Local differentiation in the defensive morphology of an invasive zooplankton species is not genetically based

by

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ABSTRACT

LOCAL DIFFERENTIATION IN THE DEFENSIVE MORPHOLOGY OF AN INVASIVE ZOOPLANKTON SPECIES IS NOT GENETICALLY BASED

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Invasive species cause widespread ecological and economic damage, but to better understand how exotic species spread to become invasive, empirical studies of the mechanisms driving phenotypic differentiation between populations of invasive species are required. This study determined whether differences in distal spine length among populations of *Bythotrephes longimanus* in lakes with or without gape-limited fish predators could be explained by local adaptation or phenotypic plasticity. I collected *Bythotrephes* from six lakes and found that distal spine lengths and natural selection on distal spine length differed among populations, but were unrelated to the gape-limitation of the dominant fish predator. A common garden experiment revealed significant genetic and maternal variation for the trait, but phenotypic differences among populations were not genetically based. Phenotypic differentiation of this ecologically important trait is, therefore, a result of plasticity and not local adaptation, despite spatially variable selection on this heritable trait.

To my parents.

Everything I accomplish is a product of your guidance, love, and support.

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1.0 Introduction

Invasive species have substantial adverse impacts on global biodiversity, community structure, and ecosystem function (Vitousek et al. 1996; Mack et al. 2000), but few exotic species spread, causing the wide-scale ecological and economic damage that we associate with "invasiveness" (Mooney and Cleland 2001). Understanding why some exotic species become invasive while others do not has been the focus of decades of ecological and ecosystem-level research (Drake et al. 1989; Novak 2007). More recently, there has been increasing interest in the evolutionary aspects of biological invasions (Mooney and Cleland 2001; Lee 2002; Parker et al. 2003; Lambrinos 2004; Facon et al. 2006), but tests for adaptive evolution remain rare.

The spread of an exotic species depends on its ability to perform well in new abiotic and biotic conditions (Shea and Chesson 2002; Facon et al. 2006). One way in which this could be achieved is through local adaptation (Lee 2002; Parker et al. 2003; Lambrinos 2004; Facon et al. 2006), which is the process whereby divergent natural selection (i.e. selection that differs among habitats) on a fitness-related trait causes populations to become genetically differentiated (Kawecki and Ebert 2004). Populations of exotic species may have considerable potential for local adaptation because species introduced into a foreign environment are outside of the region in which they evolved, and thus are likely to encounter novel selection pressures (Mooney and Cleland 2001; Lambrinos 2004). Additionally, for populations to locally adapt, there must be sufficient genetic variation underlying the traits experiencing divergent selection (Lynch and Walsh 1998). For exotic species, invasions characterized by large founder populations or a large number of founder events are expected to have high genetic variance, whereas invasions characterized by small founder populations or a small number of founder events are expected to

have reduced genetic variance, which may constrain local adaptation (Allendorf and Lundquist 2003; Lockwood et al. 2005).

Local adaptation results in phenotypic differentiation between populations, but the presence of such differentiation is not sufficient to demonstrate that populations are locally adapted. Phenotypic plasticity is the ability of a genotype to produce alternate phenotypes based on environmental conditions (Pigliucci 2005), and represents an alternative mechanism by which exotic species can adaptively respond to heterogeneity in their environment. Plasticity can, therefore, produce a pattern of phenotypic differentiation among populations that is consistent with that of local adaptation, but without the underlying genetic differences (Kawecki and Ebert 2004). Additionally, because plasticity allows different genotypes to produce the same phenotype, it can reduce the strength of selection and may subsequently constrain local adaptation (Pfennig et al. 2010). Alternatively, plasticity can allow organisms to cope with new environments where they might otherwise not be able to persist, which allows them to experience novel selection pressures that can subsequently lead to local adaptation (West-Eberhard 2003).

Despite the potential importance of local adaptation and plasticity to invasiveness (Parker et al. 2003), little is known about their relative importance with respect to the spread of exotic species. Most previous work has focused on invasive plants, where limited studies suggest that local adaptation and phenotypic plasticity are not mutually exclusive. For example, Si et al. (2014) found evidence for local adaptation and phenotypic plasticity of several growth characteristics that contributed to the successful invasion of *Wedelia trilobata* across a tropical island. Similarly, Godoy et al. (2011) found that local adaptation and phenotypic plasticity were both involved in the successful invasion of the heavily shaded understory of South American

evergreen temperate rainforest by *Prunella vulgaris* (also see: Parker et al. 2003). Dybdahl and Kane (2005) provided a rare example outside of plants, in which North American populations of invasive freshwater snails (*Potamopyrgus antipodarum*) were found to show phenotypic plasticity (but not local adaptation) for life history and growth traits that facilitated their spread. In order to better understand the general mechanisms by which exotic species spread (and hence, become invasive), further empirical studies of the mechanisms driving phenotypic differentiation between populations of invasive species for ecologically important traits are needed.

The spiny water flea, *Bythotrephes longimanus* (hereafter, *Bythotrephes*), is an invasive species in the Laurentian Great Lakes and many surrounding inland lakes where it negatively impacts lake ecosystems due to its central position in the food web as a predator of zooplankton (Bunnell et al. 2011) and prey for fish (Pothoven et al. 2007). The tail spine of *Bythotrephes* is used as a morphological defense against fish predation (Barnhisel 1991a, b), and previous work on five Canadian Shield lakes identified that *Bythotrephes* in lakes dominated by gape-limited fish predators experience natural selection for longer distal spines (i.e. the posterior-most segment of the tail spine), whereas those in lakes dominated by non-gape-limited predators experience no selection on distal spine length (Miehls et al. 2014). Gape-limited predation (GLP) occurs when predators cannot consume individuals of a focal prey species above a certain size determined by the gape-size of the predator, and is generally expected to cause natural selection for increased size in prey (Day et al. 2002; Urban 2008). In contrast, non-gape-limited predation (NGLP), in which predators are not constrained by mouth size, is expected to impose no selection on the size of prey (Urban 2007, 2008). Additionally, distal spine length of *Bythotrephes* from Lake Michigan is heritable (H^2 = 0.27-0.76; Miehls et al. 2012), and differences in mean distal spine length among Canadian Shield lakes were consistent with

differences in natural selection among lakes: *Bythotrephes* from lakes dominated by GLP were found to have 17% longer distal spines compared to lakes dominated by NGLP (Miehls et al. 2014). These findings suggest that local adaptation may explain the pattern of phenotypic differentiation among populations of *Bythotrephes*. However, cladocerans are notoriously phenotypically plastic, especially for traits involved in predator defense. For example, the cladoceran *Daphnia lumholtzi* produces neonates with longer head spines when exposed to fish predator kairomones (Dzialowski et al. 2003) (also see: Lüning 1992). This maternal induction of offspring phenotypes in response to the maternal environment is called a maternal effect (Mousseau and Fox 1998). It has also been shown that *Bythotrephes* from Lake Michigan induce longer distal spines in offspring in response to warmer water temperature (but not fish kairomones), which may act as an indirect cue for natural selection associated with GLP (Miehls et al. 2013). It is thus plausible that local differences in distal spine length among populations of *Bythotrephes* could be explained by either local adaptation in response to GLP or maternal induction of longer distal spines in offspring in response to an environmental cue associated with GLP.

