

1 **Local differentiation in the defensive morphology of an invasive zooplankton**

2 **species is not genetically based**

3

4 Giuseppe E. Fiorino^{1*} and Andrew G. McAdam¹

5

6 ¹ Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

7 * Corresponding Author (email: gefiorino@gmail.com; phone: 226-268-3993)

8 **Abstract**

9 Evolutionary changes in functional traits represent one possible reason why exotic species spread
10 to become invasive, but empirical studies of the mechanisms driving phenotypic differentiation
11 between populations of invasive species are rare. This study tested whether differences in distal
12 spine length among populations of the invasive cladoceran, *Bythotrephes longimanus*, could be
13 explained by local adaptation or phenotypic plasticity. We collected *Bythotrephes* from six lakes
14 and found that distal spine lengths and natural selection on distal spine length differed among
15 populations, but were unrelated to the gape-limitation of the dominant fish predator in the lake
16 from which they were collected. A common garden experiment revealed significant genetic and
17 maternal variation for distal spine length, but phenotypic differences among populations were
18 not genetically based. Phenotypic differences among lakes in this ecologically important trait are,
19 therefore, the result of plasticity and not local adaptation, despite spatially variable selection on
20 this heritable trait. The ability of *Bythotrephes* to plastically adjust distal spine length may
21 explain the success of this species at invading lake ecosystems with diverse biotic environments.

22

23 **Keywords:** *Bythotrephes longimanus*, Canadian Shield lakes, cladoceran, common garden
24 experiment, invasive species, local adaptation, phenotypic differentiation, phenotypic plasticity.

25 **Introduction**

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

35 The spread of an exotic species depends on its ability to perform well in new biotic and
36 abiotic conditions (Shea and Chesson 2002; Facon et al. 2006). One way in which this could be
37 achieved is through local adaptation (Lee 2002; Parker et al. 2003; Lambrinos 2004; Facon et al.
38 2006), which is the process whereby divergent natural selection (i.e. selection that differs among
39 habitats) causes populations to become genetically differentiated (Kawecki and Ebert 2004).
40 Natural selection might be particularly strong in exotic species because species introduced into a
41 foreign environment often encounter new resources, competitors, or predators (Mooney and
42 Cleland 2001; Lambrinos 2004). Additionally, for populations to locally adapt, there must be
43 sufficient genetic variation underlying the traits experiencing divergent selection (Lynch and
44 Walsh 1998). For exotic species, invasions characterized by large founder populations or a large
45 number of founder events are expected to have high genetic variance, whereas invasions
46 characterized by small founder populations or a small number of founder events are expected to

47 have reduced genetic variance, which may constrain local adaptation (Allendorf and Lundquist
48 2003; Lockwood et al. 2005).

49 Local adaptation results in phenotypic differentiation between populations, but the
50 presence of such differentiation is not sufficient to demonstrate that populations are locally
51 adapted. Phenotypic plasticity is the ability of a genotype to produce alternate phenotypes based
52 on environmental conditions (Pigliucci 2005), and represents an alternative mechanism by which
53 exotic species can adaptively respond to heterogeneity in their environment. Plasticity can,
54 therefore, produce a pattern of phenotypic differentiation among populations that is consistent
55 with that of local adaptation, but without the underlying genetic differences (Kawecki and Ebert
56 2004). Additionally, because plasticity allows different genotypes to produce the same
57 phenotype, it can reduce the strength of selection, which might constrain local adaptation
58 (Pfennig et al. 2010). Alternatively, plasticity can allow organisms to cope with new
59 environments where they might not otherwise persist, which can result in novel selection
60 pressures that subsequently cause local adaptation (West-Eberhard 2003).

61 Despite the potential importance of local adaptation and plasticity to invasiveness (Parker
62 et al. 2003), little is known about their relative importance with respect to the spread of exotic
63 species. Most previous work has focused on invasive plants, where limited studies suggest that
64 local adaptation and phenotypic plasticity are not mutually exclusive. For example, Si et al.
65 (2014) found evidence for local adaptation and phenotypic plasticity of several growth
66 characteristics that contributed to the successful invasion of *Wedelia trilobata* across a tropical
67 island. Similarly, Godoy et al. (2011) found that local adaptation and phenotypic plasticity were
68 both involved in the successful invasion of the heavily shaded understory of South American
69 evergreen temperate rainforest by *Prunella vulgaris* (also see: Parker et al. 2003). Dybdahl and

70 Kane (2005) provided a rare example outside of plants, in which North American populations of
71 invasive freshwater snails (*Potamopyrgus antipodarum*) were found to show phenotypic
72 plasticity (but not local adaptation) for life history and growth traits that facilitated their spread.
73 In order to better understand the general mechanisms by which exotic species spread (and hence,
74 become invasive), further empirical studies of the mechanisms driving phenotypic differentiation
75 between populations of invasive species for ecologically important traits are needed.

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

93 found to have 17% longer distal spines compared to lakes dominated by NGLP (Miehls et al.
94 2014). These findings suggest that local adaptation might explain the observed phenotypic
95 differentiation among populations of *Bythotrephes*. However, cladocerans are commonly
96 phenotypically plastic, especially for traits involved in predator defense. For example, the
97 cladoceran *Daphnia lumholtzi* produces neonates with longer head spines when exposed to fish
98 predator kairomones (Dzialowski et al. 2003) (also see: Lüning 1992). This maternal induction
99 of offspring phenotypes in response to the maternal environment is called a maternal effect
100 (Mousseau and Fox 1998). It has also been shown that *Bythotrephes* from Lake Michigan induce
101 longer distal spines in offspring in response to warmer water temperature (but not fish
102 kairomones), which may act as an indirect cue for natural selection associated with GLP (Miehls
103 et al. 2013). It is thus plausible that local differences in distal spine length among populations of
104 *Bythotrephes* could be explained by either local adaptation in response to GLP or maternal
105 induction of longer distal spines in offspring in response to an environmental cue associated with
106 GLP.

107 In this study, we first measured *Bythotrephes* distal spine lengths and the strength of
108 natural selection on distal spine length in six Canadian Shield lakes that differed in the presence
109 or absence of GLP. We then conducted a common garden experiment (Kawecki and Ebert 2004)
110 to evaluate the hypotheses of local adaptation and phenotypic plasticity as causes of phenotypic
111 differences among these populations. Using clonal lines (Lynch and Walsh 1998), we reared
112 individuals from the six study lakes in identical conditions for two generations. We measured
113 genetic and maternal variation for distal spine length to determine broad-sense heritability for the
114 trait and maternal effects, and determined whether phenotypic differences in distal spine length
115 among populations were genetically based. If phenotypic differences among populations were

116 due to local adaptation (i.e. genetically based differentiation), we predicted that phenotypic
117 differences in distal spine length among populations would be maintained in second-generation
118 *Bythotrephes* reared in a common garden environment. Alternatively, if phenotypic differences
119 among populations were due to plasticity, we predicted that phenotypic differences among
120 populations would no longer be present in second generation *Bythotrephes*.

121 **Material and Methods**

122 *Study Species*

123 *Bythotrephes* is a predatory cladoceran zooplankter with a widespread native distribution
124 throughout the Palearctic region (Therriault et al. 2002; Colautti et al. 2005; Kim and Yan 2013),
125 which can tolerate a wide range of pH, salinity, temperature, and conductivity (Grigorovich et al.
126 1998). *Bythotrephes* was first identified in the Laurentian Great Lakes in the early 1980s
127 (Johannsson et al. 1991), and has since spread to more than 160 inland Ontario lakes, and lakes
128 in the mid-western USA (Kelly et al. 2013). In the Canadian Shield, *Bythotrephes* can be found
129 in lakes dominated by gape-limited predators, such as rainbow smelt (*Osmerus mordax*), or non-
130 gape limited predators, such as cisco (*Coregonus artedi*) (Strecker et al. 2006; Young and Yan
131 2008).

