

Two-step dialogue between the cladoceran *Bosmina* and invertebrate predators: Induction and natural selection

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Abstract

Aquatic prey species respond to predators with fast (developmental) and slow (selective) feedbacks. Natural selection is assumed to fashion details of induction and to modify baseline morphology, but only rarely do we catch the slower (multi-generation) process in action. Laboratory experiments with *Bosmina* detected predator-mediated induction and estimated spine heritability ($h^2 = 0.2\text{--}0.5$). Third Sister Lake, Michigan, U.S.A., *Bosmina* exhibited induction to resident (*Mesocyclops*) and to two nonresident, neighborhood predators (*Epischura* and *Leptodora*). However, the magnitude of induction in Third Sister Lake *Bosmina* to nonresident predators (*Epischura*, *Leptodora*) was muted, when compared with induction and final spine lengths in *Epischura*–*Leptodora* lakes. Inadvertent escape of *Leptodora* into Third Sister Lake in 1987 created a long-term (multiyear), whole-lake experiment, where resident *Bosmina* populations fell under intense size selection. During the interval, *Leptodora* suppressed a late-season smaller, short-featured species (*B. freyii*), favored seasonal expansion of an overwintering long-featured species (*B. liederii*), and selected for longer features in the latter species. Before local extinction, defensive spines of *B. liederii* achieved lengths comparable to populations that co-occur with *Epischura* and *Leptodora*.

Given the isolated and ephemeral nature of lakes, akin to islands, to what degree are species and community responses fashioned at the local (resident lake) or regional (lake district) level? Regional dispersal is receiving attention (De Meester et al. 2002; Havel and Shurin 2004), yet how does the duration of species contact modify predator–prey interactions, species persistence, and genetic adjustments (Ricklefs and Schluter 1993; Tilman and Kareiva 1997)? Zooplankton communities are probably best approached from a regional metapopulation perspective (Kerfoot et al. 2004; Leibold and Norberg 2004), because long-term survival of species is tied both to dispersal and to local interactions (Shurin and Allen 2001). However, unraveling ecological and evolutionary responses at local and regional scales requires extended studies, although insight may be aided by fortuitous circumstances.

One of the challenges is that predator–prey interactions are complicated by two levels of response (developmental, selective). Early on, species in the cladoceran genera *Daphnia* and *Bosmina* were found capable of rapid developmental reaction to the presence of predators (*Daphnia*: Grant and Bayly 1981; Havel 1985; *Bosmina*: Kerfoot 1987). Since then, the nature of inducible defenses has been investigated from laboratory, field, and theoretical perspectives (Dodson 1989a; Tollrian and Harvell 1999; Gabriel et al. 2005). Yet there are many unanswered questions about the nature of the dialogue played out in ecological and evolutionary time

between predators and prey (Tollrian and Harvell 1999; Relyea 2002). From the standpoint of the prey species: (1) how often and quickly does the predator–prey inductive response evolve; (2) how is it maintained (physical–chemical, developmental, and genetic feedbacks); and (3) how many predators are involved? To answer these questions, we require more information about the process of natural selection in lakes, particulars of developmental and genetic responses, and details on neighborhood and historical ecological interactions.

In the mid–1980s, a series of zooplankton translocation experiments investigated predator–prey relationships at northeastern and Midwestern U.S. localities (i.e., moving predators and prey around in different lakes to explore food-web and ecological interactions [Kerfoot 1987; McNaught 1993a,b]). Near the end of the planned manipulations in Third Sister Lake, Michigan, U.S.A., during the summer of 1987, *Leptodora* inadvertently escaped from enclosures (McNaught et al. 2004). This highly efficient, size-selective predator rapidly increased in abundance, creating a long-term whole-lake experiment. Plankton samples from 1987 to 1992 documented that *Leptodora* became abundant and differentially depleted small-bodied cladoceran species (McNaught 1993a; McNaught et al. 2004). For 6 yr after 1992, as *Leptodora* reached the height of its abundance, *Bosmina* became locally extinct. When *Leptodora* crashed in the late 1990s, *Bosmina* reappeared in 1999 and became abundant again by 2004–2007. A sequence of translocation experiments, laboratory and field observations between 1987 and 2007 allow insight into developmental and selective responses by *Bosmina*.

Methods

Study system and tested hypotheses—North of Ann Arbor, Michigan, there is a lake district that contains a

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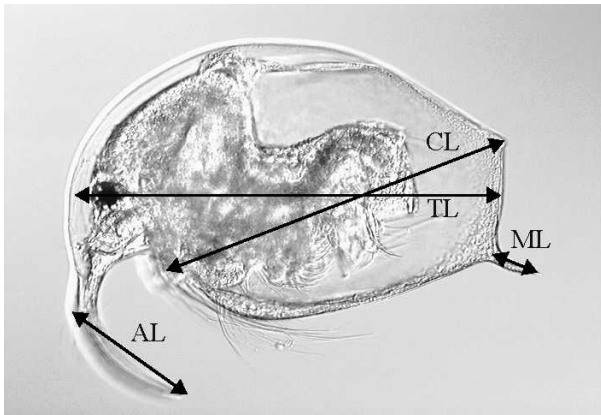


Fig. 1. *Bosmina liederii* from Third Sister Lake, illustrating position of general length (TL, total length; CL, carapace length) and defensive spine (ML, mucrones length; AL, antennule length) measurements. Mucro sutures and antennule segments are counts of cell boundaries that make up the terminal spine structures.

diverse assemblage of invertebrate predators (McNaught et al. 2004). Two species of invertebrate predators (the calanoid copepod *Epischura lacustris*, the cladoceran *Leptodora kindtii*) not previously reported from Third Sister Lake were collected at Whitmore Lake for a series of experiments. Previous geographic surveys suggested that Third Sister Lake would be suitable for both species, although the lake was relatively small (McNaught 1993a,b; Kerfoot et al. 2000). The field experiments were done in suspended enclosures (McNaught 1993a,b; McNaught et al. 2004). Community interactions included size-selective responses of planktonic communities to fish and invertebrate predators (McNaught 1993a,b; McNaught et al. 2004), whereas trait responses focused on *Bosmina* spine lengths (Fig. 1) relative to introduced *Epischura* and *Leptodora*.

Third Sister Lake was also selected because it had a history of limnological investigations. The waterbody is a small (0.04 km²) seepage lake isolated somewhat to the south of the northern district as the third kettle-hole in a series that straddles Ann Arbor, Michigan (First, Second, and Third Sister Lakes; McNaught et al. 2004). The lake is relatively deep (maximum and mean depths of 16.5 m and 7.2 m, respectively), with no permanent surface inlets or outlets, and is moderately eutrophic (Lehman and Naumoski 1986). Water-column stratification occurs in early spring, after a brief mixing period, followed by the hypolimnion quickly becoming anoxic (Bridgeman et al. 2000). By late summer the thermocline is at 5–6 m and phytoplankton and zooplankton are concentrated in the upper 5 m. Phytoplankton assemblages are dominated by diatoms, chrysophytes, and cryptomonads in the spring and autumn and by cyanobacteria in the summer.

Bosmina possess chitinous anterior (antennule) and posterior (mucrone) protuberances (Fig. 1). When attacked by predatory copepods, they fold their single pair of swimming appendages (antennae) into a groove behind the antennules and fall passively through the water column. During the motionless, dead-man behavior, the fixed

antennules protect the antennae, whereas the posterior mucrones protect rotation towards the ventral groove region, through which algae and bacteria are filtered. The early Third Sister Lake translocation experiments were designed to evaluate the relative importance of induction and natural selection in regulating defensive spine length during short-term experiments by challenging resident populations of *Bosmina* with more effective, nonresident predators. The unintentional escape and colonization of Third Sister Lake by *Leptodora* provided us an opportunity to see if natural selection over multiple generations by a highly efficient size-selective predator could push responses beyond regular induction norms. We divided the phenotypic responses into three periods: pre-*Leptodora*, *Leptodora*, post-*Leptodora*.

Seasonal pelagic sampling—*Bosmina* morphology and abundance was monitored by hauling a 0.3-m-diameter plankton net (75 μ m or 120 μ m Nitex) vertically at a 16-m-deep, central station. Larger net tows used to quantify *Leptodora* abundance (Fig. 2) are discussed in McNaught et al. (2004). All microcrustaceans were preserved in a 5% formalin solution to which 40 g L⁻¹ of sucrose were added. Around 40 *Bosmina* were haphazardly removed from each plankton sample and mounted for measurement. Individuals were placed on a glass slide in a 50% glycerin–water mixture. Slides were covered with a glass cover-slip and measurements were taken under a Zeiss Universal Microscope at 500 \times . Features measured on all individuals included: (1) total body length, (2) number of mucro sutures, (3) length of mucro, (4) number of antennule segments, and (5) length of the antennule, measured from the tooth to the tip (Fig. 1). If the antennule was curved, measurements followed the curvature.

Heritability estimates for defensive traits—Reasons for estimating the heritability (h^2) of defensive spines were two-fold. First, this information is important for determining the magnitude of selective responses, because the selective response equals the heritability times the selective differential (Lynch and Walsh 1998). Secondly, we wanted to emphasize the cost of selective responses (individuals lost) during selection relative to the neutral (no mortality) condition during induction.

Heritability estimates were attempted only on *B. liederii*, the most abundant species. Electrophoretic and sequencing studies showed that Third Sister Lake has a cryptic two-species assemblage (*Bosmina liederii* and *B. freyii*), which complicated comparisons (W. C. Kerfoot and L. J. Weider unpubl.). Headpore placement was not useful in separating the two species (Korinek et al. 1997; Taylor et al. 2002). However, a morphometric character, spacing of teeth on the postabdominal claw, previously pointed out by De Melo and Hebert (1994), could be used to distinguish the two species in formalin-preserved samples. For cross-comparisons, we also estimated trait heritability in *B. liederii* from Douglas Lake, Cheboygan County, Michigan, an *Epischura*–*Leptodora* lake.

