

# Multiple inducers in aquatic foodwebs: Counter-measures and vulnerability to exotics

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#### Abstract

Do aquatic predator and prey species interact strongly enough to foster specialized coevolutionary feedbacks, or are interactions strongly asymmetrical, with prey species responding much more strongly and to multiple threats? Here we utilize prey induction to measure the strength of interactions around a reciprocal "arms race" candidate (*Epischura-Bosmina*). When prey (*Bosmina*) are transferred from predator-poor to predator-rich environments, defensive spines increase in length to achieve a plateau after 12–16 d (1–2 generations). Spine lengths are reversible with predator addition and removal, confirming developmental induction as the major short-term response. Laboratory assays reveal major geographic variation, implying active evolution. Responses range from almost no spine elongation where *Epischura* is historically absent (Europe), to major elongation where the predator and prey are in prolonged contact (Laurentian Great Lakes). Tradeoffs (i.e., loss of competitive ability) can be related to spine lengths of the prey species. However, induction is not exclusive to *Epischura*, as a collection of invertebrate predators also induce spine elongation. *Bosmina* responses to individual predator species are different, implying active recognition of multiple predation threats in nature. The absence of induction responses to some exotics (e.g., *Bythotrephes*) may help explain disproportionate food web impacts. Both revelations underscore the importance of ongoing evolution in aquatic communities.

Some of the most spectacular examples of geographic and seasonal variation are found in the cladoceran families Bosminidae and Daphnidae. Populations from neighboring lakes may differ markedly and exhibit strong seasonal changes in shape and size (Hutchinson 1967). Early on, local selective factors such as vertebrate and invertebrate predation were implicated in structuring zooplankton community composition and in modifying species evolution (Brooks and Dodson 1965; Kerfoot 1980; Zaret 1980). Aquatic prey species were demonstrated to have evolved a host of counter-defenses against predators, including active behavioral avoidance (e.g., vertical migration), cryptic coloration (extreme transparency), rapid escape behaviors, spines and protuberances, and distasteful chemical compounds (e.g., Kerfoot and Sih 1987; Lampert and Sommer 1997). Recent interest in the tightness of predator-prey coevolution (Rauscher 2001), and in the nature and speed of evolutionary feedbacks (Yoshida et al. 2003; Kerfoot and Weider 2004; Hairston et al. 2005)

has prompted questions about interaction strengths around counter-measures in aquatic food webs.

In species interactions, one classical overbridging theory has been the "Red Queen Hypothesis" (Van Valen 1973). Morphological changes in fossils (mollusk families) originally prompted Van Valen to postulate the Red Queen Hypothesis. When estimating extinction rates for families, he obtained a straight line on a log scale, suggesting a constant rate of extinction. Van Valen interpreted morphological changes as indicative of progressive evolutionary change-that is, species must continually evolve just to stay in place. Yet he also viewed the responses as coevolutionary. That is, to maintain constant fitness, he postulated an ongoing reciprocal response between interacting pairs of species (predator and prey, competitors) through time. The environment of each species was interpreted as deteriorating at a constant rate as the species with which it interacted evolved, continually adjusting phenotypic traits. Some of Van Valen's inspiration undoubtedly came from contemporaries, for example, Janzen's (1980) arguments for coevolution in tropical species and Dawkins and Krebs (1979) characterization of the predatorprev interaction as an "arms race."

Smith (1976) criticized the "Red Queen" concept, believing it unlikely that the fitness improvement of a single

Additional Supporting Information may be found in the online version of this article.

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species was precisely balanced by a decrement in the fitness of another interacting species. Whereas arguments involving the evolution of sexual reproduction in *Daphnia* (Ebert 2008), and specialized, reciprocal examples of aquatic hostparasite and host-pathogen evolution (Decaestecker et al. 2007) may fit the Red Queen Hypothesis, the case for application to ordinary aquatic predator-prey interactions remains contentious. Here, we explore antipredator morphological traits in a prey species complex and check to what degree the responses are to a single predator or rather gauged responses to a suite of predators.

An important issue in predator-prey interactions is the question of asymmetric responses (Abrams 2000). One could argue that for many predator-prey interactions, from the perspective of an omnivorous predator, individual prey species constitute little more than a portion of a daily meal, one of many items consumed during foraging. Conversely, from the perspective of individual prey, the consumption issue is one of life or death. Such asymmetries argue that prey should evolve more rapidly away from predators than predators towards prey (Vermeij 1994). Thus, the morphological responses observed in prey species through time by Van Valen (1973) may better reflect responses to multiple threats in the aquatic community, rather than specialized responses to a single, specialized threat. If so, to what degree are prey aware of multiple predators in communities and do they modify morphology to respond to multiple threats? Do we have a way to objectively measure the variety of predators that impact individual prey species and to gauge the relative strength of responses?

An important evolved short-term phenotypic response of prey to predators in aquatic environments is developmental induction (Tollrien and Harvell 1999). Chemical substances (kairomones) released from fish and invertebrate predators may stimulate defensive helmets, spines, and body size changes in primary prey species. These are clearly evolved responses in prey, adaptations designed to reduce mortality and to counter behavioral actions of predators. Moreover, although few of the chemicals have been characterized, effects on prey species are usually rapid, and of sufficient magnitude, that they offer an important way to clarify interaction networks in aquatic food webs. By mapping induction networks, we have the opportunity to determine, from the prey standpoint, whether risk is spread over many potential consumers or is more tightly constrained to contests between pairs of species. For example, Laforsch and Tollrian (2004) showed multiple induction of Daphnia cucullata helmets to a variety of predators. The findings prompted them to assert that helmets serve "generalized" functions, and are an example of Janzen's (1980) "diffuse coevolution."

On a larger perspective, to what degree are communities compartmentalized (Krause et al. 2003; Allesina and Pascual 2009)? Are communities composed of only a few tight predator-prey "spheres of influence" scattered among weakly interacting linkages, or does induction involve ongoing adjustments between multiple members of the community? If strong interactions are limited to only a restricted set of species in the community (Paine 1980), there are important implications for models of trophic-level interactions and foodweb stability calculations (Stouffer and Bascompte 2011). Does induction in nature serve to dampen top–down effects by reducing predator-prey oscillations (Abrams and Walters 1996; Verschoor et al. 2004; Vos et al. 2004)? If invasive predatory species, ones unrecognized by prey species, penetrate well-adjusted communities, could there be dire consequences if defenses in prey are not mobilized?

In the Bosminidae, small species in the subgenus Bosmina produce protuberances (Fig. 1; anterior antennules, posterior mucrones) that act as morphological defenses against invertebrate predators, especially predatory copepods (Kerfoot 1975; Kerfoot and Peterson 1979; Post et al. 1995; Chang and Hanazato 2003). In the case of the Bosminidae, a genus of calanoid copepods (Epischura) has evolved a behavior, the so-called "somersault" maneuver, which is particularly effective against the "dead-man" antipredation tactic of Bosmina (Kerfoot 1978; Kerfoot et al. 1980; Sakamoto and Hanazato 2008). When contacted by an attacking copepod, Bosmina folds its antennae, the sole set of swimming appendages, into a protective groove behind the recurved antennules (Fig. 1). As a consequence of protecting its swimming appendages, the animal becomes motionless and falls passively through the water column. Whereas many attacking cyclopoid and calanoid copepods will lose contact with the prey, Epischura's somersault maneuver allows it to intercept the falling cladoceran and to re-engage handling tactics (Kerfoot et al. 1980). Elongated tail spines retard Epischura's attempt to rotate prey to enter the vulnerable ventral filtering region, whereas long curved antennules protect the folded swimming antennae during an attack. That is, there is a reciprocal nature of components in the Epischura-Bosmina interaction, involving both behavior and morphology, that make it an "arms race" candidate. More recently, there have been attempts to separate short-term (developmental induction) from long-term (selective) responses (Van der Stap et al. 2008; Kerfoot and McNaught 2010).

Here, we measure the magnitude and estimate the specificity of induced responses, comparing induction responses of a number of closely related bosminid species to a variety of aquatic predators. Some of the tested prey populations cooccur with primary predators, whereas others have probably not experienced contact for centuries to thousands of years. We show that there are responses to multiple native predators and that the responses are graded, that is, varying according to perceived threat. Such graded responses suggest that the prey species are identifying which predators are present in the community. Graded responses make sense if there is a cost for defenses. In contrast, the absence of response to some exotic species (e.g., *Bythotrephes*) suggests that these threats are not perceived by native prey species and that the prey species are "blind-sided." Both observations underscore the importance of ongoing evolution in zooplankton community interactions.



#### Methods

#### Induction experiments with Bosmina and Epischura

Presently, New England populations of small bosminids are assigned to a three-species complex (Bosmina longirostris, Bosmina freyi, and Bosmina liederi; DeMelo and Hebert 1994a,b; Taylor et al. 2002). DNA Sequencing established the widespread presence of all three taxa in North America and demonstrated their clustered relationship in a single subgenus (Bosmina; Taylor et al. 2002). European populations were allied to the nominal B. longirostris, which primarily occurs in smaller, eutrophic waters. In the New World, there are some habitat differences, as B. longirostris commonly prefers shallow, productive ponds and reservoirs, whereas B. freyii preferentially occurs in moderate-sized lakes and river systems, and B. liederi inhabits moderate to large lakes, including the Laurentian Great Lakes and Lake Washington. Often, there is spatial overlap, as DeMelo and Hebert found B. freyi and B. liederi together in 42% of sampled moderate-sized lakes in northeastern North America. Western populations are presently assigned to two species, B. longirostris and B. liederi, where at least in the state of Washington, B. longirostris usually occurs in small fish ponds, whereas B. liederi is more commonly found in large lakes (Manning et al. 1978; Taylor et al. 2002).