In this study, I first measured *Bythotrephes* distal spine lengths and the strength of natural selection on distal spine length in six Canadian Shield lakes that differed in the presence or absence of GLP. I then conducted a common garden experiment (Kawecki and Ebert 2004) to evaluate the hypotheses of local adaptation and phenotypic plasticity as the proximal cause of phenotypic differences among these populations. Using clonal lines (Lynch and Walsh 1998), I reared individuals from the six study lakes in identical conditions for two generations. I measured genetic and maternal variation for distal spine length to determine broad-sense heritability for the trait and maternal effects, and determined if phenotypic differences in distal

spine length among populations were genetically based. If phenotypic differences among populations were due to local adaptation (i.e. genetically based differentiation), I predicted that lab-reared *Bythotrephes* from lakes dominated by GLP would have longer distal spines than lab-reared *Bythotrephes* from lakes dominated by NGLP. Alternatively, if phenotypic differences among populations were due to phenotypic plasticity, I predicted that phenotypic differences among populations would no longer be present in lab-reared *Bythotrephes.*

2.0 Methods

2.1 Study Species

2.1.1 Invasion History

Bythotrephes is a predatory cladoceran zooplankter with a widespread native distribution throughout the Palearctic region (Therriault et al. 2002; Colautti et al. 2005; Kim and Yan 2013), and can tolerate a wide range of pH, salinity, temperature, and conductivity (Grigorovich et al. 1998). *Bythotrephes* was first identified in the Laurentian Great Lakes in the early 1980s (Johannsson et al. 1991), and has since spread to more than 160 inland Ontario lakes, and lakes in the mid-western USA (Kelly et al. 2013). In the Canadian Shield, *Bythotrephes* can be found in lakes dominated by gape-limited predators, such as rainbow smelt (*Osmerus mordax*), or non-gape limited predators, such as cisco (*Coregonus artedi*) (Strecker et al. 2006; Young and Yan 2008).

2.1.2 Morphology and Life History

The *Bythotrephes* caudal process (i.e. tail spine) consists of segments, the longest of which is the distal spine (i.e. the section from the posterior tip of the spine to the first paired articular spines; Fig. 1), which is present at birth and does not change in length with development (Burkhardt 1994). Thus, the length of the distal spine cannot respond plastically to the

environment directly experienced by offspring, but it can be maternally induced (see Determining Genetically Based Differences among Populations; Miehls et al. 2013). Only the distal spine is present in neonates (i.e. the first instar stage), but total spine length increases through development as an additional spine segment is added to the base of the spine at each instar molt (Branstrator 2005). These segments are each separated by paired articular spines (Fig. 1), which allows the instar stage to be easily identified and the length of each segment to be measured separately (Yurista 1992). Like most cladocerans, *Bythotrephes* have a cyclically parthenogenetic life cycle, reproducing apomictically (i.e. clonally) multiple times before reproducing sexually at the end of the growing season (Yurista 1992; Branstrator 2005). Apomictic reproduction produces eggs that immediately develop into young in the brood pouch, whereas sexual reproduction results in resting eggs that overwinter on the lake bottom before hatching the following spring (Yurista 1992; Branstrator 2005).

2.2 Study Lakes

Bythotrephes were collected during the middle of the growing season (July 29-31, 2014) from six lakes in the Muskoka district and County of Haliburton in south-central Ontario (Fig. 2). Predation on *Bythotrephes* in three of the lakes (Peninsula, Mary, and Fairy; hereafter, GLP lakes) is thought to be dominated by the gape-limited fish predator, rainbow smelt, while in the three other lakes (Boshkung, Harp, and Drag; hereafter, NGLP lakes) predation is thought to be dominated by the non-gape-limited fish predator, cisco (Strecker et al. 2006; Young and Yan 2008; S. J. Sandstrom and N. Lester, unpublished data). Although rainbow smelt are present in Boshkung Lake (Young and Yan 2008), cisco have been reported to be more abundant and were considered to be the dominant *Bythotrephes* predator (Strecker et al. 2006; Miehls et al. 2014), so Boshkung was classified as a NGLP lake.

These lakes were chosen because they were invaded by *Bythotrephes* over the last 30 years and are characteristic of many lakes in the Canadian Shield that are dominated by rainbow smelt or cisco (Strecker et al. 2006; Young and Yan 2008). They are similar to one another physically (in terms of depth and water temperature) and biologically (in terms of invertebrate predators, and other fish predators of *Bythotrephes*) (Hovius et al. 2006; Strecker et al. 2006; Young and Yan 2008). Although Harp Lake is much smaller than the other lakes, several studies have found that it is ecologically similar (Hovius et al. 2006; Strecker et al. 2006; Young and Yan 2008). Five of these six lakes (excluding Drag) were also previously used by Miehls et al. (2014) to test for the effects of GLP on natural selection and local differences in distal spine length. Miehls et al. (2014) sampled an additional lake (Kashagawigamog, NGLP) but sample sizes were too low to measure natural selection. I sampled Drag Lake (NGLP) instead of Kashagawigamog to balance the experimental design.

2.3 Sample Collection

Bythotrephes were collected using a conical zooplankton net with a 0.5 m diameter opening and 363 μm mesh size. To collect *Bythotrephes* to measure distal spine lengths and natural selection for each lake, the net was horizontally towed at a depth of 10-15 m, and approximately 100 individuals were haphazardly chosen and immediately preserved in 95% ethanol. For the common garden experiment, live *Bythotrephes* were collected using a vertical net tow (instead of a horizontal tow) through the top 15 m of the water column, and 30-40 actively swimming individuals without pigmented brood pouches were individually isolated in 60 mL jars containing 50 mL of lake water filtered through a 63 μm sieve. A vertical net tow was used to collect individuals for the common garden experiment because it minimizes damage to the animals associated with turbulence, whereas a horizontal tow was used to collect individuals

to measure phenotypic differences and selection because animals could be collected in greater quantity. All collection methods were based on those reported by Kim and Yan (2010) and Miehls et al. (2014).

2.4 Common Garden Experiment

I used a common garden experiment and clonal breeding design (Fig. 3) to measure genetic and maternal variation for distal spine length, and to determine if phenotypic differences among populations were genetically based. During the three-day collection period, all live *Bythotrephes* were maintained at the Dorset Environment Science Center (DESC, Dorset, Ontario) in a climate controlled facility (20°C, 14L:10D photoperiod) in lake water filtered through a 63 μm sieve from their "home" lake. Afterwards, the cultures were moved to an environmental chamber at the Hagen Aqualab (University of Guelph, Guelph, Ontario) under the aforementioned temperature and photoperiod and introduced to the common garden medium. The medium was an autoclaved mixture of lake water filtered through a 63 μm sieve from the six sampled lakes (i.e. each *Bythotrephes* was reared in water that was $1/6^{th}$ of their local environment). *Bythotrephes* received daily water changes and were fed *ad lib* with approximately 150 *Artemia* sp. nauplii that were less than 30 h old (Miehls et al. 2012).

Clonal lines were initiated using 188 wild-caught individuals (28-37 per lake), and were reared in the common garden through two apomictic generations (Fig. 3; Miehls et al. 2012, 2013). Once a female produced offspring she was preserved in 95% ethanol within 24 h. All offspring were individually transferred to 60 mL jars containing 50 mL of common garden medium, also within 24 h (Miehls et al. 2012). Of the 188 clonal lines, 12.2% produced second-generation lab-born offspring (7-37 individuals per lake; Supplementary Material, Table S1).

2.5 Measurement and Analyses

All *Bythotrephes* were photographed using a digital camera mounted to a dissecting microscope (Leica MZ8, Leica Microsystems). IMAGEJ software (Abramoff et al. 2004) was used to measure the length of the distal spine segment from the tip of the tail spine to the first paired articular spines (Fig. 1) to the nearest 0.001 mm. Instar was assessed by counting the number of paired articular spines on the total tail spine (Fig. 1).

