month and site, we estimated natural selection on distal spine length within a cohort through a comparison of distal spine length between the first two developmental (instar) stages. We predicted that selection would vary temporally, with selection for decreased distal spine length occurring early in the growing season when predators are predominantly non-gape-limited, and selection for increased distal spine length later in the growing season when predators are predominantly gape-limited. We also tested for spatial variation in selection, which could be due to the patchiness of fish abundance. Further, we looked for evidence of phenotypic change since invasion by investigating within and among season trends in first instar distal spine length of wild-captured *Bythotrephes* using data from our three sampling years (2007-2009) as well as previously published data from years soon after invasion (1989, 1992-1993, 1996). Finally, we used a paleoecological approach (Jeppesen et al. 2001) to look for additional evidence of phenotypic change since invasion by retrieving remnant distal spines from sediment cores. Previous work (Keilty 1988; Hall and Yan 1997) has shown that *Bythotrephes* spines preserve effectively in sediments, with their depth distribution reflecting time since invasion. This paleoecological approach offers a unique opportunity for us to assess phenotypic change through time in distal spine length.

Methods

Morphology and Life History of Bythotrephes

The longest section of the *Bythotrephes* tail spine is the distal spine, which extends from the tip of the spine to the first pair of instar barbs (Figure 4.1). The distal spine is present at birth and is retained throughout instar development at its initial length, whereas total spine length increases during the first two molts through the addition of intercalary segments separated by paired barbs (Burkhardt 1994; Sullivan and Lehman 1998; Branstrator 2005). Other traits, such as body size, also increase with each instar molt (Burkhardt 1994; Sullivan and Lehman 1998). *Bythotrephes* reproduce by cyclic parthenogenesis, with multiple asexual generations during the growing season culminating in sexual reproduction at the end of the season (Yurista 1992; Pothoven et al. 2001).

Natural Selection Analysis

Field Collection. We collected *Bythotrephes* monthly from July through September in 2007 and July through October in 2008-2009, time periods spanning the majority of the *Bythotrephes* growing season in Lake Michigan. For each month, we completed paired sampling trips separated by three days, with the exception of September 2007 and October 2008, for which we only completed the first collection (Table 4.1). We collected *Bythotrephes* using a mid-water trawl with a 1m x 2m opening and 1000µm mesh size towed horizontally through the epilimnion and metalimnion. Tows were completed along an 8.0km south-north transect (starting coordinates: 43° 11.76' N 86° 28.10' W; ending coordinates: 43° 15.46' N 86° 31.25' W) that encompassed six sampling sites at the 60m depth contour, approximately 12.5km west of Muskegon, MI, USA. One tow was completed at each site; tows were approximately 0.9km long and sites were separated by approximately 0.5km. All six sites were analyzed in the natural selection analysis for 2007, whereas only four of the six sites (sites 1-2, 4-5) were analyzed for 2008-2009; for one time period, October 2008, collections occurred at only one site (site 1). All *Bythotrephes* collected were immediately preserved in 95% ethanol. Water temperature 1m below the water surface was recorded during each sampling trip (Table 4.1).

Measurement of Morphological Traits. We measured *Bythotrephes* distal spine length in ImageJ (Abramoff et al. 2004) to the nearest 0.001mm from photographs taken with a digital camera attached to a dissecting microscope. Distal spine length was measured from the tip of the spine to the anterior end of the first pair of instar barbs (Figure 4.1; Burkhardt 1994; Sullivan and Lehman 1998). Although some organisms are known to decrease in length after preservation in ethanol (e.g., larval fish; Moku et al. 2004), a previous test (Miehls et al. 2012) of *Bythotrephes* preserved in 95% ethanol revealed no significant shrinkage of *Bythotrephes* distal spine length.

Natural Selection. We measured natural selection on *Bythotrephes* distal spine length through a comparison of distal spine length between the first two instar stages. Because distal spine length does not change with instar development, differences in mean distal spine length between the first and second instar stages would reflect size-specific survival as opposed to a developmentally based difference in length (Sullivan and Lehman 1998). We compared distal spine length of first instar individuals collected on the first of the paired sampling trips to distal spine length of second instar individuals collected on the second of the paired sampling trips. We assumed that these individuals were members of the same cohort because our trips were separated by three days, which is the average instar development time of *Bythotrephes* at 21°C (Branstrator 2005); this water temperature was similar to the average water temperature at the time of most collections (Table 4.1). As a result, most *Bythotrephes* in their first instar at the time of the first trip should have molted to the second instar at the time of the second trip. This estimation of selection within a cohort is important, as it provides a control for the potentially confounding effects of phenotypic plasticity. Our previous work (Chapter 3) indicated that *Bythotrephes* mothers plastically adjust distal spine length of their offspring in response to water temperature, with higher temperature inducing the production of offspring with longer distal spines. Individuals of the same cohort should have experienced the same temperature environment during development, and thus are free from possible temperature-induced differences in distal spine length, which a comparison of individuals between cohorts might entail. For example, first and second instar individuals collected on the same sampling trip might have different distal spine lengths because they experienced different water temperatures early in development.

Instar stage was determined by counting the number of paired barbs on the spine, which reliably indicates instar stage for the first two instars (Yurista 1992; Martin and Cash-Clark 1995; Sullivan and Lehman 1998). Although *Bythotrephes* pass through up to three or more instar stages (Yurista 1992), the comparison between first and second instars was chosen because survival through this early life stage likely comprises a large proportion of total fitness and because the distal segment for first instar individuals represents the entire spine length, which we suspected was the target of gape-limited fish predation.

Statistical Analysis. We statistically tested whether selection on distal spine length varied temporally and spatially with a linear model in R version 2.15.0 (R Development Core Team 2012). The model included distal spine length as a continuous response, instar (categorical for instars one and two), time period (categorical for each month within each year), site (categorical), and all two-way interactions and the three-way interaction as fixed effects. In this model, a statistically significant three-way interaction between instar, time period, and site would indicate selection (effect of instar) varies temporally (effect of time period) and spatially (effect of site). Component two-way interactions between instar and time period or instar and site would indicate only temporal or spatial variation in selection, respectively. Non-significant three- and two-way interactions but a significant main effect of instar would represent consistent directional selection.

We estimated selection differentials (Falconer and Mackay 1996) for distal spine length at each site within each month separately as the difference in mean distal spine length between first and second instar individuals. We assessed the statistical significance of the selection differentials using linear models of distal spine length as a continuous response and instar as the sole fixed effect; in these models, a statistically significant effect of instar would indicate significant selection on distal spine length.

Phenotypic Response to Selection

Wild-Captured Individuals. We looked for evidence of phenotypic change since invasion by assessing trends in average distal spine length of wild-captured individuals within and among years using our monthly collections across three years (2007-2009) as well as previously published data from years soon after invasion (1989: Burkhardt 1994; 1992-1993, 1996: Sullivan and Lehman 1998; note that Sullivan and Lehman 1998 present single yearly length estimates without corresponding sample dates). We used only first instar individuals in this investigation as their phenotype would most directly reflect an evolutionary response to selection, whereas the phenotype of older instars would also be influenced by within-generation selection. Although there are other published studies presenting historical distal spine lengths of *Bythotrephes* (e.g., Pothoven et al. 2001; 2003), we used Burkhardt (1994) and Sullivan and Lehman (1998) because of the similarity of their measurement procedure to ours for distal spine length. Using our three sample years (2007-2009), we statistically tested for a change in distal spine length through time. We used a two-step process in this analysis: first, we fit a linear model with distal spine length as a continuous response and water temperature as a continuous fixed effect, and, second, we tested the effect of Julian day (continuous covariate) and year (continuous fixed effect) on the residuals of the first model. This process tested for an effect of year on distal spine length (which assessed phenotypic change in distal spine length through time), while removing the confounding effect of phenotypic plasticity in distal spine length in response to water temperature (through the first model). Removing the effect of water temperature on distal spine length was important, as previous research (Chapter 3) indicated that mothers produce offspring with longer distal spines when reared in warmer water.

Remnant Spines. We looked for additional evidence of phenotypic change since invasion by retrieving remnant distal spines from sediment cores (Keilty 1988; Hall and Yan 1997). We collected sediment cores from Lake Michigan during 2007-2009 at the 57m depth contour (42º 52.69' N 86º 21.35' W) approximately 12km west of Grand Haven, MI. Previous work indicated that sedimentation rates near our field collection site were approximately 0.1cm per year

(Robbins and Edgington 1975), although the sedimentation rate has likely increased since the early 1990s following dreissenid mussel invasion (Thayer et al. 1997). Conservatively allowing for an increased sedimentation rate post-dreissenid invasion, and given a *Bythotrephes* invasion year of 1986 in Lake Michigan (Evans 1988), we sampled the upper 10cm of sediment during 2007. We sampled the upper 20cm of sediment during 2008-2009 to ensure the 10cm depth during 2007 was sufficient to encompass all sedimentation since *Bythotrephes* invasion. Sediment was partitioned into 1cm depth intervals on board the ship, and intact distal spines were removed from the sediment in the lab and measured according to the methods above. We used a linear model to assess whether distal spine length (continuous response) correlated with depth (proxy for time since invasion; continuous fixed effect) while accounting for variation due to our sampling years (categorical fixed effect).

Results

Natural Selection

We found net selection for increased distal spine length across all time periods (months, years) and sites (net selection differential: $S = 0.08$mm, $F_{1,\,6821} = 43.3$, $p < 0.001$; Figure 4.2). However, selection varied both temporally and spatially (significant three-way interaction between instar, time period, and site: $F_{26,\,5433} = 7.80$, $p < 0.001$; Figure 4.3; see Appendix A for mean distal spine length of each instar, selection differentials, and selection intensities for each site and time period combination). The greatest within-season variation in selection occurred in 2007, when we observed selection for decreased distal spine length predominantly early in the growing season (July), and selection for increased distal spine length later in the season (August and September; Figure 4.3a). Moreover, we also observed the strongest spatial variation in selection during 2007, as the direction of selection varied by site for two months (July, September). Significant selection for decreased distal spine length was never observed in 2008 or 2009 (Figure 4.3b;c); instead, there was consistent selection for increased distal spine length for at least one site during each time period, with the exception of October 2008 and July 2009 when we observed no significant selection (Figure 4.3b;c). Notably, there was a trend for the strongest episodes of selection to occur at the temporal extremes: the strongest selection for decreased distal spine length occurred during July 2007 (our earliest sample collection across all sample dates; Table 4.1) and the strongest selection for increased distal spine length occurred during October 2009 (the penultimate sample collection across all sample dates; Table 4.1).

Phenotypic Response to Selection

Wild-Captured Individuals. Considering our three sampling years (2007-2009) as well as one historical year (1989), within season variation in distal spine length was greater than among season variation (Figure 4.4a). During 2007 and 2008, distal spine length was smallest at the start of the growing season (July) and longest later in the season (August-October). First instar distal spine length (statistically controlled for temperature-induced plasticity) significantly increased throughout our three sampling years (total length increase across the three years: 0.08mm, $t_{6265} = 11.9$, $p < 0.001$), although the relationship between distal spine length and year was weak (model adjusted $R^2 = 0.12$). The 0.08mm increase over three years corresponds to an annual rate of 0.03mm/yr. The range of historical distal spine lengths (1989, 1992-1993, 1996) almost completely encompassed the range of distal spine lengths found in our study years (the exception being one smaller distal spine length estimate during July 2007), indicating little phenotypic change in *Bythotrephes* distal spine length since invasion (Figure 4.4a).

Remnant Spines. Distal spine length increased with depth interval for all three years (length increase across all years: 0.04mm; $t_{267} = 2.8$, $p < 0.01$), although the relationship between distal spine length and depth interval was weak (model adjusted $R^2 = 0.04$; Figure 4.4b). Note that as deeper sediments were deposited earlier in time, this relationship indicates distal spine length decreased slightly since invasion. Given that *Bythotrephes* invaded Lake Michigan in 1986 (Evans 1988), the 0.04mm decrease occurred over 23 years (using our last year of sediment collection, 2009), corresponding to an annual rate of -0.002mm/yr. Only two spines were found below the 10cm depth interval (one spine each at the 11 and 13cm depth intervals in 2008). Given the rarity of spines below 10cm deep (the 269 other spines were found 9cm or shallower), and the possibility the two deep spines resulted from contamination of samples during processing, the 2007 sediment collection (which only included the upper 10cm of sediment) likely reflected all sedimentation since *Bythotrephes* invasion.

Discussion

Although adaptive evolution is generally considered ubiquitous in nature (Kinnison and Hendry 2001), and natural selection is frequently documented in natural populations (Kingsolver et al. 2001; Hereford et al. 2004; Kingsolver and Diamond 2011), there are a surprising number of examples where an evolutionary response to selection on a heritable trait is not observed (Merilä et al. 2001). Our research has demonstrated evolutionary stasis in an important trait governing interspecific interactions that is under significant, directional selection (Figure 4.2) and has a strong genetic basis (Miehls et al. 2012). We base this finding of evolutionary stasis on a temporally and spatially replicated investigation of natural selection (47 total estimates of natural selection) and using multiple lines of evidence (historic and contemporary wild-captured individuals, paleoecology of retrieved spines).