132 The *Bythotrephes* caudal process (i.e. tail spine) consists of segments, the longest of
133 which is the distal spine (i.e. the section from the posterior tip of the spine to the first paired
134 articular spines; Fig. 1), which is present at birth and does not change in length with development
135 (Burkhardt 1994). Thus, the length of the distal spine cannot respond plastically to the
136 environment directly experienced by offspring, but it can be maternally induced (Miehls et al.
137 2013). Only the distal spine is present in neonates (i.e. the first instar stage), but total spine
138 length increases through development when an additional spine segment is added to the base of

139 the spine at each instar molt (Branstrator 2005). These segments are each separated by paired
140 articular spines (Fig. 1), which allow the instar stage to be identified and the length of each
141 segment to be measured separately (Yurista 1992). Like most cladocerans, *Bythotrephes* have a
142 cyclically parthenogenetic life cycle, reproducing apomictically (i.e. clonally) multiple times
143 before reproducing sexually at the end of the growing season (Yurista 1992; Branstrator 2005).
144 Apomictic reproduction produces eggs that immediately develop into young in the brood pouch,
145 whereas sexual reproduction results in resting eggs that overwinter on the lake bottom before
146 hatching the following spring (Yurista 1992; Branstrator 2005).

147 *Study Lakes*

148 *Bythotrephes* were collected during the middle of the growing season (July 29-31, 2014)
149 from six lakes in the Muskoka district and County of Haliburton in south-central Ontario
150 (Supplementary Material, Fig. S1). Predation on *Bythotrephes* in three of the lakes (Peninsula,
151 Mary, and Fairy; hereafter, GLP lakes) is thought to be dominated by the gape-limited fish
152 predator, rainbow smelt, while in the three other lakes (Boshkung, Harp, and Drag; hereafter,
153 NGLP lakes) predation is thought to be dominated by the non-gape-limited fish predator, cisco
154 (Strecker et al. 2006; Young and Yan 2008; S. J. Sandstrom and N. Lester, unpublished data).
155 Although rainbow smelt are present in Boshkung Lake (Young and Yan 2008), cisco have been
156 reported to be more abundant and were considered to be the dominant *Bythotrephes* predator
157 (Strecker et al. 2006; Miehl et al. 2014), so Boshkung was classified as a NGLP lake.

158 These lakes were chosen because they were invaded by *Bythotrephes* over the last 30
159 years and are characteristic of many lakes in the Canadian Shield that are dominated by rainbow
160 smelt or cisco (Strecker et al. 2006; Young and Yan 2008). They are similar to one another
161 physically (in terms of depth and water temperature) and biologically (in terms of invertebrate

162 predators, and other fish predators of *Bythotrephes*) (Hovius et al. 2006; Strecker et al. 2006;
163 Young and Yan 2008). Although Harp Lake is much smaller than the other lakes, several studies
164 have found that it is ecologically similar (Hovius et al. 2006; Strecker et al. 2006; Young and
165 Yan 2008). Five of these six lakes (excluding Drag) were previously used by Miehl et al. (2014)
166 to test for the effects of GLP on natural selection and local differences in distal spine length.
167 Miehl et al. (2014) sampled an additional lake (Kashagawigamog, NGLP) but sample sizes
168 were too low to measure natural selection. We sampled Drag Lake (NGLP) instead of
169 Kashagawigamog to balance the experimental design.

170 *Sample Collection*

171 *Bythotrephes* were collected using a conical zooplankton net with a 0.5 m diameter
172 opening and 363 μm mesh size. To measure distal spine lengths and natural selection for each
173 lake, *Bythotrephes* were collected with the net horizontally towed at a depth of 10-15 m, and
174 approximately 100 individuals were haphazardly chosen and immediately preserved in 95%
175 ethanol. For the common garden experiment, live *Bythotrephes* were collected using a vertical
176 net tow (instead of a horizontal tow) through the top 15 m of the water column, and 30-40
177 actively swimming individuals without pigmented brood pouches were individually isolated in
178 60 mL jars containing 50 mL of lake water filtered through a 63 μm sieve. A vertical net tow was
179 used to collect individuals for the common garden experiment because it minimizes damage to
180 the animals associated with turbulence, whereas a horizontal tow was used to collect individuals
181 to measure phenotypic differences and selection because animals could be collected in greater
182 quantity. All collection methods were based on those reported by Kim and Yan (2010) and
183 Miehl et al. (2014).

184 *Common Garden Experiment*

185 We used a common garden experiment and clonal breeding design (Fig. 2) to measure
186 genetic and maternal variation for distal spine length, and to determine whether phenotypic
187 differences among populations were genetically based. During the three-day collection period,
188 all live *Bythotrephes* were maintained at the Dorset Environment Science Center (DESC, Dorset,
189 Ontario) in a climate controlled facility (20°C, 14L:10D photoperiod) in lake water filtered
190 through a 63 µm sieve from their “home” lake. Afterwards, the cultures were moved to an
191 environmental chamber at the Hagen Aqualab (University of Guelph, Guelph, Ontario) under the
192 aforementioned temperature and photoperiod and introduced to the common garden medium.
193 The medium was an autoclaved mixture of lake water filtered through a 63 µm sieve from the six
194 sampled lakes (i.e. each *Bythotrephes* was reared in water that was 1/6th of their local
195 environment). *Bythotrephes* received daily water changes and were fed *ad lib* with
196 approximately 150 *Artemia* sp. nauplii that were less than 30 h old (Miehls et al. 2012).

197 Clonal lines were initiated using 188 wild-caught individuals (28-37 per lake), and were
198 reared in the common garden through two apomictic generations (Fig. 2; Miehls et al. 2012,
199 2013). Once a female produced offspring she was preserved in 95% ethanol within 24 h. All
200 offspring were individually transferred to 60 mL jars containing 50 mL of common garden
201 medium, also within 24 h (Miehls et al. 2012). Of the 188 clonal lines, 12.2% produced second-
202 generation lab-born offspring (7-37 individuals per lake; Supplementary Material, Table S1).

203 *Measurement*

204 All *Bythotrephes* were photographed using a digital camera mounted to a dissecting
205 microscope (Leica MZ8, Leica Microsystems). IMAGEJ software (Abramoff et al. 2004) was
206 used to measure the length of the distal spine segment from the tip of the tail spine to the first

207 paired articular spines (Fig. 1) to the nearest 0.001 mm. Instar was assessed by counting the
208 number of paired articular spines on the total tail spine (Fig. 1).

209 ***Comparing Distal Spine Length among Natural Populations***

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

225 ***Measuring Natural Selection***

226 Natural selection was quantified by comparing distal spine lengths between first and
227 second instar *Bythotrephes*. Because *Bythotrephes* distal spine length does not change with
228 development (Burkhardt 1994), a change in mean distal spine length from the first to the second
229 instar stage represents the relationship between distal spine length and survival between first and

230 second instar stages, and not developmentally based differences. Consequently, the magnitude
231 and direction of the difference represents the strength and direction of natural selection (Miehls
232 et al. 2014). Although *Bythotrephes* develops into a third or fourth instar stage, the comparison
233 between the first two stages was assessed because the distal spine represents the entire length of
234 the spine in first instar individuals, which was the expected target of selection (Miehls et al.
235 2014).

236 We calculated selection differentials (Falconer and Mackay 1996) for each population as
237 the difference between the mean distal spine lengths for the first two instar stages (Miehls et al.
238 2014). The statistical significance of these selection differentials for each population was
239 assessed using Welch *t*-tests (two-tailed). In these analyses, a statistically longer mean distal
240 spine for second instar individuals compared to first instar individuals would indicate significant
241 selection for longer distal spines in that lake. Additionally, to compare to other published
242 estimates of selection, standardized selection differentials (i.e. selection intensities) were
243 calculated by dividing the selection differential for a lake by the standard deviation of distal
244 spine length for first and second instar animals from that lake (Miehls et al. 2014).

245 To statistically test whether selection on distal spine length differed among lakes, we
246 used two-way ANOVA with distal spine length of wild-caught first and second instar animals as
247 the response variable, lake and instar as predictors, and a lake-by-instar interaction. In this
248 model, a significant effect of instar would indicate that there was selection on distal spine length
249 irrespective of lake; a significant effect of lake would indicate that distal length differs by lake
250 irrespective of selection; and a significant lake-by-instar interaction would indicate that selection
251 differs among lakes. To determine whether selection varied consistently with predation regime,
252 we fitted a LME model with distal spine length of wild-caught, first and second instar animals as

253 the response variable, predation regime, instar, and a predation regime-by-instar interaction as
254 fixed effects, and lake as a random effect. In this model, a significant effect of instar would
255 indicate that there was selection on distal spine length irrespective of predation regime; a
256 significant effect of predation regime would indicate that distal length differs by predation
257 regime irrespective of selection; and a significant predation regime-by-instar interaction would
258 indicate that selection depended on the gape limitation of the dominant fish predator.