All statistical analyses were conducted using R version 3.2.2 (R Core Team 2015). For all linear mixed-effects models (LME models), the statistical significance of the random effects was assessed through model comparisons using likelihood ratio tests (LRTs) in which the change in the deviance between the more complex model and the simpler model was assumed to follow a chi-squared distribution where the degrees of freedom were equal to the difference in the number of parameters between the more complex and simpler models (df = 1 in all cases here). For models with one random effect, I fitted one additional model with the same fixed effects but without the random effect. The significance of the random effect was assessed by comparing these two models. For nested models (i.e. models with multiple nested random effects), I fitted additional models with the same fixed effects but with successively fewer random effects, starting with the removal of the most nested random effect. The significance of a random effect was assessed by comparing the model that included the random effect of interest to the simpler model without that random effect. The statistical assumptions of homoscedasticity and normality were met for all models.

2.5.1 Comparing Distal Spine Length among Natural Populations

I first determined whether the mean distal spine length of wild-caught *Bythotrephes* differed among lakes using one-way ANOVA with distal spine length of wild-caught, first instar

individuals as the response variable and lake as the predictor, where a significant effect of lake would indicate that mean distal spine length differed among lakes. For this model, Tukey's multiple comparison test (Abdi and Williams 2010) was used to assess the significance of differences among pairs of lakes. To determine whether there was phenotypic differentiation between predation regimes (i.e. GLP vs. NGLP), I fitted a LME model using the *nlme* package in R (Pinheiro et al. 2015) with the distal spine length of wild-caught, first instar individuals as the response variable, predation regime as a fixed effect, and lake as a random effect (to account for variation among lakes unrelated to predation type). In this model, a significant effect of predation regime would indicate that mean distal spine length was associated with the gape-limitation of the dominant fish predator. For both models, only first instar individuals were considered because distal spine length at this stage represents the pre-selection phenotype; therefore, using only first instar *Bythotrephes* ensured that differences in distal spine length among lakes were not confounded by selection.

2.5.2 Measuring Natural Selection

Natural selection was quantified by comparing distal spine lengths between first and second instar *Bythotrephes*. Because *Bythotrephes* distal spine length does not change with development (Burkhardt 1994), a change in mean distal spine length from the first to the second instar stage represents the relationship between distal spine length and instar survival success, and not developmentally based differences. Consequently, the magnitude and direction of the difference is the strength and direction of natural selection (Miehls et al. 2014). Although *Bythotrephes* develops into a third or fourth instar stage, the comparison between the first two stages was assessed because the distal spine represents the entire length of the spine in first instar individuals, which was the expected target of selection (Miehls et al. 2014).

I calculated selection differentials (Falconer and Mackay 1996) for each population as the difference between the mean distal spine lengths for the first two instar stages (Miehls et al. 2014). The statistical significance of these selection differentials for each lake was assessed using Welch *t*-tests (two-tailed). In these analyses, a statistically longer mean distal spine for second instar individuals compared to first instar individuals would indicate significant selection for longer distal spines in that lake. Additionally, to compare to other published estimates of selection, standardized selection differentials (i.e. selection intensities) were calculated by dividing the selection differential for a lake by the standard deviation of distal spine length for first and second instar animals from that lake (Miehls et al. 2014).

To statistically test whether selection on distal spine length differed among lakes, I used two-way ANOVA with distal spine length of wild-caught first and second instar animals as the response variable, lake and instar as predictors, and a lake-by-instar interaction. In this model, a significant effect of instar would indicate that there was selection on distal spine length irrespective of lake; a significant effect of lake would indicate that distal length differs by lake irrespective of selection; and a significant lake-by-instar interaction would indicate that selection differs among lakes. To determine if selection varied consistently with predation regime, I fitted a LME model with distal spine length of wild-caught, first and second instar animals as the response variable, predation regime, instar, and a predation regime-by-instar interaction as fixed effects, and lake as a random effect. In this model, a significant effect of instar would indicate that there was selection on distal spine length irrespective of predation regime; a significant effect of predation regime would indicate that distal length differs by predation regime irrespective of selection; and a significant predation regime-by-instar interaction would indicate that selection depended on the gape limitation of the dominant fish predator.

2.5.3 Determining Genetically Based Differences among Populations

I reared *Bythotrephes* from all study lakes in a laboratory setting under identical conditions for two generations to eliminate phenotypic differences among populations that may be expressed as a result of environmental heterogeneity among lake populations, including maternal effects (Mousseau and Fox 1998). As aforementioned, the *Bythotrephes* distal spine is present at birth and its length does not change with development (Burkhardt 1994). Therefore, the distal spine lengths of second-generation lab-born individuals are expressed in response to the common lab environment experienced by their mothers, and any remaining differences among populations should be genetically based (assuming negligible grand-maternal effects).

To statistically determine if phenotypic differences in distal spine length among lakes were genetically based, I fitted a LME model with distal spine length of second-generation individuals as the response variable, lake as a fixed effect, and clonal subline nested within clonal line as random effects. In this model, a nonsignificant effect of lake would indicate that distal spine length was not genetically differentiated among lakes, which would be consistent with a phenotypic plasticity hypothesis. Alternatively, a significant effect of lake would indicate that local differences in distal spine length were genetically based, and could thus reflect local adaptation in response to GLP. To determine if phenotypic differences were genetically based between predation regimes, I fitted a LME model with distal spine length of second-generation lab-born individuals as the response variable, predation regime as a fixed effect, and clonal subline nested within clonal line nested within lake as random effects. In this model, a significant effect of predation regime would indicate that there were genetically based differences in distal spine length associated with the gape limitation of the dominant fish predator, which would

reflect local adaptation in response to the local predation regime if phenotypic differences were in the predicted direction (i.e. longer in GLP lakes).

2.5.4 Estimating Broad-sense Heritability and Maternal Effects

The clonal breeding design that I used (Fig. 3) allowed for the quantification of genetic (V_g), maternal (V_m), and environmental (V_e) variance components for distal spine length (Lynch and Walsh 1998; Miehls et al. 2012). I fitted a LME model with distal spine length of second-generation lab-born individuals as the response variable, the intercept as the only fixed effect, and clonal subline nested within clonal line as random effects. In an additional model, lake was included as a fixed effect (Supplementary Material, Table S2). In this breeding design, the variance in distal spine length among clonal lines estimates genetic variance, the variance among sublines within clonal lines estimates maternal variance, and the variance within sublines estimates environmental variance. Note, because *Bythotrephes* distal spine length is fixed from birth, the variance within sublines (i.e. environmental variance) must be due to small scale environmental differences within the brood pouch of the mother during development. Similarly, variance in distal spine length among sublines (i.e. maternal variance) could be confounded by environmental differences within the brood pouch of the grandmother during development of the mothers, or subtle differences experienced by the mothers in the lab. To assess the significance of the variance components, I obtained 95% confidence intervals around the random effects (Pinheiro and Bates 2000) and conducted model comparisons using LRTs (see Measurement and Analyses) (Miehls et al. 2014). I calculated broad-sense heritability (H^2) as the ratio of among-line variance to the total phenotypic variance (i.e. the sum of the among-line, among-subline, and within-subline variances), and calculated maternal effects (m^2) as the ratio of among-subline variance to the total phenotypic variance.

3.0 Results

3.1 Variation in Mean Distal Spine Length among Populations

The mean distal spine length of wild-caught, first instar individuals was 5.80 ± 0.02 mm (mean ± SE). Mean distal spine length differed among lakes (ANOVA: $F_{4,225} = 17.1$, $P < 0.001$), but did not differ by predation regime (Table 2; Fig. 4). Specifically, populations in Peninsula Lake (GLP) and Boshkung Lake (NGLP) had significantly longer distal spines than populations in Mary Lake (GLP), Fairy Lake (GLP) and Harp Lake (NGLP) (Tukey HSD: $P < 0.003$). There was no difference in mean distal spine length between the Peninsula and Boshkung populations (Tukey HSD: $P = 0.913$) and no differences among the Mary, Fairy, and Harp populations (Tukey HSD: $P > 0.244$). No first instar animals were collected from Drag Lake.