Our results showed evidence of temporal variation in both the magnitude and direction of selection on *Bythotrephes* distal spine length. The dominant direction of selection was for increased distal spine length, which we observed in all time periods, although selection for increased distal spine length was not significant in October 2008 or July 2009 (Figure 4.3). In 2009, when we had the greatest temporal resolution, there was a trend for strengthening selection throughout the growing season, with the strongest selection occurring during October. We only observed significant selection for decreased distal spine length during two time periods, July 2007 and September 2007. Note, however, that the selection for decreased distal spine length in July 2007 was the strongest selection (positive or negative) we observed over all time periods (Figure 4.3). Interestingly, the July 2007 sample collection was also the earliest collection we completed. It is possible the July 2008 and 2009 collections, which occurred approximately 3 and 1.5 weeks later in the growing season than the July 2007 collection, respectively, may have occurred sufficiently late to have missed seasonal episodes of selection for decreased distal spine length. In general, the data are suggestive of a trend for strong selection for decreased distal spine length early in the growing season (July 2007), followed by strengthening selection for increased distal spine length that reaches the greatest magnitude late in the season (October 2009).

The temporal pattern of selection we observed is consistent with typical seasonal shifts in gape-limitation of fish predators, suggesting fish are the likely agents of selection in our system. Specifically, the strong selection for decreased distal spine length early in the season occurred when non-gape-limited (adult) fish predation would be the strongest (Straile and Hälbich 2000;

Branstrator 2005; Pothoven et al. 2012), whereas the strengthening selection for increased distal spine length during the remainder of the growing season occurred when gape-limited fish predation would be increasing coincident with growth of juvenile fish (Straile and Hälbich 2000; Branstrator 2005; Pothoven et al. 2012). The conclusion that these changes in the direction and magnitude of selection are the result of temporally variable gape-limitation of fish predators is supported by previous research that indicates gape-limited predators typically exert selection for increased morphological size (e.g., Paine 1976, Case 1978, Day et al. 2002; Zimmerman 2007; Urban 2008), whereas non-gape-limited predators typically exert selection for decreased morphological size (e.g., Hambright 1991; Urban 2007; 2008). Further, laboratory research (Barnhisel 1991a; 1991b) indicated that gape-limited fish selectively consume *Bythotrephes* based on distal spine length, with gape-limited fish preferentially consuming *Bythotrephes* with shorter distal spines. Thus, it is likely that fish predators are the agents of selection in our system, and that temporal variation in selection is due to changes in gape-limitation, although this assertion remains to be tested more rigorously.

We also found that selection varied spatially (Figure 4.3). During seven of our eleven time periods, we observed opposing directions of selection by site, although we only observed statistically significant opposing selection by site in two time periods (July and September 2007). The spatial variation in selection is presumably due to heterogeneous distribution of fish predators, which is known to occur in Lake Michigan and other large lakes. Both adult (O'Gorman et al. 1991) and juvenile fish (Dettmers et al. 2005; Miehls and Dettmers 2011) are known to be spatially patchy at the scale at which we sampled, with patchiness of adult fish generally due to active fish movement (for, e.g., reproduction or foraging; O'Gorman et al. 1991) and patchiness of juvenile due to passive transport by currents (Dettmers et al. 2005; Miehls and

Dettmers 2011). The opposing directions of selection found in July and September 2007 could thus be due to different sites experiencing different relative strengths of predation by non-gape-limited adult and gape-limited juvenile fish. Alternatively, we note that for both July and September 2007 there were at least two significant selection differentials of consistent direction and only one significant selection differential of opposing direction (Figure 4.3a); it is possible the single opposing selection differential for each time period was a statistical artifact resulting from high power to detect a difference in means (Appendix A) and multiple comparisons that resulted in Type I error. In general, however, our data are indicative of fairly strong spatial variation in selection.

Despite net selection for increased distal spine length ($S = 0.08$mm; Figure 4.2), we found only limited evidence of phenotypic change in *Bythotrephes* distal spine length since invasion. Our wild-captured *Bythotrephes* exhibited a minor increase in length from 2007-2009 (absolute length increase: 0.08mm; annual rate: 0.03mm/yr); however, the historical data (1989, 1992-1993, 1996) almost completely overlapped with our 2007-2009 data, indicating little phenotypic change in distal spine length since invasion. Moreover, the sediment-retrieved distal spines indicated little phenotypic change in length since invasion. Contrary to the wild-captured data, the retrieved distal spines indicated a decrease in length since invasion (absolute length decrease: -0.04mm; annual rate: -0.002mm/yr). Given the conflicting directions, small magnitudes, and low annual rates of change from these sources of data, it is unlikely *Bythotrephes* distal spine length has undergone any consistent phenotypic change since invasion.

Multiple factors can cause evolutionary stasis despite directional selection on a heritable trait (Merilä et al. 2001). The most relevant potential factors in our system include: 1) temporal and spatial fluctuation in selection; 2) genetic correlations with unmeasured traits; 3) trade-offs between fitness components and/or selection that varies by life stage; and 4) phenotypic plasticity. We explore each of these factors below.

First, temporal and spatial fluctuations in the magnitude and/or direction of selection can reduce the cumulative effects of selection and constrain evolution (Merilä et al. 2001; Siepielski et al. 2009; Bell 2010; Kingsolver and Diamond 2011). Investigations in well-studied populations show that both the direction and magnitude of selection can vary considerably from year-to-year (e.g., Price et al. 1984; Grant and Grant 1993; Sinervo et al. 2000; McAdam and Boutin 2003). We found strong temporal and spatial variation in selection (Figure 4.3), but still found net selection (S = 0.08mm) for increased distal spine length (Fig 4.2). It is possible, however, that our net selection differential was biased due to missing early season selection in 2008 and 2009 (discussed above). Additionally, the spatial coverage of our transect (8.0km) was small relative to the size of Lake Michigan; it is reasonable to assume that selection may vary considerably more across the larger spatial scale of the lake. Thus, spatio-temporal variation in selection in Lake Michigan may be even greater than the variation reflected in our study, for which the net effect may be no significant selection. Indeed, although our net selection differential was statistically different from zero, its low magnitude relative to the trait standard deviation (i.e., selection intensity = 0.10) indicates only moderate net selection (Kingsolver and Diamond 2011).

Second, genetic correlations may reduce evolutionary responses to selection, even given substantial genetic variation in individual traits (Walsh and Blows 2009; Kingsolver and Diamond 2011). Previous research (Miehls et al. 2012) found that *Bythotrephes* distal spine length is highly heritable (range of H^2: 0.27-0.76), but they did not investigate possible genetic correlations between distal spine length and other traits. Our work (Appendix B) and others

(Sullivan and Lehman 1998; Branstrator 2005) indicates *Bythotrephes* distal spine length is phenotypically correlated with body size, suggesting the possibility of a genetic correlation (Conner and Hartl 2004). If there is selection on body size in the opposite direction as selection on distal spine length, an evolutionary response of distal spine length could be constrained. Alternatively, body size, and not distal spine length, may be the true target of selection; in this scenario, our selection differentials (which estimate total selection) may be overestimating direct selection on distal spine length. Estimating selection gradients for distal spine length would remedy this potential problem. However, our selection design, which involved a comparison of different instar stages, does not allow for the estimation of selection gradients because our comparisons across developmental stages do not quantify individual fitness and selection on body size cannot be determined using our method as body size always increases with each instar molt (Burkhardt 1994; Sullivan and Lehman 1998). Of note, however, recent reviews (Kingsolver et al. 2001; Kingsolver and Diamond 2011; Kingsolver et al. 2012) found that selection differentials and selection gradients were often of similar magnitude, suggesting our selection differentials may approximate direct selection.

Third, trade-offs between fitness components (Roff 2002) and/or variable selection by life stage (Schluter et al. 1991; Kingsolver et al. 2012) can also limit evolutionary responses. Specifically, trait values advantageous to one component of fitness (e.g., survival) may be disadvantageous to some other component of fitness (e.g., fecundity). Our previous work (Chapter 3) and others (Straile and Hälbich 2000; Pothoven et al. 2003) indicates *Bythotrephes* mothers face a size-number trade-off (Smith and Fretwell 1974) in their allocation of resources to distal spine defenses of offspring versus number of offspring, which might constrain evolutionary responses in distal spine length. In this case, the viability selection for increased spine length that we have documented here might be offset by fecundity selection for reduced distal spine length. Another related possibility is that a trade-off exists between investment in current defense (i.e., distal spine length of younger instar individuals) and investment in future defense (i.e., length of the segments added with future molts); however, this scenario is unlikely as our work indicates distal spine length positively correlates with the length of the first intercalary segment (Appendix C). Regarding the latter constraint to evolution (i.e., variable selection by life stage), a trait that is strongly associated with an aspect of fitness in an early developmental stage may not necessarily be as strongly associated with that aspect of fitness in a later developmental stage (Schluter et al. 1991). *Bythotrephes* pass through multiple developmental (instar) stages, but we only estimated selection on *Bythotrephes* distal spine length between the first two instar stages. For older instar individuals - whose tail spines contain up to two intercalary segments (Figure 4.1) added with the first two instar molts - selection is likely on total spine length, which may effectively weaken direct selection on distal spine length and therefore constrain evolution.

Finally, phenotypic plasticity may constrain evolutionary responses to selection if there is a genetic correlation between the trait and its degree of plasticity (Schlichting 2004; Pfennig et al. 2010), or through altered trait heritability in different environments (Wilson et al. 2006). We found that *Bythotrephes* mothers plastically altered distal spine length of offspring in response to their temperature environment (Chapter 3). Although *Bythotrephes* distal spine length is heritable (Miehls et al. 2012), the genetic basis of plasticity in *Bythotrephes* distal spine length is unknown, as is the potential for a genetic correlation between absolute length and plasticity in length. If a correlation between plasticity and absolute length exists, the correlation could constrain an evolutionary response to selection (Pfennig et al. 2010). Further, phenotypic plasticity could constrain an evolutionary response if trait heritability varies by environment (Wilson et al. 2006). Because individuals vary in their reaction norms for plastic traits (Pigliucci 2001), phenotypic variation will differ across environments even given the same genotypic variation, which in turn causes heritabilities to be environment-dependent (Pigliucci 2001). Miehls et al. (2012) found high distal spine length heritability in a single temperature environment (15°C); given a plastic response to temperature, it is possible distal spine length heritability is lower in other temperature environments. Thus, given seasonal water temperature variation in the wild (Pothoven et al. 2001), heritability of *Bythotrephes* distal spine length may vary temporally. In this manner, phenotypic plasticity can constrain an evolutionary response of *Bythotrephes* distal spine length via environment-dependent trait heritabilities.

In conclusion, our research has demonstrated evolutionary stasis in a key trait for interspecific interactions despite significant, directional selection and known genetic trait variation. Although these findings may initially appear contradictory, multiple constraints on the evolutionary response to selection could exist in this system that can readily explain evolutionary stasis. These findings have two main implications to evolutionary ecology. First, our work strongly supports the conclusions of others (e.g., Merilä et al. 2001) that selection can vary widely in time and space, and therefore, accurate predictions of the evolutionary response to selection necessitate greater replication in studies of natural section. Second, even though natural selection is common in the wild (Kingsolver et al. 2001; Hereford et al. 2004; Kingsolver and Diamond 2011), evolutionary responses to selection may be constrained, even when traits are under selection and have a significant genetic basis. Importantly, though, not all constraints may be viewed as detrimental to fitness, as some constraints (e.g., phenotypic plasticity) may themselves confer a strong fitness advantage.

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Tables

Table 4.1. Sample dates for paired monthly collections used to estimate natural selection on *Bythotrephes* distal spine length during 2007-2009 and average water temperature 1m below the surface. Note that the October 2, 2007 and November 3, 2008 collections are referred to in the text as September and October collections, respectively, given the approximate one-month interval between these collections and the previous collections. Only one sampling trip was completed for these two time periods.

Paired Sample Dates	**Temperature (°C)**
2007	
July 9, 12	19.1
August 21, 24	21.1
October 2	19.8
2008	
July 28, 31	21.6
August 26, 29	21.5
September 22, 25	19.8
November 3	11.3
2009	
July 20, 23	20.1
August 17, 19	21.3
September 21, 24	19.6
October 26, 29	10.2

Figures

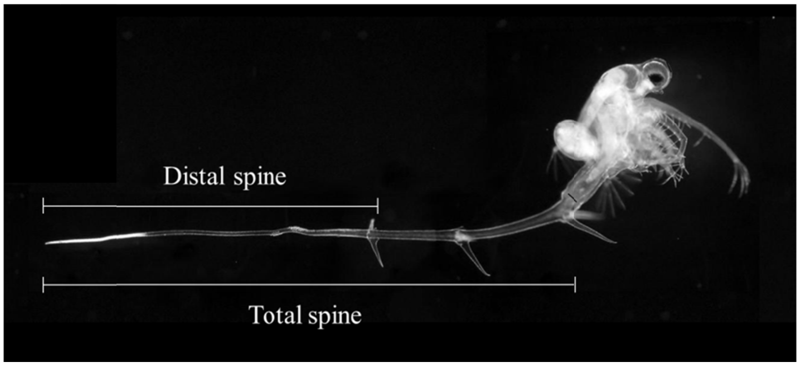


Figure 4.1. We estimated natural selection on distal spine length of *Bythotrephes longimanus*, the spiny water flea (the individual pictured is a third instar).