# Testing reversibility of defensive traits (suspended jar vs. field bag experiments)

Translocation experiments tested induction of Bosmina spine lengths in the field. The source lake for short-featured North American B. longirostris was Occom Pond, a small (19.3-ha; 2.1 m maximum depth) eutrophic lake located on the Dartmouth campus, Hanover, New Hampshire. The zooplankton assemblage is a typical fish predation-associated assemblage (Brooks and Dodson 1965) dominated by various rotifers, small cyclopoid copepods (Tropocyclops prasinus) and B. longirostris. The plankton was repeatedly sampled between 1976 and 2010, confirming that short-featured phenotypes were present over extended periods, although a few largebodied invertebrate predators were occasionally present, but usually scarce (Chaoborus, Mesocyclops). Seasonal change of B. longirostris morphology in Occom Pond was described previously in Kerfoot (1987). Allozyme studies of relationships include Manning et al. (1978) and Kerfoot and Weider

**Fig. 1.** Changes in mucro length, antennule length, and body length during 160 L plastic bag enclosure experiments compared to lengths found at the end of 4 L jar incubations. Spline regressions are fit to bag responses, with the exception of body length (linear fit), whereas examples of jar values are to the right and show means  $\pm$  95% C.L. Solid squares are predator (*Epischura*) incubations (E) whereas hollow squares are controls (C). Notice that *Bosmina* in controls also show increases in feature lengths, although not as much as in predation treatments. A *Bosmina liederi* adult is superimposed over results to show the position of measurements: total (TL), mucro (ML, tail spine), and antennule (AL, curved anterior 1<sup>st</sup> antennule) lengths.

(unpublished data), the latter run to confirm the affinity with Taylor et al.'s (2002) North American *B. longirostris*.

Field induction experiments were performed at Norford Lake, west of Norwich, Vermont, in 1980–1981 (bag), 1985–1986 (jar, first and second years) and again in 2006–2010 (lab induction tests). Norford Lake is a 10.9-ha mesotrophic lake with a maximum depth of 7.5 m. *Epischura lacustris* copepodites and adults appear in May and extend through October. Typical concentrations of *Epischura* copepodites range between 1 and 6 individuals  $\cdot L^{-1}$ , whereas adult densities range between 0.2 and 2.0 individuals  $\cdot L^{-1}$ . Maximum concentrations of copepodites occur from May through late August (Kerfoot 1987; Schulze and Folt 1990).

The 1980–1981 bag experiments were described in Kerfoot (1987). The bags were filled with 160 L of coarsely filtered (75  $\mu$ m Nitex) Lake Norford water, then secured to the inside perimeter of a floating platform. Whereas bag experiments were run between 28 July and 15 August and then again between 15 and 28 August, only results from the 28 July to 15 August experiments are shown in Fig. 1 for comparison with jar results. *Bosmina longirostris* from Occom Pond were introduced into all bags and followed with repeated subsampling. One hundred CIV to adult instar *Epischura* were added to predator treatments to achieve a total density of about 0.6 predators · L<sup>-1</sup>.

The 4-L jar follow-up experiments in Lake Norford tested reversibility of induced spine lengths. In the jar experiments, *Bosmina* were collected fresh from Occom Pond with a 75  $\mu$ m Nitex plankton net and placed into 4-L glass jars. After 1–2 d, *Bosmina* were pipetted into a single "seed" 4-L jar that contained 48  $\mu$ m Nitex filtered water from Occom Pond, creating a *Bosmina* monoculture without competitors or predators. The "seed" jar was taken to Norford Lake, where the contents were stirred gently to mix individuals, then equal portions of the original culture were distributed to 4-L glass jars filled with filtered (48  $\mu$ m Nitex) Lake Norford water or preserved as "reference" samples. The reference samples provided information about introduced numbers, initial morphology, and demography.

Jars were assigned into two categories: Predation Treatment and Controls. After placing approximately equal numbers of *Bosmina* into jars, the Predation Treatments received two 4th copepodite to adult *E. lacustris* from Norford Lake, whereas the Controls received none. A piece of 48  $\mu$ m Nitex netting was placed over the opening of each jar and secured with rubber bands stretched around the jar neck. In the jars, the initial density of advanced *Epischura* (0.50 individuals L<sup>-1</sup>) closely matched natural concentrations for Norford Lake (Kerfoot 1987; Schulze and Folt 1990) and previous bag densities, whereas introduced *Bosmina* densities ranged between 10 and 12 *Bosmina*  $\cdot$  L<sup>-1</sup>. The basic jar deployment design followed Kerfoot and Pastorok (1978), that is, 4-L glass jars placed in sets of three within wire baskets suspended at 2 m depths between floats and anchors. Water exchange rates were measured using methylene blue dye, indicating that about 26% of the volume was exchanged per day. Short-term jar experiments run the first year on 9–21 August consisted of two controls and five predation (*Epischura*) treatments. The second set of short-term experiments run the second year on 21 August–5 September included 4 controls and 13 predation (*Epischura*) treatments. In short duration (12–15 d exposure) experiments, jars were placed in the lake and retrieved at the end of the exposure period.

Longer duration experiments directly tested reversibility. The primary way to document reversibility of induction effects was to remove Epischura from jars midway during a long incubation period. In prolonged predator-removal experiments, jars were placed in Norford Lake on 10 July of the first year (8 controls, 14 Epischura treatments). On 17 July, after 7 d of exposure, seven of the 14 Epischura (EA) treatment jars were retrieved. Contents of three of these jars were preserved to indicate transformations up to that date. In the remaining four jars, Epischura were removed (ER), that is, pipetted out of jars, and the jars were returned to the lake. On 8 August, after 29 d of exposure, all four ER jars were retrieved from the lake. Three of these jars were preserved on that date. Two jars (1 Control and 1 of the Epischura Removal treatments) were transported to the laboratory and brought back into lab culture until 18-21 August, an additional 10-13 d, to check if features reverted to original (reference) lengths.

At the end of exposures, jars were removed and jar contents filtered through 75  $\mu$ m Nitex netting. All microcrustaceans were preserved in a 5% formalin solution containing 40 g  $\cdot$  L<sup>-1</sup> of sucrose. If the number of individuals surviving in the jar was less than 40, all were transferred to microscope slides for measurement. If the number was greater than 40, then 40–50 animals were removed haphazardly and placed on a glass slide in a 50% glycerin–water mixture for measurement. Slides were covered with a glass coverslip and measured under a Zeiss Universal at 500X. Features measured on individuals included: (1) total body length, (2) length of the tail spine (mucro), and (3) length of the antennule (Fig. 1).

During the 2 yr of bottle experiments, a few jars had both *Epischura* dying before the end of the experiment. To be comprehensive, we decided to include the case of both *Epischura* dead at the end of the incubation period as a separate treatment (ED) in the statistical analysis. The statistical distribution of spine lengths has been treated elsewhere in detail (Kerfoot 1988). Spine lengths appear normally distributed, with correspondence close enough to the Normal Distribution to permit ANOVA tests. However, there is a relationship between the standard deviation and mean that suggests an underlying lognormal distribution (common to many morphological variables: Wright 1968; Kerfoot and Kluge 1971). Significant differences between treatment responses in the jar experiments were evaluated using a Nested Analysis of Variance (Nested ANOVA; Systat; Wilkinson 2007) on the three

treatments (EA, ED, C) of the two short series and the four treatments of the long-term series (EA, ED, ER, C).

#### Clonal diversity in defensive responses to multiple predators (short laboratory split-clone experiments in 35 mL vials; 4 L prolonged exposures)

Prior studies established that *Bosmina* undergo spine elongation when placed in close proximity to *Epischura* (Kerfoot 1987; Kerfoot and McNaught 2010). The transmitting agent may be a chemical kairomone, but may also involve physical stimulation (Sakamoto et al. 2007). To test induction in *Bosmina* from different geographic regions and strains, and to judge specificity of responses with predators other than *Epischura*, we utilized a "split-clonal" design (Kerfoot 2006; Kerfoot and McNaught 2010).

In small vial (35 mL) exposures, we tested *Bosmina* in the subgenus *Bosmina* (*B. liederi*, *B. freyii*, *B. longirostris*) from North America and *B. longirostris* from Europe as prey, while *E. lacustris* from Portage Lake, Michigan, was the predator. A second series of tests utilized a wide variety of invertebrate predators (endogenous and exotic predatory cladocerans, copepods, and insect larvae) from Michigan lakes.

In the "split-clonal" design, stem females were removed from source lakes and placed into individual 35 mL culture vials. The stem females established clonal lines that were numbered. The culture medium was finely filtered (0.45  $\mu$ m Supor-450) and aged (6 month) Portage Lake water. Chlamydomonas reinhardii was added as food each day to 35 mL culture vials, medium was changed weekly, and new vials were started at about 1-2 month intervals. Typical concentrations for *Chlamydomonas* ranged between  $10 \times 10^3$  cells/mL  $40 \times 10^3$  cells/mL at time of feeding. After a minimum of three generations to purge maternal effects (ca. 2 weeks), a clonal population in log growth phase was stirred to mix individuals, then split into two separate 35 mL vials. Medium levels in the two vials were then returned to normal by adding aged Portage Lake water ("conditioned water"). Introduced Bosmina varied from 40-80 individuals, sufficient to satiate predators. One vial received a single predator (Predator Treatment; e.g., advanced instar cyclopoid copepod; calanoid copepod, juvenile predatory cladoceran; midge larvae), whereas the other served as a control (Control Treatment). The strengths of the "split-clonal" design include: (1) all individuals are of identical genotype, (2) the initial demographics of the two tested populations are identical, and (3) there is relatively little medium shock to transferred individuals. Moreover, introduced numbers were large enough during short exposure durations to insure sufficient Bosmina survived for measurements.

Vial exposures normally ran for 7–12 d, after which the entire vial contents were preserved in 10% formalin/sucrose. Contrasting to conditions in *Daphnia*, where feature lengths are strongly correlated with total body length, *Bosmina* spine lengths are exceptionally large at birth and are poorly corre-

lated with body length, showing weak positive or negative regression with total body length throughout ontogeny (see Kerfoot 1987, 2006; Kerfoot and McNaught 2010). Because of the weak correlations and almost constant spine lengths throughout life, ANOVA could be used to compare Predator vs. Control treatments without data transformation. However, if there was any evidence for major size-selective depletion of young during exposures (plots of size structure, see size regressions), results were not included in final tables. Additionally, if predators died during experiments, they were replaced (<20% of short, 35 mL trials). Predator condition was checked every 2 d. There was also concern that large Leptodora or Bythotrephes might deplete oxygen. This prompted us to use only immature stages of these taxa in 35 mL vial experiments. Results in the tables include 36 split-clone experiments run with Epischura, four with Limnocalanus, two with Mesocyclops, and six with Acanthocyclops. Finally, to compare average responses to different predators, mean spine elongation (Predation-Control lengths) for multiple clonal tests with various predators were calculated and plotted, with predator species differences tested by t-tests.