259 ***Determining Genetically Based Differences among Populations***

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

268 To statistically determine whether phenotypic differences in distal spine length among
269 lakes were genetically based, we fitted a LME model with distal spine length of second-
270 generation individuals as the response variable, lake as a fixed effect, and clonal subline nested
271 within clonal line as random effects. In this model, a nonsignificant effect of lake would indicate
272 that distal spine length was not genetically differentiated among lakes, which would be
273 consistent with a phenotypic plasticity hypothesis. Alternatively, a significant effect of lake
274 would indicate that local differences in distal spine length were genetically based, and could thus
275 reflect local adaptation in response to GLP.

276 *Estimating Broad-sense Heritability and Maternal Effects*

277 The clonal breeding design that we used (Fig. 2) allowed for the quantification of genetic
278 (V_G), maternal (V_M), and environmental (V_E) variance components for distal spine length (Lynch
279 and Walsh 1998; Miehl et al. 2012). We fitted a LME model with distal spine length of second-
280 generation lab-born individuals as the response variable, the intercept as the only fixed effect,
281 and clonal subline nested within clonal line as random effects. In an additional model, lake was
282 included as a fixed effect but this did not alter our conclusions (Supplementary Material, Table
283 S2). In this breeding design, the variance in distal spine length among clonal lines estimates
284 genetic variance, the variance among sublines within clonal lines estimates maternal variance,
285 and the variance within sublines estimates environmental variance. Note, because *Bythotrephes*
286 distal spine length is fixed from birth, the variance within sublines (i.e. environmental variance)
287 must be due to small scale environmental differences within the brood pouch of the mother
288 during development. Similarly, variance in distal spine length among sublines (i.e. maternal
289 variance) could be confounded by environmental differences within the brood pouch of the
290 grandmother during development of the mothers, or subtle differences experienced by the
291 mothers in the lab. To assess the significance of the variance components, we obtained 95%
292 confidence intervals around the random effects (Pinheiro and Bates 2000) and conducted model
293 comparisons using likelihood ratio tests (see *Assessing the Significance of Random Effects*)
294 (Miehl et al. 2014). We calculated broad-sense heritability (H^2) as the ratio of among-line
295 variance to the total phenotypic variance (i.e. the sum of the among-line, among-subline, and
296 within-subline variances), and calculated maternal effects (m^2) as the ratio of among-subline
297 variance to the total phenotypic variance.

298 *Assessing the Significance of Random Effects*

299 For all LME models, the statistical significance of the random effects was assessed
300 through model comparisons using likelihood ratio tests in which the change in the log-likelihood
301 between the more complex model and the simpler model was assumed to follow a chi-squared
302 distribution where the degrees of freedom were equal to the difference in the number of
303 parameters between the more complex and simpler models ($df = 1$ in all cases here). For models
304 with one random effect, we fitted one additional model with the same fixed effects but without
305 the random effect. The significance of the random effect was assessed by comparing these two
306 models. For nested models (i.e. models with multiple nested random effects), we fitted additional
307 models with the same fixed effects but with successively fewer random effects, starting with the
308 removal of the most nested random effect. The significance of a random effect was assessed by
309 comparing the model that included the random effect of interest to the simpler model without
310 that random effect. All statistical analyses were conducted using R version 3.2.2 (R Core Team
311 2015). The statistical assumptions of homoscedasticity and normality were met for all models.

312 **Results**

313 *Mean Distal Spine Lengths of Natural Populations*

314 The mean distal spine length of all wild-caught, first instar individuals was 5.80 ± 0.02
315 mm (mean \pm SE). Mean distal spine length differed among lakes (ANOVA: $F_{4,225} = 17.1$, $P <$
316 0.001), but did not differ by predation regime (Table 2; Fig. 3). Specifically, populations in
317 Peninsula Lake (GLP) and Boshkung Lake (NGLP) had significantly longer distal spines than
318 populations in Mary Lake (GLP), Fairy Lake (GLP) and Harp Lake (NGLP) (Tukey HSD: $P <$
319 0.003). There was no difference in mean distal spine length between the Peninsula and Boshkung

320 populations (Tukey HSD: $P = 0.913$) and no differences among the Mary, Fairy, and Harp
321 populations (Tukey HSD: $P > 0.244$). No first instar animals were collected from Drag Lake.

322 *Natural Selection*

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

334 *Common Garden Experiment*

335 The mean distal spine length of second-generation individuals was 5.06 ± 0.04 mm
336 (mean \pm SE), approximately 87% of the mean length observed in wild-caught individuals. Mean
337 distal spine length of second-generation lab-born individuals did not differ among lakes (Table 5;
338 Fig. 4). There was, however, significant genetic variation in *Bythotrephes* distal spine length,
339 corresponding to a H^2 estimate of 0.24. Likewise, there was significant maternal variation in
340 distal spine length, corresponding to a m^2 estimate of 0.61 (Table 6).

341 Discussion

342 The goal of this study was to determine whether phenotypic differences in distal spine
343 length among populations of *Bythotrephes* in Canadian Shield lakes could be explained by local
344 adaptation or phenotypic plasticity. We found that *Bythotrephes* from two study lakes (Peninsula
345 and Boshkung) had long mean distal spine lengths compared to those from three other study
346 lakes (Mary, Fairy, and Harp); however, the mean distal spine lengths of second-generation lab-
347 born individuals reared in a common environment did not differ among populations (Fig. 4).
348 Differences in distal spine length among populations were, therefore, a result of phenotypic
349 plasticity in response to the maternal environment, and not local adaptation, despite spatially
350 variable selection on this heritable trait.

351 The absence of genetically based differences in distal spine length among *Bythotrephes*
352 populations was surprising because natural selection varied among populations. Specifically, we
353 identified significant selection for longer distal spines in two of three GLP lakes (Mary and
354 Fairy, but not Peninsula) and one of two NGLP lakes (Boshkung, but not Harp). A recent review
355 of selection in wild populations identified that the median magnitude of directional selection
356 (measured as the absolute value of standardized linear selection gradients) for survival was 0.08
357 (Kingsolver and Diamond 2011). Selection on *Bythotrephes* distal spine length that we measured
358 in the three lakes with significant selection (Mary: $i = 0.71$; Fairy: $i = 0.74$; Boshkung: $i = 0.82$;
359 Table 3) was thus very strong, falling within the top 10% of previously reported estimates
360 (Kingsolver et al. 2001). Although Kingsolver and Diamond's (2011) review reported selection
361 using standardized selection gradients and we reported standardized selection differentials, these
362 selection metrics have been found to often be similar in magnitude (Kingsolver and Diamond
363 2011). Miehl et al. (2014) also found strong selection for increased distal spine length in

364 *Bythotrephes* in Mary Lake ($i = 0.79$) and Fairy Lake ($i = 0.53$) in the summer of 2008,
365 suggesting that selection on *Bythotrephes* distal spine length in these lakes has been consistently
366 strong.

367 Directional selection for increased distal spine length (which was observed in three of
368 five lakes) should cause an evolutionary response if the trait is heritable (Falconer and Mackay
369 1996). We found significant genetic and maternal variation for *Bythotrephes* distal spine length,
370 corresponding to a moderate broad-sense heritability and large maternal effect (Table 6;
371 Mousseau and Roff 1987), which are the first such estimates for *Bythotrephes* in Canadian
372 Shield lakes. Our estimates of genetic variation and heritability for *Bythotrephes* distal spine
373 length were very similar to previous estimates in Lake Michigan in July ($V_G = 0.06$, $H^2 = 0.27$;
374 Miehl et al. 2012), indicating that most of the genetic variation in distal spine length has been
375 maintained since *Bythotrephes* invasion from the Laurentian Great Lakes, and that the spread of
376 *Bythotrephes* has not limited their potential for adaptive evolution. Despite this adaptive
377 potential, and significant differences in selection among lakes, we found no evidence of genetic
378 differentiation for distal spine length among populations of *Bythotrephes*. Previous work on
379 *Bythotrephes* used historic and contemporary wild-caught animals and remnant distal spines
380 retrieved from sediment cores to test for a response to selection on distal spine length since
381 *Bythotrephes* invasion of Lake Michigan, and found little evidence of phenotypic change through
382 time (Miehl et al. 2015). Together, our results and those of Miehl et al. (2015) provide clear
383 examples of selection on a heritable trait not leading to an evolutionary response temporally (in
384 Lake Michigan) or spatially (in these Canadian Shield lakes).