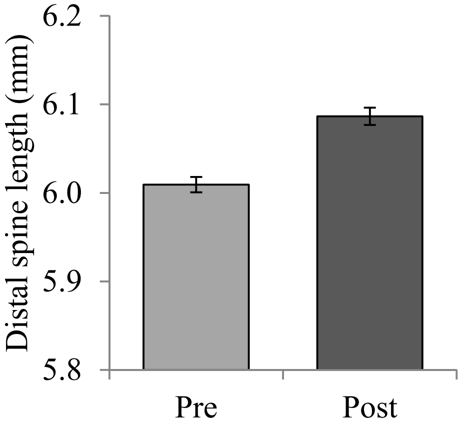
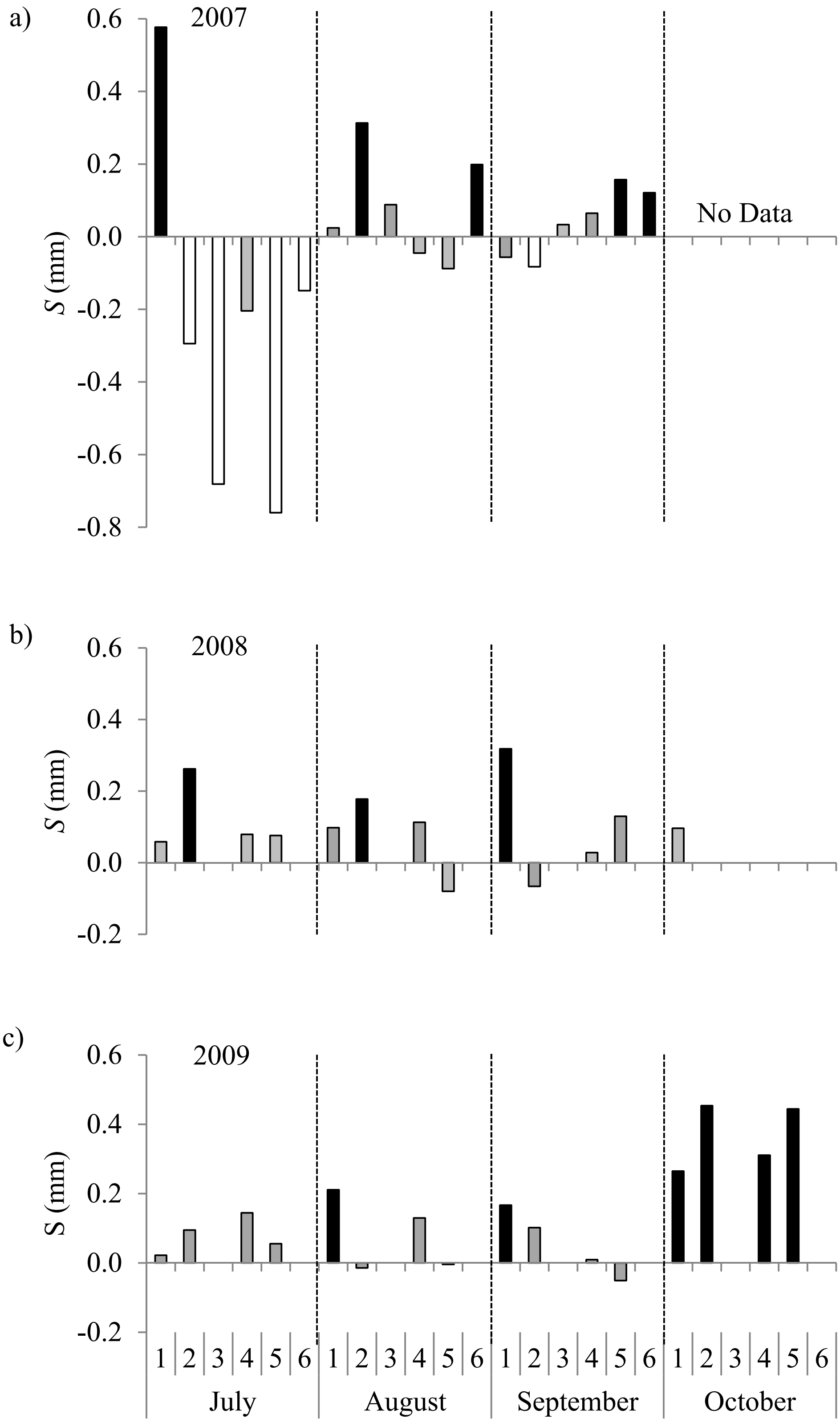


Figure 4.2. Net selection on *Bythotrephes* distal spine length as illustrated by the change in average distal spine length pre-selection (first instar individuals) and post-selection (second instar individuals) across all years and sites (net selection differential: *S* = 0.08mm). Error bars are standard errors.

Figure 4.3. Selection differentials (*S*) on *Bythotrephes* distal spine length for multiple sites along an 8.0km transect in Lake Michigan during: a) 2007 (six sites: July-September); b) 2008 (four sites: July-September; 1 site: October); and c) 2009 (four sites: July-October). Note the different y-axis in panel (a). Black bars: statistically significant ($p < 0.05$) selection differentials for increased distal spine length; white bars: statistically significant ($p < 0.05$) selection differentials for decreased distal spine length; gray bars: non-statistically significant selection differentials.

Figure 4.3.


a)
2007
No Data
b)
2008
c)
2009
S (mm)
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
1 2 3 4 5 6
July
August
September
October

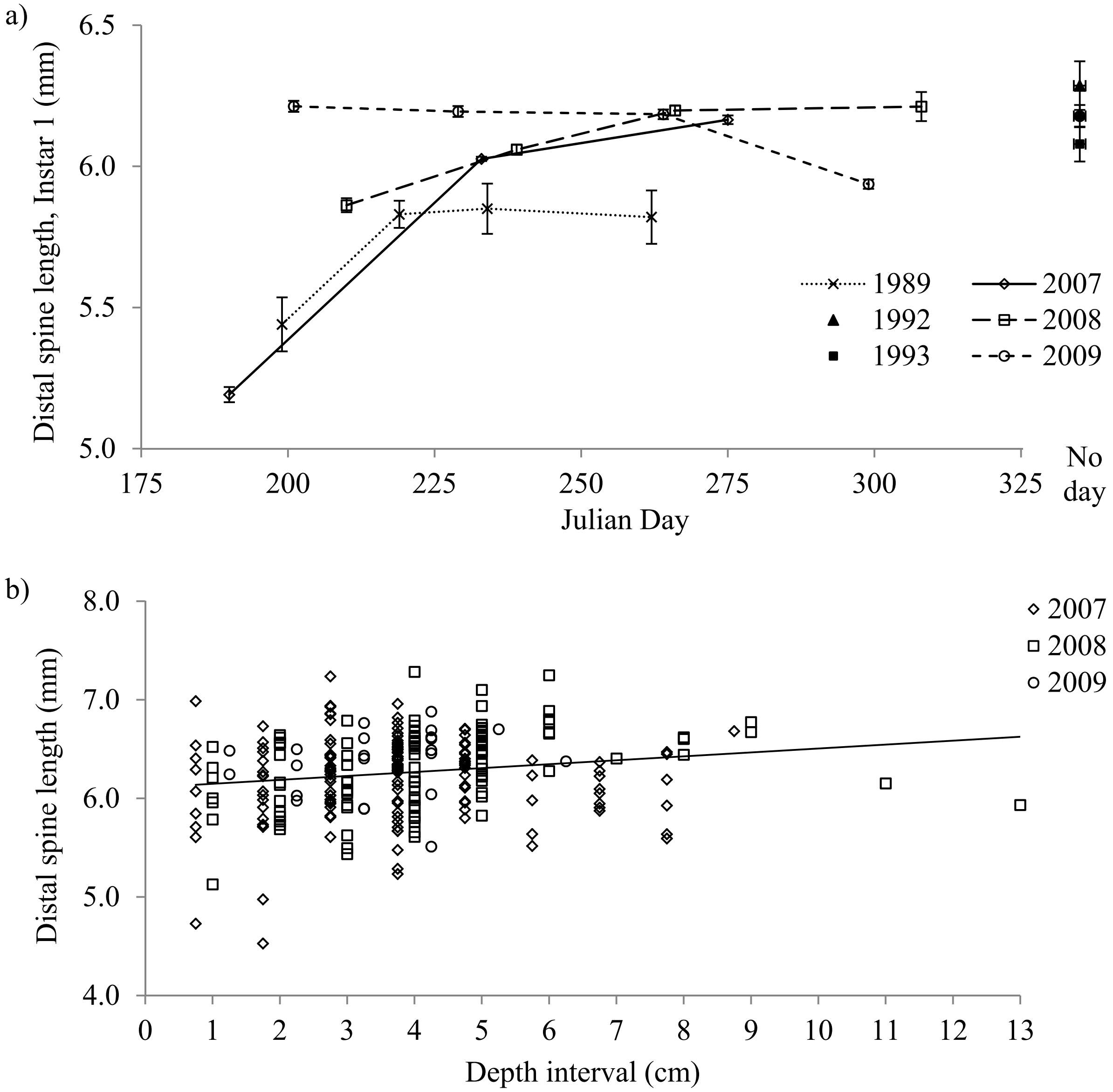


Figure 4.4. Distal spine length of: a) first instar wild-captured *Bythotrephes* from this study (2007-2009) and previously published studies (1989: Burkhardt 1994; 1992-1993, 1996: Sullivan and Lehman 1998); and b) retrieved spines collected from sediment cores (2007-2009); note that the 2007 and 2009 sediment core data are shown offset from the depth interval for clarity. The solid line in panel (b) is the linear regression across all three years (adjusted $R^2 = 0.04$).

APPENDICES

Appendices

Appendix A. Selection differentials and intensities by time period and site

Table 4.2. Natural selection on distal spine length of *Bythotrephes longimanus* between the first two developmental (instar) stages in Lake Michigan for multiple sites in an 8.0km transect from 2007-2009. Ave: average distal spine length (mm); *SD*: standard deviation; *N*: sample size; *SE*: standard error; *S*: selection differential (mm); SD_p: propagated standard deviation (calculated as the square root of the sum of the squared standard deviation of distal spine length for each instar); *i*: selection intensity (calculated as the selection differential divided by the propagated standard deviation); *p*: *p*-value (*$p < 0.05$, **$p < 0.01$; ***$p < 0.001$).

		Instar 1 length				**Instar 2 length**				**Natural selection**			
Month	**Site**	**Ave**	***SD***	***N***	***SE***	**Ave**	***SD***	***N***	***SE***	***S***	SD_p	***i***	***p***
2007													
Jul	1	5.23	0.54	36	0.09	5.81	0.79	71	0.09	0.58	0.95	0.60	***
	2	4.91	0.34	12	0.10	4.62	0.40	50	0.06	-0.29	0.53	-0.55	*
	3	5.56	0.47	36	0.08	4.87	0.44	45	0.07	-0.68	0.65	-1.05	***
	4	4.93	0.34	13	0.09	4.72	0.45	92	0.05	-0.20	0.56	-0.36	NS
	5	5.44	0.78	32	0.14	4.68	0.42	139	0.04	-0.76	0.89	-0.86	***
	6	4.92	0.40	49	0.06	4.77	0.45	160	0.04	-0.15	0.60	-0.25	*
Aug	1	6.04	0.34	62	0.04	6.07	0.28	63	0.04	0.02	0.44	0.05	NS
	2	5.85	0.34	103	0.03	6.16	0.32	146	0.03	0.31	0.47	0.67	***
	3	5.96	0.35	134	0.03	6.04	0.34	63	0.04	0.09	0.49	0.18	NS
	4	6.01	0.33	163	0.03	5.97	0.27	119	0.02	-0.05	0.43	-0.11	NS
	5	6.24	0.40	71	0.05	6.15	0.29	99	0.03	-0.09	0.49	-0.18	NS
	6	5.95	0.35	66	0.04	6.15	0.37	267	0.02	0.20	0.51	0.39	***
Sep	1	6.39	0.41	114	0.04	6.33	0.39	182	0.03	-0.06	0.56	-0.10	NS
	2	6.33	0.36	136	0.03	6.25	0.34	188	0.02	-0.08	0.49	-0.17	*
	3	6.14	0.35	114	0.03	6.18	0.35	203	0.02	0.03	0.50	0.07	NS
	4	6.15	0.38	91	0.04	6.22	0.37	265	0.02	0.06	0.53	0.12	NS
	5	5.98	0.35	112	0.03	6.14	0.35	239	0.02	0.16	0.50	0.32	***
	6	5.95	0.37	114	0.04	6.07	0.34	222	0.02	0.12	0.51	0.24	**
2008													
Jul	1	5.76	0.45	48	0.06	5.81	0.59	56	0.08	0.06	0.74	0.08	NS
	2	5.58	0.54	49	0.08	5.85	0.55	48	0.08	0.26	0.77	0.34	*
	4	5.83	0.45	51	0.06	5.91	0.66	45	0.10	0.08	0.80	0.10	NS
	5	5.81	0.49	50	0.07	5.89	0.55	46	0.08	0.08	0.74	0.10	NS

Table 4.2 (con't)

