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REVIEW

The cost of predator-induced morphological defense in rotifers: experimental studies and synthesis

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In many small planktonic rotifers, predator kairomones induce the development of longer spines, which can provide effective post-contact defense against the predators. This review briefly discusses potential trade-offs associated with pronounced spine development, focuses on an evaluation and synthesis of experimental work comparing the reproductive potential of basic (non-induced) and *Asplanchna*-induced morphs and suggests areas for future research. To date, the only convincing evidence for an association between induced spine development and reduced reproduction is for *Keratella testudo*. This rotifer has a dense lorica (skeleton), so that elongated spines increase its sinking rate and thus presumably an offsetting swimming effort. In contrast, several studies with *Brachionus calyciflorus* and *Keratella tropica*, which have a thin lorica, showed that morphs with pronounced spine development reproduced at the same rate as basic morphs. In other studies with several different species, and also with *B. calyciflorus*, the presence of *Asplanchna* did inhibit reproduction. However, this inhibition generally was not clearly related to *Asplanchna* density or the degree of spine development, and could not be attributed to a defense cost. While predator-induced morphs of some species do not have a

reduced reproductive potential in the laboratory, they may be at a disadvantage in certain interactions or conditions occurring in more complex, natural ecosystems. Such environmental costs, or plasticity costs resulting from the evolution of mechanisms for detecting and responding to predators, may explain why long spines in some rotifers are an inducible rather than a constitutive defense.

KEYWORDS: induced defense; rotifers; defense costs; phenotypic plasticity

INTRODUCTION

Some planktonic invertebrates are phenotypically plastic and respond to predator chemicals, called kairomones, by developing defenses that reduce the risk of predation. Defenses induced by various invertebrate or vertebrate predators have been extensively studied in *Daphnia* and several genera of rotifers (for general reviews, see Larsson and Dodson, 1993; De Meester *et al.*, 1999; Gilbert, 1999; Tollrian and Dodson, 1999; Lass and Spaak, 2003). In *Daphnia*, the defenses may be changes in morphology (e.g. body size, development of neckteeth, elongated tail spine, enlarged helmet), behavior (e.g. initiation of diel vertical migration, enhanced escape response, aggregation) or life history (e.g. age or size at first reproduction, fecundity, initiation of diapause). In rotifers, all known predator-induced defenses are morphological and involve the development and elongation of spines.

It is generally believed that these predator-induced defenses must occur at some cost, and thus are expressed only when the predator is present and the benefit of the defense exceeds the cost (Gilbert, 1980a, 1999; Tollrian and Harvell, 1999; Riessen, 2012). Otherwise, the defense would not affect fitness in the absence of the predator and could be fixed or constitutive rather than inducible. Clearly, understanding the evolution and ecological effects of induced defenses requires knowledge of both benefits and costs.

Potential costs of predator-induced defense have been extensively investigated in *Daphnia*, especially regarding the induction of neckteeth by predatory larvae of the dipteran *Chaoborus* (Tollrian and Dodson, 1999; Riessen, 1999, 2012). More than 20 studies employed life table experiments to compare the reproduction of individuals with and without neckteeth. Most of them show an association between neckteeth induction and a lower reproductive rate due to delayed reproduction. However, some show the reverse or no association.

Many fewer studies have attempted to determine the cost of predator-induced defense in rotifers. However, an analysis and review of published research at this time seems appropriate for several reasons. Firstly,

conclusions from laboratory experiments testing the effect of induced defense on rotifer reproduction are contradictory, even for a given species. Some studies report decreased reproduction (Stemberger, 1988; Pavón-Meza *et al.*, 2008; Aránguiz-Acuña *et al.*, 2010; Sarma *et al.*, 2011b); others report no effect (Gilbert 1980a, 2009, 2012a) or enhanced reproduction (Stemberger, 1990). Some inconsistencies probably reflect differences among rotifer species; others appear due to problems in experimental design and the interpretation of experimental results.

Secondly, more extensive research should be conducted, because rotifers are excellent model organisms for investigating the evolution of predator-controlled phenotypic plasticity. Species from several genera exhibit pronounced morphological responses, and these persist throughout the lifetime of the individual. In contrast, the well-studied induction of neckteeth in *Daphnia* involves a very modest morphological response that occurs during a small part of an individual's lifetime (Tollrian and Dodson, 1999). Therefore, costs associated with induced morphological defense may be more likely in rotifers. Furthermore, the rotifers exhibiting this plasticity are small, easily cultured in defined media in the laboratory and reproduce very rapidly via female parthenogenesis.