Parthenogenetic cladocerans present favorable options for measuring heritability. The heritability (h^2) of a trait is

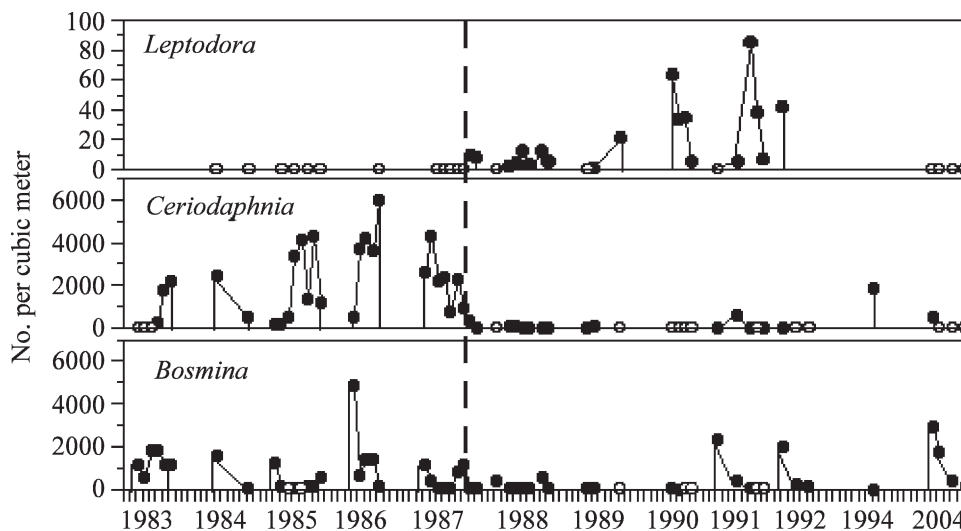


Fig. 2. As *Leptodora* became abundant, it depressed densities of small cladocerans, especially *Ceriodaphnia* and *Bosmina*. *Leptodora*'s appearance in Third Sister Lake is marked by the dashed vertical line.

the fraction of the phenotypic variance that is attributable to genetic variation (Falconer 1960; Lynch and Walsh 1998). Under controlled laboratory conditions, interactions between genotype and environment are assumed to be negligible; hence, $V_P = V_E + V_G$ (V_P = phenotypic variance; V_E = variance due to environmental influences; V_G = variance due to the different genotypes). We sampled lake populations and randomly transferred stem females to establish individual clones (i.e., a number of genotypes, grown together under common-garden conditions). Prior to cloning, lake water was collected, millipore-filtered, aerated, then stored for weeks to lower any residual kairomones. *Bosmina* were collected from the lake with a 120- μm Nitex plankton net and individual females pipetted into ~ 50 35-mL shell vials filled with millipore-filtered and conditioned lake water. Cultures were fed daily with *Chlamydomonas* sp., and medium exchanged weekly. Clones were numbered and followed for three generations to purge maternal effects. Vial populations were preserved in a sucrose–formalin mixture and measured at 500 \times under a Zeiss Universal.

Morphometric data were analyzed via a single classification ANOVA (Sokal and Rohlf 1995). To estimate h^2_B , the variance among the genotype means, the average variance among clones was compared to the variance within clones (Lynch and Walsh 1998). Within each clone, only one genotype is considered; hence, the observed variation is entirely environmental in origin. Heritability in the broad sense is defined as: $h^2_B = V_G/(V_G + V_E)$. Thus the mean square within clones (MS_{win}) provides an estimate of V_E . The mean square (MS_{btw}) between clones has environmental as well as genotypic components, essentially $V_E + nV_G$ (when n is the number of twins or clone-mates per vial; Parsons 1973, Lynch and Walsh 1998). So V_G is estimated from $(MS_{\text{btw}} - MS_{\text{win}})/n$. In addition, use of common-garden cultures during the interval that bridged the *Leptodora* introduction allowed us to determine if field populations were pushed beyond typical reaction norms.

Laboratory induction experiments—*Bosmina* show developmental induction when placed in close proximity to certain predators (Kerfoot 1987, 2006), although the transmitting agent in different species is not always a chemical kairomone, but may also involve physical stimulation (Sakamoto et al. 2007). Laboratory induction experiments were carried out with Third Sister Lake *Bosmina* and resident (rotifer *Asplanchna* sp., cyclopoid *Mesocyclops edax*, *Chaoborus punctipennis* midge larvae) or neighborhood (*Epischura lacustris*, *Leptodora kindtii*, *Polyphemus pediculus*, *Eurytemora affinis*, *Bythotrephes longimanus*) invertebrate predators. The laboratory experiments utilized a split clonal design. The paired design takes into account close proximity effects on individuals (common environment effects on identical genotype) and demographic composition, making the tests very sensitive (Kerfoot 2006). The exposure included both chemical and physical stimuli. *Bosmina* stem females were cloned within 35-mL glass shell vials on a medium that contained finely filtered (0.4- μm Millipore) and aged lake water. *Chlamydomonas reinhardtii* was added as food each day, medium was changed weekly, and new vials were started at about 2–3-month intervals. After a minimum of three generations to purge maternal effects, around 2 weeks, a growing clone was split into two vials. One vial received a single predator (e.g., advanced instar *Mesocyclops edax*, *Epischura lacustris*, or juvenile *Leptodora kindtii*), whereas the other served as a control.

There was concern that large *Leptodora* could deplete oxygen in small containers, so only immature instars were utilized. The vial experiments were run for 7–12 d to test for induction, after which the entire vial contents were preserved in 10% formalin–glucose. We observed predators in the vials, to check on condition. If predators died during the experiment, they were replaced (only 21% of trials). If there was obvious evidence for size-selective depletion of young during the brief exposure, the vial results were not included in ANOVA tests (9% of tests).

Table 1. Heritability estimates for defensive spine traits in *Bosmina leideri* based on single-classification clonal ANOVA (Sokal and Rohlf 1995; Lynch and Walsh 1998). Shown are results from Third Sister Lake, Washtenaw County, Michigan, (1986) and two separate clonal rearing experiments from Douglas Lake, Emmet County, Michigan, U.S.A. (1989). In the table, degrees of freedom for between-clone mean squares (MS) is the number of clones - 1. An example ANOVA analysis and heritability estimate is shown in 1B.

A. Heritability estimates					
	Clones	Individuals	Heritability	F	p
Third Sister Lake					
Mucro length	8	47	0.38	4.5	8.80×10^{-4}
Ant length	8	51	0.25	3.2	0.008
Douglas Lake—first test					
Mucro length	28	227	0.40	6.3	1.33×10^{-15}
Ant length	28	188	0.33	4.4	1.88×10^{-9}
Mucro sutures	28	198	0.44	6.5	2.95×10^{-15}
Ant segments	28	192	0.43	6.1	4.52×10^{-14}
Douglas Lake—second test					
Mucro length	22	112	0.53	6.8	3.63×10^{-11}
Ant length	22	114	0.21	2.4	0.002
Mucro sutures	23	115	0.51	6.1	2.03×10^{-10}
Ant segments	22	113	0.22	2.4	0.002
B. Example ANOVA (Douglas Lake, mucro length); $MS = SS/df$; $SS = \sum(X - \bar{x})^2$					
	SS	df	MS	F	p
Between clones	14.9054	21	0.70978	6.8	3.63×10^{-11}
Within clones	9.3852	90	0.10428		
$n = 5.1$					
$V_E = 0.10428$					
$V_G = (0.70978 - 0.10428) / 5.1 = 0.11873$					
$Heritability = V_G / (V_G + V_E) = 0.11873 / (0.11873 + 0.10428) = 0.53$					

Field induction experiments—To test induction responses under lake conditions, we used suspended transparent polyethylene bags. Populations of *Bosmina* respond differently to size-selective *Mesocyclops*, *Epischura*, and *Leptodora* because these taxa differ in body size and effectiveness of prey removal (Kerfoot 1978; Hanazato and Yasuno 1989). Of the three, *Leptodora* is the most effective predator on *Bosmina*, capable of depleting populations (Lunte and Luecke 1990; Branstrator and Lehman 1991; McNaught et al. 2004). *Mesocyclops* and *Epischura* were used primarily in the mid-1980s because small-bag methods were perfected (Kerfoot 1987), whereas a larger, deeper enclosure design for *Leptodora* was being tested (McNaught 1993a,b). The smaller enclosures consisted of polyethylene bags (160 liters; 50- μ m wall thickness) suspended from floating platforms. The platforms were buoyed by styrofoam slabs, arranged in rows, and anchored at their ends via cinder blocks to the lake floor. Wooden cross-braces allowed placement of four bags per floating platform, secured by pressure clamps. The bags draped down a meter into the water column.

Moored bag experiments with *Mesocyclops* removal and *Epischura* addition were run in 1985 and 1986. The bags were filled with 160 liters of coarsely filtered (75- μ m Nitex) lake water pumped from mid-epilimnion depth. Once bags were filled, water was not renewed, nor was there exchange with lake water via a mesh boundary (i.e., closed bag). Using a vertical plankton tow, zooplankton were sampled from Third Sister Lake and placed in 4-liter glass jars. The

jars were returned to the laboratory, where *Bosmina* were pipetted individually from the mixed-species field sample into a single jar with filtered Third Sister Lake water. After we returned to the lake, the *Bosmina* culture was stirred to mix individuals evenly throughout the vessel, and equal portions were distributed to enclosure bags and collection jars (Reference Samples). The reference samples provided estimates of introduced numbers and initial morphology. *Epischura* from Whitmore Lake were introduced into half the bags at initial densities of 0.5 individuals L^{-1} , whereas the other bags served as resident-predator removal treatments (see below). Bags were sampled periodically by stirring and removing 10.5-liter subsamples to estimate densities and *Bosmina* morphology. At the end of the experiment, all remaining water was filtered through a 75- μ m Nitex plankton net and organisms preserved in 10% formalin and 40 g L^{-1} sugar.