		Instar 1 length				Instar 2 length				Natural selection			
Month	**Site**	**Ave**	***SD***	***N***	***SE***	**Ave**	***SD***	***N***	***SE***	***S***	SD_p	***i***	***p***
Aug	1	6.00	0.35	50	0.05	6.10	0.29	46	0.04	0.10	0.45	0.22	NS
	2	6.03	0.44	50	0.06	6.21	0.32	40	0.05	0.18	0.54	0.33	*
	4	6.03	0.32	51	0.05	6.15	0.29	48	0.04	0.11	0.43	0.26	NS
	5	6.16	0.32	49	0.05	6.08	0.33	43	0.05	-0.08	0.46	-0.17	NS
Sep	1	6.11	0.28	50	0.04	6.42	0.30	45	0.04	0.32	0.41	0.77	***
	2	6.28	0.38	50	0.05	6.22	0.33	49	0.05	-0.07	0.50	-0.13	NS
	4	6.12	0.39	50	0.05	6.14	0.28	47	0.04	0.03	0.48	0.06	NS
	5	6.16	0.31	50	0.04	6.29	0.37	49	0.05	0.13	0.48	0.27	NS
Oct	1	6.21	0.34	44	0.05	6.31	0.39	50	0.06	0.10	0.52	0.19	NS
2009													
Jul	1	6.23	0.36	21	0.08	6.25	0.25	45	0.04	0.02	0.44	0.05	NS
	2	6.15	0.42	50	0.06	6.25	0.31	48	0.04	0.09	0.52	0.18	NS
	4	6.07	0.45	50	0.06	6.21	0.36	49	0.05	0.14	0.58	0.25	NS
	5	6.27	0.37	50	0.05	6.32	0.37	50	0.05	0.06	0.53	0.11	NS
Aug	1	6.14	0.39	49	0.06	6.35	0.28	47	0.04	0.21	0.48	0.44	**
	2	6.20	0.31	47	0.04	6.19	0.25	49	0.04	-0.01	0.39	-0.04	NS
	4	6.27	0.34	48	0.05	6.40	0.35	49	0.05	0.13	0.49	0.27	NS
	5	6.30	0.44	51	0.06	6.30	0.26	50	0.04	0.00	0.51	-0.01	NS
Sep	1	6.20	0.32	49	0.05	6.37	0.37	46	0.05	0.17	0.49	0.34	*
	2	6.12	0.30	52	0.04	6.22	0.24	49	0.03	0.10	0.39	0.26	NS
	4	6.28	0.35	48	0.05	6.29	0.35	51	0.05	0.01	0.49	0.02	NS
	5	6.39	0.29	53	0.04	6.34	0.30	49	0.04	-0.05	0.42	-0.12	NS
Oct	1	6.01	0.33	53	0.05	6.28	0.23	45	0.03	0.26	0.40	0.66	***
	2	5.96	0.29	50	0.04	6.41	0.32	47	0.05	0.45	0.44	1.04	***
	4	5.92	0.33	47	0.05	6.23	0.37	48	0.05	0.31	0.50	0.62	***
	5	5.85	0.34	54	0.05	6.29	0.30	50	0.04	0.44	0.45	0.98	***

Appendix B. Correlation between distal spine and body length of *Bythotrephes longimanus*.

Many phenotypic correlations result from underlying genetic correlations (Conner and Hartl 2004), which may constrain evolution, even given substantial genetic variation in individual traits (Walsh and Blows 2009; Kingsolver and Diamond 2011). Previous studies (Sullivan and Lehman 1998; Branstrator 2005) have shown a positive phenotypic correlation between distal spine length and body size of *Bythotrephes*. If this phenotypic correlation has an underlying genetic basis, then evolution of distal spine length may be constrained given countering selective forces on body length. We, therefore, tested for the presence of a correlation between distal spine and body length using first and second instar *Bythotrephes* collected from Lake Michigan during 2007-2009 (see main text for details on sample collection).

We measured distal spine length from the tip of the spine to the anterior end of the first set of instar barbs and body length from the anterior edge of the eye to the base of the tail spine along the midline of the body with segments spanning the head and eye region, thorax, and abdomen (Figure 4.1; Miehls et al. 2012). We tested for a correlation between distal spine and body length using a linear mixed effects model (LME) with the nlme package (Pinheiro et al. 2009) in R version 2.15.0 (R Development Core Team 2012). The model included distal spine length as a continuous response, body length (continuous), instar (categorical for instars one and two), and their interaction as fixed effects, and month (July-October) nested within year (2007-2009) as random effects. Note that this random effects structure tested for a correlation between distal spine and body length within a time period, as opposed to among time periods. We also investigated correlations for each instar separately using linear models of distal spine length (continuous response) predicted by body length (continuous fixed effect).

Distal spine length was positively correlated with body length (slope = 0.40, $t_{14,057}$ = 46.2, $p < 0.001$) across both instars, but there was a significant interaction between body length and instar ($F_{1,\ 14,056}$ = 13.9, $p < 0.001$). Considering each instar separately, first instar individuals exhibited a stronger correlation between distal spine and body length (slope = 0.48, t_{6300} = 31.7, $p < 0.001$) than second instar individuals (slope = 0.33, t_{7746} = 31.6, $p < 0.001$). Given the presence of a phenotypic correlation between distal spine and body length for first and second instar individuals, it is possible a genetic correlation might also be present which could constrain evolution of distal spine length.

Appendix B Figures

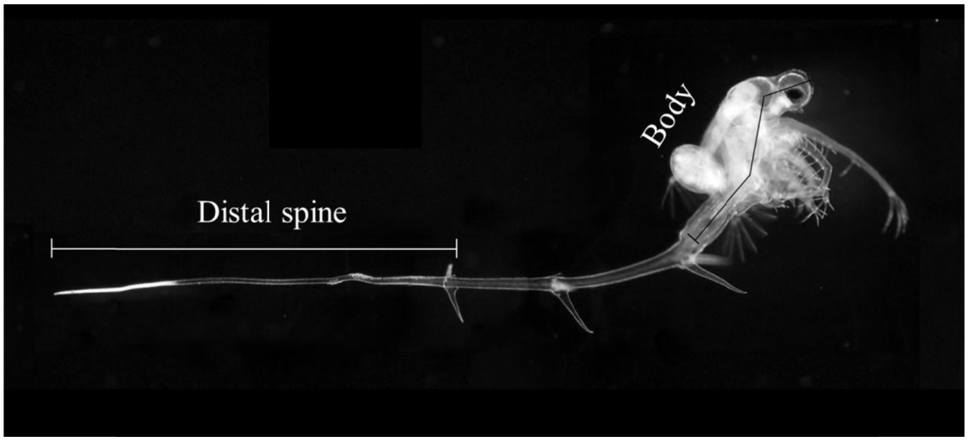


Figure 4.5. Morphological measurements used to test for a correction between distal spine and body length of *Bythotrephes longimanus*. The individual pictured is a third instar.

Appendix C. Correlation between spine segments of *Bythotrephes longimanus*.

Variable selection by life stage may constrain an evolutionary response to selection (Schluter et al. 1991). One way in which this constraint may manifest is when a trait that is strongly associated with an aspect of fitness in an early developmental stage trades-off with another trait that is associated with fitness later in development (Schluter et al. 1991). *Bythotrephes longimanus* are born with a long spine (the distal spine; Figure 4.1), which they retain through instar development at its original length (Burkhardt 1994; Sullivan and Lehman 1998). Although distal spine length does not change during development, total spine length increases with the addition of two intercalary segments (S2 and S3; Figure 4.1) during the first two molts (Burkhardt 1994; Sullivan and Lehman 1998). Owing to its long length and presence at birth, the distal spine is considered to be the spine segment providing the greatest protection from gape-limited fish predation (Branstrator 2005); the additional intercalary segments might also function as defenses against gape-limited fish predation, although their adaptive value is less clear (Sullivan and Lehman 1998). We investigated whether distal spine length (a trait conferring protection early in development) exhibits a trade-off with segment two length (a trait conferring protection later in development) for second instar *Bythotrephes* collected from Lake Michigan during 2007-2009 (see main text for details on sample collection).

We measured distal spine length from the tip of the spine to the anterior end of the first set of instar barbs; we measured segment two length from the anterior end of the first set of instar barbs to the anterior end of the second set of instar barbs (Figure 4.1; Miehls et al. 2012). We tested for a correlation between distal spine length and segment two length using a linear mixed effects model (LME) with the nlme package (Pinheiro et al. 2009) in R version 2.15.0 (R Development Core Team 2012). The model included distal spine length as a continuous response, segment two length as a continuous fixed effect, and month (July-October) nested within year (2007-2009) as random effects. Note that this random effects structure tested for a correlation between distal spine and segment two length within a time period, as opposed to among time periods. A negative correlation between distal spine and segment two length would suggest a trade-off between these two traits.

We found that distal spine length was positively correlated with segment two length (slope = 0.65, $t_{7,765} = 27.1$, $p < 0.001$). The positive correlation indicates that protection from predation early in development (i.e., distal spine length) does not trade-off with protection from predation later in development (i.e., segment two length), indicating little likelihood for a constraint on evolution owing to varying associations of these traits with fitness by life stage.

Appendix C Figures

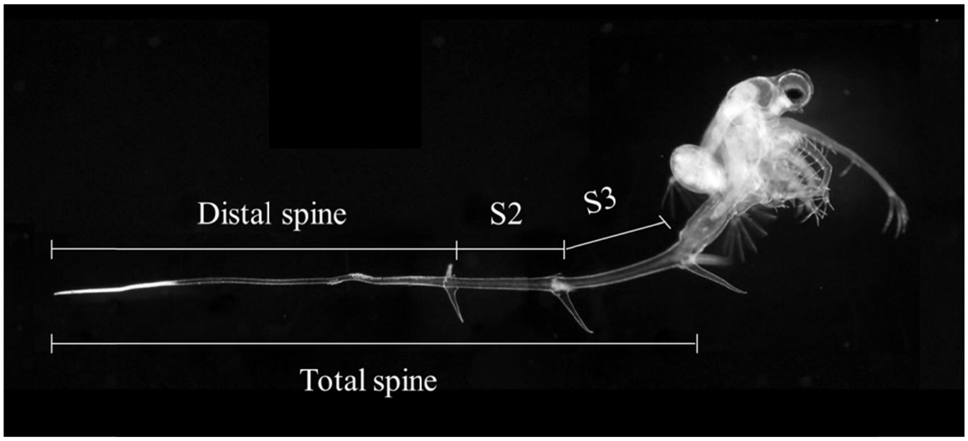


Figure 4.6. Spine morphology of *Bythotrephes longimanus*. The distal spine is present at birth, whereas the second (S2) and third (S3) spine segments are added with the first and second instar molts, respectively. We tested for a trade-off between distal spine and segment two length using the morphological measurements indicated below. The individual pictured is a third instar.

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CHAPTER 5

Gape-limited predators as agents of selection on the defensive morphology of an invasive species

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Abstract

Evolution of invasive species is causing pronounced effects on ecosystems, yet we still have limited knowledge of the agents of natural selection on invasive species and the rate of adaptive evolution in invasive species traits. *Bythotrephes longimanus*, called the spiny water flea because of its conspicuous distal spine that serves as a morphological defense against gape limited (GLP) fish predation, is an invasive predatory zooplankton in North America that invaded multiple lakes in the Canadian Shield over the past several decades. We estimated natural selection on distal spine length of *Bythotrephes* collected from six replicate lakes that varied in the dominance of gape-limited (GLP) and non-gape-limited (NGLP) fish predation. Using a between cohort analysis, we found that natural selection varied by gape-limitation, with strong selection (selection intensity: 0.20-0.79) for increased distal spine length in lakes dominated by GLP, and no significant selection in lakes dominated by NGLP. Further, distal spine length was 20% longer in lakes dominated by GLP, suggesting the possibility of local adaptation in response to selection. As all study lakes were invaded less than twenty years prior to our collections, these results suggest rapid divergence in a defensive morphological phenotype

Introduction

Invasive species are one of the world’s greatest threats to biodiversity and ecosystem function, and there is mounting evidence that evolution plays a key role in their pronounced ecological effects (Mooney and Cleland 2001; Lambrinos 2004). Invasive species may evolve upon introduction into new environments due to novel selection pressures, population bottlenecks, founder effects, and hybridization in the invaded range, as well as freedom from selection pressures in the native range (Lee 2002; Lambrinos 2004). Evolution via population bottlenecks, founder effects, and hybridization is often rapid, frequently occurring within a few years or decades of introduction (Lee 2002; Lambrinos 2004; Prentis et al. 2008). Comparatively less is known about the pace of evolution by natural selection in invasive species (especially invasive animals), although preliminary work indicates the pace is variable, involving years, decades, or even centuries (Reznick and Ghalambor 2001; Kristjánsson et al. 2002; Lee 2002).

In addition to limited knowledge of the pace of evolution by natural selection in invasive species, we more generally often lack explicit knowledge of the agents of selection on traits of prey in the wild. Presumably predators are important agents of selection (Darwin 1859), but our knowledge of predators as agents of selection in the wild is relatively limited. The work that is available suggests one of the primary determinants of the direction of selection exerted by predators is the size of prey relative to the mouth (i.e., gape) size of the predator. Many predators cannot capture prey above a certain gape-size threshold (Wilson 1975; Paine 1976; Hambright 1991), which allows predators to be functionally categorized as either gape-limited (i.e., predator is constrained by mouth size from consuming larger size classes of prey) or non-gape-limited (i.e., predator is unconstrained by mouth size and can consume any size class of prey) with respect to any particular prey species (Urban 2007; 2008). Gape-limited predation (GLP) typically selects for increased morphological size of prey (e.g., Paine 1976; Day et al. 2002; Urban 2008), whereas non-gape-limited predation (NGLP) may yield no selection on morphological size (Scharf et al. 2000; Urban 2007; 2008) or select for decreased morphological size if larger individuals are preferentially consumed (e.g., Hambright 1991; Urban 2007; 2008). There is evidence to suggest many prey have evolved to combat GLP through morphological defenses that provide a size refuge (e.g., Tollrian 1995; Young et al. 2004; Lescak and von Hippel 2011). Conversely, prey experiencing NGLP are often small, lack defenses, or exhibit reduced morphological defense compared to conspecifics experiencing GLP (Gross 1978; Kristjánsson et al. 2002). Thus, we have reasonable expectations for the direction of selection based on gape-limitation of potential predators, but we rarely have adequate data to test these predictions.