In addition, to check responses over longer intervals in the laboratory, long-term induction experiments with Portage Lake Epischura were run in larger containers with mixed clones under batch-culture conditions For Epischura, two replicate 4-L glass beakers were filled with conditioned Portage Lake water to 3.5 L volume and kept at 20°C. Initial cultures were established by mixing 10-15 clones from different laboratory 35 mL shell-vial cultures into a common vessel, then distributing this mixture in equal amounts to each beaker and a preserved sample (Reference). Advanced copepodite to adult Epischura were removed from Portage Lake plankton samples and placed in two predator-treatment beakers, at a density of 1-2 predators/beaker (again 0.3-0.6 Epischura ·  $L^{-1}$ , similar to previous field jar experiments). Predator condition was checked every 2-3 d. If Epischura died, they were replaced with fresh individuals. The cultures were fed Chlamydomonas reinhardtii (U.Tex 90) daily and maintained for 3-4 months. Every 4-15 d, a 500 mL subsample was preserved from the 3.5 L culture (i.e., 14% volume). After sampling events, at approximately weekly intervals, conditioned water was added to maintain beaker volume at 3.5 L. Because of mixed clonal populations and extended durations, the longer experiments would permit both induction and natural selection, but are important in that they more closely matched the longer durations, makeup, and volume (4 L) of the Lake Norford suspended jar experiments. Significance was tested using ANOVA on Predation vs. Control feature lengths, after values had plateaued.

#### Trade-off experiment (4-L Beaker)

Is there a trade-off between spine elaboration and competitive ability? Previous field experiments in Union Bay, Lake Washington, with B. longirostris and B. liederi (Kerfoot 1977; Kerfoot and Pastorak 1978) suggested that species with increased spine lengths and presumably other associated antipredator modifications suffer in competitive ability. Here we tested "trade-offs," in the laboratory by comparing two separate stocks: small-spined Grosser Plöner See B. longirostris against long-spined Portage Lake B. liederi. We pooled individuals from 10 Portage Lake clones, grown under culture for at least 3 week, into a combined "PL population." We pooled individuals from 21 Grosser Ploener See clones, also grown for at least 3 weeks, into a "GPS population." The mixtures were then subsampled to determine densities, and introduced into 4-L glass beakers filled to 3.5 L with milliporefiltered (0.45 um Gelman Supor-450) Portage Lake water in the following combinations: a 50: 50 mixture into two replicated Predation Treatments ("Pred A, B") and two replicated Competition Treatments ("Comp A, B"). A "Reference" sample was taken initially to check on the 50: 50 mixture introduced into predation and competition beakers.

Combinations were set up initially on 18 May, and two Epischura were added to predator treatments on 28 May. Beakers were stirred and subsampled periodically over a span of 2-3 months (1/10 volume; i.e., 350 mL) at room temperature (20°C). If trade-offs exist between susceptibility to predation and competitive ability, the ratio of GPS: PL should diverge under the two treatments. Well-defended longspined individuals (PL) should show a relative increase in the presence of predators, whereas short-spined individuals (GPS) should increase under pure competition conditions. Notice that the test for competitive loss is conservative, because it does not examine loss of competitive ability under maximum expression of spine length (Predation Treatment), but only repercussions during the relaxed condition (predator absent, competition dominant). Spine lengths of the two stocks were checked during the experiment. The cultures were fed Chlamydomonas reinhardtii (U.Tex 90) daily. After sampling events, conditioned water was added to maintain volume at 3.5 L. Divergence of treatments was tested against the Reference sample using chi square tests on the time sequence (Systat; Wilkinson 2007).

#### Results

#### New England field induction experiments

The New England source population (Occom Pond, Hanover, NH) of *B. longirostris* was composed of small-bodied, short-featured clones typical of fish-dominated, low invertebrate predation sites. The seasonal phenotypic patterns described in Kerfoot (1987) remained similar throughout the study (1976–2010). That is, over a typical season, mean mucro lengths (ML) varied between 10  $\mu$ m and 23  $\mu$ m, whereas mean antennule lengths (AL) ranged from 53  $\mu$ m to 93  $\mu$ m. Both mucrones and antennules were proportionately longer in newly born individuals. The morphology of Occom

Pond *Bosmina* introduced into Lake Norford jar experiments initially had small features (Reference samples, Supporting Information). Mean mucro lengths ranged between 17  $\mu$ m and 20  $\mu$ m, whereas mean antennule lengths ranged from 78  $\mu$ m to 93  $\mu$ m. The introduced Occom Pond clones survived relatively well in control and predation jar treatments. Densities began at 10–12 individuals  $\cdot$  L<sup>-1</sup> and ended up at 2.2– 12 individuals  $\cdot$  L<sup>-1</sup>. In the presence of *Epischura*, populations progressively increased spine lengths (Figs. 1, 2; Tables 1, 2). Individuals in control jars, exposed to exchange of predator-conditioned water, also showed modest to slight elongation of mucrones and antennules.

Maximum spine responses in jar treatments reached values found in the prior 160-L polyethylene enclosures (Fig. 1), suggesting little container size effect. When placed with Epischura in 160-L enclosures, mucro lengths increased in 12-16 d from 14  $\mu$ m to 18  $\mu$ m to between 44  $\mu$ m and 49  $\mu$ m, whereas antennule lengths increased from 68  $\mu$ m to 72  $\mu$ m to between 118  $\mu$ m and 122  $\mu$ m. Body length responses were weaker and variable (258–262  $\mu$ m to 310–343  $\mu$ m). The jar manipulations showed similar responses (Figs. 1, 2). In Epischura-added (EA) jar treatments, mucrones and antennules also elongated to maximum values at 12-14 d exposure (Fig 2a), with little additional increase in 29-d exposures (Fig. 2b). Mucro lengths elongated from 18  $\mu$ m to 20  $\mu$ m to between 34  $\mu$ m and 48  $\mu$ m, whereas antennule lengths elongated from 78  $\mu$ m to 93  $\mu$ m to between 107  $\mu$ m and 119  $\mu$ m. Body lengths increased from 269–296  $\mu$ m to 315–373  $\mu$ m, but were highly variable in jars (see Supporting Information).

We used a Nested ANOVA (see Methods) to evaluate changes in the jar experiments. An example is presented in Table 1 (left mucro length, 15-d exposure; 21 August–5 September), followed by a case-by-case summary of results (Table 2). *F*-values indicate that mucro lengths increased significantly in five of six contrasts (right, left side  $\times$  3 time periods = 6 comparisons), whereas antennule lengths increased significantly in all six contrasts (Table 2). Mean body length increased in all predation treatments, yet the differences were significant in only one of the three contrasts. Spine lengths in control treatments increased above reference values, that is, the original introduced condition, indicating influence of a water-borne agent. There was also higher variance for spine lengths in predation treatment jars compared to much greater uniformity in control jars (Table 1; between jars).

The longer-term manipulation series (Fig. 2b; also Supporting Information) showed clear differences between predatoralive (EA), predator-removed (ER), and control (C) treatments. *F* ratios again indicated that mucro and antennule responses were highly significant in the predator treatments (Table 2). Mean mucro lengths moved 3–11 standard deviations beyond reference samples, whereas antennule lengths moved 2–6 standard deviations (standard deviation moved = Reference mean—Observed mean divided by the average SD; giving a distance measure). Given a rough generation time of 6–8 d,



**Fig. 2.** Spine responses in field jars: (A) Responses in a typical field jar experiment (09–21 August, 12 d incubation). Treatments include: Reference (R); *Epischura* alive (EA); and Control (C). Symbols include mean (solid square), 95% C.L. of mean (plus or minus brackets around mean), minimum (blue square) and maximum (red square). (B) Responses in manipulated, longer (10 July–August 8, 29 d) field jar experiments. Symbols the same, but including additional treatments: ER (*Epischura* removed, see Methods), EL (*Epischura* treatment returned to lab, 18 August, kept 10 d), CL (Control returned to lab, 21 August, kept 13 d).

**Table 1.** Nested ANOVA for left mucro length, second year, 21 August–5 September, after 15 d exposure (n = 339). Significance of *F*-ratios = ns, not significant;  $p < 0.001^{***}$ . "Between treatments" contrasts Predation treatments with Controls, whereas "Between jars within treatments" compares jars within treatments.

		Degrees			
Source	Sum-of-squares	of freedom	Mean-square	F-ratio	р
Between treatments	19,247.8	2	9623.9	16.3	0.000***
Between jars	7066.4	12	588.9	10.3	0.000***
Alive	4641.4	7	663.1	11.6	0.000***
Dead	2371.3	2	1185.7	20.8	0.000***
Control	53.7	3	17.9	1.3	0.816 <sup>ns</sup>
Between all individuals	18,498.6	324	57.1		

the responses spanned approximately 1–3 generations. Feature lengths reduced when *Epischura* were removed during the long incubation interval (Fig. 1b), relaxing towards lake water-treatment controls. Again lake water-exposed Control treatments showed slight increases in feature lengths over Reference samples during short exposure durations, with significant departures over the 26-d exposure period (Supporting Information; *t*-test, mucro, *t* = 7.1–14.7; all five jars *p* < 0.001; antennule, *t* = 2.0–11.9; three jars *p* < 0.01, two jars *p* < 0.05), strongly suggesting water stimulation. When individuals from

*Epischura* removal treatments and from control jars were returned to the laboratory, their morphology relaxed back toward the initial Occom Pond reference sample appearance (Fig. 2b). A comparison between the reference and returned feature lengths indicated convergence (*t*-test, p = 0.21-0.93 mucro and antennule lengths, Control jar; p = 0.07-0.53 for mucro and p = 0.03-0.11 for antennule, ER jar). The removal experiments and lab return results underscored that the transformations were largely developmental (i.e., phenotypic) and reversible.

**Table 2.** Nested ANOVA results (*F*-values) for Occom Pond *Bosmina* in jars exposed to Norford Lake *Epischura*. Between-treatment and between-jar values are listed for the three jar incubation experiments. The degrees of freedom for *F*-values are below the *F*-values. Significance levels = ns, not significant; \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001).