385 There are several reasons why selection on a heritable trait may not cause evolutionary
386 change (i.e. evolutionary stasis; Merilä et al. 2001). For example, temporal fluctuations in

387 selection can influence the direction and strength of selection overall (Siepielski et al. 2009; Bell
388 2010; Kingsolver and Diamond 2011). In GLP lakes, predation risk for *Bythotrephes* increases
389 through the growing season because juvenile gape-limited fish grow from sizes too small to
390 consume any *Bythotrephes* to sizes that can consume some *Bythotrephes* depending on gape-size
391 (Straile and Halbich 2000; Branstrator 2005; Pothoven et al 2012; Miehl et al. 2015). Our study
392 only looked at a single snapshot of selection for each study lake, but previous work on
393 *Bythotrephes* in Lake Michigan found strong temporal variation in selection within a growing
394 season, which reduced net selection (Miehl et al. 2015). Selection might also fluctuate across
395 years. Miehl et al. (2014) found significant selection for increased distal spine length in
396 Peninsula, Mary, and Fairy (GLP lakes; $i = 0.20-0.79$) in 2008, but no selection in Boshkung and
397 Harp (NGLP lakes) in 2008, whereas we found significant selection in Mary, Fairy, and
398 Boshkung ($i = 0.71-0.82$; Table 3), but no selection in Peninsula and Harp in 2014.

399 It is also possible that *Bythotrephes* experience a tradeoff between components of fitness
400 (i.e. survival vs. fecundity; Roff 2002) or that selection varies among life stages (Schluter et al.
401 1991) such that our selection estimates based on a fitness component (i.e. survival between first
402 and second instar) does not represent overall selection. Previous work suggested that
403 *Bythotrephes* exhibit a tradeoff between clutch size and offspring distal spine length (i.e. females
404 that produced offspring with longer distal spines had smaller clutches; Straile and Halbich 2000;
405 Pothoven et al. 2003; Miehl et al. 2013), which means that viability selection favouring longer
406 distal spines could be opposed by fecundity selection favouring shorter distal spines. Lastly, we
407 measured selection between first and second instar individuals, but selection on later instar stages
408 is likely to occur on the length of the total spine, rather than just the distal spine (Fig. 1), which
409 might reduce the strength of selection on distal spine length if selection on the total spine is

410 weaker or in the opposite direction (see Miehl et al. 2015 for further discussion of potential
411 causes of stasis in *Bythotrephes*).

412 In contrast with previous findings (i.e. Miehl et al. 2014), the differences among lakes in
413 mean distal spine length and selection that we observed were inconsistent with GLP as an agent
414 of selection. In particular, our results from two lakes did not match our expectations. First, the
415 population in Peninsula Lake (GLP) had a long mean distal spine length, but weak selection
416 (Table 3; Fig. 3). It is possible that individuals from Peninsula did not experience selection
417 because distal spine lengths were already long enough to provide defense against GLP.
418 Comparing GLP lakes, individuals from Fairy had the shortest mean distal spines (5.54 mm) but
419 the strongest selection ($i = 0.74$), whereas individuals from Peninsula had the longest mean distal
420 spines (5.87 mm) but the weakest selection ($i = 0.03$; Fig. 3; see also Miehl et al. 2014). These
421 results suggest the possibility of a threshold distal spine length that provides refuge from GLP,
422 which is consistent with “hard” natural selection (Wallace 1975).

423 The second unexpected finding was that the *Bythotrephes* in Boshkung Lake had long
424 distal spines and experienced strong selection, despite Boshkung being classified as a NGLP lake
425 (Table 3; Fig. 3). The most obvious explanation for this finding is that Boshkung may no longer
426 be dominated by NGLP. Rainbow smelt (the dominant gape-limited fish predator) were
427 previously found to be present in Boshkung (Young and Yan 2008), but cisco (the dominant non-
428 gape-limited fish predator) were thought to be the dominant predator of *Bythotrephes* (Table 1;
429 Strecker et al. 2006; Miehl et al. 2014). Unfortunately, there has not been a recent fish survey of
430 Boshkung to provide further insights into whether the strong selection measured in this study
431 could be explained by an increase in the abundance of smelt relative to cisco since the last
432 survey. Interestingly, Miehl et al. (2014) also found that the *Bythotrephes* population in

433 Boshkung had a longer mean distal spine length compared to Harp (a NGLP lake with no smelt),
434 but their primary analysis yielded no evidence of selection. However, an alternative analysis
435 found the occurrence of reasonably strong (though statistically insignificant) selection ($i = 0.46$)
436 that was stronger than selection in Peninsula ($i = 0.32$), which is somewhat consistent with our
437 findings. More recent fish surveys in Boshkung Lake will be needed to determine whether
438 Boshkung is in fact a GLP lake, and more replication of NGLP and GLP lakes will be needed to
439 assess the importance of gape-limited predation as an agent of selection on *Bythotrephes* distal
440 tail spine length.

441 Overall, our results strongly support the hypothesis that phenotypic differences among
442 *Bythotrephes* populations in these study lakes are a result of plasticity, but the way in which
443 plasticity causes these differences remains unclear. In general, there are two ways in which
444 plasticity can result in the phenotypic differences among populations that we observed (Fig. 3).
445 First, it is possible that the reaction norm for distal spine length is the same in all populations,
446 and that phenotypic differences among populations are a result of differences in the level of an
447 environmental cue among lakes (Fig. 5a). Alternatively, phenotypic differences among
448 populations of *Bythotrephes* could have resulted from the evolution of different reaction norm
449 slopes among lakes in response to spatial variation in selection (Fig. 5b).

450 The environmental cue that caused the plastic response that we observed is also unclear.
451 Our finding that the mean distal spine length of common garden *Bythotrephes* was smaller than
452 that of wild caught *Bythotrephes* for all lakes (Fig. 4) suggests that the cue was not present in the
453 water that we collected to create the common garden medium. Had the cue been present in the
454 common medium, we would have expected the phenotypes from lab-reared *Bythotrephes* to
455 converge on an intermediate phenotype (Fig. 5a), or to maintain phenotypic differences among

456 lake populations as observed in wild-caught animals (Fig. 5b). Miehls et al. (2013) found that
457 *Bythotrephes* from Lake Michigan induce longer tail spines in their offspring in response to
458 warmer water temperature, and the water temperature in our common environment (20°C) was
459 slightly lower than lake temperatures at the time of collection (range = 20.5°C to 21.7°C; Table
460 1). However, the magnitude of the differences in water temperature among lakes were quite
461 small (Table 1), and these temperature differences were inconsistent with phenotypic differences
462 in distal spine length among lakes: Fairy and Mary had shorter mean distal spine lengths than
463 Peninsula, but water temperature was cooler in Mary (21.2°C) and warmer in Fairy (21.7°C)
464 compared to Peninsula (21.5°C). Miehls et al. (2013) also found that *Bythotrephes* distal spine
465 length did not change in response to kairomones from yellow perch (*Perca flavescens*), so it is
466 unlikely that differences in the presence or concentration of fish kairomones among Canadian
467 Shield lakes caused the observed phenotypic differences. It is possible that *Bythotrephes* respond
468 to kairomones of specific fish species (as opposed to fish kairomones in general) and that this
469 cue somehow degraded in the lab, but this cue would need to be specific to gape-limited
470 predators for which the induction of longer distal spines would be beneficial, and not a generic
471 cue of predation risk (Miehls et al. 2013). Clearly, further work is needed to identify the causes
472 of phenotypic differences in distal spine lengths among lakes. This would involve identifying the
473 environmental variable causing plastic responses in distal spine lengths as well as the degree to
474 which this plasticity varies among lakes.