Thirdly, experimental studies indicating that predator-induced defense in rotifers may occur without a decrease in reproductive potential have not been sufficiently appreciated, and are not mentioned in some general review papers considering the cost of induced defense in metazooplankton (see Lass and Spaak, 2003; Van Donk, 2007). Evidence for the absence of any appreciable reproductive cost is now strong for some rotifers, and suggests that future work should investigate costs that might occur in some environments and ecological interactions, but are unlikely to be revealed in simple laboratory experiments.

Here, I summarize predator-induced spine development in rotifers, briefly discuss the types of costs that may be associated with it, analyze methodological procedures and the interpretation of results in published

studies and then present a synthesis of present knowledge. I hope the review will encourage, and provide a firm foundation for, future research.

PREDATOR-INDUCED SPINE DEVELOPMENT

Many rotifers develop long spines (hypodermal evaginations), and in some cases a larger lorica (hypodermal skeleton of the body), in response to kairomones released into the environment by certain invertebrate predators and interference competitors (reviewed by Gilbert, 1999). Spine development involves the production of new spines, the elongation of pre-existing spines or both, and can provide very effective post-contact defense against the predator that induces it.

All polymorphic species known to have a predator-induced defense respond to the large, predatory rotifer *Asplanchna* (Table I), with induced morphs of *Brachionus calyciflorus* (Fig. 1), *Keratella slacki*, *Keratella testudo* and *Keratella tropica* showing particularly pronounced spine development, especially of the posterior spines (Table II). The induced morphs of these four species also have longer anterior spines, and three of them have larger loricas. In all species that respond to *Asplanchna*, the total length of the induced morph is much greater than that of the non-induced, or basic morph. In one experiment with *B. calyciflorus*, the mean total length (lorica, plus right posterior spine, plus anterolateral spines) of adult individuals of these morphs was 360 and 210 μm , respectively (Gilbert, 1967), and in an

Table I: *Asplanchna*-induced spine development in planktonic rotifers

Genus	Species	Reference(s)
<i>Brachionus</i>	<i>bidentatus</i>	Pourriot (Pourriot, 1974)
	<i>calyciflorus</i>	De Beauchamp (De Beauchamp, 1952); Gilbert (Gilbert, 1966, 1967)
	<i>havanaensis</i>	Pavón-Meza et al. (Pavón-Meza et al., 2007)
	<i>quadridentatus</i>	Gilbert (Gilbert, 2001)
	<i>sericus</i>	Pourriot (Pourriot, 1964)
<i>Filinia</i>	<i>passa</i>	Pourriot (Pourriot, 1964)
<i>Keratella</i>	<i>cochlearis</i>	Stemberger and Gilbert (Stemberger and Gilbert, 1984)
	<i>morenoi</i>	Gilbert (unpublished)
	<i>slacki</i>	Gilbert and Stemberger (Gilbert and Stemberger, 1984)
	<i>testudo</i>	Stemberger and Gilbert (Stemberger and Gilbert, 1987a)
	<i>tropica</i>	Gilbert (Gilbert, 2009)
<i>Platyonus</i>	<i>patulus</i>	Sarma (Sarma, 1987); Sarma et al.
	<i>macracanthus</i>	(Sarma et al., 2011b)

Species names conform to Segers (2007).

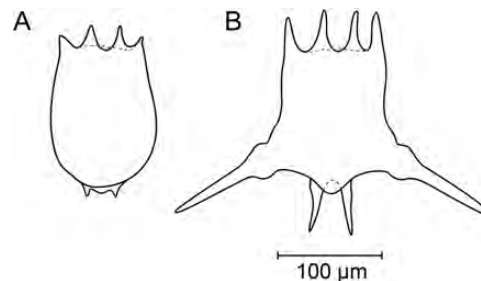


Fig. 1. *Asplanchna*-induced defense in *Brachionus calyciflorus*. (A) Basic or non-induced morph. (B) *Asplanchna*-induced morph, showing elongation of two pairs of anterior spines, elongation of posteromedian spines and *de novo* development, and lateral extension, of posterolateral spines. Lorica outlines from Gilbert (1967).