Source	Total length	Left mucro	Right mucro	Left antennule	Right antennule
Jource	Total length	length	length	length	length
First Year, 21 Aug, after 12	d				
Between treatments	1.72 <sup>ns</sup>	6.40 <sup>ns</sup>	7.40*	15.32*	14.57*
	2,4	2,4	2,4	2,4	2,4
Between jars	2.39 <sup>ns</sup>	36.70***	34.43***	13.93***	15.70***
	4,272	4,272	4,268	4,262	4,259
Second Year, 8 Aug, after 2	29 d				
Between treatments	0.90 <sup>ns</sup>	11.93***	10.63**	8.51**	10.08**
	3,13	3,13	3,13	3,13	3,13
Between jars	1.10 <sup>ns</sup>	4.72***	4.95***	4.93***	4.10***
	13,286	13,286	13,280	13,278	13,273
Second Year, 5 Sep, after 1	5 d				
Between treatments	5.38*	16.34***	19.06***	16.52***	14.77***
	2,12	2,12	2,12	2,12	2,12
Between jars	2.25**	10.31***	8.89***	7.17***	7.56***
	12,329	12,324	12,308	12,288	12,274

### Laboratory induction experiments (New England, Occom Pond clones)

When Occom Pond clones were brought into the laboratory and cultured in 35 mL vials, they also responded strongly to the presence of Epischura in split-clonal incubations (Fig. 3a). For example, after acclimation to laboratory conditions, clones had mucro and antennule lengths varying between 20-23 µm and 86-103 µm, respectively. When placed in the presence of Epischura for 7-8 d, Bosmina elongated mucro and antennule lengths up to 25–41  $\mu$ m and 99-127 µm, respectively, all highly significant responses (Table 3; mucrones: F values 19.6–106.4, p < 0.001; antennules: F values 11.7–87.0, p < 0.005). Spines increased in newly borne individuals and between molts, influencing the entire size spectrum of populations (Fig. 3a). In Bosmina, recall that growth relationships of spines are atypical (relatively large size at birth, low correlation with body size). In general, body size relationships explain only 12-14% of the spine total variance, with regressions that often fluctuate between slightly positive to slightly negative (Fig. 3). For the exposure duration (7-8 d), the elongation responses closely matched values seen in field enclosures and suspended jars (Figs. 1, 2). As a check for longer exposures with Occom clones, an experiment was run in a 300 mL beaker for 16 d, to see if feature differences increased to plateau levels. They did, as mucro lengths reached  $41 \pm 3 \ \mu m$  and antennule lengths reached  $127 \pm 3 \ \mu m$  (Fig. 3a, Table 3; mucrones: F = 121.2, p < 0.001, antennules F = 218.5, p < 0.001). Thus, the predator-related responses observed in field enclosures could be replicated in laboratory experiments. The lack of a

container effect was also evident, as the 35 mL vials were again much smaller containers than the suspended 4 L jars or the original 160 L bags, respectively.

## Lab induction experiments with bosmina from different geographic regions and with different predators

In the laboratory, Bosmina clonal responses were judged by two comparisons: (1) degree of spine regression, that is, the spine length when the predator was absent (Control Treatment), and (2) the spine elongation in the presence of Epischura (Predation Treatment). There were clear differences for Bosmina taken from lakes where Epischura occur naturally vs. lakes where the association was only nearby (neighborhood) or where there was little historic contact (Fig. 4; Table 3). For example, induction experiments using B. liederi and Epischura from Portage Lake, Houghton County, Michigan, where Bosmina coexist with multiple invertebrate predators (the calanoid copepods E. lacustris, Limnocalanus macrurus; the cyclopoid copepods Mesocyclops edax and Acanthocyclops vernalis, the predatory cladoceran Leptodora kindtii), showed the largest size of features. Even in the regression condition, spines remained moderately long (mean- $\pm$  SD = mucro 36.0  $\pm$  4.9  $\mu$ m; antennule 89.0  $\pm$  9.0  $\mu$ m). In Predation Treatments, there was strong induction (mucro  $49.5 \pm 5.8 \ \mu\text{m}$ ; antennule  $109.7 \pm 10.2 \ \mu\text{m}$ ; Figs. 3, 4; Table 3). ANOVA comparisons were always highly significant between the Predation and Control split-clonal comparisons.

When Portage Lake *E. lacustris* were introduced into 4-L beakers with Portage Lake prey (*Bosmina liederi*) for long-term exposures, over the first 8–15 d there was a rapid



**Fig. 3.** Spine induction responses in laboratory 35 mL vial experiments: (A) Responses to *Epischura lacustris*. Hollow square symbols represent Controls, whereas solid diamonds represent predation (*Epischura*) treatments. The  $r^2$  values given with regressions. Examples include: Occum Pond (8-d), Portage Lake (8-d), and Portage Lake long term (16-d). B) Responses to various cyclopoid copepods, illustrating less response in mucrones and antennules (*Mesocyclops* and *Acanthocyclops* 8-d exposures; 16-d *Acanthocyclops*). The significance of differences is tested by ANOVA in Tables 4 and 5.



Fig. 3. (Continued)

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**Table 3.** ANOVA results for split-clone laboratory induction experiments (35 mL vials) with the copepod predator *Epischura lacustris*. Locations (PL, Portage Lake; TSL, Third Sister Lake; OP, Occom Pond; LW, Lake Washington; GPS, Grosser Ploener See; SEL, Selenter See) and taxonomic status of tested populations (last two digits of clonal line give year of lab tests). Exposure durations are 7–8 d in 35 mL vials unless otherwise noted. Table indicates the number of individual *Bosmina* measured (*n*), the nature of the treatment (Control or Predator Treatment for split-clone), mean spine length ( $\mu$ m) with SD in parenthesis, *F* (df) value for ANOVA, and probability (*p* value).

Region							
and taxa	Clone	Variable	n	Treatment	Mean	F	<i>p</i> value
Portage Lake, Houg	hton, MI, USA						
B. liederi	PLA-05	Mucro	42	Control	37(5.4)	163.4	3.25 E −21
			42	Predator	55(6.8)	(1,82)	
		Antennule	42	Control	93(9.9)	86.7	1.71 E -14
			42	Predator	112(8.9)	(1,82)	
B. liederi	PL1-05	Mucro	72	Control	37(5.2)	436.3	8.67 E -47
			84	Predator	54(5.2)	(1,154)	
		Antennule	72	Control	90(8.4)	345.5	3.46 E -41
			84	Predator	115(8.4)	(1,154)	
B. liederi	PL2-05	Mucro	42	Control	37(5.4)	168.3	1.15 E -21
			43	Predator	55(6.7)	(1,83)	
		Antennule	42	Control	93(9.9)	89.8	7.33 E -15
			43	Predator	112(8.8)	(1,83)	
B. liederi	PL135-08	Mucro	42	Control	34(4.1)	60.0	2.39 E -11
			41	Predator	43(5.8)	(1,81)	
		Antennule	42	Control	79(5.7)	40.6	1.08 E -08
			41	Predator	91(11.4)	(1,81)	
B. liederi	PL3EC-05	Mucro	46	Control	45(6.2)	109.7	2.61 E -17
			45	Predator	56(3.6)	(1,91)	
		Antennule	46	Control	104(12.6)	64.5	3.29 E -12
			45	Predator	124(11.3)	(1,91)	
B. liederi	PL181-08	Mucro	22	Control	29(4.8)	49.4	1.21 E -07
			8	Predator	44(5.9)	(1,28)	
		Antennule	22	Control	86(6.4)	67.5	6.11 E -09
			8	Predator	108(6.1)	(1,28)	
B. liederi	PL-135B-08	Mucro	41	Control	33(4.3)	115.5	7.73 E -17
			36	Predator	44(4.7)	(1,75)	
		Antennule	41	Control	78(5.4)	188.0	4.00 E -22
			36	Predator	99(8.2)	(1,75)	
B. liederi	PLA-05	Mucro	42	Control	3/(5.4)	163.4	3.25 E −21
			42	Predator	55(6.8)	(1,82)	
		Antennule	42	Control	93(9.9)	86./	1./1 E -14
	214.05		42	Predator	112(8.9)	(1,82)	
B. liederi	PL1-05	Mucro	/2	Control	37(5.2)	436.3	8.67 E -47
			84	Predator	54(5.2)	(1,154)	
		Antennule	/2	Control	90(8.4)	345.5	3.46 E -41
	21.0.05		84	Predator	115(8.4)	(1,154)	
B. liederi	PL2-05	Mucro	42	Control	3/(5.4)	168.3	1.15 E -21
			43	Predator	55(6./)	(1,83)	
		Antennule	42	Control	93(9.9)	89.8	7.33 E -15
			43	Predator	112(8.8)	(1,83)	
B. liederi	PL135-08	Mucro	42	Control	34(4.1)	60.0	2.39 E -11
			41	Predator	43(5.8)	(1,81)	1 00 5 55
		Antennule	42	Control	/9(5./)	40.6	1.08 E -08
			41	Predator	91(11.4)	(1,81)	