475 In conclusion, we have demonstrated that phenotypic differences in a key trait involved
476 in interspecific interactions are a result of phenotypic plasticity and not local adaptation, despite
477 spatially variable selection on a heritable trait. This evolutionary stasis (Merilä et al. 2001) serves
478 as a reminder that adaptation cannot be inferred from phenotypic divergence even when this

479 divergence is consistent with natural selection. Furthermore, this finding highlights the potential
480 importance of phenotypic plasticity as a mechanism by which invasive species might respond to
481 environmental heterogeneity (e.g. Dybdahl and Kane 2005). General lessons about the
482 importance of local adaptation and phenotypic plasticity to the spread of exotic species, however,
483 require further explicit tests of their relative importance across a wider range of taxa.

484 **Acknowledgements**

485 We thank Teresa Crease and Beren Robinson for insightful comments, and for providing field
486 and lab equipment. The Dorset Environmental Science Centre (DESC) provided a climate
487 controlled facility. James Rusak (DESC) and Matt Cornish (Hagen Aqualab) assisted with lab
488 set-up. Andrea Miehl helped with *Bythotrephes* collection and culturing protocols. Guang
489 Zhang assisted with field work. Emily De Freitas, Evan McKenzie, Kaileigh Watson, Katelyn
490 Cross, Kirsten Bradford, Marissa Skinner, Mary Paquet, Meera Navaratnam, Ronena Wolach,
491 and Yu Jin Song assisted with data collection. This work was funded by an Ontario Ministry of
492 Research and Innovation Early Researcher Award to Andrew McAdam, a NSERC Discovery
493 Grant to Andrew McAdam, and an Ontario Graduate Scholarship to Giuseppe Fiorino.

494 **Literature Cited**

- 495 Abramoff, M. D., P. J. Magelhaes, and S. J. Ram. 2004. Image processing with ImageJ.
496 Biophotonics International **11**:36–42.
- 497 Allendorf, F. W., and L. L. Lundquist. 2003. Introduction: population biology, evolution, and
498 control of invasive species. Conservation Biology **17**:24–30.
- 499 Barnhisel, D. R. 1991a. The caudal appendage of the cladoceran *Bythotrephes cederstroemi* as
500 defense against young fish. Journal of Plankton Research **13**:529–537.
- 501 Barnhisel, D. R. 1991b. Zooplankton spine induces aversion in small fish predators. Oecologia
502 **88**:444–450.
- 503 Bell, G. 2010. Fluctuating selection: the perpetual renewal of adaptation in variable
504 environments. Philosophical Transactions of the Royal Society B **365**:87–97.
- 505 Branstrator, D. K. 2005. Contrasting life histories of the predatory cladocerans *Leptodora kindtii*
506 and *Bythotrephes longimanus*. Journal of Plankton Research **27**:569–585.
- 507 Bunnell, D. B., B. M. Davis, D. M. Warner, M. A. Chriscinske, and E. F. Roseman. 2011.
508 Planktivory in the changing Lake Huron zooplankton community: *Bythotrephes* consumption
509 exceeds that of *Mysis* and fish. Freshwater Biology **56**:1281–1296.
- 510 Burkhardt, S. 1994. Seasonal size variation in the predatory cladoceran *Bythotrephes*
511 *cederstroemii* in Lake Michigan. Freshwater Biology **31**:97–108.
- 512 Colautti, R. I., M. Manca, M. Viljanen, H. A. M. Ketelaars, H. Bürgi, H. J. Macisaac, and D. D.
513 Heath. 2005. Invasion genetics of the Eurasian spiny waterflea: evidence for bottlenecks and
514 gene flow using microsatellites. Molecular Ecology **14**:1869–1879.
- 515 Day, T., P. A. Abrams, and J. M. Chase. 2002. The role of size-specific predation in the
516 evolution and diversification of prey life histories. Evolution **56**:877–887.
- 517 Drake, J. A., H. A. Mooney, F. di Castri, R. H. Groves, F. J. Kruger, M. Rejmanek, and M.
518 Williamson. 1989. Biological Invasions: A Global Perspective. John Wiley and Sons, New York,
519 NY.
- 520 Dybdahl, M. F., and S. L. Kane. 2005. Adaptation vs. phenotypic plasticity in the success of a
521 clonal invader. Ecology **86**:1592–1601.
- 522 Dzialowski, A. R., J. T. Lennon, W. J. O'Brien, and V. H. Smith. 2003. Predator-induced
523 phenotypic plasticity in the exotic cladoceran *Daphnia lumholtzi*. Freshwater Biology **48**:1593–
524 1602.

- 525 Facon, B., B. J. Genton, J. Shykoff, P. Jarne, A. Estoup, and P. David. 2006. A general eco-
526 evolutionary framework for understanding bioinvasions. *Trends in Ecology & Evolution* **21**:130–
527 135.
- 528 Falconer, D. S., and T. F. C. Mackay. 1996. *Introduction to Quantitative Genetics*, 4th edn.
529 Longman, Essex.
- 530 Godoy, O., A. Saldana, N. Fuentes, F. Valladares, and E. Gianoli. 2011. Forests are not immune
531 to plant invasions: Phenotypic plasticity and local adaptation allow *Prunella vulgaris* to colonize
532 a temperate evergreen rainforest. *Biological Invasions* **13**:1615–1625.
- 533 Grigorovich, I. A., O. V Pashkova, Y. F. Gromova, and C. D. A. van Overdijk. 1998.
534 *Bythotrephes longimanus* in the Commonwealth of Independent States: variability, distribution
535 and ecology. *Hydrobiologia* **379**:183–198.
- 536 Hovius, J. T., B. E. Beisner, and K. S. McCann. 2006. Epilimnetic rotifer community responses
537 to *Bythotrephes longimanus* invasion in Canadian Shield lakes. *Limnology and Oceanography*
538 **51**:1004–1012.
- 539 Johannsson, O. E., E. L. Mills, and R. O’Gorman. 1991. Changes in the nearshore and offshore
540 zooplankton communities in Lake Ontario: 1981-88. *Canadian Journal of Fisheries and Aquatic*
541 *Sciences* **48**:1546–1557.
- 542 Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology Letters*
543 **7**:1225–1241.
- 544 Kelly, N. E., N. D. Yan, B. Walseng, and D. O. Hessen. 2013. Differential short- and long-term
545 effects of an invertebrate predator on zooplankton communities in invaded and native lakes.
546 *Diversity and Distributions* **19**:396–410.
- 547 Kim, N., and N. D. Yan. 2013. Food limitation impacts life history of the predatory cladoceran
548 *Bythotrephes longimanus*, an invader to North America. *Hydrobiologia* **715**:213–224.
- 549 Kim, N., and N. D. Yan. 2010. Methods for rearing the invasive zooplankter *Bythotrephes* in the
550 laboratory. *Limnology and Oceanography: Methods* **8**:552–561.
- 551 Kingsolver, J. G., and S. E. Diamond. 2011. Phenotypic selection in natural populations: what
552 limits directional selection? *American Naturalist* **177**:346–357.
- 553 Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A.
554 Hoang, P. Gilbert, and P. Beerli. 2001. The strength of phenotypic selection in natural
555 populations. *The American Naturalist* **157**:245–261.
- 556 Lambrinos, J. G. 2004. How interactions between ecology and evolution influence contemporary
557 invasion dynamics. *Ecology* **85**:2061–2070.