Table II: Length of lorica and posterior spine(s) of basic and *Asplanchna*-induced morphs of four rotifers

Rotifer and morph	Spine(s)	Mean length (μm)	
		Lorica	Spine(s)
<i>B. calyciflorus</i>	Both posterolaterals	190	0
		216	98
<i>K. slacki</i>	Right posterior	126	29
		137	72
<i>K. testudo</i>	Both posteriors	115	0
		118	35
<i>K. tropica</i>	Right posterior	127	25
		139	105

Brachionus calyciflorus (adults; from Gilbert, 1967, Table 1a and b), *Keratella slacki* (mixed ages; from Gilbert and Stemberger, 1984, Table 1), *Keratella testudo* (mixed ages; from Stemberger and Gilbert, 1987a, Fig. 3) and *Keratella tropica* (juveniles; from Gilbert, 2009, Table 1).

experiment with *K. tropica*, the mean total length of these morphs was 274 and 165 μm , respectively (Gilbert, 2009).

Asplanchna commonly eats smaller rotifers that it can trap in its pharynx (Gilbert, 1980b; Gilbert and Stemberger, 1985). Prey exceeding a certain size or with long spines either cannot enter the pharynx through the mouth or cannot be maneuvered from the pharynx into the esophagus and stomach and are rejected. Thus, larger, and more spinous, induced morphs usually are much less likely to be captured and ingested by this predator than basic morphs (Gilbert, 1967, 2009; Gilbert and Stemberger, 1984; Stemberger and Gilbert, 1987a). The posterolateral spines of *B. calyciflorus* articulate at the base and spread out laterally (see Fig. 1B) when the rotifer is contacted by *Asplanchna* and retracts its corona (Gilbert, 1966, 1967).

Some *Keratella* species (*cochlearis*, *testudo* and *tropica*) also respond to copepods and cladocerans, producing longer spines that can reduce the risk of predation (Stemberger and Gilbert, 1984, 1987a; Marinone and Zagarese, 1991; Zagarese and Marinone, 1992; Gilbert, 2009). The responses of *K. cochlearis* and *K. testudo* are similar to those induced by *Asplanchna*, but the response of *K. tropica* to crustaceans is different and much less exuberant than its response to *Asplanchna* (Gilbert, 2009).

The chemical identity and mechanism of action of predator kairomones are unknown. The *Asplanchna* and crustacean kairomones clearly are different, since some rotifers such as *B. calyciflorus* and *K. slacki* respond only to the former. The *Asplanchna* kairomone is pronase sensitive and probably a protein, and it affects rotifer development only when the rotifer is in the oocyte stage within the maternal body cavity (Gilbert, 1967, 1999). Once an egg is oviposited and begins cleavage, the phenotype of the individual is fixed. Thus, induced spine development persists from birth to death, whether or not the predator remains in the environment, and can be increased or reduced only in the next generation. This differs from the situation in *Daphnia*, where an induced defense can appear after the birth of an individual and then disappear before its death. For example, neckteeth induced after birth in early instars by *Chaoborus* are lost in later ones, even if the predator is present (Tollrian and Dodson, 1999).

It is important to note that spine development in planktonic rotifers may be induced by other factors besides predator kairomones. Both low temperature and low food availability can stimulate spine elongation in some species of *Brachionus* and *Keratella*, but to a much lesser extent than *Asplanchna* kairomone (Gilbert, 1967, 2011a; Halbach, 1970; Stemberger, 1990). The ecological significance of these responses most likely is a reduced sinking rate (Stemberger, 1990; Gilbert, 2011a). The effects of low temperature, low food availability and *Asplanchna* kairomone can be additive in both *B. calyciflorus* and *K. tropica* (Halbach 1970; Stemberger, 1990; Gilbert, 2011a).

Furthermore, in the absence of any environmental condition that promotes spine development, clonal populations of some strains of *B. calyciflorus* contain individuals with posterolateral spines of very different length—from absent to as long as those induced by *Asplanchna* (Gilbert and Schröder, 2004; Schröder and Gilbert, 2009). This variation is due to a birth-order effect. As females age, they produce daughters with increasingly long spines (Schröder and Gilbert, 2009; Gilbert, 2012a).

POTENTIAL COSTS OF PREDATOR-INDUCED DEFENSE

Costs that may be associated with predator-induced defenses have been categorized and described (Tollrian and Dodson, 1999; Tollrian and Harvell, 1999). A *plasticity cost* may occur as a result of the evolution of an inducible defense. Genetic, sensory and developmental mechanisms allowing predator detection and the expression of different morphs may have evolved at some expense. If so, the basic, or non-induced, morph of a rotifer clone with the potential to develop long spines in the presence of *Asplanchna* may have a lower reproductive rate in the absence of *Asplanchna* than the basic morph of a clone without this potential.