3.2 Natural Selection

Differences in distal spine length between first and second instar *Bythotrephes* differed by lake (i.e. a significant lake-by-instar interaction; ANOVA: $F_{4,459} = 3.5$, $P = 0.008$; Fig. 4), indicating that strength of natural selection differed among populations in the study lakes. However, differences in selection among lakes were not consistently relatable to predation regime (i.e. a nonsignificant predation regime-by-instar interaction; Table 4). Of the GLP lakes, there was significant directional selection for increased distal spine length in Mary and Fairy (i.e. the mean distal spine length in second instar individuals was larger than that of first instar individuals), but selection on distal spine length in Peninsula was not significant. Of the NGLP lakes, selection was not significant in Harp, but there was significant directional selection for increased distal spine length in Boshkung (Table 3). Natural selection could not be assessed for Drag Lake because no first instar animals were collected from this lake.

3.3 Common Garden Experiment

The mean distal spine length of second-generation individuals was 5.06 ± 0.04 mm (mean ± SE), approximately 87% of the mean length observed in wild-caught individuals. Mean distal spine length of second-generation lab-born individuals did not differ among lakes or between predation regimes (Table 5; Fig. 5). There was, however, significant genetic variation in *Bythotrephes* distal spine length, corresponding to a H^2 estimate of 0.24. Likewise, there was significant maternal variation in distal spine length, corresponding to a m^2 estimate of 0.61 (Table 6).

4.0 Discussion

The goal of this study was to determine whether phenotypic differences in distal spine length among populations of *Bythotrephes* in Canadian Shield lakes (that differ in the presence or absence of gape-limited fish predators) could be explained by local adaptation or phenotypic plasticity. I found that *Bythotrephes* from two study lakes (Peninsula and Boshkung) had long mean distal spine lengths compared to those from three other study lakes (Mary, Fairy, and Harp), and that there was strong selection for increased distal spine length in three of the five study lakes (Mary, Fairy, and Boshkung). A common garden experiment with a clonal breeding design revealed significant genetic and maternal variation for distal spine length (Table 6), but the mean distal spine lengths of second-generation lab-born individuals did not differ among populations (Fig. 5), indicating that phenotypic differences were not genetically based. Differences in distal spine length among populations were, therefore, a result of phenotypic plasticity in response to the maternal environment, and not local adaptation, despite spatially variable selection on a heritable trait.

The absence of genetically based differences in distal spine length among *Bythotrephes* populations was surprising because natural selection varied among populations. Specifically, I identified significant selection for longer distal spines in two of three GLP lakes (Mary and Fairy, but not Peninsula) and one of two NGLP lakes (Boshkung, but not Harp). It is worth noting that the standardized selection differentials I calculated for lakes with significant selection were very large (Mary: $i = 0.71$; Fairy: $i = 0.74$; Boshkung: $i = 0.82$; Table 3). A recent review of selection in wild populations identified that the median magnitude of directional selection (measured as the absolute value of standardized linear selection gradients) for survival was 0.08 (Kingsolver and Diamond 2011), indicating that selection on *Bythotrephes* distal spine length in these three lakes was very strong. In fact, the three significant standardized selection differentials that I measured were in the top 10% of those previously reported (Kingsolver et al. 2001). Although Kingsolver and Diamond's (2011) review reported selection using standardized selection gradients and I reported standardized selection differentials, these selection metrics have often been found to be similar in magnitude (Kingsolver and Diamond 2011). Miehls et al. (2014) also found strong selection for increased distal spine length in *Bythotrephes* in Mary Lake ($i = 0.79$) and Fairy Lake ($i = 0.53$) in the summer of 2008, suggesting that selection on *Bythotrephes* distal spine length in these lakes has been consistently strong.

Directional selection for increased distal spine length (which was observed in three of five lakes) should cause an evolutionary response if the trait is heritable (Falconer and Mackay 1996). There was significant genetic and maternal variation for *Bythotrephes* distal spine length, corresponding to a moderate broad-sense heritability and large maternal effect (Table 6; Mousseau and Roff 1987), which are the first such estimates for *Bythotrephes* in Canadian Shield lakes. My estimates of genetic variation and heritability for *Bythotrephes* distal spine

length were very similar to previous estimates in Lake Michigan in July (V_g = 0.06, H^2 = 0.27; Miehls et al. 2012), indicating that most of the genetic variation in distal spine length has been maintained since *Bythotrephes* invasion from the Laurentian Great Lakes, and that the spread of *Bythotrephes* has not limited their potential for adaptive evolution. Despite this adaptive potential, and significant differences in selection among lakes, I found no evidence of genetic differentiation for distal spine length among populations of *Bythotrephes.* Previous work on *Bythotrephes* used historic and contemporary wild-caught animals and remnant distal spines retrieved from sediment cores to test for a response to selection on distal spine length since *Bythotrephes* invasion of Lake Michigan, and found little evidence of phenotypic change through time (Miehls et al. 2015). Together, my results and those of Miehls et al. (2015) provide clear examples of selection on a heritable trait not leading to an evolutionary response through time (in Lake Michigan) or across space (in these Canadian Shield lakes).

There are several reasons why selection on a heritable trait may not cause evolutionary change (i.e. evolutionary stasis; Merilä et al. 2001). For example, temporal fluctuations in selection can influence the direction and strength of selection overall (Siepielski et al. 2009; Bell 2010; Kingsolver and Diamond 2011). In GLP lakes, predation risk for *Bythotrephes* increases through the growing season because juvenile gape-limited fish grow from sizes too small to consume any *Bythotrephes* to sizes that can consume some *Bythotrephes* depending on gape-size (Straile and Halbich 2000; Branstrator 2005; Pothoven et al 2012; Miehls et al. 2015). Although my study only looked at a single snapshot of selection for each study lake, previous work on *Bythotrephes* in Lake Michigan found strong temporal variation in selection on distal spine length that was consistent with the increase in predation risk over the growing season, which greatly reduced net selection (Miehls et al. 2015). Furthermore, the differences in selection

among lakes and magnitudes of selection that I measured differed from previous estimates of selection measured during the summer of 2008 (Miehls et al. 2014). Specifically, Miehls et al. (2014) found significant selection for increased distal spine length in Peninsula, Mary, and Fairy (GLP lakes; i = 0.20-0.79), but no selection in Boshkung and Harp (NGLP lakes), whereas I found significant selection in Mary, Fairy, and Boshkung (i = 0.71-0.82; Table 3). Second, it is possible that *Bythotrephes* experience a tradeoff between components of fitness (i.e. survival vs. fecundity; Roff 2002) or that selection varies among life stages (Schluter et al. 1991). Previous work suggests that *Bythotrephes* exhibit a tradeoff between clutch size and offspring distal spine length in *Bythotrephes* (i.e. the larger the clutch, the shorter the distal spines of the offspring; Straile and Halbich 2000; Pothoven et al. 2003; Miehls et al. 2013), which means that viability selection favouring longer distal spines could be opposed by fecundity selection favouring shorter distal spines. Lastly, I measured selection between first and second instar individuals, but selection on later instar stages is likely to occur on the length of the total spine, rather than just the distal spine (Fig. 1), which may reduce the strength of selection on distal spine length if selection on the total spine is weaker or in the opposite direction. Overall, it is plausible that the episodes of selection that I documented may have been offset by some other component of fitness (e.g. fecundity or life stage) or temporal variation in selection (see Miehls et al. 2015 for a thorough review of the potential limits of adaptive evolution with respect to *Bythotrephes*).