We encountered difficulties measuring *Bosmina* induction responses in the larger *Leptodora* enclosure experiments. *Leptodora* proved such an efficient predator on small-bodied cladocerans that only one *Bosmina* was retrieved from predator treatments (McNaught 1993a; A. Scott McNaught unpubl.). Hence for testing *Leptodora* induction responses, we relied on laboratory tests.

Differences between treatment responses in bag experiments were examined using a nested Analysis of Variance (Nested ANOVA; SYSTAT; Wilkinson 1989) on the two treatments (*Epischura* addition, predator removal). The nested ANOVA design incorporated three levels: (1)

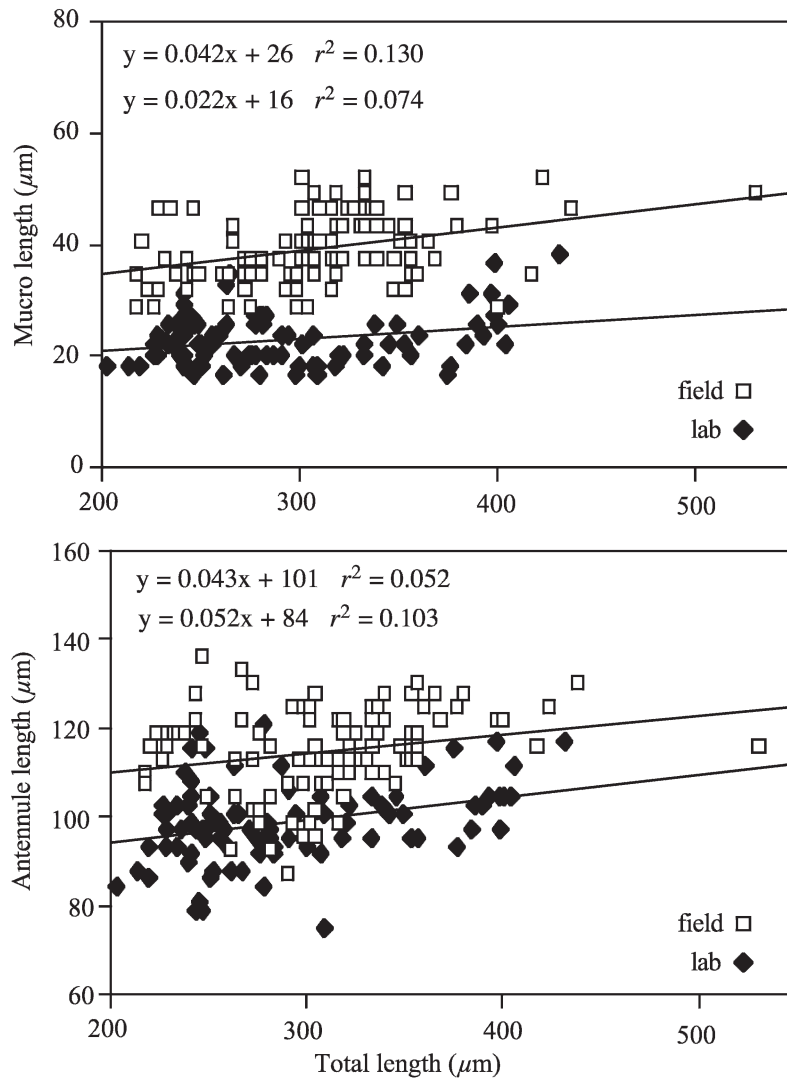


Fig. 3. Reduction in defensive spine lengths when individuals are removed from lake waters and placed in isolated laboratory culture. Hollow symbols are field lengths, whereas solid symbols are lengths in laboratory cultures (after at least three generations, to purge maternal effects). Linear regressions are fit to the points and equations shown above scatter plots. Note that the regression slopes are quite low. Comparison done on transfers from August 1986.

measurements within individual bags, (2) differences among replicate bags in treatments, and (3) evaluation of treatment effects. The nested ANOVA examined only trait sizes at the end of each experiment. Mucro and antennule segment counts were excluded from the nested ANOVA analysis because these were discrete measurements. We emphasize that controls served more as a predator-removal treatment, than a strict control, because all resident invertebrate predators were removed via filtration at the start of the experiment and only *Bosmina* were reintroduced. At the end of an experiment, individuals were preserved in formalin-sucrose and enumerated as before.

Results

Heritability of features—Third Sister Lake tests utilized eight clonal lineages and 47–51 individuals. Douglas Lake tests were more extensive, using 22–28 separate clonal

lineages, with 112–227 individuals for each estimate. An example ANOVA table is given in Table 1B, which lists the mean square (MS) for between and within clones. Variation between clones was much greater than variation within a single clone, because F -values were significant ($p < 0.05$) to highly significant ($p < 0.01$) for all comparisons. Heritability for mucro features (mucro length, mucro sutures) ranged between 0.38 and 0.53 (mean = 0.45), whereas heritability for antennule features (antennule length, segments) was lower, ranging between 0.21 and 0.43 (mean = 0.29; Table 1). Under directional selection, for traits with a mean $h^2 = 0.50$, a two standard-deviation selective differential (the amount that the mean is moved during the generation of selection; Futuyma 1986; Lynch and Walsh 1998) would produce around a one standard-deviation selective response, and would cost the population about 95% mortality. Consequently, rapid selection in enclosure experiments and in the field should be accompanied by a

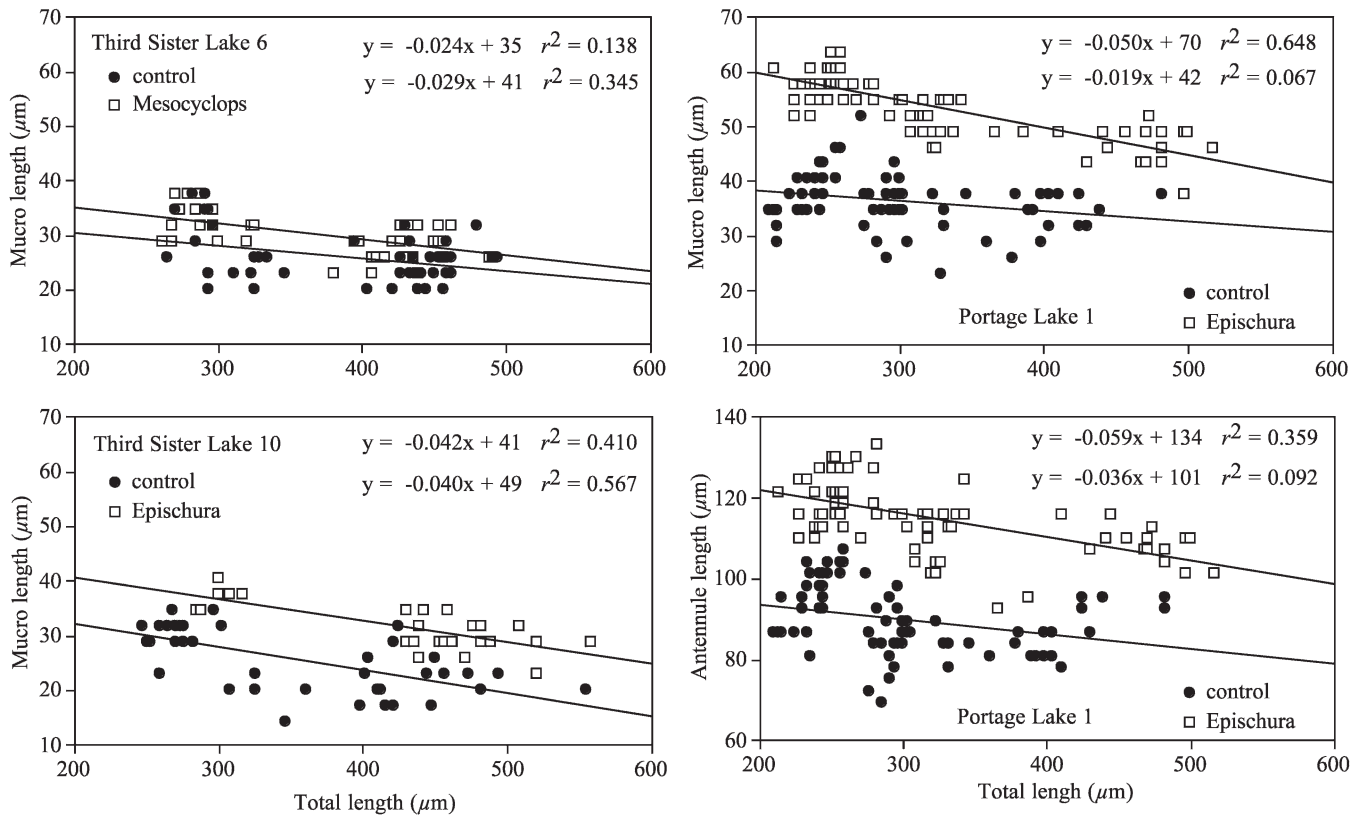


Fig. 4. Results of laboratory induction experiments. (Left panels) In *Bosmina liederii* from Third Sister Lake, there is modest elongation of spines in the presence of *Mesocyclops* (resident predator) and *Epischura* (nonresident predator). (Right panels) In *B. liederii* from Portage Lake (*Epischura* and *Leptodora* present), the elongation is much greater. Linear regression lines are fit to scattered points and equations listed above. See Tables 2–3 for more examples.

major reduction in population density and an increased risk of local population collapse.

Laboratory induction experiments—When brought in from the field, *Bosmina* clones decreased mucrone and antennule spine lengths, a response seen before in New Hampshire field-lab transfers (Kerfoot 1987). Figure 3 compares August 1986 field populations of *B. liederii* with mixed laboratory cultures drawn from the lake during summer and held in culture for over three generations to purge maternal effects. In the lake, mean mucrone lengths ranged between 35 μm and 41 μm and mean antennule lengths = 108–119 μm, whereas after three generations in the laboratory in the absence of predators, mucrone lengths of clones ranged between 20 μm and 25 μm and antennule lengths between 91 μm and 102 μm. The observed regression amounted to a 45% reduction in mucrone length (2.4 standard deviations [SD]) and a 17% reduction in antennule length (1.5 SD), although the absolute reduction (~15–20 μm) was similar for both features. Differences between field and lab were highly significant (two-sample *t*-test with unequal variance, mean ± 95% confidence limits (C.L.): mean field mucrone length, 39 ± 1 μm; lab 23 ± 1 μm, $t = 21.5$, $df = 187$, $p = 8.6 \times 10^{-53}$; mean field antennule length = 115 ± 2 μm, lab 99 ± 2 μm, $t = 12.0$, $df = 192$, $p = 2.5 \times 10^{-25}$).