- 558 Abdi, H., and L. J. Williams. 2010. Tukey's honestly significant difference (HSD) test. In N. J.
559 Salkind, ed. Encyclopedia of Research Methods. Sage, Thousand Oaks, CA.
- 560 Lee, C. E. 2002. Evolutionary genetics of invasive species. Trends in Ecology & Evolution
561 **17**:386–391.
- 562 Lockwood, J. L., P. Cassey, and T. Blackburn. 2005. The role of propagule pressure in
563 explaining species invasions. Trends in Ecology & Evolution **20**:223–228.
- 564 Lünig, J. 1992. Phenotypic plasticity of *Daphnia pulex* in the presence of invertebrate
565 predators: morphological and life history responses. Oecologia **92**:383–390.
- 566 Lynch M., and B. Walsh. 1998. Genetics and Analysis of Quantitative Traits. Sinauer,
567 Sunderland, MA.
- 568 Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000.
569 Biotic invasions: causes, epidemiology, global consequences, and control. Ecological
570 Applications **10**:689–710.
- 571 Merilä, J., B. C. Sheldon, and L. E. B. Kruuk. 2001. Explaining stasis: Microevolutionary studies
572 in natural populations. Genetica **112-113**:199–222.
- 573 Miehls, A. L. J., A. G. McAdam, P. E. Bourdeau, and S. D. Peacor. 2013. Plastic response to a
574 proxy cue of predation risk when direct cues are unreliable. Ecology **94**:2237–2248.
- 575 Miehls, A. L. J., S. D. Peacor, and A. G. McAdam. 2014. Gape-Limited Predators As Agents of
576 Selection on the Defensive Morphology of an Invasive Invertebrate. Evolution **68**:2633–2643.
- 577 Miehls, A. L. J., S. D. Peacor, and A. G. McAdam. 2012. Genetic and maternal effects on tail
578 spine and body length in the invasive spiny water flea (*Bythotrephes longimanus*). Evolutionary
579 Applications **5**:306–316.
- 580 Miehls, A. L. J., S. D. Peacor, L. Valliant, and A. G. McAdam. 2015. Evolutionary stasis despite
581 selection on a heritable trait in an invasive zooplankton. Journal of Evolutionary Biology
582 **28**:1091–1102.
- 583 Mooney, H. A., and E. E. Cleland. 2001. The evolutionary impact of invasive species.
584 Proceedings of the National Academy of Sciences of the United States of America **98**:5446–
585 5451.
- 586 Mousseau, T. A., and C. W. Fox. 1998. The adaptive significance of maternal effects. Trends in
587 Ecology & Evolution **13**:403–407.
- 588 Mousseau, T. A., and D. A. Roff. 1987. Natural selection and the heritability of fitness
589 components. Heredity **59**:181–197.

- 590 Novak, S. J. 2007. The role of evolution in the invasion process. *Proceedings of the National*
591 *Academy of Sciences of the United States of America* **104**:3671–3672.
- 592 Parker, I. M., J. Rodriguez, and M. E. Loik. 2003. An evolutionary approach to understanding
593 the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum*
594 *thapsus*. *Conservation Biology* **17**:59–72.
- 595 Pfennig, D. W., M. A. Wund, E. C. Snell-Rood, T. Cruickshank, C. D. Schlichting, and A. P.
596 Moczek. 2010. Phenotypic plasticity's impacts on diversification and speciation. *Trends in*
597 *Ecology & Evolution* **25**:459–467.
- 598 Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? *Trends in*
599 *Ecology & Evolution* **20**:481–486.
- 600 Pinheiro, J. C, and D. M. Bates. 2000. *Mixed-Effects Models in S and S-PLUS*. Springer-Verlag,
601 New York, NY.
- 602 Pinheiro, J. C., D. M. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2015. nlme: Linear and
603 Nonlinear Mixed Effects Models. R package version 3.1–121.
- 604 Pothoven, S.A., G. L. Fahnenstiel, and H. A. Vanderploeg. 2003. Population characteristics of
605 *Bythotrephes* in Lake Michigan. *Journal of Great Lakes Research* **29**:145–156.
- 606 Pothoven, S. A., H. A. Vanderploeg, D. M. Warner, J. S. Schaeffer, S. A. Ludsin, R. M.
607 Claramunt, and T. F. Nalepa. 2012. Influences on *Bythotrephes longimanus* life-history
608 characteristics in the Great Lakes. *Journal of Great Lakes Research* **38**:134–141.
- 609 Pothoven, S. A., H. A. Vanderploeg, J. F. Cavaletto, D. M. Krueger, D. M. Mason, and S. B.
610 Brandt. 2007. Alewife planktivory controls the abundance of two invasive predatory cladocerans
611 in Lake Michigan. *Freshwater Biology* **52**:561–573.
- 612 R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for
613 Statistical Computing, Vienna, Austria.
- 614 Roff, D. 2002. *Life History Evolution*. Sinauer Associates, Sunderland, MA.
- 615 Shea, K., and P. Chesson. 2002. Community ecology theory as a framework for biological
616 invasions. *Trends in Ecology & Evolution* **17**:170–176.
- 617 Schluter, D., T. D. Price, and L. Rowe. 1991. Conflicting selection pressures and life history
618 trade-offs. *Proceedings of the Royal Society of London B* **246**:11–17.
- 619 Si, C.-C., Z.-C. Dai, Y. Lin, S.-S. Qi, P. Huang, S.-L. Miao, and D.-L. Du. 2014. Local
620 adaptation and phenotypic plasticity both occurred in *Wedelia trilobata* invasion across a tropical
621 island. *Biological Invasions* **16**:2323–2337.

- 622 Siepielski, A.M., J. D. DiBattista, and S. M. Carlson. 2009. It's about time: the temporal
623 dynamics of phenotypic selection in the wild. *Ecology Letters* **12**:1261–1276.
- 624 Straile, D., and A. Halbich. 2000. Life History and Multiple Antipredator Defenses of an
625 Invertebrate Pelagic Predator, *Bythotrephes longimanus*. *Ecology* **81**:150–163.
- 626 Strecker, A. L., S. E. Arnott, N. D. Yan, and R. Girard. 2006. Variation in the response of
627 crustacean zooplankton species richness and composition to the invasive predator *Bythotrephes*
628 *longimanus*. *Canadian Journal of Fisheries and Aquatic Sciences* **63**:2126–2136.
- 629 Therriault, T. W., I. A. Grigorovich, M. E. Cristescu, H. A. M. Ketelaars, M. Viljanen, D. D.
630 Heath, and H. J. Macisaac. 2002. Taxonomic resolution of the genus *Bythotrephes* Leydig using
631 molecular markers and re-evaluation of its global distribution. *Diversity and Distributions* **8**:67–
632 84.
- 633 Urban, M. C. 2007. Predator size and phenology shape prey survival in temporary ponds.
634 *Oecologia* **154**:571–580.
- 635 Urban, M. C. 2008. Salamander evolution across a latitudinal cline in gape limited predation
636 risk. *Oikos* **117**:1037–1049.
- 637 Vitousek, P. M., C. M. D'Antonio, L. L. Loope, and R. Westbrooks. 1996. Biological Invasions
638 as Global Environmental Change. *American Scientist* **84**:468–478.
- 639 Wallace, B. 1975. Hard and Soft Selection Revisited. *Evolution* **29**:465–473.
- 640 West-Eberhard, M. J. 2003. *Developmental Plasticity and Evolution*. Oxford University Press,
641 Oxford.
- 642 Young, J. D., and N. D. Yan. 2008. Modification of the diel vertical migration of *Bythotrephes*
643 *longimanus* by the cold-water planktivore, *Coregonus artedii*. *Freshwater Biology* **53**:981–995.
- 644 Yurista, P. M. 1992. Embryonic and postembryonic development in *Bythotrephes cederstoemii*.
645 *Canadian Journal of Fisheries and Aquatic Sciences* **49**:1118–1125.

646 **Tables**

Table 1. Field information, dominant predation regime, and water temperature at the time of sampling for each lake from which *Bythotrephes longimanus* were sampled.

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

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

647

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

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

Results are reported for a linear mixed-effects model in which 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.

648

Table 3. Natural selection on *Bythotrephes longimanus* distal spine length in lakes where fish predation was dominated by gape-limited predation (GLP) or non-gape-limited predators (NGLP).

Predation	Lake	t	df	P	S	95% CI of S		SD	i
						Lower	Upper		
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

Selection was measured as the difference in mean distal spine length between second instar animals and first instar animals for each population. 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).

649

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 in which 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.

650

Table 5. The mean distal spine length of second-generation lab-born *Bythotrephes longimanus* did not differ among lakes.

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

Results are reported for a linear mixed-effects model in which only the distal spine lengths of second-generation lab-born individuals were included as the response variable. The significance of the random effects was assessed using likelihood ratio tests that compared models with successively fewer random effects. $F = F$ -statistic; df = degrees of freedom; $P = P$ -value; $\sigma^2 =$ variance; $\chi^2_1 =$ Chi-square value with 1 degree of freedom.