The differences among lakes in mean distal spine length and selection that I observed were inconsistent with GLP as a strong agent of selection, which suggests that the influence of gape-limited fish predation on distal spine length may be less important than previously hypothesized. However, this finding contradicts previous work in Canadian Shield lakes (Miehls et al. 2014), and challenges the importance of the *Bythotrephes* tail spine as a morphological

defense against fish predators (Barnhisel 1991a, b). In my study, the absence of a consistent effect of predation regime on mean distal spine lengths and natural selection on distal spine length was a result of unexpected findings for two lakes. First, the population in Peninsula Lake (GLP) had a long mean distal spine length but selection was weak and not statistically significant, which was contrary to my expectations (Table 3; Fig. 4). It is possible that individuals from Peninsula may not have experienced selection because they were born with a distal spine long enough to deter GLP. Comparing GLP lakes, individuals from Fairy had the shortest mean distal spines (5.54 mm) but the strongest selection (i = 0.74), whereas individuals from Peninsula had the longest mean distal spines (5.87 mm) but the weakest selection (i = 0.03; Fig. 4). These results suggest the possibility of a threshold distal spine length that provides refuge from GLP, which is consistent with "hard" natural selection (Wallace 1975). In general, this finding of a possible threshold distal spine length is consistent with previous work. Among GLP lakes in the Canadian Shield, Miehls et al. (2014) also found that populations with the shortest mean distal spine experienced the strongest selection (Mary), whereas the population with the longest mean distal spine experienced the weakest selection (Peninsula). Contrary to my results, however, Miehls et al. (2014) found the relatively weak selection in Peninsula to be statistically significant.

The second unexpected finding was that the *Bythotrephes* population in Boshkung Lake (NGLP) had a long mean distal spine length but selection was strong and statistically significant (Table 3; Fig. 4). The most obvious explanation for this finding is that Boshkung may no longer be dominated by NGLP. Recall that rainbow smelt (the dominant gape-limited fish predator) are present in Boshkung (Young and Yan 2008), but cisco (the dominant non-gape-limited fish predator) are thought to be the dominant predator of *Bythotrephes* (Table 1; Strecker et al. 2006;

Miehls et al. 2014). Unfortunately, there has not been a recent fish survey of Boshkung, so the strong selection measured in this study could be explained by an increase in the abundance of smelt relative to cisco since the last survey. Interestingly, Miehls et al. (2014) found that the *Bythotrephes* population in Boshkung had a longer mean distal spine length compared to Harp (NGLP, with no smelt), though their primary analysis yielded no evidence of selection. However, an alternative analysis found the occurrence of reasonably strong (though statistically insignificant) selection (i = 0.46) that was stronger than selection in Peninsula (i = 0.32), which is somewhat consistent with my findings. Furthermore, if Boshkung is in fact a GLP lake, then my results for Boshkung were consistent with GLP as an agent of selection on distal spine length (i.e. long distal spines and strong selection), but fish surveys are needed to assess the relative abundances of smelt and cisco. It is also possible that there is a newly introduced fish predator of *Bythotrephes* with a larger gape size than smelt in Boshkung that is not present in the other study lakes. A predator with a larger gape could impose selection for a longer distal spine length compared to the other study lakes, which would be consistent with my results (Fig. 4). Lastly, it is conceivable that the developmental rate of smelt differs among lakes, such that smelt gape size differs among lakes at any one time of the growing season. If, for example, smelt in Boshkung have a relatively advanced developmental rate (and thus had a larger gape when I sampled), and smelt in Peninsula have a relatively slower developmental rate (and thus had a smaller gape when I sampled), then this could explain the strong selection measured in Boshkung and lack of selection measured in Peninsula. However, the possibility of differing rates of development for smelt populations may also apply to the other GLP lakes, and thus may have confounded my selection results. In sum, my results suggest that these lakes may be more ecologically different

with respect to predation regime than initially hypothesized. With such variability, determining the influence of GLP on *Bythotrephes* distal spine length will require more replication of lakes.

Overall, my results strongly support the hypothesis that phenotypic differences among the study lakes are a result of plasticity, but the way in which plasticity causes these differences remains unclear. In general, there are two ways in which plasticity can result in the phenotypic differences among populations that I observed (Fig. 4). First, it is possible that the reaction norm for distal spine length is the same in all populations, and that phenotypic differences among populations are a result of differences in the presence or absence of an environmental trigger among lakes. Second, different populations may have evolved different reaction norms in response to spatial variation in selection. In this instance, the presence of a trigger could be similar in two populations that differed in distal spine length, but the population with the longer distal spine length would have a steeper reaction norm slope.

There are two environmental triggers that need to be considered as possible causes for the phenotypic differences among populations that I observed. First, as previously mentioned, *Bythotrephes* from Lake Michigan were previously found to produce offspring with longer distal spines in response to warmer water temperature, which may represent a proxy cue for seasonal changes in predation risk (Miehls et al. 2013). If the reaction norm for distal spine length is the same in all of the study lakes, then it is possible that phenotypic differences among lakes reflect differences in water temperature among lakes. Miehls et al. (2013) found that *Bythotrephes* from Lake Michigan reared at 15°C produced offspring with distal spines ~1.5 mm longer than those reared at 9°C. However, the water temperatures of the study lakes during sampling were between 20.5°C and 21.7°C (Table 1), so it is unlikely that such small temperature differences could account for the phenotypic differences that I observed. Furthermore, differences in water

temperature among lakes do not match the differences in distal spine length. For example, populations from Fairy and Mary had shorter mean distal spine lengths than Peninsula, but water temperature was cooler in Mary (21.2°C) and warmer in Fairy (21.7°C) compared to Peninsula (21.5°C). Additionally, inducing longer distal spines in response to warmer water in NGLP lakes would presumably serve no benefit with respect to fish predation, and *Bythotrephes* would likely be better off allocating resources elsewhere (e.g. clutch size). Thus, I would expect the slope of the reaction norm for distal spine length in response to temperature to be steeper is lakes dominated by GLP, compared to those dominated by NGLP. However, this notion is not supported by the long distal spines observed in Boshkung, which was classified as an NGLP lake and was relatively cool compared to the other study lakes (20.5°C). Therefore, plasticity in response to water temperature likely cannot explain the observed phenotypic differences among lakes. Second, Miehls et al. (2013) also found that *Bythotrephes* distal spine length did not change in response to kairomones from the gape-limited fish predator, yellow perch (*Perca flavescens*), so differences in the presence or concentration of fish kairomones among lakes is also an unlikely explanation for the observed phenotypic differences. It is possible that *Bythotrephes* only respond to specific fish kairomones, such as those from smelt or other fish species from Canadian Shield lakes, or that the reaction norm for distal spine length in response to fish kairomones differs among lakes, but these possibilities require further investigation.