The matched comparison (Fig. 3) illustrates another curious feature of defensive spines. Spines show atypical growth patterns and are proportionally largest in immature individuals. Growth is not proportional to body length, because there is only a slight positive or slight negative allometry from immature to mature instars ($Y = Ax^k$; k ranges from -0.32 to $+0.68$ for mucrones and -0.25 to $+0.18$ for antennules).

Spine lengths did not respond to several invertebrate predators (*Asplanchna*, *Polyphemus*, *Eurytemora*, *Chaoborus*, *Bythotrephes*) that reside in Third Sister Lake (TSL) or in neighborhood regions. However, when placed in the presence of resident *Mesocyclops* or neighborhood *Epischura* and *Leptodora*, defensive spines (mucrones and antennules) showed significant elongation (Fig. 4; Table 2). Within 7–8 d of exposure, mucrones increased from 19–28 μm to 29–35 μm ($n = 9$ split clone experiments, mean difference ± 95% C.L. = 6.1 ± 1.6 μm), whereas antennules increased from 88–101 μm to 102–122 μm ($n = 9$, mean difference = 13.6 ± 3.4 μm). Paired ANOVAs confirmed highly significant increases between predator treatments and controls in almost all individual experiments (Table 2). Mucrone length increased 1.0–3.1 SD (mean = 1.5 ± 0.5 SD) and antennule length 1.2–3.8 SD (mean = 1.9 ± 0.6 SD).

Yet how do the Third Sister Lake responses compare with *Bosmina* responses in lakes where the three taxa

Table 2. Laboratory induction experiments (split-clonal in 35-mL shell vials) from Third Sister Lake, Michigan, U.S.A. Results listed by predator, source of clone and date, trait (mucro, antennule length), number of individuals measured (n) and treatment (predator, control), mean length (Mean) and standard deviation (SD) of trait. ANOVA compares controls vs. predator treatment in split-clone tests; F -values, degrees of freedom (df), and p -values are given after trait. Predators are *Mesocyclops edax*, *Epischura lacustris*, and *Leptodora kindtii*. Clone TSL10 is *B. freyii*, whereas TSL4-6 clones are *B. liederii*.

Predator	Clone	Variable	n	Treatment	Mean	F	p -value
<i>Mesocyclops</i>	TSL4-05	Mucro	44	Control	28(4.8)	38.7	1.90×10^{-8}
			41	Predator	34(3.6)	(1,83)	
		Antennule	44	Control	101(9.4)	112.8	3.88×10^{-17}
			41	Predator	122(8.5)	(1,83)	
<i>Mesocyclops</i>	TSL6-05	Mucro	46	Control	26(4.6)	17.8	5.93×10^{-5}
			43	Predator	30(3.7)	(1,87)	
		Antennule	46	Control	100(7.1)	27.9	9.31×10^{-5}
			43	Predator	109(8.3)	(1,87)	
<i>Epischura</i>	TSL5-05	Mucro	42	Control	27(5.3)	17.7	8.00×10^{-5}
			25	Predator	32(4.4)	(1,65)	
		Antennule	42	Control	99(7.9)	30	7.55×10^{-7}
			25	Predator	111(11.1)	(1,65)	
<i>Epischura</i>	TSL6-05	Mucro	32	Control	24(2.9)	120.4	1.11×10^{-14}
			18	Predator	35(4.2)	(1,48)	
		Antennule	32	Control	98(8.6)	21.3	2.63×10^{-5}
			21	Predator	108(4.2)	(1,51)	
<i>Epischura</i>	TSL10-05	Mucro	42	Control	26(5.7)	17.7	8.00×10^{-5}
			25	Predator	32(4.3)	(1,65)	
		Antennule	42	Control	99(8.2)	30.0	7.55×10^{-7}
			25	Predator	112(11.0)	(1,65)	
<i>Epischura</i>	TSL6-06	Mucro	35	Control	26(4.2)	37.2	8.32×10^{-8}
			27	Predator	32(3.6)	(1,60)	
		Antennule	35	Control	93(8.5)	47.5	3.84×10^{-9}
			27	Predator	107(6.4)	(1,60)	
<i>Epischura</i>	TSL6-06	Mucro	35	Control	23(3.4)	38.5	3.72×10^{-8}
			35	Predator	30(4.8)	(1,68)	
		Antennule	35	Control	95(7.1)	86.0	1.10×10^{-13}
			35	Predator	110(6.2)	(1,68)	
<i>Epischura</i>	TSL10-06	Mucro	34	Control	23(4.2)	37.2	5.71×10^{-8}
			36	Predator	29(4.1)	(1,68)	
		Antennule	34	Control	91(5.8)	118.7	1.47×10^{-16}
			36	Predator	110(8.9)	(1,68)	
<i>Leptodora</i>	TSL5-05	Mucro	34	Control	23(4.0)	35.8	1.17×10^{-7}
			30	Predator	29(3.4)	(1,62)	
		Antennule	34	Control	88(9.9)	40.3	2.86×10^{-8}
			30	Predator	102(8.0)	(1,62)	
<i>Leptodora</i>	TSL10-06	Mucro	36	Control	24(4.0)	8.8	3.88×10^{-3}
			49	Predator	27(3.2)	(1,84)	
		Antennule	36	Control	97(9.0)	0.4	0.54×10^{-1}
			49	Predator	96(15.8)	(1,84)	
<i>Leptodora</i>	TSL5-06	Mucro	12	Control	23(3.2)	18.7	1.27×10^{-4}
			24	Predator	31(5.3)	(1,35)	
		Antennule	12	Control	88(8.3)	5.6	2.39×10^{-2}
			24	Predator	100(15.9)	(1,35)	

(*Bosmina*, *Epischura*, *Leptodora*) naturally co-occur? Induction experiments using *B. liederii* and *Epischura* from Portage Lake, Houghton County, Michigan, where all three taxa co-exist, show two pronounced differences. First, regressed mucro lengths were shorter in Third Sister clones than in Portage Lake clones (Third Sister, 20–25 μm ; Portage, 37 μm). Second, induction in TSL clones increased spine length only 40–60% the amount of induction observed in Portage Lake clones (Figs. 3,4; Table 2; $n = 3$, Portage Lake mucro mean difference $\pm 95\%$ C.L. = 18.3 \pm

3.8 μm ; antennule 21.0 \pm 8.6 μm). Comparisons of the mean magnitude of induction (Third Sister Lake vs. Portage Lake) demonstrated significantly greater responses in *Bosmina* in an *Epischura*–*Leptodora* lake (ANOVA, $n = 11$; mucro responses $F_{1,10} = 85.0$, $p = 3.3 \times 10^{-6}$; antennule $F_{1,10} = 7.0$, $p = 0.02$). In terms of SDs, mean mucro length in Portage Lake induction tests increased 3.1 SD, whereas mean antennule length increased 2.3 SD.

What if one predator is entirely absent from the regional neighborhood? For this comparison, split-clone induction

Table 3. Induction responses of *Bosmina* from an *Epischura*–*Leptodora* lake (*Bosmina liedereri*; Portage Lake, Houghton County, Michigan, U.S.A.) and from Europe (*Bosmina longirostris*; *Epischura* regionally absent). *Epischura* added to 35-mL vial for 7–10-d exposure (clone number and year of test). Variables include: sample size (*n*), treatment (predator, control), mean trait length (SD).*

Region and clone	Variable	<i>n</i>	Treatment	Mean	<i>F</i>	<i>p</i> -value
North American						
PLA-05	Mucro	42	Control	37(5.4)	163.4	3.25×10^{-21}
		42	<i>Epischura</i>	55(6.8)	(1,82)	
	Antennule	42	Control	93(9.9)	86.7	1.71×10^{-14}
		42	<i>Epischura</i>	112(8.9)	(1,82)	
PL1-05	Mucro	72	Control	37(5.2)	436.3	8.67×10^{-47}
		84	<i>Epischura</i>	54(5.2)	(1,154)	
	Antennule	72	Control	90(8.4)	345.5	3.46×10^{-41}
		84	<i>Epischura</i>	115(8.4)	(1,154)	
PL2-05	Mucro	42	Control	37(5.4)	168.3	1.15×10^{-21}
		43	<i>Epischura</i>	55(6.7)	(1,83)	
	Antennule	42	Control	93(9.9)	89.8	7.33×10^{-15}
		43	<i>Epischura</i>	112(8.8)	(1,83)	
European						
GPS1-06 Grosser Ploener See	Mucro	43	Control	15(3.9)	0.3	0.58
		45	<i>Epischura</i>	15(4.9)	(1,86)	
	Antennule	43	Control	75(11.0)	1	0.33
		45	<i>Epischura</i>	77(8.9)	(1,86)	
GPS54-07 Grosser Ploener See	Mucro	42	Control	16(3.2)	6.3	0.01
		41	<i>Epischura</i>	18(4.2)	(1,81)	
	Antennule	42	Control	80(8.7)	11.7	9.97×10^{-4}
		41	<i>Epischura</i>	86(7.6)	(1,81)	
SEL2-06 Selenter See	Mucro	42	Control	22(6.2)	0.2	0.65
		42	<i>Epischura</i>	21(4.9)	(1,82)	
	Antennule	42	Control	90(13.7)	1.4	0.24
		42	<i>Epischura</i>	94(11.6)	(1,82)	

* ANOVA results on control vs. predator treatment in split-clone experiments includes *F*-value (df), and probability (*p*). The *Epischura* came from the same source (Portage Lake).

experiments were carried out with *B. longirostris* from German lakes, where *Epischura* does not occur, and Portage Lake *Epischura lacustris*. When placed in laboratory culture without predators, the small European bosminid regressed to the shortest spine lengths of all *Bosmina* tested (15–22 μm mucro; 75–90 μm antennule). In the presence of *Epischura*, two of three clones showed no significant elongation of mucrones. Mean antennule elongation was only 4 μm , the lowest response in *Bosmina* tests, and was only significant in one of three tests (Table 3). In terms of SDs, the mean response for mucro length was 0.1 SD and 0.4 SD for antennule length (i.e., hardly any induction at all).