Additionally, we know particularly little about selection and adaptive evolution of invasive prey in response to predation. One of the best-studied systems documenting evolution of an invasive prey is the threespine stickleback (*Gasterosteus aculeatus*). Threespine sticklebacks evolved reduced defenses (number of armor plates, dorsal spines) after invading freshwater habitats where predation pressure was reduced (Kristjánsson et al. 2002). Of course, this example is not an instance of evolution in response to selection from predators, *per se*, but rather evolution resulting from the relaxation of predation (i.e., reverse evolution; Porter and Crandall 2003). Examples of invasive prey evolving due to direct selection from predators are limited, although experimental introductions of species into habitats with high predation indicate evolution can occur (Reznick et al. 1990).

Recent species introductions into multiple lakes of the Canadian Shield in Ontario, Canada, offer an opportunity to investigate predators as agents of selection in the wild and the potential for adaptation of invasive species to predation. *Bythotrephes longimanus* (the spiny water flea; hereafter *Bythotrephes*; Figure 5.1a) are cladoceran zooplankton native to Eurasia that were first found in the North American Great Lakes in the early 1980s (Mills et al. 1993). Within the following two decades, *Bythotrephes* spread through all of the Great Lakes and invaded more than 90 surrounding lakes, including many Canadian Shield lakes (Strecker et al. 2006). *Bythotrephes* are a dominant predator of zooplankton (Yan et al. 2002; Vanderploeg et al. 2012) and may have widespread negative effects on food webs through trophic cascades (Hoffman et al. 2001; Strecker and Arnott 2008). *Bythotrephes* are also a common prey for fish (Pothoven et al. 2007), which might serve as a source of size-selective predation on *Bythotrephes* traits.

The *Bythotrephes* invasion in multiple lakes of differing GLP fish risk provides a natural experiment allowing for the measurement of selection from GLP and NGLP predators in the wild. Previous research suggests that *Bythotrephes* defensive distal spine (Figure 5.1a) experiences selection for increased length (Sullivan and Lehman 1998), but the agents of selection are unknown. Diet analysis of wild-caught fish (Schneeberger 1991; Barnhisel and Harvey 1995) and evidence for selective feeding of GLP fish on *Bythotrephes* based on distal spine length (Barnhisel 1991a; 1991b) suggest GLP fish may act as selective agents on *Bythotrephes* distal spine length, but natural selection on this important trait has not yet been measured. We, therefore, measured natural selection on *Bythotrephes* distal spine length in six lakes dominated by either GLP or NGLP fish. We predicted that there would be selection for increased distal spine length in lakes dominated by GLP fish, but no selection on distal spine length in lakes dominated by NGLP fish.

Methods

Methodological Overview

We collected *Bythotrephes* in three lakes dominated by a GLP (rainbow smelt, *Osmerus mordax*) and three lakes dominated by a NGLP (cisco, *Coregonus artedi*) during June 2008. We measured natural selection on distal spine length occurring between two cohorts and within a single cohort (Figure 5.1b) using two closely spaced *Bythotrephes* collections (within one week). Viability selection was estimated through a comparison of distal spine length between the first two developmental (instar) stages.

Morphology and Life History of Bythotrephes

The longest section of the *Bythotrephes* tail spine is the distal spine, which extends from the tip of the spine to the first pair of instar barbs (Figure 5.1a). The distal spine is present at birth and does not change with instar development, although other traits, such as body size, increase with each instar molt (Sullivan and Lehman 1998). Instead, during the first two molts, total spine length increases through the addition of intercalary segments separated by paired barbs (Branstrator 2005). *Bythotrephes* reproduce by cyclic parthenogenesis, with multiple asexual generations (up to ten or more) during the growing season culminating in sexual reproduction at the end of the season (Yurista 1992; Pothoven et al. 2001). *Bythotrephes* typically reproduce at either the second or third instar stage (Pothoven et al. 2001); each instar lasts approximately 3-5 days, depending on water temperature (Branstrator 2005).

Study Lakes

We sampled *Bythotrephes* from six lakes in the districts of Muskoka and Haliburton in south-central Ontario, Canada that were recently (less than twenty years prior to this study) invaded by *Bythotrephes* (Table 5.1). In three of these lakes (Fairy, Mary, Peninsula; hereafter collectively referred to as GLP lakes) predation on *Bythotrephes* is dominated (in terms of predator abundance) by rainbow smelt (*Osmerus mordax*), which are GLP of *Bythotrephes* as juveniles (Barnhisel and Harvey 1995), but exhibit little predation on *Bythotrephes* as adults (Young and Yan 2008). In the other three lakes (Harp, Boshkung, and Kashagawigamog; hereafter collectively referred to as NGLP lakes) predation on *Bythotrephes* is dominated by cisco (*Coregonus artedi*), which are important NGLP of *Bythotrephes* (Young and Yan 2008). Although smelt are also present in two of the NGLP lakes (Boshkung: Young and Yan 2008; Kashagawigamog: Strecker et al. 2006), the abundance of cisco in these lakes is greater than that of smelt, and cisco are considered to be the dominant predator of *Bythotrephes* (Table 5.1; Strecker et al. 2006; S.J. Sandstrom and N. Lester, Ontario Ministry of Natural Resources, unpublished data). The physical and biological characteristics of the six lakes are otherwise similar (Strecker et al. 2006; Young and Yan 2008).

Field Collection of Bythotrephes

We collected *Bythotrephes* (Ontario Ministry of Natural Resources License to Collect Fish for Scientific Purposes No. 1046358) during June 23-28, 2008 (Table 5.1), a time period during the middle of the *Bythotrephes* growing season. We completed two collections from each lake separated by three days (hereafter, paired trips), with the exception of Kashagawigamog, for which we only completed the first collection due to low *Bythotrephes* abundance. We used a conical zooplankton net with a 0.5m diameter opening and 363μm mesh size towed horizontally in 1m steps through the epilimnion and metalimnion. All *Bythotrephes* collected were immediately preserved in 95% ethanol. Water temperature 1m below the water surface was collected during each sampling trip. We also obtained additional temperature data for Harp Lake for the entire 2008 growing season (Figure 5.1c; A. Paterson, Dorset Environmental Science Centre, Ontario Ministry of the Environment, Dorset, ON, Canada, unpublished data).

We measured *Bythotrephes* distal spine length (Figure 5.1a) using ImageJ (Abramoff et al. 2004) to the nearest 0.001mm from photographs taken with a digital camera attached to a dissecting microscope. Distal spine length was measured from the tip of the spine to the anterior end of the first pair of instar barbs (Figure 5.1a; Miehls et al. 2012). Although some organisms are known to decrease in length after preservation in ethanol (e.g., larval fish; Moku et al. 2004), a previous test (Miehls et al. 2012) of *Bythotrephes* preserved in 95% ethanol revealed no statistically significant shrinkage of *Bythotrephes* distal spine length.

Statistical Analysis: Natural Selection between Cohorts

We estimated natural selection on *Bythotrephes* distal spine length through a comparison of distal spine length between the first two instar stages collected on the same trip (Figure 5.1b); note that this method for estimating natural selection involves a comparison between different cohorts of *Bythotrephes*, as individuals from different instar stages collected on the same trip would have been born during different time periods. Because distal spine length does not change with instar development, different distal spine length between the first and second instar stages reflects a difference in survival as opposed to a developmentally based difference in length (Sullivan and Lehman 1998). Instar stage was determined by counting the number of paired barbs on the spine, which reliably indicates instar stage for the first two instars (Yurista 1992; Sullivan and Lehman 1998). Although *Bythotrephes* pass through up to three or more instar stages (Yurista 1992), the comparison between first and second instars was chosen because survival through this early life stage likely comprises a large proportion of total fitness and because the distal segment for first instar individuals represents the entire spine length, which we suspected was the target of GLP.

We statistically tested whether selection on distal spine length differed by lakes dominated by GLP versus NGLP with a linear mixed effects model (LME) using the nlme package (Pinheiro et al. 2009) in R version 2.15.0 (R Development Core Team 2012). The LME model included distal spine length as a continuous response, instar (categorical for instars one and two), fish predator (categorical for GLP and NGLP), trip (categorical for trip one and two), and instar-by-fish predator and fish predator-by-trip interactions as fixed effects, and lake (six replicate lakes) as a random effect. In this model, a statistically significant effect of instar would indicate significant selection on distal spine length irrespective of fish predator. A significant interaction between instar and fish predator (GLP vs. NGLP) would indicate that selection differed by fish predator, which was the primary result of interest in our study. A significant interaction between fish predator and trip would indicate *Bythotrephes* distal spine length in lakes of each fish predator type varied by trip, a result which would suggest the effect of fish predator may be confounded by phenotypic plasticity (see Natural Selection within a Cohort).

We estimated selection differentials (Falconer and Mackay 1996) for distal spine length as the difference in mean distal spine length between first and second instars within each lake. To compare our estimates of selection to other published studies (Kingsolver et al. 2001; Kingsolver and Diamond 2011), we calculated standardized selection differentials (i.e., selection intensities) by dividing the selection differential for each lake by the pooled standard deviation of distal spine length within the lake. The statistical significance of our selection differentials was determined using linear models that included distal spine length as a continuous response and instar as a fixed effect for every lake. In these models, a statistically significant effect of instar would indicate significant selection on distal spine length.

Statistical Analysis: Natural Selection within a Cohort

In addition to the above method for estimating natural selection between cohorts, our paired trip design allowed for estimation of natural selection within a cohort (Figure 5.1b), providing a control for the potentially confounding effects of phenotypic plasticity (Pigliucci 2001). Previous work (Chapter 3) indicated that *Bythotrephes* mothers produce offspring with longer distal spines at higher temperature. If first and second instar *Bythotrephes* collected on the same sampling trip experienced different water temperatures during development, estimates of selection could be confounded by temperature-induced plasticity in distal spine length. Comparing *Bythotrephes* between sampling trips (i.e. comparing first instar individuals collected on trip 1 to second instar individuals collected on trip 2) allowed us to control for these potential confounding effects of plasticity by tracking a single cohort through time. We assumed that first instar individuals from trip one and second instar individuals from trip two were members of the same cohort because our trips were separated by three days, which is the average instar development time of *Bythotrephes* at 21°C (Branstrator 2005). This water temperature is similar to the average water temperature in our sampled lakes at the time of collection (Table 5.1). As a result, most *Bythotrephes* in their first instar at the time of the first trip should have molted to the second instar by the time of the second trip.

We statistically tested whether selection on distal spine length within a cohort differed between lakes dominated by GLP versus NGLP using a LME model with distal spine length as the response, instar, fish predator, and an instar-by-fish predator interaction as fixed effects, and lake as a random effect. This analysis included only first instar individuals collected during trip one and second instar individuals collected during trip two. In this analysis, the inclusion of a fixed effect for trip was not necessary and would have been redundant with the instar effect. As in the LME above, the interaction between instar and fish predator was the primary result of interest, as this interaction examines whether selection differed between GLP and NGLP lakes. We also calculated selection differentials and selection intensities for each lake separately except Kashagawigamog (for which we only had the single collection on trip one, prohibiting a within cohort analysis). The statistical significance of our selection differentials was determined using linear models identical to the single-lake models above. As above, a significant effect of instar indicated significant selection on distal spine length.

Results

Average Distal Spine Length

Average distal spine length of first instar individuals (i.e., pre-selection phenotype) was 20% longer in the GLP lakes (average length ± 1 SD: GLP lakes: 5.94 ± 0.48mm; NGLP lakes: 4.97 ± 0.41mm; $t_4 = 5.20$, $p < 0.01$) and exhibited less among-lake variation than the NGLP lakes (Figure 5.2a). There was no overlap in average length between the GLP and NGLP lakes. That is, the GLP lake with the shortest average distal spine length (Mary) exhibited longer mean length than the NGLP lake with the longest average distal spine length, Boshkung (Figure 5.2a).

Natural Selection between Cohorts

Natural selection on *Bythotrephes* distal spine length differed significantly between GLP and NGLP lakes (i.e., significant instar-by-fish predator interaction: Table 5.2), and lake predator types did not differ in the effect of trip (i.e., non-significant fish predator-by-trip interaction: Table 5.2). There was significant selection for increased distal spine length in all GLP lakes (range of selection intensities: 0.20-0.79) and no significant selection in NGLP lakes (Table 5.3; Figure 5.2b). Within the GLP lakes, selection differentials were smallest in Peninsula and greatest in Mary (Table 5.3). Although not significantly different from zero, the strength of selection on distal spine length in one NGLP lake, Harp, was greater than the strength of selection in one GLP lake, Peninsula (Table 5.3). Note that the data for Kashagawigamog should be interpreted with caution as the pre- and post-selection sample sizes were only eight and four individuals, respectively, which we consider too low to provide reliable estimates of selection (Hersch and Phillips 2004).