The finding that the distal spine lengths of second-generation lab-born *Bythotrephes* converged on a smaller mean value for all lakes (Fig. 5) provides insight into the cause of the phenotypic differences that I observed among populations. For example, if populations from the six study lakes shared a common reaction norm for distal spine length, then this would indicate that the phenotypic differences among lakes are in response to a trigger that is unrelated to

chemical differences among lakes. This is because if a chemical cue was present at a higher concentration in populations with long mean distal spine lengths compared to populations with shorter mean distal spine length, then the common garden medium (which was an equal parts mixture of water from each lake) should have caused individuals from short-spined populations to express longer distal spines compared to wild-caught individuals, which was not observed. However, it is also possible that the cue was diluted to a point where it was no longer effective. One possibility is that lakes differ in the abundance of prey available to *Bythotrephes,* and mothers from populations with longer distal spines simply have more resources to invest into the development of distal spines in their offspring. However, this hypothesis neglects the importance of *Bythotrephes* tail spine as a defense against fish predators, and the fact that *Bythotrephes* interactions with fish should fundamentally differ in GLP lakes compared to NGLP lakes. Alternatively, if populations do not share a common reaction norm, then the convergence on a smaller mean distal spine length for all lakes indicates that the common garden conditions represent an environment in which the different reaction norms intersect (on a common value for distal spine length). All told, the next step for future research is to solidify the relationship between *Bythotrephes* distal spine length and fish predation in Canadian Shield lakes, which could be achieved by measuring distal spine lengths and selection throughout the growing season in GLP and NGLP lakes. Future work could then investigate alternative causes for the plastic response that I observed, and the possibility that there is variation in the reaction norm for distal spine length among *Bythotrephes* populations in Canadian Shield lakes.

In conclusion, this study has shown: (1) local differences in distal spine length among populations of *Bythotrephes* in Canadian Shield lakes; (2) differences in selection on distal spine length among lakes; (3) significant genetic and maternal variation for distal spine length; and (4)

that local differences among populations are not genetically based. Thus, I have demonstrated that phenotypic differences in a key trait involved in interspecific interactions are a result of phenotypic plasticity in response to the maternal environment, and not local adaptation, despite spatially variable selection on a heritable trait. Although this conclusion is interesting, it is not altogether surprising. In their native range, *Bythotrephes* are heavily preyed upon by a variety of fish species (Grigorovich et al. 1998), so it is plausible that phenotypic plasticity of distal spine length in response to predation risk is a pre-adaptation to the biotic heterogeneity of Canadian Shield lakes, but this remains to be tested. In order to spread across a new landscape (and subsequently become invasive), an exotic species must be able to perform well in new environmental conditions. Two well documented ways in which species are able to respond to variable environmental conditions are through local adaptation and phenotypic plasticity, yet we know little regarding the importance of these processes with respect to invasiveness. To address this knowledge gap, we must build a critical mass of empirical studies that investigate the cause of phenotypic differentiation for ecologically important traits among populations of invasive species. In this study, I have started to address this gap by demonstrating that phenotypic plasticity, and not local adaptation, causes phenotypic differentiation in distal spine length among populations of *Bythotrephes.* Furthermore, this finding highlights the potential importance of phenotypic plasticity as a mechanism of responding to environmental heterogeneity for invasive species, but a greater body of work is required to substantiate its prevalence. Lastly, I found strong evidence of evolutionary stasis (Merilä et al. 2001), which emphasizes that an evolutionary response may be constrained despite strong selection on a heritable trait.

5.0 Data Archiving Statement

Data for this study will be available at the Dryad Digital Repositor

6.0 References

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7.0 Tables

Table 1. Field information, dominant predation regime, and water temperature at the time of sampling for each study lake.

Lake	Sample Date	GPS Coordinates	Dominant Predation[1]	Surface Area (ha)[2]	Max Depth (m)[2]	Temp. (°C)
Peninsula	July 28, 2014	45°21.1'N, 79°06.1'W	GLP	864.8	34.1	21.5
Mary	July 29, 2014	45°13.5'N, 79°16.2'W	GLP	1065.4	56.4	21.2
Fairy	July 29, 2014	45°19.4'N, 79°11.3'W	GLP	711.5	69.5	21.7
Boshkung	July 30, 2014	45°03.1'N, 78°43.3'W	NGLP	715.8	71.0	20.5
Harp	July 28, 2014	45°22.5'N, 79°08.1'W	NGLP	71.4	37.5	21.0
Drag	July 30, 2014	45°05.2'N, 78°24.2'W	NGLP	1002.6	55.5	20.5

GLP = lake dominated by gape-limited predation (i.e. rainbow smelt); NGLP = lake dominated by non-gape-limited predation (i.e. cisco). Water temperature was taken 1 m below the surface immediately prior to sampling.

Sources: [1]S. J. Sandstrom and N. Lester, Ontario Ministry of Natural Resources, unpublished data.

[2]Hovius et al. (2006), Strecker et al. (2006), Young and Yan (2008).

Table 2. The mean distal spine length of wild-caught *Bythotrephes longimanus* did not differ between predation regimes (i.e. GLP vs. NGLP).

Fixed	*F*	df	*P*
Predation Regime	0.001	1,3	0.979
Random	σ^2	χ^2_1	*P*
Lake	0.06	37.3	< **0.001**

Results are reported for a linear mixed-effects model. Only the distal spine lengths of first instar individuals were included as the response variable. The significance of the lake random effect was assessed using a likelihood ratio test that compared the model to a model with the same fixed effect but with no random lake effect. *F* = *F*-statistic; df = degrees of freedom; *P* = *P*-value; σ^2 = among-lake variance; χ^2_1 = Chi-square value with 1 degree of freedom.

Table 3. Natural selection on *Bythotrephes longimanus* distal spine length measured by comparing wild-caught first instar animals to second instar animals for each population.

						95% CI of *S*			
Predation	Lake	*t*	df	*P*	*S*	Lower	Upper	SD	*i*
GLP	Peninsula	0.17	116	0.868	0.01	-0.09	0.11	0.28	0.03
	Mary	3.31	68	**0.002**	0.19	0.07	0.30	0.26	0.71
	Fairy	3.15	51	**0.003**	0.21	0.08	0.35	0.29	0.74
NGLP	Boshkung	3.33	28	**0.002**	0.28	0.11	0.45	0.34	0.82
	Harp	0.30	8	0.769	0.03	-0.21	0.27	0.24	0.13

Natural selection for Drag Lake could not be calculated because no first instar animals were collected. *t* and *P* are derived from t-tests comparing distal spine length of first and second instar animals. *t* = *t*-statistic; df = degrees of freedom; *P* = *P*-value; S = selection differential; CI = confidence interval; SD = pooled standard deviation for first and second instar individuals; *i* = standardized selection differential (i.e. selection intensity).

Table 4. Natural selection on *Bythotrephes longimanus* distal spine length did not differ between predation regimes (i.e. GLP vs. NGLP).

Fixed	*F*	df	*P*
Predation Regime	0.1	1,3	0.819
Instar	21.9	1,462	< **0.001**
Predation Regime-by-Instar	1.6	1,462	0.204

Random	σ^2	χ^2_1	*P*
Lake	0.08	129.9	< **0.001**

Results are reported for a linear mixed-effects model. Only the distal spine lengths of first and second instar individuals were included as the response variable. The significance of the lake random effect was assessed using a likelihood ratio test that compared the model to a model with the same fixed effects but with no random lake effect. *F* = *F*-statistic; df = degrees of freedom; *P* = *P*-value; σ^2 = among-lake variance; χ^2_1 = Chi-square value with 1 degree of freedom.

Table 5. The mean distal spine length of second-generation lab-born *Bythotrephes longimanus* did not differ among lakes or between predation regimes (i.e. GLP vs. NGLP).