Field enclosure experiments—Induction was also tested in field enclosure experiments (Tables 4–5) with predator-removal and *Epischura* treatments. *Epischura* were introduced at a density of 0.5 L⁻¹ and were present in all predation bags at the end of the experiment. *Bosmina* survived the duration of the experiment as 10.5-liter subsamples on 19 July 1985, retrieved a mean of 41(SD = 9.9) *Bosmina* from controls and 31(SD = 27.8) from predation treatments.

Figure 5 illustrates trait responses in bags with addition of *Epischura lacustris*, relative to predator-removal treatments. Mean mucro lengths began at around 15–29 μm

(Reference Samples). By the end of the 9-d exposure, mean lengths reduced to 20–23 μm in removal treatments (25% reduction), yet increased to 35–46 μm in predator-addition treatments (29% increase). Antennule lengths began at 68–112 μm (Reference Samples). Mean antennule lengths declined to 84–90 μm in removal treatments (28% reduction), yet were maintained at 102–116 μm in *Epischura* addition treatments. A nested ANOVA (Table 4) run on the midsummer end samples (19 Jul) showed significant treatment effects on body length ($F_{1,6} = 3.6$, $p = 0.002$), mucro length ($F_{1,6} = 4.7$, $p < 0.000$) and antennule length ($F_{1,6} = 5.6$, $p < 0.000$).

An identical enclosure experiment run later in the year (02–19 Aug) gave very similar results (Table 4). Although *Bosmina* density was greater in late-season bags and the duration of predator exposure longer, the outcome was nearly identical. Defensive spine lengths (mucrones and antennules) were significantly shorter in predator-removal treatments and longer in *Epischura* addition treatments (ANOVA, mucrones $F_{1,4} = 76.7$, $p = 4.9 \times 10^{-42}$; antennules $F_{1,4} = 44.3$, $p = 6.2 \times 10^{-28}$). Mean mucro lengths were 22–23 μm in removal treatments and 36–40 μm in *Epischura* addition treatments. Mean antennule lengths were 88–93 μm in predator-removal treatments and 105–109 μm in predator-addition treatments.

Table 4. Results of in situ enclosure (plastic bag) experiments, Third Sister Lake, summer 1985. Reference sample represents a subsample of the *Bosmina* placed into enclosure bags. Only results from end sample dates are shown. Controls are actually a *Mesocyclops*- (predator) removal treatment, where there was no water exchange between bag and lake. All lengths (L) are in micrometers. Trait values are means \pm 95% C.L. (SD) with ranges underneath (n =sample size).

Date	Sample	n	Total L	Mucro L	Antennule L
First enclosure experiment (11–19 Jul 1985)					
11 Jul	Reference	50	353 \pm 20(71) 224–465	28 \pm 1(4) 15–29	116 \pm 3(10) 68–112
19 Jul	Control 2	47	310 \pm 19(63) 224–465	20 \pm 1(4) 15–29	90 \pm 3(8) 68–112
19 Jul	Control 3	38	309 \pm 14(43) 232–403	22 \pm 1(4) 14–32	84 \pm 3(9) 67–101
19 Jul	Control 5	28	337 \pm 19(48) 226–438	22 \pm 1(3) 14–32	84 \pm 3(7) 72–99
19 Jul	Control 6	50	287 \pm 17(59) 203–429	23 \pm 1(4) 14–32	85 \pm 3(10) 64–104
19 Jul	<i>Epischura</i> 1	13	318 \pm 43(71) 215–476	35 \pm 5(8) 23–49	103 \pm 9(14) 75–121
19 Jul	<i>Epischura</i> 4	23	340 \pm 31(71) 254–492	37 \pm 5(12) 13–51	102 \pm 7(14) 78–126
19 Jul	<i>Epischura</i> 7	15	328 \pm 53(96) 238–483	46 \pm 4(7) 32–55	116 \pm 8(13) 97–132
19 Jul	<i>Epischura</i> 8	72	296 \pm 16(70) 216–520	39 \pm 2(7) 18–53	110 \pm 2(9) 92–130
Second enclosure experiment (02–19 Aug 1985)					
02 Aug	Reference	50	332 \pm 17(59) 249–551	23 \pm 1(4) 15–31	105 \pm 3(11) 77–124
14 Aug	Control 2	50	302 \pm 18(62) 205–434	23 \pm 1(5) 12–33	93 \pm 3(10) 75–113
14 Aug	Control 4	52	285 \pm 9(32) 217–383	22 \pm 2(6) 14–55	88 \pm 3(9) 72–122
19 Aug	<i>Epischura</i> 1	50	339 \pm 17(61) 223–447	38 \pm 2(6) 26–53	109 \pm 3(10) 86–128
14 Aug	<i>Epischura</i> 3	45	327 \pm 18(60) 235–495	40 \pm 3(10) 14–55	105 \pm 4(12) 78–128
14 Aug	<i>Epischura</i> 8	52	301 \pm 17(61) 209–444	36 \pm 2(7) 17–49	105 \pm 2(8) 84–122

These experiments confirm that the timing and magnitude of field responses corresponded closely to laboratory induction experiments, resulting in 2–3 SD differences between predator-removal and predation addition treatments within 7–17 d, or about 1–2 generations. The morphological responses were reversible, increasing when predators were added and reducing when they were removed. This correspondence argues that field responses were due largely to short-term developmental (phenotypic) induction, rather than to directional selection.

No major population reduction was observed in field enclosures, as *Bosmina* density decreased only slightly in July predation treatments, from 0.8 (SD = 0.4) to 0.5 (0.2) individuals L⁻¹, whereas control densities were increasing from 0.9 (0.4) to 3.9 (0.5) individuals L⁻¹. In August enclosures, *Bosmina* densities increased in both control (from 4.1 [1.2] to 32.8 [38.1] individuals L⁻¹) and in predation treatments (from 4.3 [2.1] to 45.6 [46.3] individuals L⁻¹).

Morphological variance patterns among bags provided another insight (Gabriel 1999; Gabriel et al. 2005). The nested ANOVA at the end of the field experiment revealed

that morphological variance between individuals was less in predator-removal treatments (controls) than in predator-addition treatments (Table 5; Fig. 5). Note that the variance observed between individuals in predator-addition treatment bags also compared favorably with the variance observed in nature (e.g., in Reference or routine field samples). Hence, in the absence of a localized stimulus from a predator, clones regressed back towards a more uniform morphology.

Seasonal sampling of Third Sister Lake—Pre-Leptodora period: Total length tended to be longer in late winter and spring (371–445 μ m), and shorter in late summer (298–401 μ m). Some of the seasonal trends were due to demographics, because early spring populations were dominated by overwintering females of *B. liederii*. Morphological patterns for 1984–1985 (before *Leptodora* introduction) indicated that *Bosmina* spines were longer in early spring (Fig. 6). Both features began a decline in June that continued through July and early August. Detailed inspection of seasonal dynamics disclosed that the resident population was composed of two very cryptic,

Table 5. Nested ANOVA run for end samples (19 Jul 1985) of first enclosure experiment, Third Sister Lake. Treatments were addition of *Epischura lacustris* (Invertebrate Predator, initial density = 0.5 L⁻¹) and Control (predator removal) in suspended plastic bags. Hypothesis is a test of significance between control and predation treatments, whereas control and *Epischura* are tests for significant differences between bags in that category. Traits measured included total body length, mucro length, and antennule length. Note that, at the end of the experiment, mucro and antennule lengths in control bags are much more uniform than in predator treatments.

Source	Sum of squares	df	Mean-sq	F	p
Total body length					
Hypothesis	87,233	6	14,539	3.6	0.002
Control	46,162	3	15,387	3.8	0.011
<i>Epischura</i>	41,071	3	13,690	3.3	0.020
Error	1,139,633	278	4099		
Mucro length					
Hypothesis	1084	6	181	4.7	0.000
Control	189	3	63	1.6	0.181
<i>Epischura</i>	894	3	298	7.7	0.000
Error	10,670	277	39		
Antennule length					
Hypothesis	3192	6	532	5.6	0.000
Control	1024	3	341	3.6	0.014
<i>Epischura</i>	2168	3	723	7.6	0.000
Error	24,678	260	95		

but morphologically and electrophoretically distinguishable species (W. C. Kerfoot and L. J. Weider unpubl.). Differentiating the species helped clarify some of the observed seasonal trends. Both species were present in early spring samples, but one was much more abundant (*B. liederii*). The relative abundance of *B. freyii* increased in late summer and autumn, moving from 2% to 11% in April–May to 55% to 93% by August–September (Table 6). Early season heterogeneity was evident in the relative variance of features measured during April and May. Prior to 1987, Coefficients of Variation (SD divided by the mean) for traits were high throughout the season (mucro length, 17.0–27.2%; antennule length, 9.1–14.0%), partly reflecting the mixture of species. Spine lengths in both species were greatest in early spring and declined over the course of the summer, although *B. freyii* had relatively shorter mucrones and antennule lengths.