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### TABLE 3. Continued

Region							
and taxa	Clone	Variable	n	Treatment	Mean	F	p value
B. liederi	PL3EC-05	Mucro	46	Control	45(6.2)	109.7	2.61 E −17
			45	Predator	56(3.6)	(1,91)	
		Antennule	46	Control	104(12.6)	64.5	3.29 E −12
			45	Predator	124(11.3)	(1,91)	
B. liederi	PL181-08	Mucro	22	Control	29(4.8)	49.4	1.21 E −07
			8	Predator	44(5.9)	(1,28)	
		Antennule	22	Control	86(6.4)	67.5	6.11 E -09
			8	Predator	108(6.1)	(1,28)	
B. liederi	PL-135B-08	Mucro	41	Control	33(4.3)	115.5	7.73 E −17
			36	Predator	44(4.7)	(1,75)	
		Antennule	41	Control	78(5.4)	188.0	4.00 E −22
			36	Predator	99(8.2)	(1,75)	
B. liederi	PL-189-08	Mucro	42	Control	47(7.4)	103.3	3.54 E −16
			42	Predator	63(7.5)	(1,82)	
		Antennule	42	Control	102(15.6)	34.9	7.72 E −08
			42	Predator	122(15.7)	(1,82)	
B. liederi	PLE1-14	Mucro	41	Control	36(5.3)	25.4	2.76 E −06
			43	Predator	43(7.6)	(1.82)	
		Antennule	41	Control	99(11.5)	15.4	0.00018
			43	Predator	111(15.9)	(1.82)	
Third Sister Lake. An	n Arbor, MI, USA					('''''	
B. liederi	TSL5-05	Mucro	42	Control	27(5.3)	17.7	8.00 E −05
D. neden			25	Predator	32(4.4)	(1.65)	
		Antennule	42	Control	99(7.9)	30	7.55 E -07
		, arcennare	25	Predator	111(11.1)	(1.65)	,
B. liederi	TSI 6-05	Mucro	32	Control	24(2.9)	120.4	1.11 F -14
D. neden	1320 03	Macro	18	Predator	35(4.2)	(1.48)	
		Antennule	32	Control	98(8.6)	21.3	2 63 E -05
		<i>internate</i>	21	Predator	108(4.2)	(1.51)	2.05 2 05
R frevii	TSI 10-05	Mucro	42	Control	26(5.7)	17 7	8 00 F -05
21			25	Predator	32(4 3)	(1.65)	0.00 2 00
		Antennule	42	Control	99(8.2)	30.0	755 F - 07
		<i>internate</i>	25	Predator	112(11.0)	(1.65)	7.55 E 07
R liederi	TSI 6-06	Mucro	35	Control	26(4.2)	37.2	8 32 F -08
D. neden	1320 00	Macro	27	Predator	32(3.6)	(1.60)	0.52 2 00
		Antennule	35	Control	93(8.5)	47.5	384 F - 09
		/ intermate	27	Predator	107(6.4)	(1.60)	5.012 07
R liederi	TSI 6-06	Mucro	35	Control	23(3.4)	38.5	3 72 F 08
D. neden	1320 00	Widero	35	Predator	30(4.8)	(1.68)	5.72 2 00
		Antennule	35	Control	95(7.1)	86.0	1 10 F -13
		Antennuie	35	Predator	110(6.2)	(1.68)	1.10 2 15
R frevii	TSI 10-06	Mucro	34	Control	23(4.2)	37.2	5 71 E -08
D. Heyn	15210-00	Widero	36	Predator	29(4.2)	(1.68)	5.7TE 00
		Antonnulo	34	Control	27(4.1)	119.7	1 47 5 -16
		Antennule	24	Dradator	110(2.0)	(1 69)	1.47 L = 10
Occom Pond Hanov	ver NH USA		20	FIEUALUI	110(0.7)	(1,00)	
B. lonairostris	OP11-10A	Mucro	18	Control	20(2.3)	20.5	4.14 E −05
			30	Predator	26(5.4)	(1.46)	
		Antennule	18	Control	91(9.0)	11.7	1.31 F -03
			30	Predator	103(13.4)	(1.46)	
			20			(.,)	

#### TABLE 3. Continued

and taxa	Clone	Variable	n	Treatment	Mean	F	p value
B. longirostris	OP14-06	Mucro	42	Control	23(3.3)	106.4	1.77 E −16
			42	Predator	34(5.7)	(1,82)	
		Antennule	42	Control	103(7.9)	87	1.79 E −14
			42	Predator	122(10.4)	(1,81)	
B. longirostris	OP12-10	Mucro	42	Control	20(3.5)	56.1	1.99 E −10
			27	Predation	30((7.2)	(1,67)	
		Antennule	42	Control	86(11.2)	47.7	2.19 E −09
			27	Predation	107(14.5)	(1,67)	
B. longirostris	OP11-10B	Mucro	38	Control	22(3.1)	50.4	2.36 E −09
			20	Predation	31(6.7)	(1,56)	
		Antennule	38	Control	92(7.6)	19.6	4.42 E −05
			20	Predation	105(16.1)	(1,56)	
B. longirostris	OP21-10(18-d)	Mucro	42	Control	23(4.3)	121.2	6.63 E -18
			43	Predation	41(10.1)	(1,83)	
		Antennule	42	Control	96(10.3)	218.5	5.74 E -25
			43	Predation	127(8.7)	(1,83)	
Union Bay, Seattle, WA	A, USA						
B. longirostris	UB3-06	Mucro	42	Control	19(3.0)	0.79	0.38
-			32	Predator	19(4.4)	(1,72)	
		Antennule	42	Control	84(8.5)	18.8	4.67 E −05
			32	Predator	94(9.5)	(1,72)	
B. lonairostris	UB1-06	Mucro	42	Control	18(3.1)	9.9	2.22 E −03
5			43	Predator	20(3.3)	-1.83	
		Antennule	42	Control	73(8.0)	11.8	9.38 E −04
			43	Predator	79(6.2)	(1,83)	
B. longirostris	UB10-06	Mucro	41	Control	19(3.7)	0.52	0.47
J.			42	Predator	19(3.2)	(1,81)	
		Antennule	41	Control	77(6.8)	20.4	2.16 E −05
			42	Predator	83(4.9)	(1,81)	
B. longirostris	UB2-06	Mucro	42	Control	18(3.1)	0.52	0.47
5			42	Predator	18(2.8)	(1,82)	
		Antennule	42	Control	81(6.6)	11.2	0.001
			42	Predator	87(7.6)	(1,82)	
Grosser Ploner See, Plo	en, Germany, Europe						
B. longirostris	GPS-06	Mucro	43	Control	15(3.9)	0.3	0.58
5			45	Predator	15(4.9)	(1,86)	
		Antennule	43	Control	75(11.0)	1	0.33
			45	Predator	77(8.9)	(1.86)	
B. lonairostris	GPS-07	Mucro	42	Control	16(3.2)	6.3	0.01
			41	Predator	18(4.2)	(1.81)	
		Antennule	42	Control	80(8.7)	11.7	9.97 E −04
			41	Predator	86(7.6)	(1.81)	
B lonairostris	GP\$21-06	Mucro	43	Control	12(4.0)	16	0.21
	0.02.00		43	Predator	11(2.8)	(1.85)	
		Antennule	43	Control	75(9.9)	0.5	0.47
Selenter See Ploen		, intermute	43	Predator	76(9 1)	(1.85)	,
Germany, Europe						()/	

TABLE 3. Conti	nued
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Region	Clana	Variable	2	Treatment	Moon	E	n valuo
	Clotte	variable	п	Treatment	Iviean	Г	<i>p</i> value
B. longirostris	SEL2-06	Mucro	42	Control	22(6.2)	0.2	0.65
			42	Predator	21(4.9)	(1,82)	
		Antennule	42	Control	90(13.7)	1.4	0.24
			42	Predator	94(11.6)	(1,82)	
B. longirostris	SEL4-10	Mucro	36	Control	23(5.9)	0.05	0.83
			40	Predator	23(6.7)	(1,74)	
		Antennule	36	Control	92(10.8)	0.05	0.82
			40	Predator	92(10.7)	(1,74)	

increase in mucro and antennule spine lengths (Fig. 5; Table 4). Mucrones moved from 33  $\mu$ m to 54–59  $\mu$ m, whereas antennules increased from 92  $\mu$ m to 106–116  $\mu$ m, achieving a plateau after about 16 d. Curiously, control mucro lengths fell below Reference levels to 25–31  $\mu$ m over the same interval. After plateauing, the differences between predation and control treatments were highly significant (ANOVA, 8/2 mucro PRED = 58.5  $\mu$ m, CONT = 31.4  $\mu$ m;  $F_{1,91}$ = 388.7, p = 1.32 E -34; antennule PRED = 116.1  $\mu$ m, CONT = 83.5  $\mu$ m;  $F_{1.90} = 183.5$ , p = 1.94 E - 23). For mucro lengths, comparisons of predator treatment mean with control mean showed a 4.3 standard deviation (SD) difference. The same pattern occurred in antennule lengths, but the relative difference was less, around 2.8 standard deviations (SD). These experiments verified that the substantial induction differences observed with Epischura in field experiments could also be shown in the laboratory under controlled conditions both in 1-week 35 mL split-clonal design experiments and in long-term 4L exposures with mixed clones.

What if *Epischura* is in the neighborhood, but not in the lake, that is, a circumstance of probable historic, but not sustained, contact. When *Epischura* were introduced into 35 mL vials with Third Sister *Bosmina*, both mucro and antennule lengths increased, but not as much as with Portage Lake *Bosmina*. Mucro lengths increased from a mean of 24.8  $\mu$ m to 31.7  $\mu$ m, an increase of 6.9  $\mu$ m (1.6 SD increase), whereas antennule lengths increased from a mean of 95.8  $\mu$ m to 109.7  $\mu$ m, an increase of 13.9  $\mu$ m (1.8 SD increase).

What if *Epischura* is mostly or entirely absent from the regional neighborhood? For this comparison, split-clone induction experiments were performed with *B. longirostris*: (1) from the state of Washington, where *B. longirostris* populations are known to be found mostly in small fish ponds, but occasionally colonize shallow bays (Union Bay, Lake Washington), and (2) in German lakes, where *Epischura* does not occur regionally (Table 3). The Seattle pond and Union Bay *B. longirostris* had relatively small regressed mucrones (18–19  $\mu$ m) and antennules (73–84  $\mu$ m). When placed in the presence of *E. lacustris*, there were insignificant to only minor responses in mucrones (0–0.6 SD) and only moderate

responses in antennules (0.9–1.1 SD). When placed in laboratory culture without predators, the small European bosminid *B. longirostris* regressed down to the shortest of spine lengths (15–22  $\mu$ m mucro; 75–90  $\mu$ m antennule). In the presence of *E. lacustris*, four of five clones from the two German lakes (Grosser Ploener See, Selenter See) showed no significant elongation of mucrones, the lowest response observed (Table 3). Mean antennule elongation in the presence of *Epischura* was only 4  $\mu$ m, also the lowest response in *Bosmina* tests, and was only significant in one of three tests. In terms of standard deviations, the mean response for mucro length was 0.1 SD and 0.4 SD for antennule length, that is, hardly any induction at all.

These results suggest that intimate contact between large invertebrate predators and *Bosmina* is important for maintaining maximum spine lengths, especially tail spine (mucro) lengths. Plotting all responses to *Epischura* on the same graph (Fig. 4), illustrates a rather simple pattern for the two features (mucrones and antennules). Populations from the three species vary geographically. Feature lengths are smallest where *Epischura* are absent or scarce, intermediate lengths where *Epischura* is common in the neighborhood, and longest when *Epischura* show prolonged residency. Induction of mucrones greatly increases when *Epischura* is more abundant, whereas the induction pattern is a bit more uniform for antennules.