*Lake Model: Distal Spine Length = Lake + (1|Line/Subline)**

Fixed	*F*	df	*P*
Lake	0.6	5,17	0.724
Random	σ^2	χ^2_1	*P*
Subline in Line	0.10	62.2	**< 0.001**
Line	0.05	22.2	< **0.001**

*Predation Regime Model: Distal Spine Length = Pred. Regime + (1|Lake/Line/Subline)**

Fixed	*F*	df	*P*
Predation Regime	0.5	1,4	0.511
Random	σ^2	χ^2_1	*P*
Subline in Line in Lake	0.10	61.8	**< 0.001**
Line in Lake	0.04	20.3	**< 0.001**
Lake	0	0.008	0.930

Results are reported for two linear mixed-effects models. Only the distal spine lengths of second-generation lab-born individuals were included as the response variable for both models. The significance of the random effects in each model were assessed using likelihood ratio tests that compared models with successively fewer random effects. *F* = *F*-statistic; df = degrees of freedom; *P* = *P*-value; σ^2 = variance; χ^2_1 = Chi-square value with 1 degree of freedom.

*Model format is: response variable = fixed effect + (1| nested random effects)

Table 6. Genetic (V_g), maternal (V_m), and environmental (V_e) variance components, broad-sense heritability (H^2), and maternal effects (m^2) for *Bythotrephes longimanus* distal spine length in Canadian Shield lakes.

		95% CI of σ^2			
Component	σ^2	Lower	Upper	H^2	m^2
V_g	0.039	0.008	0.192	0.238	0.612
V_m	0.099	0.050	0.197		
V_e	0.024	0.017	0.035		

All populations were combined for this analysis. Variance components were extracted from a linear mixed-effects model with only the intercept as a fixed effect. Only the distal spine lengths of second-generation lab-born individuals were included as the response variable. The variance in distal spine length among clonal lines is V_g; the variance among sublines within clonal lines is V_m; and the variance within sublines is V_e. Likelihood ratio tests indicate that genetic ($\chi^2_1 = 19.2$, $P < 0.001$) and maternal ($\chi^2_1 = 61.4$, $P < 0.001$) variance components were statistically significant. σ^2 = variance; CI = confidence interval.

8.0 Figures

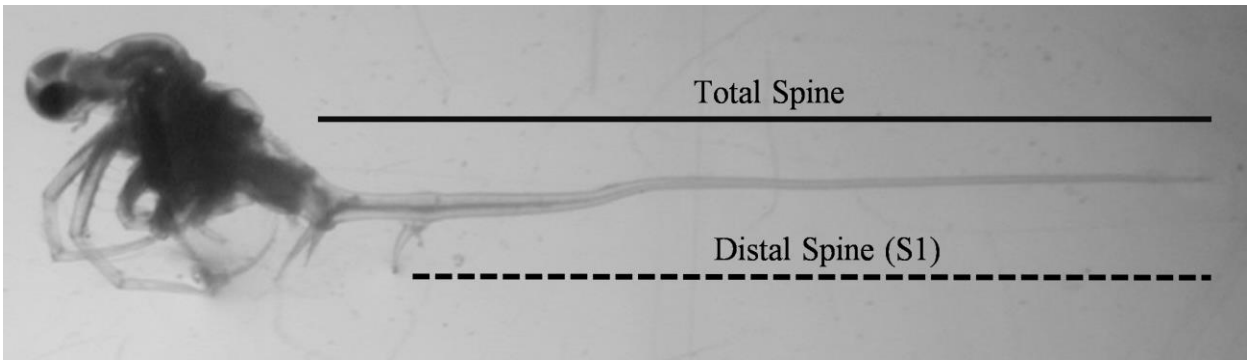

Figure 1. Photograph of *Bythotrephes longimanus.* The total tail spine (solid line) is composed of several segments. The distal spine segment (i.e. the section from the posterior tip of the spine to the first paired articular spines) is present at birth and does not grow. Total spine length increases during development through the production of additional spine segments at each instar molt. The photographed individual can be identified as a second instar animal because it has two spine segments separated by two pairs of articular spines.

Figure 2. Map of study lakes (Google My Maps 2016). Predation on *Bythotrephes longimanus* in Peninsula Lake (PL), Mary Lake (ML), and Fairy Lake (FL) is thought to be dominated by the gape-limited fish predator, rainbow smelt (dark markers), whereas predation in Boshkung Lake (BL), Harp Lake (HL), and Drag Lake (DL) is thought to be dominated by the non-gape-limited fish predator, cisco (light markers) (Strecker et al. 2006; Young and Yan 2008).

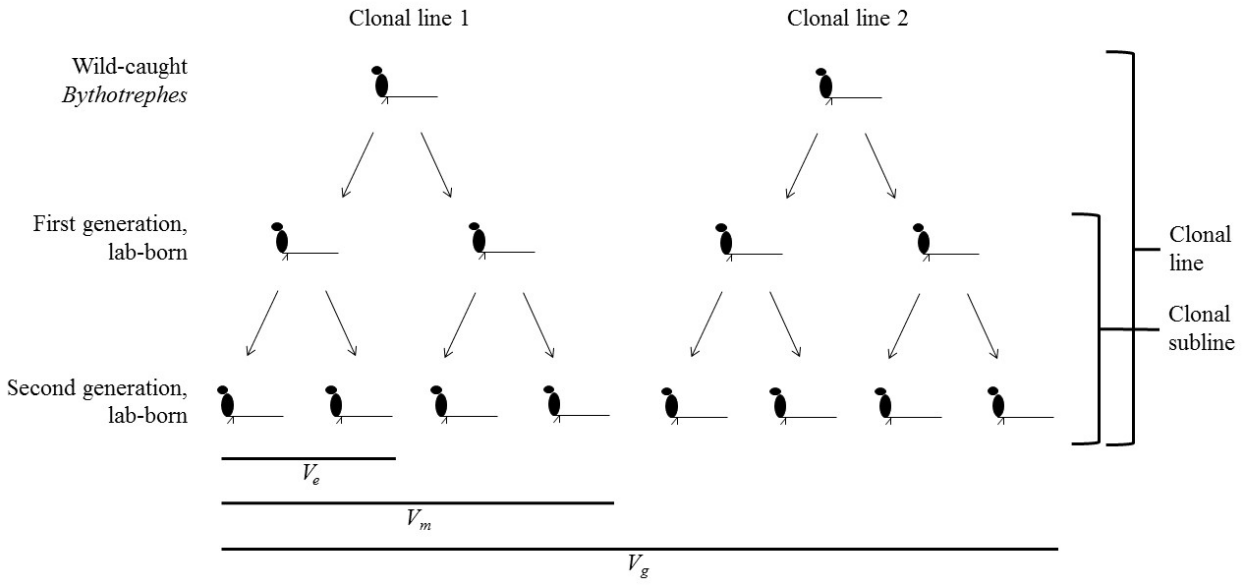

Figure 3. Schematic diagram of *Bythotrephes longimanus* clonal analysis design. Wild-caught individuals were used to initiate clonal lines. All offspring from wild-caught individuals (first generation lab-born) were used to initiate clonal sublines. Distal spine length measurements of second-generation lab-born animals were analyzed to estimate variance components and to determine if populations were genetically differentiated. The variance in distal spine length among clonal lines represents the genetic variance (V_g), the variance among sublines within clonal lines represents maternal variance (V_m), and the variance among individuals within clonal sublines is the environmental variance (V_e). This figure is modified from Lynch and Walsh (1998) and Miehls et al. (2012).