Leptodora period: Inadvertent introduction of *Leptodora* in 1987 resulted in dramatic declines in small-bodied cladocerans (Fig. 2; *Bosmina*, *Ceriodaphnia*). After *Leptodora* introduction, the seasonal pattern of *Bosmina* traits also changed (Fig. 6). Seasonal samples from 1990 to 1991 showed that *Bosmina* populations maintained long features throughout the year, with no indication of a late-season decline. Lengths of both traits (defensive spines) also increased. Mean mucro lengths ranged between 45 μm and 52 μm , whereas antennule lengths ranged between 150 μm and 158 μm . The smaller featured species (*B. freyii*) was severely depressed or not present in late season samples (Table 6). The observed decline of coefficients of variation (mucro length, 10.0–17.2%; antennule length 5.1–8.9%) in *Leptodora* samples also suggested a single species (i.e., *B. liederii*). Because *Leptodora* reached maximum abundance in August to early September

(Fig. 5; McNaught et al. 2004), *Bosmina* became seasonally truncated compared to pre-*Leptodora* years. Late-season truncation could have contributed to the observed population collapse in 1992, because *Bosmina* typically produce overwintering eggs in late September to November.

When viewed over a sequence of years (1983–1991), summer samples document increased mucro and antennule lengths following *Leptodora* introduction (Fig. 7). Populations had relatively short mucrones and antennules in 1983, and variable moderate lengths between 1984 and 1987. After introduction of *Leptodora* (arrow), mucro lengths increased up to around 45–52 μm and antennule lengths to 150–158 μm , similar to lakes where *Bosmina* co-occur with *Epischura* and *Leptodora* (Table 2), remaining long until *Bosmina* population collapse in 1992.

Evidence for long-term selective responses came from laboratory common-garden culture comparisons. As part of induction and heritability studies, *Bosmina* were brought into the laboratory and cultured periodically, raised under nearly identical rearing procedures. Comparison of 15 common-garden cultures from 1986 with 26 from 1989 and 3 from 1991 indicated significant differences between pre-*Leptodora* and *Leptodora* years. Some individual culture morphologies are given in Table 7, whereas 1986 vs. 1991 clones are contrasted in Fig. 8. Comparing 1986 with 1989 lab cultures, laboratory-acclimated cultures exhibited highly significant increases in mucro and antennule lengths (*t*-test 1986 vs. 1989; mucro df = 120, *t* = 21.1, *p* = 1.58 $\times 10^{-42}$; antennule df = 93, *t* = 14.9; *p* = 1.22 $\times 10^{-26}$). Increases in body length were additionally seen, although the responses were less impressive than observed for spine lengths. Using pair-wise comparisons with the difference expressed in SD units, the amount of morphological shift between pre-*Leptodora* (1986) and *Leptodora* period (1989–1991) in common-garden

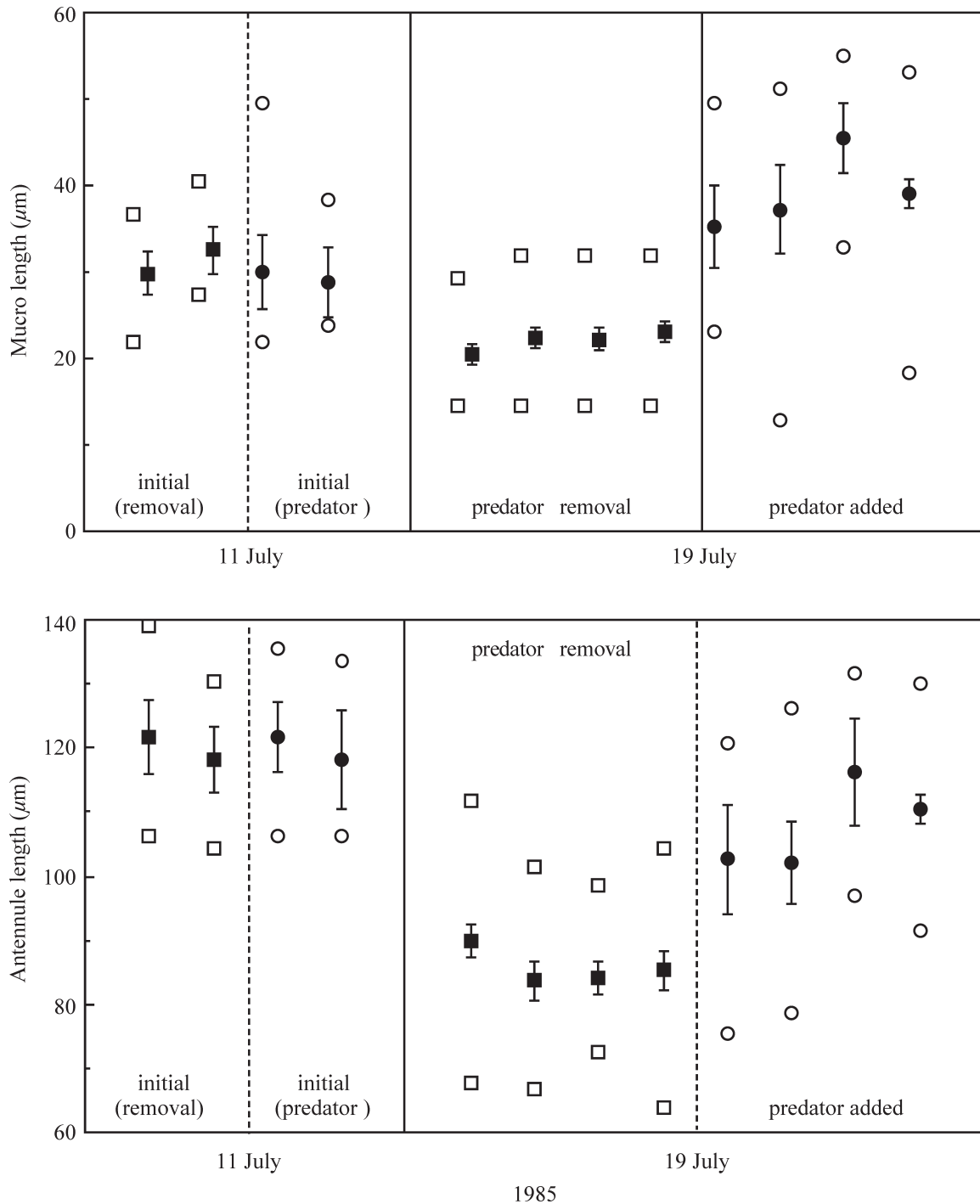


Fig. 5. Results of induction experiments in field enclosures. The right two panels show responses of spines in removal and addition experiments. In control bags where all predators were removed, spine lengths decreased, whereas in bags where *Epischura* were added, spines remained long or even increased in length. Initial lengths of spines in the treatments are shown in the left insert panels. Plotted symbols are means, \pm SD, and ranges (hollow symbols). Table 4 gives additional measurements.

cultures was 1.7–3.7 (mean = 2.8) SDs for body length, 2.2–5.7 (mean = 3.8) SDs for mucro length, and 1.5–4.8 (mean = 3.3) SDs for antennule length. Responses of about 1.5–2.0 SDs in field populations for spine lengths were expected from induction alone (Table 2). However, the common-garden mean shifts indicate directional selection also underlying spine length shifts of *B. liederii* prior to population collapse in

1992. The mean size of mucrones and antennules in 1989–1991 cultures corresponded closely to *B. liederii* populations that naturally co-exist with *Epischura* and *Leptodora* (Fig. 4b; Table 3).

Post-Leptodora period. After *Leptodora* crashed, *Bosmina* appeared again in 1999. Both species (*B. liederii*, *B. freyii*) reappeared, with *B. liederii* most abundant in spring

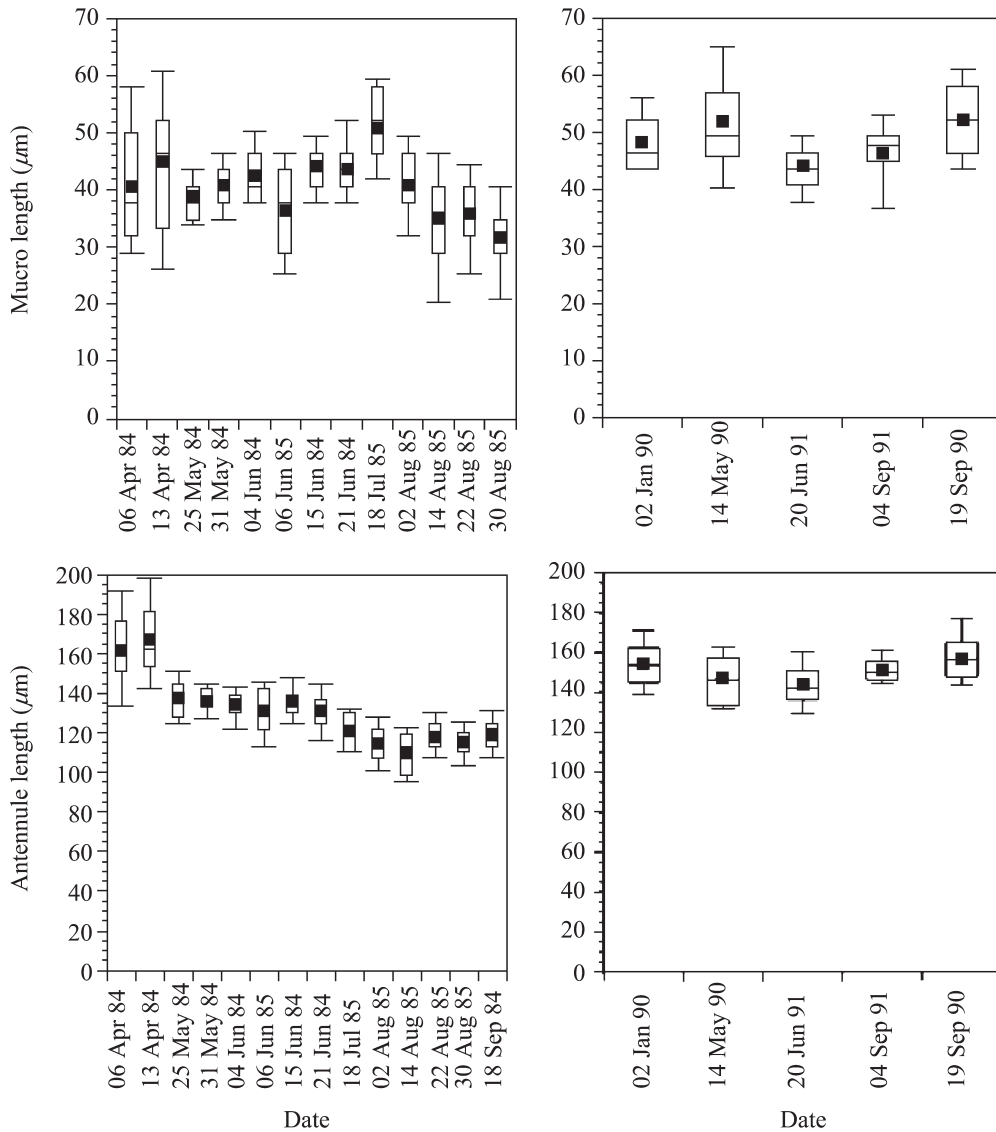


Fig. 6. Seasonal pattern for *Bosmina* spine lengths (mucrones, antennules) in Third Sister Lake, before and after *Leptodora* colonization. Left and right figures present composite diagrams for seasonal samples 2 yr before (1984–1985) and after (1990–1991) *Leptodora* introduction. Box plots give mean (square), median, 25th, and 75th percentiles; whiskers range from 10th percentile (bottom) to 90th percentile (top).