Tests with different predators (Table 5) showed that spines did not respond exclusively to *Epischura*, nor to all local invertebrate predators, but to a select set of invertebrate predators (suite of 2–5 local predators). Spine lengths of *Bosmina* did not respond to several invertebrate predators that are natural residents (e.g., *Asplanchna, Polyphemus, Chaoborus*), nor to certain exotic species (Figs. 6, 7; *Eurytemora or Bythotrephes*). Examples of split-clone tests with *Asplanchna* (mucro d.f. = 1, 76; F = 3.04, p = 0.09; antennules d.f. = 1,76, F = 0.10, p = 0.76); *Chaoborus* (mucro; d.f. = 1,76, F = 0.274, p = 0.60; antennules; d.f. = 1,76, F = 2.80; p = 0.10), *Polyphemus* (mucro d.f. = 1, 46; F = 2.25, p = 0.14; antennules, d.f. = 1,121, F = 0.162, p = 0.69; antennules, N = 1,121, F = 2.83, p = 0.10),



**Fig. 4.** Induction responses across the size range of spine features, indicating position of clones from various *Bosmina* species (see shading). Each point represents the regressed condition (*x*-axis) plotted against the induced condition (*y*-axis). The dashed line would represent the null condition, that is, if regressed and induced morphology were identical. The regression departure from the dashed line shows that clones with longer features in the regressed condition are more likely to show more induction when *Epischura* are present.

and water mites (*Unionicola*, mucro d.f. = 1, 64; F = 0.789, p = 0.38; antennules, N = 1, 64; F = 0.266, p = 0.61) illustrate predatory species that did not elicit induction of spines.

Bosmina species did respond to Leptodora, Limnocalanus, Epischura, and a suite of cyclopoids (Mesocyclops, Acanthocyclops, and Diacyclops; Fig. 3b; Table 5). For example, B. liederi in Third Sister Lake responded to Mesocyclops, Epischura, and Leptodora, whereas B. liederi in Portage Lake increased spine lengths to Epischura, Leptodora, Limnocalanus, Mesocyclops, Acanthocyclops, and Diacyclops (Fig. 6, Table 5). The response to Limnocalanus has previously not been reported. Responses to the secondary suite of invertebrate predators were significant, but generally less than to Epischura (Fig. 3b, 6; Table 5). In the case of multiple experiments with Portage Lake taxa, when mean spine increases are plotted in microns (Fig. 7), clear differences are evident. Bosmina failed to show significant responses in mucrones and antennules to the exotic species *Bythotrephes* (mean spine increase  $\pm$  95%C.L.; *N* = 8, mucro  $1.4 \pm 2.8 \ \mu\text{m}$ ; antennule  $1.0 \pm 3.5 \ \mu\text{m}$ ). In contrast, there were moderate, yet intermediate, spine increases to Acanthocyclops (N = 11, mucro increased  $3.9 \pm 1.1 \mu m$ , antennule  $10.9 \pm 1.5 \ \mu\text{m}$ , Mesocyclops (N = 4, mucro  $5.0 \pm 1.7 \ \mu\text{m}$ , antennule  $12.3 \pm 8.1 \ \mu m$ ), and *Limnocalanus* (N = 4, mucro  $6.0 \pm 2.2 \ \mu\text{m}$ ; antennule  $11.3 \pm 2.8 \ \mu\text{m}$ ). Spine increases were again greatest with *Epischura* (N = 14, mucro increased) $13.9 \pm 2.2 \ \mu m$ , antennule  $17.6 \pm 2.6 \ \mu m$ ). Antennule lengths increased proportionally more than mucro lengths to cyclopoid and calanoid copepods (Fig. 7).

#### Trade-off hypothesis

To test if there was a trade-off between predator resistance and competitive ability among the set of three species, shortfeatured Bosmina (Grosser Ploener See B. longirostris) were placed together in beakers with longer-featured Bosmina (Portage Lake B. liederi) at nearly even (50: 50 ratio) initial concentrations. One set of beakers was subjected to active predation, whereas the other set was allowed to compete. In the predation treatments, the ratio of short- vs. long-featured individuals shifted strongly towards the longer-featured Portage Lake (PL) B. liederi clones (GPS 18-11% and PL 86-89% after 44 d; Table 6). In contrast, competition treatments shifted short- vs. long-featured ratios strongly in the reverse direction, towards short-featured Grosser Ploener See (GPS) B. longirostris clones (GPS 70-81%, PL 39-36% by 78 d; Table 6). In predation treatments, divergence from initial ratios was significant by day 24,  $x^2 = 100.3$ , p < 0.01, whereas divergence in the opposite direction was seen by day 78 ( $x^2 = 69.6$ , p < 0.01). At the end of the series, species determinations were checked with morphological measurements and with allozyme markers phosphoglucose isomerase (PGI). A check on spine lengths at the beginning and end of the experiment showed that the Portage Lake population retained longer spine features than the Grosser Ploener See populations in the competition test (e.g., ANOVA; d.f. <sub>5,120</sub>; mucro, F = 51.5, p = 2.909 E - 28). In conclusion, although conferring strong resistance towards predation (Epischura), Bosmina species with long spines appear to have a lower competitive ability. Thus, the traits (longer spines) that confer greater advantage in the presence of a predator appear to detract from competitive ability, explaining why there would be counter-selection against longer spines when predators are absent.

#### Discussion

Field jar and laboratory experiments confirm the latent ability of most North American *Bosmina* species to elongate



**Fig. 5.** Long-term laboratory exposure (4 L beaker) of Portage Lake *Bosmina liederi* to *Epischura*, illustrating responses of mucrones and antennules. Labels on *x*-axis indicate Reference (REF), *Epischura* predation treatment (EP) vs. control (C, no predator). Box and whisker diagrams show mean (black box), median (horizontal line), quartiles (horizontal box ends), and ranges (end whiskers), whereas sampling dates are indicated along the *x*-axis. Near the end, the differences between predation and control treatments are measured in standard deviation units (SD).

**Table 4.** Long-term induction experiment with *Epischura* and *Bosmina liederi* (Portage Lake; N = number of individuals measured). Treatments include Reference (initial condition), Control or Predation Treatment, with date of sample. Measurements as mean 95% C.L. (TL, total length; ML, mucro length; AL, antennule length;  $\mu$ m).

Treatment	N	TL	ML	AL
Reference 6/20	62	$344\pm20$	$33\pm3$	92±3
Control				
6/27	88	$334\pm44$	$33\pm4$	$90\pm 6$
7/10	66	$280\pm20$	$28\pm2$	$76\pm5$
7/19	62	$304 \pm 19$	$28\pm2$	$84\pm4$
7/23	55	$311 \pm 17$	$27\pm2$	$81\pm4$
8/02	77	$317\pm23$	$31\pm2$	$84\pm3$
8/17	67	$322\pm21$	$31\pm3$	$82\pm4$
Predation (Replica	ites A,B)			
A 6/26	69	$281\pm38$	$40\pm7$	$98\pm9$
A 6/30	68	$339\pm25$	$45\pm3$	$99\pm5$
A 7/9	71	$331\pm23$	$55\pm2$	$106\pm4$
A 7/19	55	$308 \pm 17$	$53\pm2$	$113\pm4$
A 7/23	58	$323\pm18$	$56\pm2$	$114\pm 6$
A 8/2	64	$367\pm19$	$59\pm1$	$115\pm4$
A 8/17	75	$362\pm22$	$56\pm2$	$113\pm5$
B 6/30	58	$319 \pm 24$	$53\pm2$	$109\pm5$
B 7/10	67	$336\pm17$	$53\pm1$	$102\pm3$
B 7/19	77	$327\pm24$	$52\pm3$	$109\pm5$
B 7/23	65	$325\pm21$	$53\pm2$	$114\pm5$
B 8/2	70	$316\pm23$	$53\pm2$	$114\pm5$
B 8/17	66	$334\pm21$	$51\pm2$	$106\pm4$

defensive features (elongated mucro and elongated, curved antennule, sometimes larger body size) in the presence of Epischura and to reduce features when risk is removed. Field jar experiments closely matched morphological transformations observed earlier in 160-L polyethylene bag experiments (Kerfoot 1987). Reversibility of transformations in both the field and laboratory underscored the developmental (phenotypic plasticity) nature of the response to predators. Generally, the North American small bosminid species (B. longirostris, B. freyii, and B. liederi) formed a series of populations with increasing feature lengths (Kerfoot 1987; Kerfoot and McNaught 2010) and most populations exhibited induction to a variety of predatory copepods and predatory cladocerans. A competitive trade-off for two species with contrasting short or long spines was demonstrated in the laboratory, supplementing previous field observations (Kerfoot 1977). If strong repercussions on competition are present, flexible developmental responses make sense, especially if predation risk is variable in time (seasonal pattern) or in space (regional spatial occurrence). Not surprisingly, one widely discussed prerequisite for the evolution of induction is temporal or spatial variability (Gabriel 1999; Gabriel et al. 2005).

In predation exposures, the response time of 15–16 d for maximal expression indicated that adjustments occurred over multiple (1–3) generations. During induction, developmental responses moved features several standard deviations [mucro 1.5–4.3 SD; antennule 1.1–3.7 SD]. Multigeneration responses in parthenogenetic populations suggest "maternal" effects (Lynch and Walsh 1998; Agrawal et al. 1999) and



**Fig. 6.** Testing *Bosmina* induction responses on a variety of predators in split-clone experiments. Control and Predator treatments are plotted as box and whisker diagrams for mucro and antennule lengths (see Fig. 5). Nonsignificant comparisons are shown in the left panel, whereas significant comparisons are illustrated on the right. Data and ANOVA tests for the right-hand cases are listed in Tables 4 and 5.



**Fig. 7.** Plotting mean spine (mucro, antennule) elongation responses to *Bythotrephes* (N = 8 experiments), *Acanthocyclops* (N = 11), *Mesocyclops* (N = 4), *Limnocalanus* (N = 4), and *Epischura* (N = 14). Mean length responses are recorded in microns (Control—Predation) and displayed as box and whisker diagrams (see Fig. 5). Notice how responses to *Acanthocyclops, Mesocyclops,* and *Limnocalanus* are less than to *Epischura*.

resemble findings from *Daphnia* (Ebert 1993; Tollrian 1995; De Meester et al. 1998). Populations within net-covered control jars in the field also showed modest responses. The water-only responses suggest sensitivity to a chemical (kairomone) signal associated with water exchange, although mechanical (sound, wave) stimuli cannot be ruled out (Sakamoto et al. 2007). Individuals in control jars were much more morphologically uniform, whereas those in pre-

dation vessels were more highly variable. High variability in the presence of predators was noticed earlier in Third Sister Lake, Michigan, bag experiments (Kerfoot and McNaught 2010), and suggests that cues (chemical and/or mechanical signals) are related to physical proximity of predators and promote variable responses.