Natural Selection within a Cohort

The within-cohort analysis for individual lakes corroborated the between-cohort analysis. We found significant selection for increased distal spine length in all GLP lakes (range of selection intensities: 0.32-0.93) and no significant selection in NGLP lakes (Table 5.3, Figure 5.2c). The selection differentials were again smallest in Peninsula and greatest in Mary, and one NGLP lake (Boshkung) had a non-significant selection differential higher than that of one GLP lake (Peninsula). These selection differentials, however, did not significantly differ between GLP and NGLP lakes (i.e., non-significant instar-by-fish predator interaction: Table 5.2), which is likely due to the lower sample sizes for these more restrictive subsets of the data (Table 5.3).

Discussion

Our analysis identified GLP fish as an agent of selection on the defensive morphology of an invasive species. Natural selection on *Bythotrephes* distal spine length was strong compared to other published studies of selection (Kingsolver et al. 2001; Kingsolver and Diamond 2011). A recent review of phenotypic selection measured in natural populations by Kingsolver and

Diamond (2011) found that the median directional selection (estimated as the absolute value of standardized linear selection gradients, $|\beta|$) via survival was 0.08. Even the lowest standardized selection differentials (i.e., selection intensities) on *Bythotrephes* distal spine length in the GLP lakes (range: 0.20-0.79; between cohort analysis: Table 5.1) exceeded the median selection via survival. The highest standardized selection differentials (those greater than 0.75) fell within the top 5% previously reported (Kingsolver et al. 2001). Although Kingsolver and Diamond (2011) reported standardized selection gradients and we reported standardized selection differentials, their review found congruence between standardized selection gradients and differentials.

We observed a few notable differences in selection among lakes within each fish predator type. In Peninsula, where average distal spine length (6.05mm; Figure 5.2a) was the longest among the GLP lakes, we found the weakest selection for increased length, whereas in Mary, where average distal spine length (5.79mm; Figure 5.2a) was the shortest among the GLP lakes, we found the strongest selection for increased length. These results suggest that a threshold distal spine length provides protection from GLP consistent with the concept of “hard” natural selection (Wallace 1975). We found no significant selection in any of the NGLP lakes, but note that in Boshkung, where average distal spine length (5.31mm; Figure 5.2a) was the greatest of all the NGLP lakes, we found a (non-significant) selection differential ($S = 0.13$; Table 5.3) for the within cohort analysis that was greater than the selection differential in Peninsula. Although the primary predators of *Bythotrephes* in Boshkung (cisco) are NGLP, smelt are present at low abundance and could be a source of GLP maintaining longer average distal spine length in this lake as compared to the other NGLP lakes. However, we found a (non-significant) negative selection differential ($S = -0.03$; Table 5.3) in Boshkung for the between cohort analysis, which we feel is the more rigorous of the two analyses based on the greater sample size. Thus, the presence of a GLP in Boshkung may explain the greater average distal spine length in this lake than the other NGLP lakes, although evidence for selection by the GLP is weak.

Although we did not find a significant effect of fish predator on selection in the within cohort analysis, we found a significant effect in the between cohort analysis and, further, all within-lake analyses indicated significant selection on *Bythotrephes* distal spine length by GLP, but not NGLP; thus, we are confident in our conclusion that selection differs by fish gape-limitation. The non-significant instar-by-fish predator interaction in the within cohort analysis (Table 5.2) was likely due to poor sample size in the NGLP lakes (Hersch and Phillips 2004); because we had considerably greater power with which to detect a difference in selection by fish predator in the between cohort analysis (Table 5.2), we regard these results as the more reliable of the two comparisons. Regarding the within-lake estimates, the selection differentials were quantitatively similar for the between and within cohort analyses in all GLP lakes, providing strong support for our conclusion of natural selection due to GLP. Whereas the selection differentials were quantitatively similar for the between and within cohort analyses for one NGLP lake (Harp), there was a qualitative difference in the direction of selection for one NGLP lake (Boshkung). Despite the less consistent results in the NGLP lakes, we found no significant selection in any of the NGLP lakes for the between (where we had strong statistical power) or within cohort analysis, supporting our conclusion of a lack of natural selection with NGLP. Of note, our test of GLP and NGLP as agents of selection was observational (i.e., we assumed the effect of each fish predator type based on their presence in lakes); experimental manipulation of fish communities would be needed to conclusively identify GLP as the agent of selection.

We completed both between and within cohort analyses due to the greater rigor of the latter approach for assessing selection free from the potential confounding effects of phenotypic plasticity (Pigliucci 2001). Whereas the former approach is not as rigorous of a control for phenotypic plasticity, we are confident that the between cohort analysis yielded accurate estimates of selection given three lines of evidence against plasticity. First, in our between cohort analysis, we found no interaction between the effect of fish predator on distal spine length and trip (Table 5.2). There was a significant difference in distal spine length between the first and second collection trip (Table 5.2) indicating that average distal spine length increased between the first and second collection trip. However, the non-significant interaction between fish predator and trip indicates that plastic differences in distal spine length between trips did not differ by fish predator. Therefore, our conclusions regarding selection by the fish predator types (i.e., GLP vs. NGLP) should be robust to overall differences in mean distal spine length between the first and second collection trip. Second, the only identified cue of plasticity in *Bythotrephes* distal spine length, water temperature (Chapter 3), was relatively stable both within one of our study lakes prior to collection (Harp Lake, the only lake for which we have seasonal temperature data; Figure 5.1c) and among our study lakes at the time of collection (Table 5.1). Thus, temperature-induced differences in distal spine length were likely minimal. Third, we (Chapter 3) also tested fish kairomones as a cue of plasticity in *Bythotrephes* distal spine length, but found no effect; thus, plasticity in response to the different fish predators present in our study lakes was unlikely to affect our results. Therefore, our estimates of natural selection were unlikely to be biased by phenotypic plasticity.

The large difference in average distal spine length between lakes dominated by GLP and NGLP is consistent with the direction of selection we observed and suggests rapid local adaptation of distal spine length in response to predation. Average distal spine length was 20% longer (almost 1mm greater in absolute length) in GLP lakes, where we found strong selection for increased distal spine length. Previous work in Lake Michigan (Miehls et al. 2012) indicated that *Bythotrephes* distal spine length is moderately to highly heritable (range of H^2: 0.27-0.76) and concluded that *Bythotrephes* could rapidly respond to selection, especially given the multiple asexual generations produced during the growing season (Yurista 1992; Pothoven et al. 2001). Heritability of *Bythotrephes* distal spine length in the lakes that we sampled here is unknown, but the strength of selection that we have documented in GLP lakes is sufficient to account for our observed differences in mean spine length between lake types in only 73 generations assuming a broad-sense heritability of 0.1 and using the average of the GLP selection differentials from the between cohort analysis. Seventy-three generations could correspond to as few as 7 years if *Bythotrephes* in these lakes are capable of completing approximately 10 generations per season, which seems like a reasonable estimate based on a six-month growing season and a time to maturity of 11d at moderate water temperatures (Drake et al. 2006). Indeed, as *Bythotrephes* invaded the three GLP lakes in the early 1990s (almost 20 years prior to this study), sufficient time has passed for local adaptation comparable to observed differences in mean phenotype based on our estimates of strength of contemporary natural selection. Our results are suggestive of local adaptation in response to predation, although additional research (e.g., common garden experiments) is needed to confirm the genetic basis of these phenotypes (see Mooney and Cleland 2001 for a review of local adaptation of invasive species).

In conclusion, our study found strong directional selection on the defensive morphology of an invasive species exerted by gape-limited predators, and no selection by non-gape-limited predators. Previous research identifying predators as agents of selection in the wild is limited, particularly for invasive species. We also found a large difference (20%) in average distal spine length between lakes dominated by GLP and NGLP that was consistent with local adaptation in response to selection over a relatively short time scale (less than twenty years). Thus, our findings indicate predators are capable of exerting strong selection on invasive prey, despite a relatively short interaction history. Our findings are also suggestive that local adaptation of invasive species can be rapid, occurring in as little as a few years or decades, providing additional support that evolution by natural selection in invasive species can occur as quickly as evolution by other well-documented mechanisms (e.g., population bottlenecks, founder effects, and hybridization; Lee 2002; Lambrinos 2004; Prentis et al. 2008). Thus, as we investigate the ongoing ecological effects of invasive species on ecosystems, it is important to view invasive species as dynamic populations capable of rapidly adapting to selective pressures in their invaded ecosystems.

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Tables

Table 5.1. *Bythotrephes* invasion year, categorical fish abundance, and field information for study lakes. Smelt are considered a gape-limited predator, whereas cisco are considered a non-gape-limited predator of *Bythotrephes*. Temperatures were taken 1m below the water surface at the time of *Bythotrephes* collection. A: absent; L: low; M: medium; H: high.

Lake	**Invasion**	**Smelt**	**Cisco**	**Sample Dates**	**Latitude**	**Longitude**	**Depth (m)**	**Temp (°C)**
Fairy	1990[1]	M[3]	A[3]	June 24, 27	45° 19.90' N	79° 10.49' W	38.7	22.4
Mary	1990[1]	M[3]	A[3]	June 25, 28	45° 14.39' N	79° 15.77' W	50.3	20.9
Peninsula	1991[1]	M[3]	A[3]	June 24, 27	45° 20.94' N	79° 6.28' W	32.9	21.8
Boshkung	2003[1]	L[4]	M[4]	June 24, 27	45° 3.06' N	78° 43.76' W	40.8	20.3
Harp	1993[1]	A[3]	H[3]	June 23, 26	45° 22.79' N	79° 7.98' W	30.5	21.3
Kashagawigamog	1993[2]	L/M[3]	M/H[3]	June 25	45° 59.91' N	78° 36.07' W	33.2	20.6

[1] Young and Yan 2008; [2] Muirhead and MacIsaac 2005; [3] Strecker et al. 2006; [4] S.J. Sandstrom and N. Lester, Ontario Ministry of Natural Resources, unpublished data

Table 5.2. Statistical results for the between and within cohort analyses of natural selection on *Bythotrephes* distal spine length. The instar-by-fish interaction assessed whether natural selection (effect of instar) differed by fish predator. Note that the within cohort analysis did not include trip as a fixed effect (see text). Lake (i.e., the six replicate study lakes) was modeled as a random effect; the significance of lake was determined through a likelihood ratio test. *F*: *F*-statistic; *df*: degrees of freedom; *p*: *p*-value; *Var*: among-lake variance; *Ratio*: likelihood ratio.

	Between cohorts			**Within cohort**		
Fixed	***F***	***df***	***p***	***F***	***df***	***p***
Instar	48.86	1, 1899	***	78.09	1, 1012	***
Fish	48.32	1, 4	***	78.81	1, 4	***
Trip	28.08	1, 1899	**	N/A	N/A	N/A
Instar:Fish	9.02	1, 1899	***	0.01	1, 1012	N.S.
Fish:Trip	0.18	1, 1899	N.S.	N/A	N/A	N/A
Random	***Var***	***Ratio***	***p***	***Var***	***Ratio***	***p***
Lake	0.18	98.0	***	0.14	39.1	***

**p ≤ 0.01; ***p ≤ 0.001; N.S.: non-significant; N/A: not applicable

Table 5.3. Natural selection on *Bythotrephes* distal spine length estimated within six replicate study lakes; we found significant selection (i.e., significant effect of instar) in all GLP lakes (Fairy, Mary, Peninsula) and no significant selection in NGLP lakes (Boshkung, Harp, and Kashagawigamog). *t* and *p* values are for the effect of instar on distal spine length. *df*: degrees of freedom; *S*: selection differential (mm); *SD*: pooled standard deviation within a lake; *i*: standardized selection differential (i.e., selection intensity). Note that a second sample collection was not completed in Kashagawigamog, precluding the estimation of selection within a cohort.

	Between cohorts						**Within cohort**					
Lake	***t***	***df***	***p***	***S***	***SD***	***i***	***t***	***df***	***p***	***S***	***SD***	***i***
Fairy	4.8	436	***	0.14	0.26	0.53	4.2	265	***	0.15	0.26	0.58
Mary	6.9	438	***	0.22	0.28	0.79	7.4	227	***	0.28	0.30	0.93
Peninsula	2.8	743	**	0.05	0.23	0.20	3.2	384	**	0.07	0.22	0.32
Boshkung	-0.8	186	N.S.	-0.03	0.27	-0.12	1.1	87	N.S.	0.13	0.28	0.46
Harp	1.5	84	N.S.	0.08	0.25	0.32	0.5	39	N.S.	0.06	0.22	0.26
Kashagawigamog	-1.5	10	N.S.	-0.20	0.24	-0.85	N/A	N/A	N/A	N/A	N/A	N/A

**$p \leq 0.01$; ***$p \leq 0.001$; N.S.: non-significant; N/A: not applicable

Figures

Figure 5.1. We estimated natural selection on: a) distal spine length of *Bythotrephes longimanus*, the spiny water flea (the individual pictured is a third instar); through b) a comparison between first and second instar *Bythotrephes* between cohorts (solid arrows) and within a cohort (dashed arrow) using paired sampling trips. The collection trips were separated by 3d, which was equivalent to the duration of a single instar at the water temperature sampled (~21°C). c) Epilimnion water temperature (°C) in Harp Lake during the 2008 growing season. The open diamond represents the date of first sample collection in Harp Lake.

Figure 5.1.