Table 6. Frequency of *Bosmina freyii* and *B. liederii* in Third Sister Lake samples before (1984–1985), during (1989–1991), and after (1999–2004) *Leptodora* colonization. NR = no record.

Month	1984–1985			1989–1991			1999–2004		
	<i>n</i>	<i>B. freyii</i>	<i>B. liederii</i>	<i>n</i>	<i>B. freyii</i>	<i>B. liederii</i>	<i>n</i>	<i>B. freyii</i>	<i>B. liederii</i>
Apr	70	11%	89%	42	0%	100%	59	3%	97%
May	67	2%	99%	101	1%	99%	60	22%	78%
Jun	148	16%	84%	42	0%	100%	NR	NR	NR
Jul	84	5%	94%	43	2%	98%	70	21%	79%
Aug	40	55%	45%	41	0%	100%	119	28%	61%
Sep	28	93%	7%	98	1%	99%	NR	NR	NR

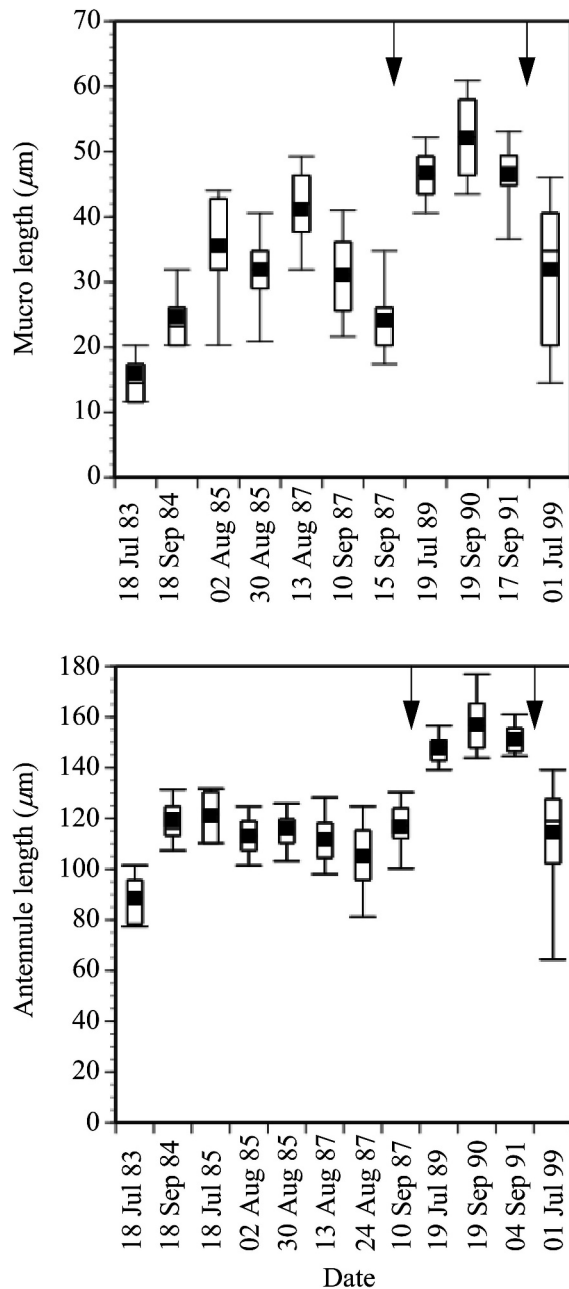


Fig. 7. Yearly mid- to late-seasonal spine (antennule, mucron) responses before, during, and after *Leptodora* appearance. The times of *Leptodora* presence are indicated by arrows. Symbols as in Fig. 6.

and *B. freyii* present in late summer to autumn (Table 6). Mucro and antennule lengths varied, but reverted to pre-*Leptodora* patterns (Fig. 7).

Discussion

In terms of a dialogue, prey sense the presence of danger via kairomones or physical cues, and modify behavior or development within minutes to days to reduce individual risk (Dodson 1989b; Tollrian and Harvell 1999). However, over longer spans (multi-generation), selection tailors

details of spine development. The observed atypical growth (bradytelic pattern) of defensive spines produces lengths that are proportionately largest in the first few instars. This aberrant developmental growth pattern is shared among all tested species within the subgenus *Bosmina* (*B. liederi*, *B. freyii*, *B. longirostris*). Such a growth pattern emphasizes greatest risk in the smallest instars, and reduced risk in large, mature individuals. The size-dependent risk pattern of *Bosmina* fits the observed size-selective tactics of predatory copepods (*Mesocyclops*, *Epischura*; Kerfoot 1978; Chang and Hanazato 2003) and *Leptodora* (Chang and Hanazato 2004). Strong size-dependent morphological patterns are also found in *Chaoborus* predation on *Daphnia* (Tollrian 1995).

Short-term laboratory experiments demonstrate that *Bosmina* in Third Sister Lake respond to resident *Mesocyclops edax* by developmentally increasing defensive spine lengths within days. Induction experiments with neighborhood predators indicated that Third Sister *Bosmina* also respond to larger bodied nonresident predators (*Epischura lacustris*, *Leptodora kindtii*), present in nearby lake districts. Our induction experiments closely resembled earlier (1980–1986) induction results in Vermont and New Hampshire (U.S.A.) lakes, where *Bosmina* transferred from a small pond to an *Epischura* lake responded to this predator by rapidly increasing spine lengths (Kerfoot 1987). One possible explanation for neighborhood sensitivity is that responses reflect a historical legacy fueled by regular regional encounters. Over the northeastern United States and Canada, *Bosmina* co-occur with *Mesocyclops edax*, *Epischura lacustris*, and *Leptodora kindtii* in 72%, 65%, and 45% of regional lakes (Carter et al. 1980). Co-occurrence with *Leptodora* is more restricted because this species generally inhabits deeper lakes (Carter et al. 1980; McNaught 1993b).

Induction was not stimulated by several other regional predators (*Asplanchna*, *Chaoborus*, *Polyphemus*), nor by two nonindigenous genera (*Eurytemora*, *Bythotrephes*). Although a temorid of similar body size to *Epischura*, *Eurytemora affinis* is a poor predator on *Bosmina*, is an introduced species, and occurs in only 0.1% of lakes (Carter et al. 1980). *Polyphemus* occurs in only 13% of lakes and also is not a particularly efficient predator on *Bosmina* (Carter et al. 1980). *Asplanchna* and *Chaoborus* are much more efficient predators on rotifers and *Daphnia*, respectively, than on *Bosmina*. *Bosmina longirostris* from Europe, which does not co-occur with any known *Epischura* species, showed a very weak induction response to *Epischura*, underscoring that prolonged lack of contact can result in loss of the developmental feedback.

Previous regional comparisons of *Bosmina* demonstrated a strong geographic correlation between morphological features (antennule, mucro, and body size lengths) and risk related to invertebrate predators (Kerfoot 1980, 1987; Sprules et al. 1984). Modification of predators within Third Sister Lake enclosures showed that rapid developmental responses (induction) dominated short-term interactions. Features rapidly increased in length in the presence of large predatory invertebrates, and would diminish down to small sizes when all predators were removed. The shortening of

Table 7. Comparison of spine features in 1986, 1989, and 1991 common garden cultures (laboratory regressed) from Third Sister Lake. Measurements shown below are examples from 26 (1986), 25 (1989), and 4 (1991) preserved cultures. Lengths are in microns with mean \pm 95% C.L. (SD) and range below.