651

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 from which *Bythotrephes* were sampled.

Component	σ^2	95% CI of σ^2		H^2	m^2
		Lower	Upper		
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		

Variance components were estimated from second-generation lab-born offspring in a common garden clonal analysis where the variance in distal spine length among clonal lines is the genetic variance (V_G), the variance among sublines within clonal lines is the maternal variance (V_M), and the variance within sublines is the environmental variance (V_E). Variance components were extracted from a linear mixed-effects model with the intercept as the only fixed effect. Likelihood ratio tests indicated that the variance in distal spine length among clonal lines ($\chi^2_1 = 19.2$, $P < 0.001$) and the variance among sublines within lines ($\chi^2_1 = 61.4$, $P < 0.001$) were statistically significant. $\sigma^2 =$ variance; CI = confidence interval.

652

653 **Figure Legends**

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

660 **Figure 2.** Schematic diagram of *Bythotrephes longimanus* clonal analysis design. Wild-caught
661 individuals were used to initiate clonal lines. All offspring from wild-caught individuals (first
662 generation lab-born) were used to initiate clonal sublines. Distal spine length measurements of
663 second-generation lab-born animals were analyzed to estimate variance components and to
664 determine if populations were genetically differentiated. The variance in distal spine length
665 among clonal lines represents the genetic variance (V_G), the variance among sublines within
666 clonal lines represents maternal variance (V_M), and the variance among individuals within clonal
667 sublines is the environmental variance (V_E). This figure is modified from Lynch and Walsh
668 (1998) and Miehl et al. (2012).

669 **Figure 3.** Mean distal spine length of first and second instar wild-caught *Bythotrephes*
670 *longimanus* for all study lakes. Mean distal spine lengths for first instar animals labelled with
671 different letters were significantly different from one another (Tukey HSD: $P < 0.003$). The
672 difference in mean distal spine length between first and second instars represents the selection
673 differential for that lake. Asterisks represent significant directional selection for increased distal
674 spine length for that lake (Welch t -tests: $P < 0.003$; Table 3). No first instar animals were

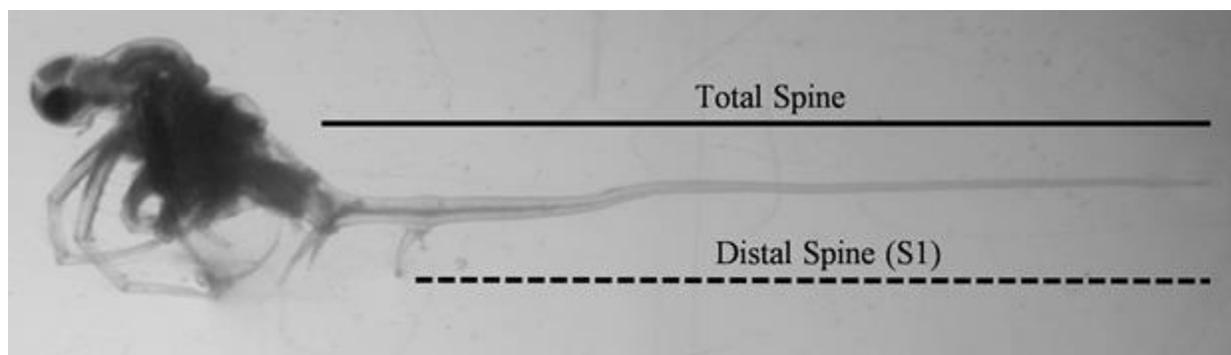
675 collected from Drag Lake. The number in each bar represents the sample size for that lake. Error
676 bars represent ± 1 standard error.

677 **Figure 4.** Mean distal spine length of wild-caught *Bythotrephes longimanus* and second-
678 generation lab-born *Bythotrephes* for all study lakes. The mean distal spine length of second-
679 generation lab-born individuals did not significantly differ among lakes (Table 5). Wild-caught
680 animals were all first instar individuals so differences among lakes were not confounded by
681 selection. The number in each bar represents the sample size for that lake. Error bars represent \pm
682 1 standard error.

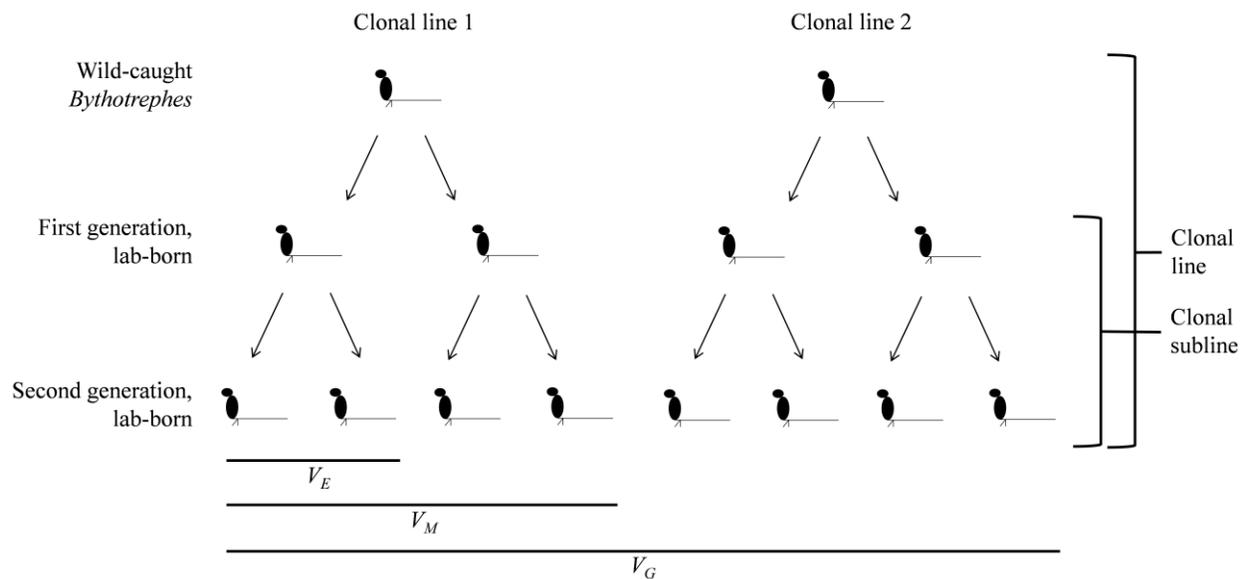
683 **Figure 5.** (A) Hypothetical reaction norm for *Bythotrephes longimanus* in Canadian Shield lakes
684 if the reaction norm for distal spine length is the same in all study populations. The “lab” value is
685 the mean distal spine length for second-generation lab-born individuals pooled across lakes. The
686 white triangle represents the mean phenotype that would be expected for lab-reared *Bythotrephes*
687 if the environmental cue for plasticity was present in the common garden medium. (B)
688 Hypothetical reaction norms for *Bythotrephes longimanus* populations from each study lake if
689 populations evolved different reaction norm slopes in response to spatial variation in selection.