Outside New England, induction responses of *Bosmina* to *Epischura* varied widely according to region, predator geographic

**Table 5.** Examples of significant induction responses to predators other than *Epischura*. Only significant species are included (predator and prey species listed). Variables include posterior (mucro) and anterior (antennule) spines, sample size (*n*), treatment, mean spine size, *F*-ratio and *p* values. All tests, except for *Limnocalanus* (3.5 L), are from split-culture 35 mL vials.

Region and predator/taxa	Clone	Variable	n	Treatment	Mean	F	n value
Portage Lake Houghton		Vullubic		incutinent	Wiculi	1	p value
Limnocalanus/B. liederi	PI A19-07	Mucro	41	Control	31(5.8)	24.2	4,49 F −06
Emmocaranas, b. neach		Macro	41	Predator	37(6.3)	(1.80)	1.17 2 00
		Antennule	41	Control	85(10.0)	12 7	0.0006
		/ intermate	41	Predator	95(14.4)	(1.80)	0.0000
	PI B29-07	Mucro	34	Control	32(4 3)	11.2	0.0014
		Macro	34	Predator	36(4.9)	(1.66)	0.0011
		Antennule	34	Control	81(5.7)	63.5	3 01 F -11
		/ intermate	34	Predator	94(8.0)	(1.66)	5.01 2 11
	PI A29-07	Mucro	41	Control	31(5.8)	28	1 09 F -06
		Macro	38	Predator	37(4.8)	(1.77)	1.07 2 00
		Antennule	41	Control	85(10.0)	15.2	0.0002
		Antennule	28	Predator	94(8.3)	(1 77)	0.0002
	DI C 20 07	Mucro	12	Control	30(4 4)	37.5	3 17 E _08
	16629-07	Mucro	40	Predator	38(6.8)	(1.80)	J.17 L 00
		Antonnulo	40	Control	SS(0.8) 85(12.0)	(1,00)	152E_05
		Antennule	42	Drodator	09(12.5)	(1.80)	1.52 L =05
Masoguelons/P		Mucro	40	Control	90(12.0) 21(5.1)	(1,60)	1075 04
liederi	PLDII-14	Mucro	45	Control	31(3.1)	20.9	1.97 E -00
neden		Antonnulo	2U 42	Control	37(3.4)	(1,71)	0.006
		Antennule	43	Control	85(12.4)	8	0.006
			20	Predator	94(13.0)	(1,67)	0.000
	PLBM17-14	Mucro	41	Control	34(6.9)	7.4	0.008
			42	Predator	38(6.4)	(1,81)	
		Antennule	41	Control	91(14.9)	10.8	0.002
			42	Predator	101(13.7)	(1,81)	
Acanthocyclops/B.	PLB1-14	Mucro	40	Control	32(6.0)	10	0.002
liederi			42	Predator	37(6.7)	(1,80)	
		Antennule	40	Control	87(12.6)	18.8	4.25 E −05
			42	Predator	99(12.7)	(1,80)	
	PLB3-14	Mucro	37	Control	30(6.7)	25.7	2.72 E -06
			42	Predator	37(6.1)	(1, 77)	
		Antennule	37	Control	85(14.8)	24.5	4.33 E -06
			42	Predator	101(13.4)	(1,77)	
	PLB9-14	Mucro	39	Control	32(5.7)	6.1	0.015
			42	Predator	35(4.4)	(1,79)	
		Antennule	39	Control	85(12.3)	19	3.89 E -05
			42	Predator	95(8.9)	(1,79)	
	PLBA4-14	Mucro	41	Control	26(3.9)	33.5	1.30 E -07
			42	Predator	31(4.2)	(1,81)	
		Antennule	41	Control	80(10.4)	23.8	5.33 E -06
			42	Predator	91(11.4)	(1,81)	
	PLBM-20	Mucro	41	Control	32(6.5)	8.8	0.004
			42	Predator	35(4.4)	(1,81)	
		Antennule	41	Control	91(13.9)	19.5	3.06 E −05
			42	Predator	103(11.0)	(1,81)	
	M1-14 (16-d)	Mucro	40	Control	28(3.8)	11.9	0.0009

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TABLE 5.	Continued
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Region and							
predator/taxa	Clone	Variable	n	Treatment	Mean	F	p value
			39	Predator	31(3.3)	(1,77)	
		Antennule	40	Control	82(9.4)	14.8	0.00024
			39	Predator	93(14.2)	(1,77)	
Diacyclops/B. liederi	PLBA10-14	Mucro	40	Control	29(5.0)	22.8	7.78 E −06
			43	Predator	34(5.8)	(1,81)	
		Antennule	40	Control	83(10.2)	21	1.62 E -05
			43	Predator	93(10.8)	(1,81)	
Tropocyclops/B. liederi	PLBU1-14	Mucro	41	Control	32(4.4)	9.7	0.003
			41	Predator	35(4.0)	(1,80)	
		Antennule	41	Control	88(12.7)	19.2	3.60 E -05
			41	Predator	98(6.5)	(1,80)	
Third Sister Lake, Ann Arb	or, MI., U.S.A.						
Mesocyclops/B. liederi	TSL4-05	Mucro	44	Control	28(4.8)	38.7	1.90 E -08
			41	Predator	34(3.6)	(1,83)	
		Antennule	44	Control	101(9.4)	112.8	3.88 E −17
			41	Predator	122(8.5)	(1,83)	
	TSL6-05	Mucro	46	Control	26(4.6)	17.8	5.93 E -05
			43	Predator	30(3.7)	(1,87)	
		Antennule	46	Control	100(7.1)	27.9	9.31 E −05
			43	Predator	109(8.3)	(1,87)	
Leptodora/B. liederi	TSL5-05	Mucro	34	Control	23(4.0)	35.8	1.17 E -07
			30	Predator	29(3.4)	(1,62)	
		Antennule	34	Control	88(9.9)	40.3	2.86 E -08
			30	Predator	102(8.0)	(1,62)	
Leptodora/B. freyii	TSL10-06	Mucro	36	Control	24(4.0)	8.8	3.88 E -03
			49	Predator	27(3.2)	(1,84)	
		Antennule	36	Control	97(9.0)	0.4	5.40 E -01
			49	Predator	96(15.8)	(1,84)	
Leptodora/B. liederi	TSL5-06	Mucro	12	Control	23(3.2)	18.7	1.27 E −04
			24	Predator	31(5.3)	(1,35)	
		Antennule	12	Control	88(8.3)	5.6	2.39 E −02
			24	Predator	100(15.9)	(1,35)	

exposure, and historical contingencies. The geographic variations in responses provide strong arguments that the induction trait is rapidly evolving, and subject to dampening by competitive trade-offs. Recent studies (Kerfoot and McNaught 2010; this article) suggest that about half the response to *Epischura* may be lost if the predator is not present in the lake for an appreciable time (e.g., hundreds of years; Third Sister Lake, Michigan), whereas almost all the response may be lost if *Epischura* is not present for thousands of years, as in the case of European lakes (e.g., Schleswig-Holstein region, Germany).

In laboratory tests, induction was not stimulated by a variety of naturally occurring invertebrate predators (*Chaoborus, Polyphemus, Asplanchna, water mites*), nor by two nonindigenous genera (*Eurytemora, Bythotrephes*). Induction responses came from a select suite of predatory microinvertebrates (cyclopoid copepods including *Mesocyclops, Acanthocyclops,*  Diacyclops and Tropocyclops; the calanoids Epischura and Limnocalanus, and the predatory cladoceran Leptodora). Most of these invertebrate predators pose a great risk to small bosminids. Large-bodied predatory calanoid copepods (Heterocope, Epischura) and cladocerans (Leptodora) are recognized to depress Bosmina in microcrustacean communities (Kerfoot, 1987; O'Brien 2001; McNaught et al. 2004; O'Brien et al. 2004). Over the northeastern North America, Bosmina coexist with M. edax, E. lacustris, and L. kindtii in 72%, 65%, and 45% of regional lakes (Carter et al. 1980), strongly suggesting that induction responses are associated with local and regional risk. Using a combination of mucro length and body length, Sprules et al. (1984) found that discriminate analysis could assign bosminid spine length to high- or low-predation lakes with 95.5% success. Sprules' composite index of invertebrate predation included Epischura, Limnocalanus, four cyclopoid

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**Table 6.** Trade-off experiment: results from 4 L competition and predation experiments (GPS *B. longirostris* vs. PL *B. liederi*). Days are from start of experiment. Reference is the introduced numbers and ratio (Day = 0). Tallies are individuals in 10% volume *subsamples*. Per cent GPS is the percentage of *B. longirostris* in the sample. Note how predation reduces *B. longirostris*, whereas competition favors this species.

Treatment	Days	GPS	PL	Total	% GPS
Reference	0	40	39	79	51
Predation A	14	76	141	217	35
	24	23	154	177	13
	44	5	30	35	14
Predation B	14	121	116	237	51
	29	34	115	149	23
	44	2	16	18	11
Competition A	14	77	71	148	52
	29	69	45	114	61
	44	58	37	95	61
	78	33	14	47	70
Competition B	14	70	60	130	54
	29	94	41	135	70
	44	101	57	158	64
	78	149	34	183	81



**Fig. 8.** Schematic food web representation of induction by multiple predators. The figure emphasizes that different species of predators stimulate defenses in an assortment of prey and that interactions are related to relative size. Zooplankton prey include the rotifer *Brachionus* and the two cladocerans *Bosmina* and *Daphnia*, whereas predators include *Asplanchna*, *Cyclops*, *Mesocyclops*, *Epischura*, *Leptodora*, notonectids, and fish.

copepods, *Mysis*, and *Chaoborus*. Six of these predators, *Limno-calanus*, *Leptodora*, *Mesocyclops*, *Acanthocyclops*, *Diacyclops*, and *Tropocyclops* are now recognized to stimulate spine elongation directly in the laboratory (Kerfoot and McNaught 2010; this article). Studies by Sakamoto and Hanazato (2008, 2009) also found induction in *Bosmina* stimulated by a variety of cyclopoid copepods: *Mesocyclops*, *Acanthocyclops*, and *Thermocyclops*.