Date	<i>n</i>	Total length	Mucro length	Mucro sutures	Antennule segments	Antennule length
1986 No. 6	13	285 \pm 29(49) 242–390	21 \pm 2(4) 16–29	0.2 \pm 0.3(0.4) 0–1	9.8 \pm 0.7(1.1) 8–12	96 \pm 4(7) 79–106
1986 No. 8	14	287 \pm 31(54) 227–392	24 \pm 3(5) 18–33	0.5 \pm 0.4(0.7) 0–2	9.5 \pm 0.5(0.9) 8–11	92 \pm 4(8) 79–102
1986 No. 9	21	277 \pm 22(48) 203–393	21 \pm 1(3) 16–26	0.1 \pm 0.2(0.4) 0–1	8.9 \pm 0.4(0.8) 7–10	96 \pm 4(9) 75–121
1986 No. 11	15	280 \pm 30(53) 227–384	23 \pm 2(3) 16–27	0.2 \pm 0.2(0.4) 0–1	9.5 \pm 0.6(1.1) 8–12	99 \pm 5(5) 81–115
1989 No. 1	13	271 \pm 15(26) 220–313	38 \pm 3(5) 32–52	2.5 \pm 0.4(0.7) 2–4	11.8 \pm 0.5(0.9) 11–14	130 \pm 5(8) 113–142
1989 No. 2	20	279 \pm 7(16) 244–319	37 \pm 2(4) 32–46	2.6 \pm 0.2(0.5) 2–3	12.4 \pm 0.5(1.1) 11–15	127 \pm 6(13) 90–142
1989 No. 3	8	292 \pm 40(48) 252–371	41 \pm 4(4) 35–46	2.5 \pm 0.4(0.5) 2–3	12.8 \pm 1.2(1.5) 10–15	128 \pm 11(13) 104–145
1989 No. 4	9	260 \pm 27(35) 215–325	36 \pm 4(6) 26–44	2.7 \pm 0.7(0.9) 1–4	12.2 \pm 1.4(1.4) 9–14	117 \pm 15(19) 93–145
1989 No. 5	15	269 \pm 17(30) 226–336	37 \pm 2(4) 29–44	2.2 \pm 0.4(0.7) 1–3	11.9 \pm 0.8(1.5) 9–14	122 \pm 6(11) 102–142
1991 No. 1	13	425 \pm 27(45) 351–516	41 \pm 3(6) 29–49	2.3 \pm 0.4(0.6) 1–3	12.5 \pm 0.6(1.1) 11–14	126 \pm 7(12) 107–148
1991 No. 2(B)	24	392 \pm 30(71) 287–534	44 \pm 4(9) 20–55	2.8 \pm 0.4(0.9) 1–4	12.9 \pm 0.4(1.1) 11–15	135 \pm 5(13) 99–154
1991 No. 3(A)	9	447 \pm 34(44) 371–513	42 \pm 5(7) 32–49	2.4 \pm 0.6(0.7) 1–3	13.0 \pm 0.9(1.1) 11–14	129 \pm 7(9) 113–139

features observed in field enclosures (predator-removal treatment) closely resembled the reversible induction responses seen in laboratory culture, which occurred when prey were separated from predators.

However, Third Sister Lake clones paired with *Epischura* in short-term trials initially did not achieve spine lengths observed in *Epischura* and *Leptodora* lakes, suggesting that natural selection also modifies lengths over geographic regions, finely tuning responses to resident predators. We suspect that reverse selection from competition may also relax the response. Both feature lengths (mucro, antennule) showed moderate heritability ($h^2 = 0.2$ – 0.5) in the *Mesocyclops* lake (Third Sister) and in an *Epischura*–*Leptodora* lake (Douglas Lake), indicating potential for selective responses. Third Sister *Bosmina* could maintain population density in the presence of *Mesocyclops* and moderate concentrations of introduced *Epischura*, illustrating the importance of induced spine lengths reducing mortality and protecting individuals. However, natural selection could not be tested effectively in enclosure experiments, for exposures were short, no more than 1–2 generations (7–14 d). The inadvertent escape and colonization of Third Sister by *Leptodora* afforded an opportunity to look for selection on *Bosmina* during an episode of enhanced size-selectivity. After establishment, the density of the invertebrate predator increased, producing a 5-yr size-selective episode that coincided with severe depression of small cladocerans (*Bosmina* and *Ceriodaphnia*). Common garden cultures verified genetic shifts in *B. liederii*, pushing means to levels commonly observed in *Epischura* and *Leptodora* lakes.

After *Leptodora* crashed, *Ceriodaphnia* and *Bosmina* are beginning to return to prior abundance. The *Bosmina* community is reverting to earlier patterns, both in species composition (*B. liederii*, *B. freyii*) and seasonal incidence. Rapid return may have been aided by dormant eggs that bridged the decade interval (sediment core studies; W. C. Kerfoot unpubl.).

Reductions in bosminid spine lengths when individuals are shifted from the field to the laboratory are usually interpreted as evidence for metabolic or demographic (e.g., molting) costs (Kerfoot 1977, 1987, 2006). Early studies on *Bosmina* provided evidence for reduced clutch size and population growth associated with longer featured species relative to shorter featured species (Kerfoot 1977; Kerfoot and Pastorok 1978). Recent work with *Eubosmina* demonstrated increased drag associated with very long antennules or dorsal humps (Lagergren et al. 1997; Lord et al. 2006). In long-term laboratory selection experiments with *Epischura*, morphological divergence of *Bosmina* lineages occurs under predation and control treatments, because predation selects for longer spines at the same time as competition in controls selects for diminished length (W. C. Kerfoot unpubl.). Loss of predator-resistant characteristics is commonplace for lineages of algae reared for extended periods of time in the laboratory, presumably because competition favors reduced anti-consumption features (W. R. DeMott unpubl.).

A widely discussed prerequisite for the evolution of induction is temporal variability (Gabriel 1999; Tollrian and Harvell 1999; Gabriel et al. 2005). Several levels of temporal variability were evident in Third Sister Lake

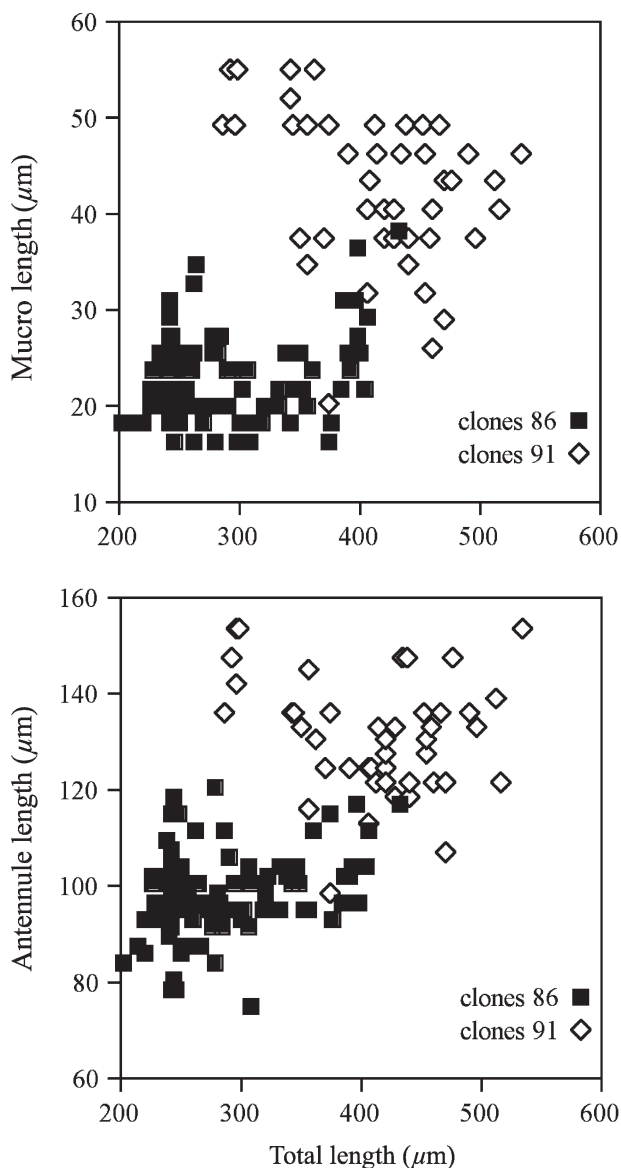


Fig. 8. Comparison of spine lengths (mucrones, antennules) on individuals from laboratory common-garden clones before (1986) and after (1991) *Leptodora* colonization. For additional examples, see Table 7.

(seasonal, yearly, decadal). A seasonal component to risk from invertebrate predators was reflected in significant month-to-month and year-to-year variability, due to the resident mix of invertebrate predators and fish. The *Leptodora* perturbation seemed superimposed upon a general increase in resident invertebrate predators (*Mesocyclops*, *Chaoborus*). An additional ecological cost of defense may involve adjustments to the variety of co-existing predators, a consideration mentioned in Tollrian and Harvell (1999). High incidence of short-featured forms (*B. freyii*) in 1983 coincided with low densities of *Mesocyclops* (McNaught et al. 2004) and other invertebrate predators. Others have noted evidence for depressed *Bosmina* in the presence of *Epischura* and *Leptodora* (Kerfoot and Peterson 1979; Branstrator and Lehman

1991; Chang and Hanazato 2003). Additional excellent long-term examples of rapid evolutionary responses from sediment core studies include Cousyn et al. (2001) and Kerfoot and Weider (2004), where both studies showed that *Daphnia* develop anti-predator traits in the presence of predators and then lose them when predation is relaxed. Thus the predator-prey dialogue involves a restricted number of invertebrate predators, is spatially and temporally dynamic on a regional scale, and seems fine-tuned by resident predators on a local scale.

Bosmina liederii is the largest of the North American small-bodied species in the subgenus *Bosmina*, and often co-occurs with *Leptodora* and *Epischura* (Taylor et al. 2002). The observation that high concentrations of *Leptodora* may eliminate *B. freyii* and severely suppress *Bosmina liederii*, suggests that long-term coexistence between this predator and the two prey species would be enhanced by even larger body size and longer spines. Evidence for a similar circumstance is evident in Lake Suwa, Japan, enclosure experiments, where *Leptodora* induced elongated features in the larger *B. fatalis*. *Leptodora* did not induce feature elongation in the smaller *B. longirostris* and severely depleted this species (Chang and Hanazato 2004).

Across the Baltic region of Europe, there is growing evidence that *Leptodora* predation may also have driven evolution of feature lengths in the larger bodied subgenus *Eubosmina*. *Eubosmina* are slightly larger in body size (total length, 300–700 μm) than *Bosmina* (200–500 μm) and co-occur abundantly in European lakes with *Leptodora*. Recent induction experiments suggest that *Leptodora* and *Mesocyclops* stimulate elongation of antennules in various *Eubosmina* forms (Lagergren and Stenson 2000; Kerfoot 2006). Microfossil records over thousands of years' duration appear to show the onset of seasonal cyclomorphosis in *Eubosmina* about 5000 yr ago in certain European lakes (e.g., in Grosser Ploener See and Schohsee, northern Germany; Hofmann 1978). Paleolimnological work on these lakes confirms that *Leptodora* was present during the cyclomorphosis interval, based on the incidence of *Leptodora* mandibles and tail spines in core sediments with *Eubosmina* remains (Kerfoot 2006).

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