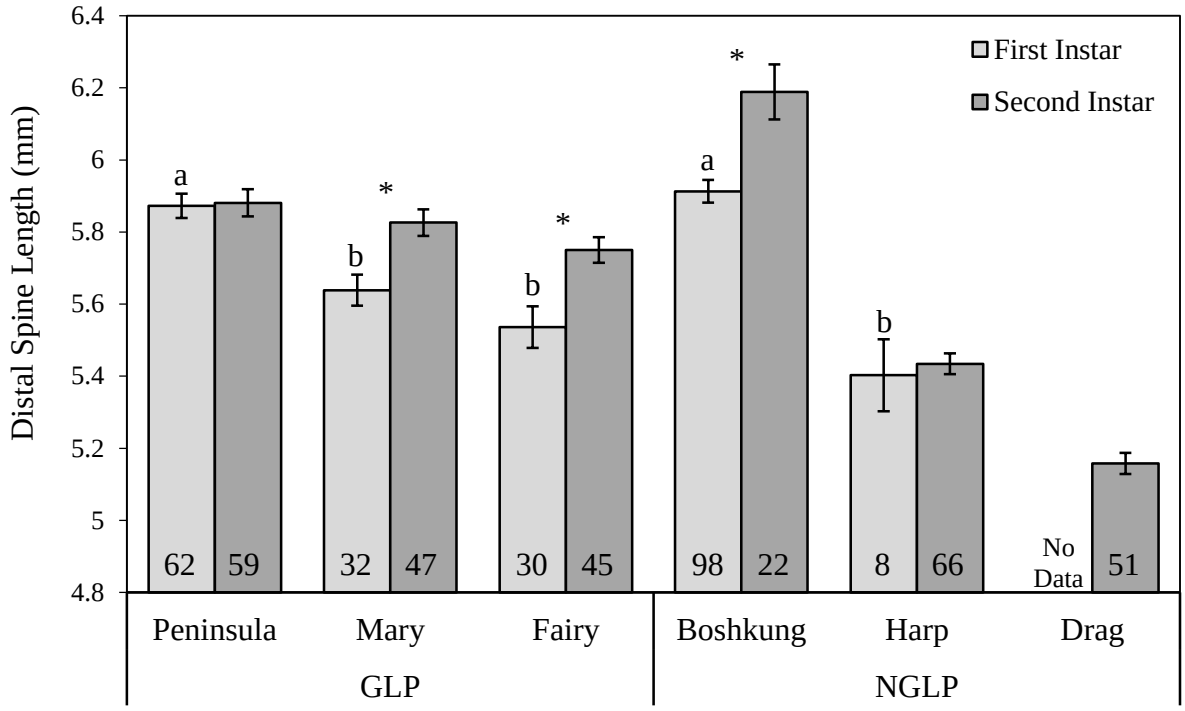

Figure 4. Mean distal spine length of first and second instar wild-caught *Bythotrephes longimanus* for all study lakes. Mean distal spine lengths for first instar animals with different letters were significantly different from one another (Tukey HSD: $P < 0.003$). The difference in mean distal spine length between first and second instars represents the selection differential for that lake. Asterisks represent significant directional selection for increased distal spine length for that lake (Welch *t*-tests: $P < 0.003$; Table 3). No first instar animals were collected from Drag Lake. The number in each bar represents the sample size for that lake. Error bars represent ± 1 standard error.

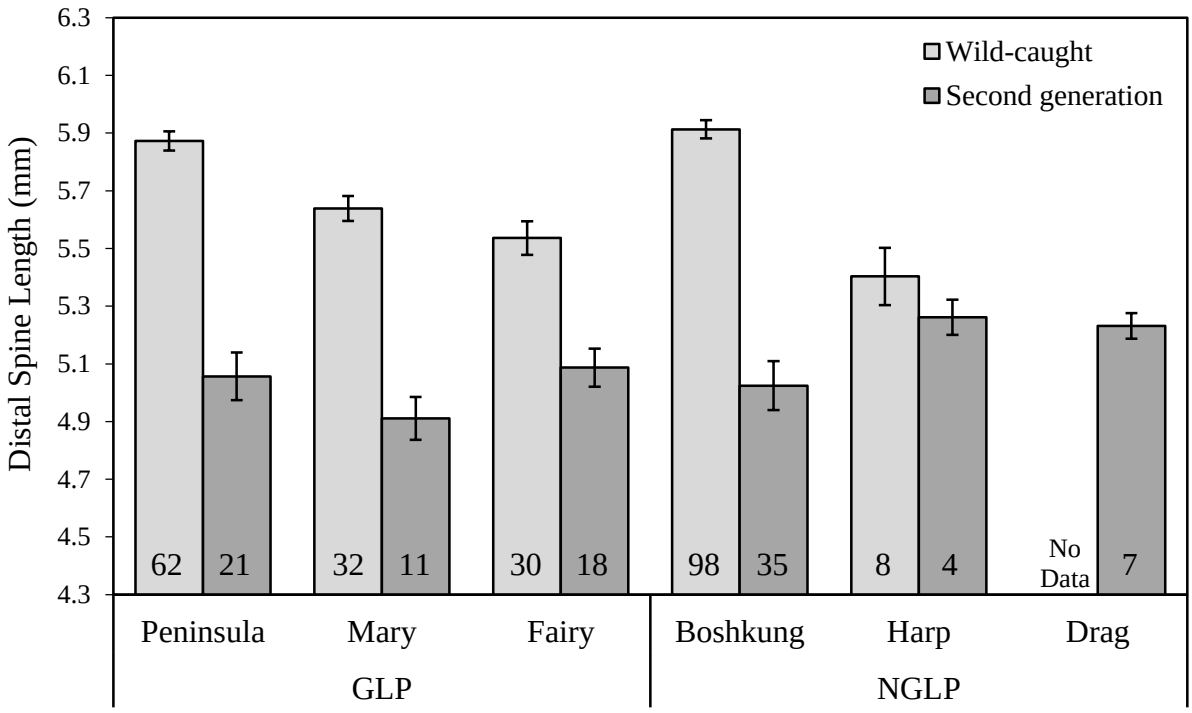

Figure 5. Mean distal spine length of wild-caught *Bythotrephes longimanus* and second-generation lab-born *Bythotrephes* for all study lakes. The mean distal spine length of second-generation lab-born individuals does not significantly differ among lakes (LME: $P = 0.724$; Table 5). Wild-caught animals were all first instar individuals so differences among lakes are not confounded by selection. The number in each bar represents the sample size for that lake. Error bars represent ± 1 standard error.

9.0 Supplementary Material

Table S1. Surviving number of *Bythotrephes longimanus* clonal lines and individuals in each generation of the common garden experiment.

			Clonal lines		Individuals	
Predation	Lake	Wild-caught	First generation	Second generation	First generation	Second generation
GLP	Peninsula	37	10	5	19	25
	Mary	32	12	3	33	14
	Fairy	31	10	6	22	20
NGLP	Boshkung	29	12	4	49	37
	Harp	31	11	3	20	7
	Drag	28	8	2	27	8

Wild-caught animals were collected from each of the study lakes and used to initiate clonal lines. First generation animals were the offspring of wild-caught individuals and were used to initiate clonal sublines. Second generation animals were the offspring of first generation individuals.

Table S2. Genetic (V_g), maternal (V_m), and environmental (V_e) variance components, broad-sense heritability (H^2), and maternal effects (m^2) for *Bythotrephes longimanus* distal spine length in Canadian Shield lakes, where variance components were extracted from a model that included lake as a fixed effect.

		95% CI of σ^2			
Component	σ^2	Lower	Upper	H^2	m^2
V_g	0.051	0.011	0.225	0.288	0.574
V_m	0.101	0.050	0.204		
V_e	0.024	0.017	0.035		

All populations were combined for this analysis. Only the distal spine lengths of second-generation lab-born individuals were included as the response variable. The variance in distal spine length among clonal lines is V_g; the variance among sublines within clonal lines is V_m; and the variance within sublines is V_e. Likelihood ratio tests indicate that genetic and maternal variance components were statistically significant ($P < 0.001$). σ^2 = variance; CI = confidence interval.