690 **Figures**

691 **Figure 1**

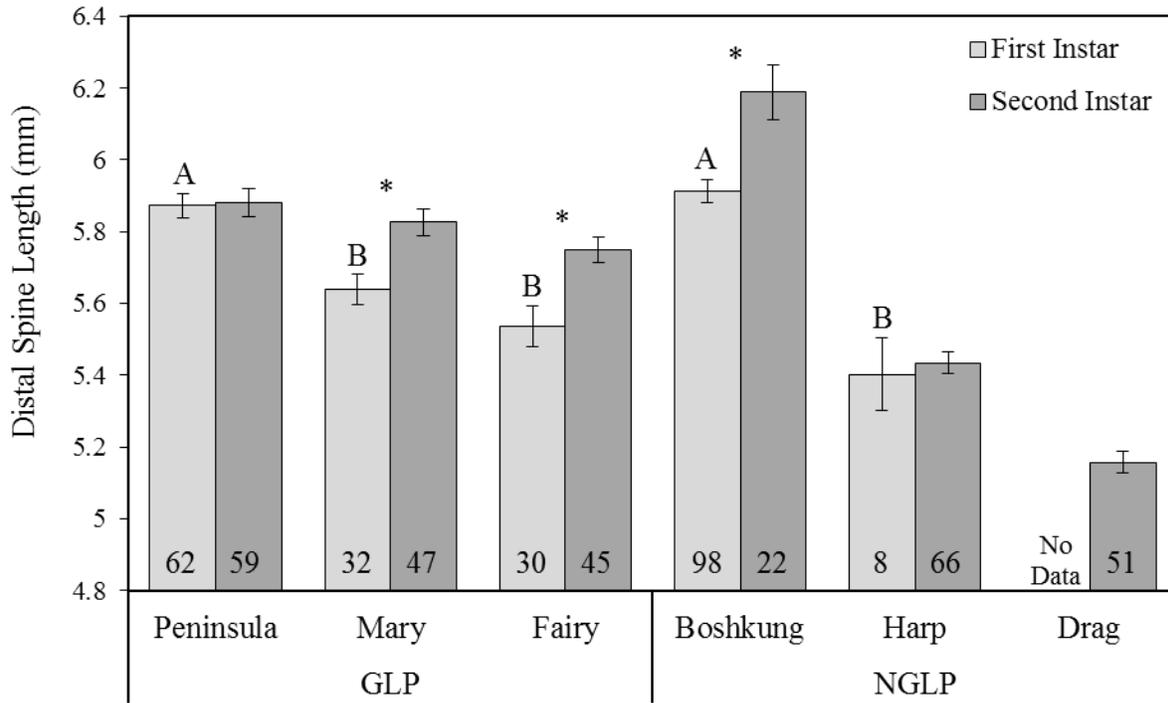


693 **Figure 2**



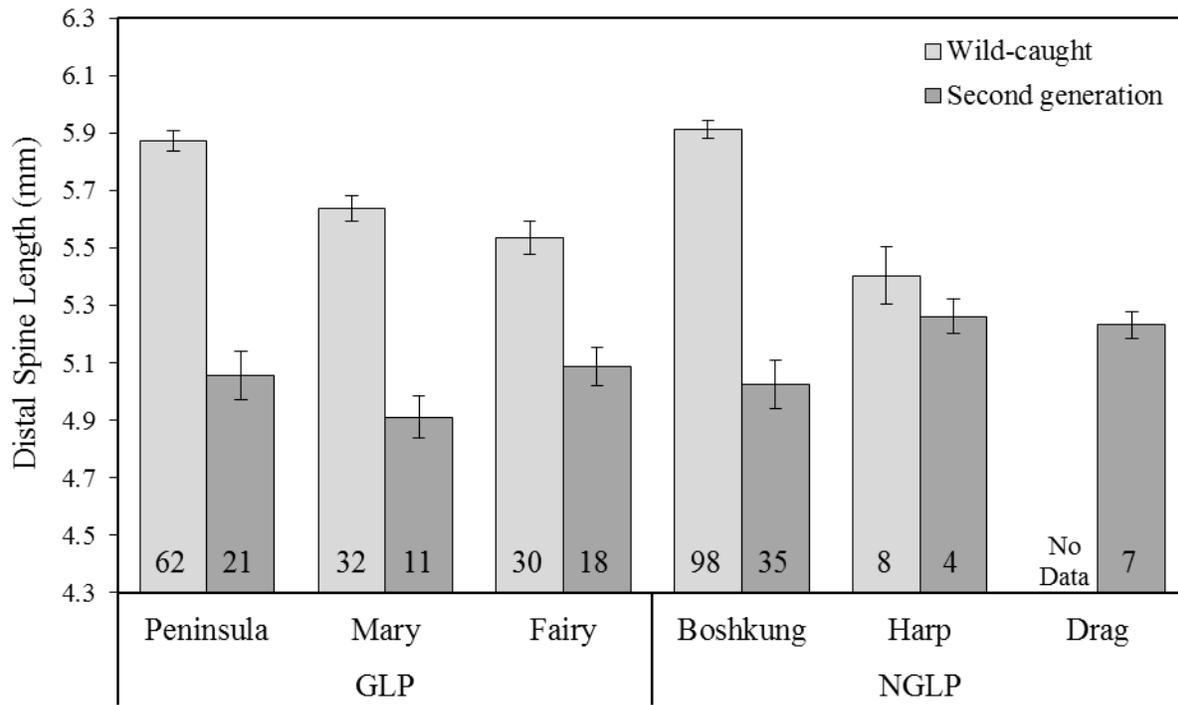
694

695 **Figure 3**



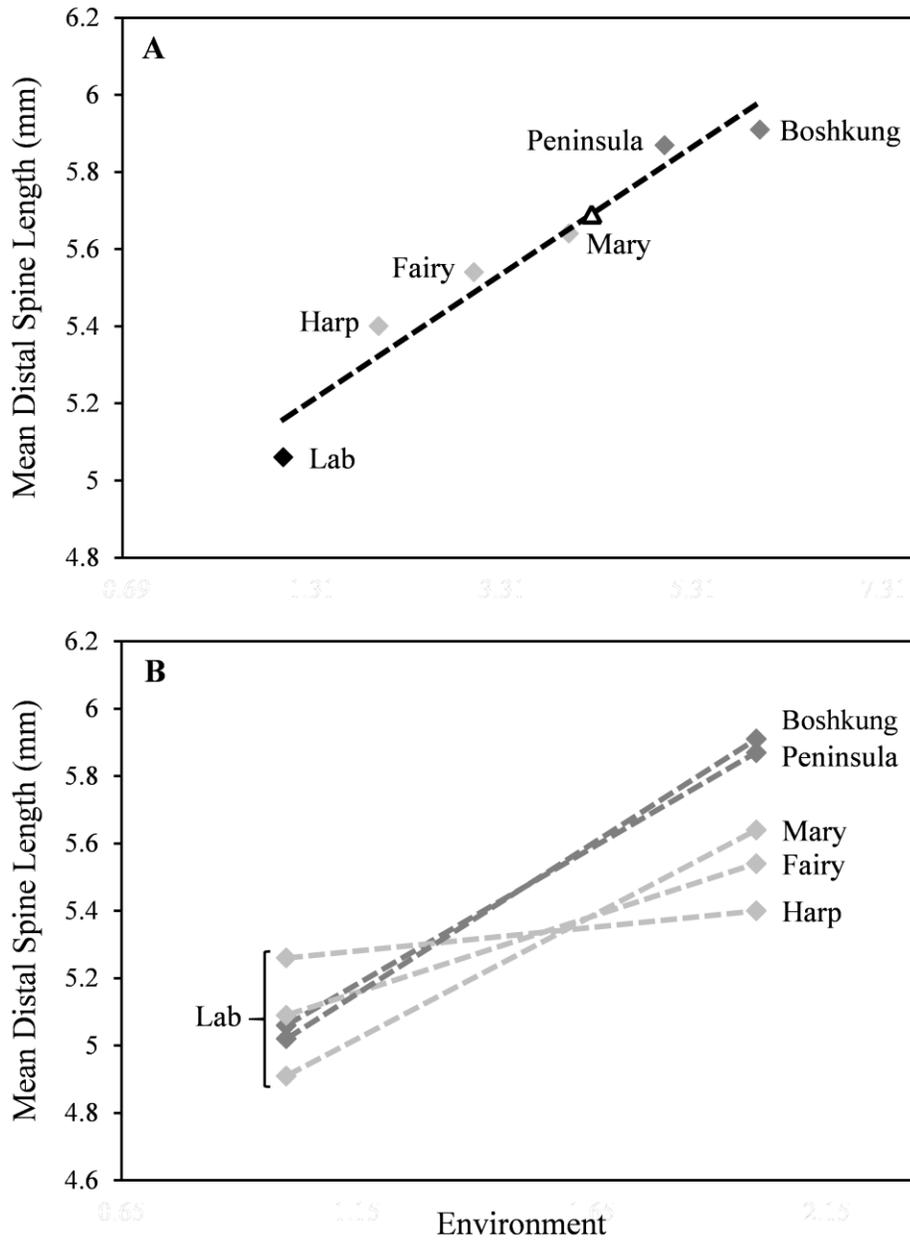
696

697 **Figure 4**



698

699 **Figure 5**



700