The competition and predation laboratory experiments between Grosser Ploener See *B. longirostris* and Portage Lake *B. liederi* in 4L beakers support a "tradeoff" between predator resistance and competitive ability. These findings complement earlier results (Kerfoot 1977; Kerfoot and Pastorok 1978) performed with populations of B. longirostris and B. liederi from Union Bay, Lake Washington. In Union Bay, short-spined B. longirostris had a decided competitive advantage over long-spined B. liederi in shallow bay waters, where Epischura nevadensis were absent because of elevated fish predation. Towards the deeper bay margins, Epischura was present and would shift prey species ratios strongly towards B. liederi (Kerfoot and Peterson 1980). In the deepest waters of Lake Washington, B. liederi was the only species present, with very long tail spines and curved antennules. B. liederi in Union Bay carried fewer eggs and was competitively inferior in suspended jars with B. longirostris (Kerfoot 1977). Our recent laboratory "trade-off" experiments demonstrated that typical European B. longirostris with short features were superior competitors against long-spined B. liederi from Michigan, but were much more poorly protected against predation from E. lacustris. So why is there a "trade-off"? Field observations suggest induction also favors greater size at sexual maturity correlated with prolonged development and reduced population growth rate, and that resistant forms have thicker carapaces (Kerfoot and Peterson 1979). Working with related Baltic Eubosmina, Lagergren et al. (1997) argued that elongated spines act to greatly increase hydrodynamic drag, a hypothesis additionally supported by experiments of Lord et al. (2006). Trade-offs between competitive ability and helmet/spine lengths have also been shown for Daphnia (Riessen 1984; Engel and Tolrian 2009).

In 1-week induction experiments, Bosmina liederi spine length responded much more to Epischura than to Limnocalanus or cyclopoid copepods (Fig. 7). Long-term induction experiments in larger vessels (Tables 5, 6; 16-d, 250 mL beakers: Acanthocyclops, Epischura) also confirmed different responses of B. liederi to Epischura and Acanthocyclops. Responses of mucro length to the cyclopoid copepod were only about half that found from Epischura. These findings suggest much more interspecies communication in waters than presently recognized, that is, that Bosmina prey may be assessing relative threat from different predators, and that induction is not a simple "on-off switch." Finding that induction comes from multiple predators and differs between predators means that the interactions are more widespread and complicated than currently thought. As mentioned earlier, Laforsch and Tollrian (2004) showed that Daphnia cucullata helmets and tail spines also respond to a variety of predators (Chaoborus, Leptodora, Mesocyclops). Lack of induction response to Bythotrephes may also be very important, for it may explain the exceptional, major effects of that exotic on food webs in inland lakes and the Great Lakes. Prey may be "blind-sided" by Bythotrephes. That is, because there is limited historic exposure, Bosmina may be unaware of its presence (not sense its kairomones) and therefore not deploy typical protective measures.

In Europe and Asia, there is accumulating evidence for widespread, multipredator induction responses. Kappes and Sinsch (2002) found that a combination of temperature and predation-induced factors influence Bosmina body size and spine lengths. Certain European forms of B. longirostris (e.g., long-spined "pellucida" form) appear to be morphotypes induced by proximity to predatory copepods (e.g., Acanthocyclops, Mesocyclops). Studies of Bosmina in various Japanese lakes by Chang and Hanazato (2003) and Sakamoto et al. (2007) again emphasized that cyclopoid copepods are an important size-selective risk factor, and Sakamoto et al. (2007) and Sakamoto and Hanazato (2009) provide evidence that a combination of temperature, kairomonal cues, and physical exposure to predatory copepods alter both the length and shape of Bosmina antennae in a fashion similar to North American populations.

In Eurasia, a combination of temperature and predationinduced variables also induce phenotypic transformations in the related, slightly larger-bodied subgenus Eubosmina. Multiple predators are again involved, as both Leptodora and Mesocyclops stimulate spine elongation in Eubosmina (Hellsten et al. 1999; Langergren and Stenson 2000; Kerfoot 2006), yet transformations span greater changes in body shape. Induction in the Eubosmina species complex of the Baltic region includes several lineages now treated as partially or completely isolated species: E. cederstroemi, E. coregoni, E. thersites, E. gibbera, E. kessleri, E. longispina, E. crassicornis, E. longicornis, E. reflexa, and E. mixta (Faustova et al. 2011). Mitochondrial DNA studies suggest relatively rapid evolution of the Baltic lineages from a single basal stock (European E. longispina; Faustova et al. 2010, 2011). Populations from Baltic lakes show dramatic differences in spine (mucro, antennule) length, curvature, and additional dorsal carapace features (humps). Departing from generalized "multipurpose" responses, the myriad of forms suggest alternative, perhaps equivalent, phenotypic ways of frustrating resident predator's handling techniques. For example, in the German lake, Grosser Ploener See, paleolimnological remains document that one lineage (E. reflexa) elongated its posterior spine (mucro) while reducing the anterior spine (antennule), whereas another co-occurring species (E. kessleri) elaborated the anterior spine while decreasing the posterior spine (Kerfoot 2006).

Evidence for chemical specialization also comes from Japanese studies. In Lake Suwa, Japan, induction in *B. longirostris* was stimulated primarily by *Mesocyclops*, whereas induction in larger-bodied *Eubosmina fatalis* was stimulated primarily by *Leptodora* (Chang and Hanazato 2003; Sakamoto and Hanazato 2008). In this case, the form of the mucro and antennule transformations differed markedly between the two prey taxa, making *E. fatalis* more susceptible to *Mesocyclops* predation. Specificity and biased removal by an alternative predator creates a second option, separate from competitive cost, that may work against particular induction responses (an idea originally mentioned in Tollrian and Harvell 1999).

In lake communities, our finding that multiple predators modify prey morphology through induction increases the importance of this phenomenon in food webs. The very phenomenon of antipredator "induction" emphasizes evolutionary tailoring of morphological adjustments in prey species, strengthening the long-term perspective of Van Valen (1973) that continual modification is necessary to insure a place in nature. Yet is the induction scenario characterized as coevolutionary (i.e., pair-wise specialized and reciprocal)? There are only a few examples of specialized coevolution in inducible defenses (e.g., how ciliate predators adjust their size to induced defensive morphologies of their prey; Kopp and Tollrian 2003). Our findings of stimulation through multiple predators suggests more the "asymmetrical" proposal by Vermeij (1994), that is, that prey are responding in nature more strongly to predators than vice-versa. Whereas predators may be driven more by optimal foraging constraints, prey species are actively assessing threats from several directions (Fig. 8). Janzen (1980) suggested the term "diffuse coevolution" for the circumstance where "an array of populations generate a selective pressure as a group." Whether the term "diffuse coevolution" proves useful, we tend to agree with Vermeij that the variety of mollusk shell bumps or morphologies followed through time by Van Valen (1973) probably represents selective responses to a diversity of marine predators, rather than a tight interaction with a single, highly coevolved predator.

How important is "induction" in aquatic communities and how would you characterize predator "spheres of influence" and "prey counter-measures"? Inclusion of multiple predators suggests more pervasive influence of "induction" than presently acknowledged. Body size appears important in the influence spectrum (Fig. 8). For example, among relatively small-bodied taxa, several species of rotifers respond to a peptide-like substance produced by several species of Asplanchna (A. girodi, A. sieboldi, A. brightwelli). In these interactions, initially documented by Beauchamp (1952), Gilbert (1966), and Halbach and Jacobs (1971), the induction greatly reduces risk from Asplanchna predation, as Brachionus with no posterolateral spines are very susceptible to ingestion, whereas those with long spines are usually well protected. However, only a handful of prey species (Brachionus calyciflorus, B. bidentata, B. urceolaris, Filinia mystacina; Pourriot 1974; Gilbert 1980), all small-bodied rotifers, are recognized to respond to the Asplanchna compound. Here again, the prey spine responses are not exclusive to predatory rotifers, but also include small, predatory cyclopoid copepods (Tropocyclops, Mesocyclops; Stemberger and Gilbert 1987, 2006). Induction in the cladoceran genus Daphnia involves a separate set of predator-prey interactions. Several "helmeted" or "neck-teeth" species (D. ambigua, D. cephalata, D. hyalina, D. longispina, D. magniceps, D. pulex, D. cuculata, D. galeata) are

induced by substances from much larger predators, i.e., larval insects (midge larvae in the genus *Chaoborus*), notonectids (*Anisops* and *Notonecta*), and fish. However, the currently recognized "induced" prey species constitute only a small fraction of all currently described *Daphnia* species (e.g., 100+ species; North American total of 30–40 species; Colbourne and Hebert 1996), and the predators are decidedly larger than those involved in the rotifer and bosminid examples. Again, findings from Laforsch and Tollrian (2004) suggest that induction is also more widespread in *Daphnia* interactions.

A cautionary note should be mentioned. In most of our experiments, the stimuli appeared to come primarily from the predators. However, if "alarm" chemicals are produced by injured prey and induce antipredator responses in conspecifics, there might be a misleading impression of multiple predators stimulating induction of defenses. Preliminary tests with artificially injured Bosmina (broken antennules, mucrones, struck bodies) failed to stimulate feature elongation in cohorts (Kerfoot, personal commun.). In addition, we have documented diverse predators that do not induce spine elongation although they injure and consume Bosmina. However, evidence for "alarm" chemicals has been noted in a few Daphnia species (D. magna, Pijanowska 1997) and evidence for an interaction between latent "alarm" chemicals and various predator guts has also been suggested (Daphnia spp., Stabell et al. 2003).

The recognition of multiple inducing predators emphasizes the importance of "induction" as a risk-reducing tactic in planktonic food webs and the possibility that prey are continually tracking multiple predator species in nature. Although scattered throughout the plankton community, induction may promote consumer-prey stability by reducing predator-prey oscillations, enhance prey diversity by promoting coexistence of resistant and susceptible species, or dampen transmission of ecological perturbations down food webs. Theoretical and laboratory investigations by Vos et al. (2004) and van der Stap and colleagues (Verschoor et al. 2004; Van der Stap et al. 2008) point towards some fundamental consequences of induction for food webs in general. If induction is a widespread adaptation in aquatic food webs, the absence of induction responses to exotic predators may help explain some of the disproportionate consequences when these predators are introduced into naïve natural communities.

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