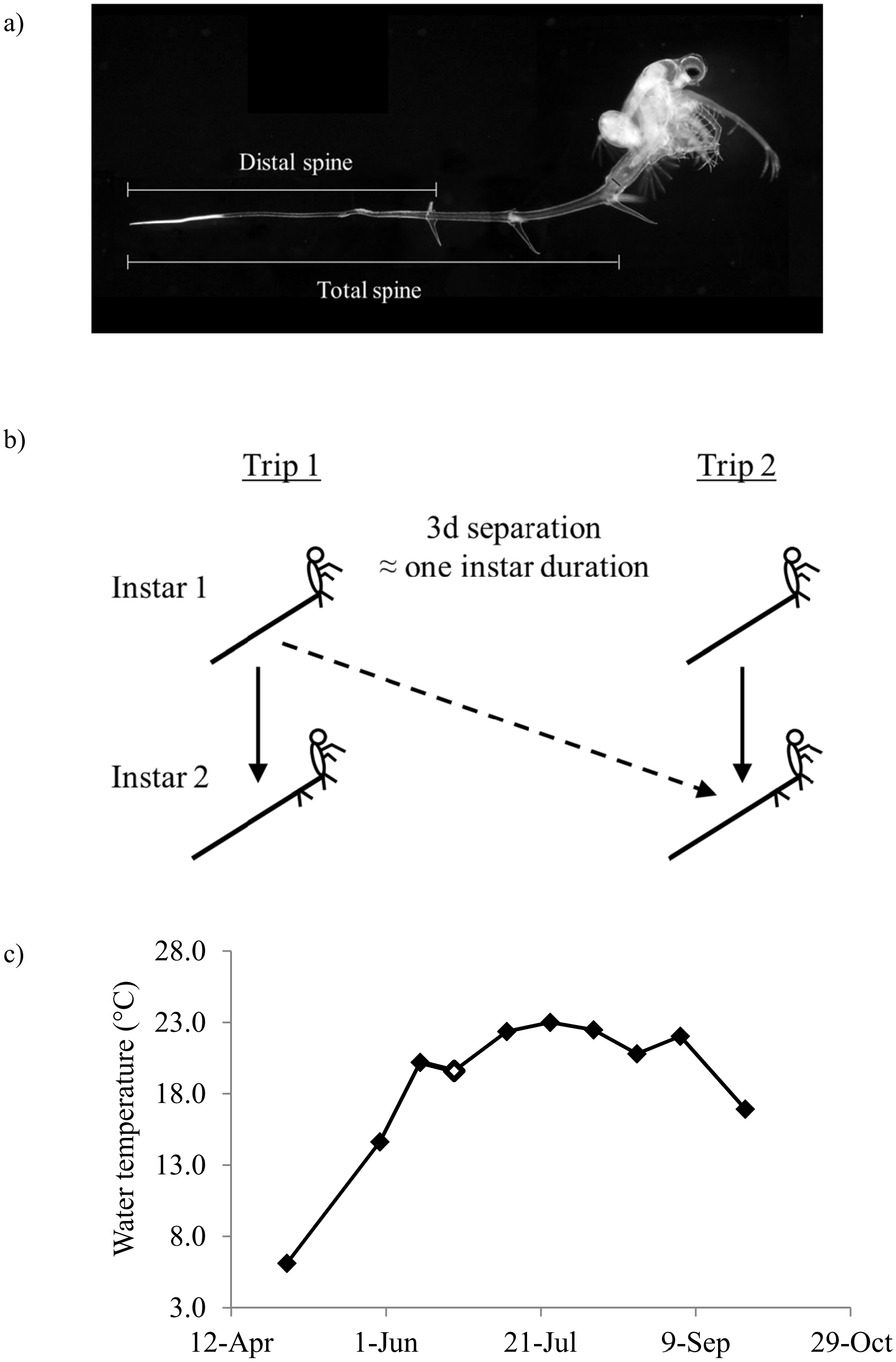
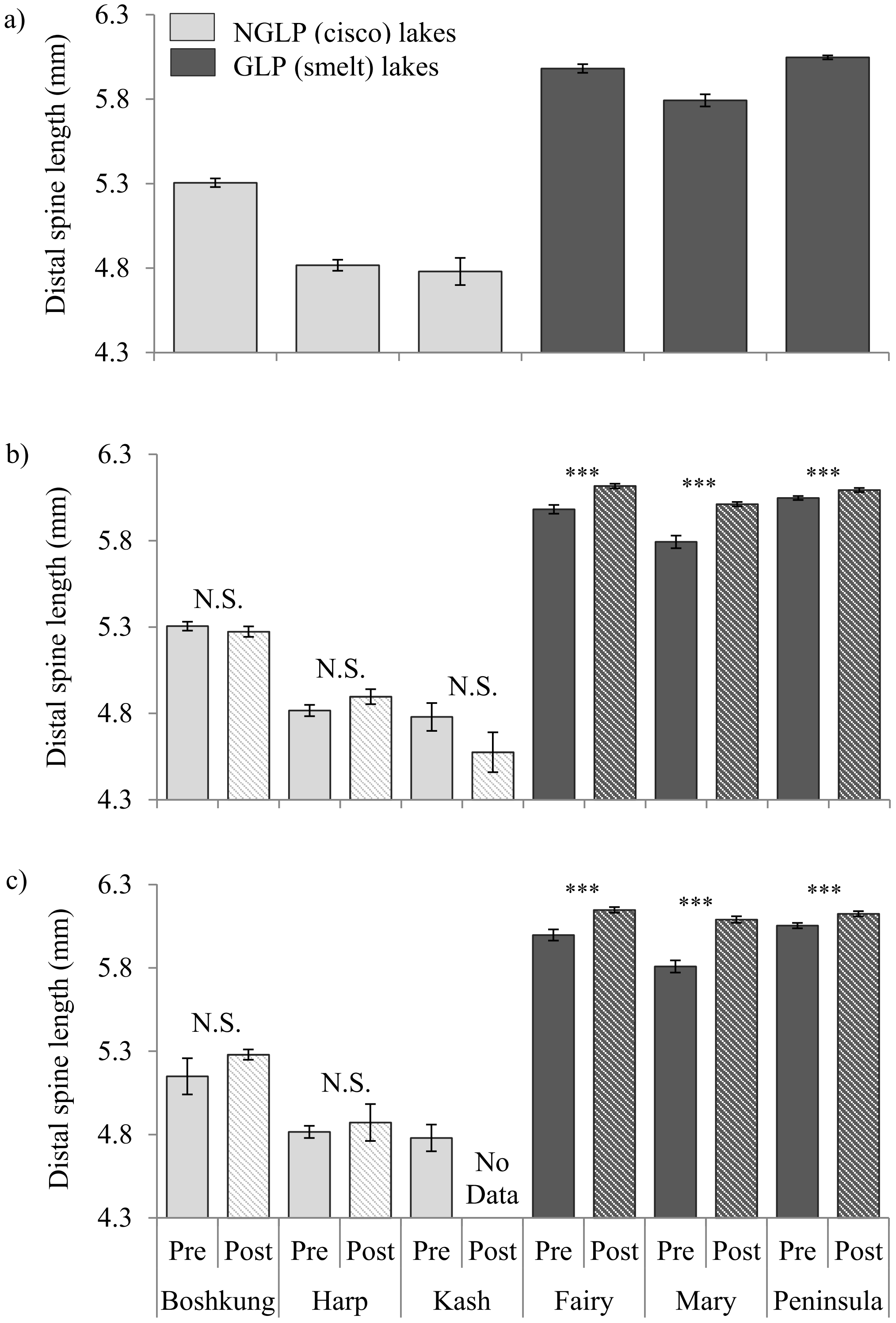

a)
Distal spine
Total spine
b)
Trip 1
Trip 2
3d separation
≈ one instar duration
Instar 1
Instar 2
c)
28.0
23.0
18.0
13.0
8.0
3.0
Water temperature (°C)
12-Apr
1-Jun
21-Jul
9-Sep
29-Oct

Figure 5.2. a) Average distal spine length of first instar *Bythotrephes* within each lake. b) and c) Natural selection on distal spine length assessed through a comparison of first instar individuals (pre-selection phenotype) to second instar individuals (post-selection phenotype) between cohorts (b) and within a cohort (c). The difference between the pre- and post-selection phenotype for each lake in panels b) and c) is equivalent to the selection differential. There are no post-selection data for Kashagawigamog for the within cohort analysis (c) because a second collection trip was not completed. Error bars are standard errors. Kash: Kashagawigamog; ***$p \leq 0.001$; N.S.: non-significant.

Figure 5.2.


a)
b)
c)
Distal spine length (mm)
6.3
5.8
5.3
4.8
4.3
NGLP (cisco) lakes
GLP (smelt) lakes
N.S.
N.S.
N.S.
***
***
***
No Data
Pre
Post
Boshkung
Harp
Kash
Fairy
Mary
Peninsula

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CHAPTER 6

Concluding thoughts: What insight has the *Bythotrephes* invasion provided to evolutionary ecology and what more can we learn?

Andrea Lynn-Jaeger Miehls

Summary of Findings

Six years of studying the evolutionary ecology of *Bythotrephes* and what have we learned? I found that distal spine and body length of *Bythotrephes* from Lake Michigan are highly heritable and that mothers influence the phenotype of these traits in their offspring (Chapter 2). I also found that genetic and maternal variation in *Bythotrephes* distal spine length varied among but not within years, and that distal spine length was always heritable but was not always influenced by maternal effects. In contrast, genetic and maternal variation in body length varied both within and among years, but likewise body length was always heritable and not always influenced by maternal effects. Further exploring the influence of the environment on *Bythotrephes* traits, my experimental research using *Bythotrephes* from Lake Michigan showed that spine length, body size, and clutch size respond plastically to temperature but not to fish predator cues, with mothers producing fewer, but larger offspring (longer distal spine and body length) that are better defended (greater ratio of distal spine-to-body length) against predation at higher temperature (Chapter 3). Although *Bythotrephes* use temperature as the proximate cue of plasticity, the trait changes likely represent adaptations to varying non-gape-limited (dominant at colder temperature) and gape-limited (dominant at higher temperature) fish predation risk.

I also found temporally and spatially variable natural selection on *Bythotrephes* distal spine length in Lake Michigan across multiple months during three growing seasons and between multiple spatial replicates within the lake (Chapter 4). Temporal variation in selection is consistent with seasonal changes in the relative strength of non-gape-limited and gape-limited fish predation, with selection for decreased distal spine length early in the growing season when predation is dominated by non-gape-limited fish, and strengthening selection for increased distal spine length later in the growing season when predation is increasingly dominated by gape-limited fish. Selection also varied spatially consistent with well-known heterogeneity of fish predators in large lakes. Yet, despite net selection for increased distal spine length occurring across the three years of my study, I observed little evidence of an evolutionary response to selection based on comparisons of historic and contemporary wild-captured individuals and retrieved spines from sediment cores. Finally, using a companion study in six Canadian Shield lakes, I was able to identify the agents of selection on *Bythotrephes* distal spine length (Chapter 5). I found selection for increased distal spine length in lakes dominated by a gape-limited fish predator and no significant selection in lakes dominated by a non-gape-limited fish predator. Average distal spine length in the lakes dominated by gape-limited predation was 20% longer than in the lakes dominated by non-gape-limited predation, suggesting possible local adaptation of distal spine length to gape-limited fish predation.

Contributions to our understanding of invasion biology and evolutionary ecology

Chapter 2. My investigation into the sources of phenotypic variation in *Bythotrephes* distal spine and body length indicates that these traits have heritable variation that would enable an evolutionary response to natural selection. Moreover, maternal effects in *Bythotrephes* distal spine and body length indicate mothers can be a source of phenotypic variation in offspring, suggesting (but not directly showing) the possibility of maternally-induced phenotypic plasticity (Mousseau and Fox 1998; Agrawal et al. 1999). Importantly, genetic and maternal variation in traits influence phenotypic change over different temporal scales, with genetic variation allowing for an evolutionary response to persistent environmental change across multiple generations and maternal variation providing phenotypic variation across a single generation (Lee and Gelembiuk 2008; Svanbäck et al. 2009). Thus, these results indicate that *Bythotrephes* can morphologically respond to environmental change over both the short-term (though maternal effects) and long-term (through an evolutionary response to selection). This capacity for phenotypic change could explain, in part, the ability of *Bythotrephes* to invade lake ecosystems with variable biotic and abiotic conditions (Bilkovic and Lehman 1997; Sullivan and Lehman 1998; Young and Yan 2008). More generally, this research indicates traits of invasive species may contain quantitative genetic variation comparable to native species (Pfrender and Lynch 2000), despite evidence that many invasive species (Dlugosch and Parker 2008), including *Bythotrephes* (Colautti et al. 2005), experience bottlenecks or founder effects that should reduce genetic variation. Thus, evolution of invasive species traits may not be limited by insufficient genetic variation.

Chapter 3. My study of phenotypic plasticity in *Bythotrephes* morphological and life history traits indicates that *Bythotrephes* can respond (through plasticity) to seasonal fluctuations (Straile and Hälbich 2000; Branstrator 2005; Pothoven et al. 2012) in the strength of gape-limited and non-gape limited fish predation that correlate with water temperature. Of note, the finding that plastic responses occur through maternal effects supports the conclusion of Chapter 2 that mothers can influence the phenotype of their offspring. Plasticity in *Bythotrephes* traits likely confers a strong fitness advantage, as *Bythotrephes* are able to capitalize on abundant prey resources and low gape-limited predation risk early in the growing season through the production of many, individually low-cost (i.e., morphologically small) offspring, but also provide protection during high gape-limited predation risk later in the growing season through the production of well-defended (i.e., high distal spine-to-body length ratio), but fewer offspring (i.e., an adaptive size-number trade-off; Smith and Fretwell 1974). *Bythotrephes*' ability to plastically adjust morphology and life history to defend against predation has likely contributed to their ability to establish in nonnative ecosystems, many of which have similar seasonal variation in gape-limitation of fish predators (Straile and Hälbich 2000; Branstrator 2005). More generally, this research provides the first experimental evidence for prey use of a proxy cue of plasticity to reduce predation risk, when direct cues from the predator are unreliable. As proxy cues are likely less often investigated than more obvious potential direct cues of plastic responses, organismal use of proxy cues in nature may be more common than previously realized. This conclusion raises an exciting prospect for investigations of phenotypic plasticity by broadening the potential set of cues that organisms may use for adaptive plasticity.

Chapter 4. My investigation of natural selection on *Bythotrephes* distal spine length in Lake Michigan showed that selection can strongly fluctuate in magnitude and direction across time and space. Many studies of selection lack replication in one or both of these dimensions (discussed in Kingsolver et al. 2001), which may inaccurately characterize selection, depending on the magnitude of fluctuations. Thus, my research supports the conclusions of others (e.g., Merilä et al. 2001) that accurate predictions of the evolutionary response to selection necessitate greater replication in studies of natural section. Of note, temporal fluctuation in the direction of selection corroborates phenotypically plastic responses observed in *Bythotrephes* distal spine length (Chapter 3). Selection favored shorter distal spine length early in the growing season, when plastic response to water temperature would induce production of smaller offspring; conversely, selection favored longer distal spine length later in the season, when plastic response to temperature would induce production of larger, better defended offspring. The directions of selection occurring in these temperature environments thus provide support that the plastic responses I observed are adaptive to seasonally fluctuating gape-limitation of fish predators.

The finding that net selection for increased distal spine length failed to result in an evolutionary response despite the genetic basis of distal spine length (Chapter 2) is supported by other studies (reviewed in Merilä et al. 2001) that found evolutionary stasis in the wild despite selection on heritable traits. Given the success of the *Bythotrephes* invasion in Lake Michigan, as evidenced by their persistence at high annual abundance (Pothoven et al. 2001) since invading almost 25 years ago (Evans 1988), evolutionary stasis in *Bythotrephes* distal spine length – a trait considered critical to their food web interactions (Branstrator 2005) – was initially surprising. I expected fish predators in Lake Michigan would act as agents of strong directional selection on *Bythotrephes* distal spine length, resulting in adaptive evolution since invasion. The lack of an evolutionary response raises the possibility that *Bythotrephes* distal spine length was pre-adapted (Schlaepfer et al. 2009) to fish predation in Lake Michigan. Pre-adaptation is reasonable, given the almost immediate explosion in *Bythotrephes* abundance after invasion (Lehman 1987; Evans 1988; Pothoven et al. 2001). Many species that are not pre-adapted to the invaded ecosystem experience a lag between invasion and population explosion (Facon et al. 2006); adaptive evolution during the lag has been implicated as an important cause of subsequent population explosion (Strayer et al. 2006). The lack of a lag between *Bythotrephes* invasion and population explosion suggests adaptation may not have been necessary. Thus, the success of the *Bythotrephes* invasion in Lake Michigan, in part, may be explained by pre-adaptation.

Chapter 5. The identification of gape-limited fish predators as agents of selection on *Bythotrephes* distal spine length provides support for the conclusion of Chapter 4 that temporal variation in selection on distal spine length in Lake Michigan is due to seasonal variation in gape-limitation of fish predators. Previous research identifying predators as agents of selection in the wild is limited, particularly for invasive species; thus, my research fills an important gap in our understanding of natural selection in the wild. Moreover, my finding of longer average distal spine length in lakes with gape-limited predators, which select for increased distal spine length, is suggestive of local adaptation. Given that *Bythotrephes* invaded these lakes less than twenty years prior to my study, local adaptation would have occurred rapidly (but note, additional work is needed to determine the genetic basis of phenotypic differences between lakes; see Future Directions). If the phenotypic differences between lakes are due to local adaptation to predator gape-limitation, this finding would be among the first documenting evolution by natural selection in an invasive species in response to predation in the wild. Moreover, this finding would also indicate that evolution by natural selection in invasive species traits can occur as quickly as evolution by other well-documented mechanisms for invasive species (e.g., population bottlenecks, founder effects, and hybridization; Lee 2002; Lambrinos 2004; Prentis et al. 2008).

Notably, the findings of (possible) adaptive evolution of *Bythotrephes* distal spine length in response to predation in the Canadian Shield lakes (Chapter 5) and phenotypic plasticity that is likely adaptive to temporal variation in predation risk in Lake Michigan (Chapter 3) indicate that the widespread success of the *Bythotrephes* invasion may be due to both evolution and phenotypic plasticity. Evolution of *Bythotrephes* traits may be common during invasion, due to attributes of their life cycle as cyclic parthenogens (Pothoven et al. 2001; Branstrator 2005). Clonal reproduction allows for rapid within-season response to selection (assuming selection on traits with a genetic basis), whereas sexual reproduction maintains genetic variation in traits across seasons (Lynch 1984). The *Bythotrephes* life cycle can thus allow for rapid adaptation to current environmental conditions, while maintaining sufficient genetic variation to respond to future environmental change: a combination that likely grants high evolvability (Hansen 2006). Thus, I would expect rapid adaptation of *Bythotrephes* to the ecosystems they invade, which in turn likely promotes their invasion success. Additionally, phenotypic plasticity may promote the invasion success of *Bythotrephes* in many ecosystems. My research was not designed to determine whether *Bythotrephes* trait plasticity was present prior to invasion, or whether *Bythotrephes* evolved phenotypic plasticity *de novo* in Lake Michigan. In the former situation, plasticity in *Bythotrephes* traits may pre-adapt *Bythotrephes* for invasion in any ecosystem where the relative risk of gape-limited and non-gape-limited predation is correlated with water temperature; this correlation likely exists in most large lakes that *Bythotrephes* invade (e.g., Straile and Hälbich 2000; Branstrator 2005). In the latter situation, the same aspects of the *Bythotrephes* life cycle that promote evolution of absolute trait magnitude would also promote evolution of plasticity in traits. Thus, evolution of plasticity in *Bythotrephes* traits may occur after invasion, which would likely promote their persistence. Together, high evolvability and phenotypic plasticity (whether present prior to invasion or rapidly evolved after invasion) are likely important to the widespread success of the *Bythotrephes* invasion.

Future Directions

As is the case for most any scientific endeavor, my dissertation research has raised many more questions than it has answered. Below, I explore multiple directions for future work based on the findings of my dissertation research.

Is heritability of Bythotrephes *distal spine and body length environment dependent?*

I found that *Bythotrephes* distal spine and body length are heritable in a 15°C temperature environment (Chapter 2). My work has shown that these traits plastically respond to temperature (Chapter 3), and others have shown (e.g., Wilson et al. 2006; discussed in Pigliucci 2001) that the heritability of plastic traits is environment dependent. Thus, heritability of *Bythotrephes* distal spine and body length may depend on their temperature environment. Investigating heritability of distal spine length in different temperature environments is important, as environment-dependent heritability will affect evolutionary response to selection.

Is there a genetic correlation between Bythotrephes *distal spine and body length?*

I measured broad-sense heritability (Lynch and Walsh 1998) of *Bythotrephes* distal spine and body length (Chapter 2), a univariate measure that does not account for possible genetic correlations between these traits. My work (Chapter 4) and others (Sullivan and Lehman 1998; Branstrator 2005) indicate *Bythotrephes* distal spine length is phenotypically correlated with body size, suggesting the possibility of a genetic correlation (Conner and Hartl 2004). Investigating a genetic correlation between these traits would be important, as a correlation might constrain an evolutionary response to selection (Walsh and Blows 2009; Kingsolver and Diamond 2011).

Is Bythotrephes *phenotypic plasticity in response to temperature adaptive to fish predation?*

My investigation of phenotypic plasticity in *Bythotrephes* life history and morphological traits (Chapter 3) identified a plastic response to temperature that is likely adaptive for seasonal variation in gape-limitation of fish predators. An important caveat to this finding is that I did not test the adaptive value of *Bythotrephes* trait plasticity *per se*. Exposing temperature-induced *Bythotrephes* to predators of variable gape-limitation would be helpful for establishing the adaptive value of plasticity in response to temperature. Addressing the following question would also be useful for establishing the adaptive value of plasticity…

Does Bythotrephes *phenotypic plasticity have a genetic basis?*

I argued (Chapter 3) that plasticity in *Bythotrephes* traits is adaptive based on the framework of Doughty and Reznick (2004) who present an argument for adaptation based on six indirect lines of evidence (criteria). One criterion of Doughty and Reznick (2004) which I was unable to meet was establishing the genetic basis of plasticity. Thus, measuring genetic variation in plasticity would be beneficial to establishing the adaptive value of plasticity.

Do Bythotrephes *plastically respond to the presence of prey?*

I investigated temperature and fish kairomones as proximate cues of plasticity in *Bythotrephes* traits (Chapter 3) due to their correlation with *Bythotrephes* seasonal trait variation. A third possible proximate cue of plasticity in *Bythotrephes* traits is the presence of a dominant prey item, *Daphnia*. Previous work has demonstrated plastic response of predators to prey (Kishida et al. 2006), and other researchers (Burkhardt 1994; Pothoven et al. 2003) have suggested that *Bythotrephes* traits may plastically respond to *Daphnia* presence in Lake Michigan. Thus plastic response to prey presence is a possible explanation for *Bythotrephes* seasonal trait variation.

Are Bythotrephes *morphology and life history phenotypically plastic in the native range?*

I identified phenotypic plasticity in *Bythotrephes* morphology and life history in Lake Michigan (Chapter 3); an ensuing question was whether *Bythotrephes* evolved plasticity *de novo* in Lake Michigan or whether plasticity was already present at invasion. Completing experiments exploring plasticity in response to temperature (similar to those in Chapter 3) with *Bythotrephes* from their native range would be valuable to determining when plasticity evolved.

Are Bythotrephes *body size and fecundity under selection?*

Trade-offs between different components of fitness (e.g., survival vs. fecundity; Roff 2002) and genetic correlations among traits (Walsh and Blows 2009; Kingsolver and Diamond 2011) are known to constrain evolutionary responses to selection. My finding of evolutionary stasis in *Bythotrephes* distal spine length (Chapter 4) raised the possibility that opposing selection on body size (given a positive genetic correlation with distal spine length) and fecundity (given a size-number trade-off) could constrain an evolutionary response to selection. Estimating selection on both of these traits would, thus, be helpful for understanding evolutionary stasis of *Bythotrephes* distal spine length in Lake Michigan.

What is the genetic basis of phenotypic differences in Bythotrephes *distal spine length between Canadian Shield lakes dominated by gape-limited and non-gape-limited predation?*

My finding of a large difference in average distal spine length between lakes dominated by gape-limited versus non-gape-limited predation (Chapter 5) was suggestive of local adaptation, but my study did not test for local adaptation *per se*. Key to asserting local adaptation of distal spine length is the determination of genetic variation underlying the phenotypic variation I observed. Clonal analyses similar to those I completed for Chapter 2 would readily establish whether the observed phenotypic variation has a genetic basis.

Do Bythotrephes *life history and morphological traits exhibit phenotypic plasticity in the Canadian Shield lakes, and are there differences in phenotypic plasticity in lakes dominated by gape-limited and non-gape-limited predation?*

I found phenotypic plasticity in *Bythotrephes* morphological and life history traits that is likely adaptive to seasonal variation in predator gape-limitation in Lake Michigan (Chapter 3). An extension of this finding is that no plasticity should be observed in lakes lacking strong temporal variation in predator gape-limitation. This prediction could be tested using Canadian Shield lakes (Chapter 5) that experience comparatively less temporal variation in predator gape-limitation than Lake Michigan. I would expect that *Bythotrephes* in Shield lakes dominated by gape-limited predation may not exhibit phenotypic plasticity, but instead, would exhibit constitutive expression of large size and morphological defenses. Similarly, *Bythotrephes* in Shield lakes dominated by non-gape-limited predation should exhibit constitutive expression of small size and morphological defenses.

Concluding Thoughts

My study of the evolutionary ecology of *Bythotrephes* has uncovered how evolution and phenotypic plasticity likely promote their invasion success in nonnative ecosystems, advancing our understanding of the *Bythotrephes* invasion in North America. Yet, my goal in studying *Bythotrephes* was not just to learn about the evolutionary ecology of this alien organism, but also to provide illumination on broader concepts in evolutionary ecology. To borrow a simple, but insightful description from Quammen (2000), *Bythotrephes* are “a small organism through which we can address big questions”. Although Quammen (2000) wrote this statement while describing the ecology of slime molds, the statement reflects how I view my work on *Bythotrephes*. I would argue that the characteristics that make any invasive species successful are the same as (or at least are similar to) the characteristics that make any species successful. And so, by studying invasive species, we achieve the dual purpose of understanding the impacts of species invasions specifically, while at the same time generating knowledge that is ultimately useful to conserving biodiversity.

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