The possibility of such a cost has not yet been investigated. Strains of *B. calyciflorus* from many geographic locations have been cultured with *Asplanchna*, and all have responded by producing longer spines. However, some genetic variation for the degree of *Asplanchna*-induced spine development has been reported (De Beauchamp, 1952; Halbach and Jacobs, 1971). Finding genotypes with low propensities for spine development perhaps could be facilitated if the genes encoding for this process were identified. Then, gene expression techniques, already used in rotifers (see Dahms 2011), could screen large numbers of individuals for their response to *Asplanchna*.

Most studies designed to identify a cost to predator-induced defense have compared the ability of basic and induced morphs of the same clone to reproduce in simple laboratory environments. Such costs would occur under all conditions and could be termed *fixed costs* (Howard P. Riessen, Buffalo, personal communication). The most obvious type of fixed cost would be an *allocation cost*, in which there is a diversion of energy or material from reproduction to the production and maintenance of the defense. Any increase in energy expenditure required to offset altered hydrodynamics of the induced morph also would be an allocation cost. Another possible type of fixed cost would be an *opportunity cost*, in which the induced defense has some unavoidable deleterious effect but does not involve an energy cost. For example, long predator-induced spines could cause some mortality during embryogenesis or hatching.

An allocation cost would most likely result from the elongation of spines. However, the induced defense may also involve a larger body size, which could increase reproductive potential at high food concentrations and reduce it at low ones (see Stemberger and Gilbert, 1984, 1987b; Sarma *et al.*, 1999). Thus, effects of spine development and body size may be difficult to separate.

Table III: Methods used to compare reproductive rate (r) of basic and *Asplanchna*-induced morphs

Rotifer	Reference	Method for estimating r	Experimental treatment		
			Initial morph	Initial rotifer density	<i>Asplanchna</i>
Bc	1	LT	Induced	1 (n) in 0.5 mL	Absent
			Basic	1 (n) in 0.5 mL	Live
Bc	2	LT	Induced	1 (n) in 2 mL	Absent
Bh	3	LT	Basic	20 (n) in 50 mL	Live (in mesh)
		PG	Basic	50 (m) in 50 mL	Live (in mesh)
Bc, Bh	4	PG	Basic	20 (m) in 20 mL	CM
Bc, Ppm	5	LT	Basic	20 (n) in 20 mL	Live (in mesh)
		PG	Basic	20 (m) in 20 mL	Live (in mesh)
Bc, Ktr	6	PG	Induced	8 (j) in 45 mL	Live
		PG	Induced	2 (j) in 15 mL	Live
Kte	7	LT	Induced	1 (n) in 2 mL	Absent
Ktr	8	LT	Induced	1 (n) in 1.5 mL	Absent

References: [1] Gilbert (Gilbert, 1980b); [2] Stemberger (Stemberger, 1990); [3] Pavón-Meza *et al.* (Pavón-Meza *et al.*, 2008); [4] Aránguiz-Acuña *et al.* (Aránguiz-Acuña *et al.*, 2010); [5] Sarma *et al.* (Sarma *et al.*, 2011b); [6] Gilbert (Gilbert, 2012a); [7] Stemberger (Stemberger, 1988); [8] Gilbert (Gilbert, 2009).

Some studies used two rotifer species or two different methods. Rotifers (Table I) are *B. calyciflorus* (Bc), *B. havanaensis* (Bh), *K. testudo* (Kte), *K. tropica* (ktr) and *Platyonus patulus macracanthus* (Ppm). Method for determining r is life-table (LT) or population growth (PG). Initial population consists of neonates (n), juveniles (j) or individuals of mixed or unspecified ages (m). In experimental treatment with induced morph: initial morph is induced, or basic morph produces induced daughters; *Asplanchna kairomone* is absent, produced by living *Asplanchna* cultured with the rotifer (live)[sometimes separated by mesh], or present in medium conditioned by *Asplanchna* (CM). Control treatments (not shown) with basic morph without *Asplanchna* kairomone.

The *Asplanchna*-induced morph had a larger lorica than the basic morph in some studies with *B. calyciflorus* (Gilbert, 1967; Aránguiz-Acuña *et al.*, 2010; Sarma *et al.*, 2011b) but not in others (Gilbert, 1967, 1980b; Halbach, 1970), in *B. havanaensis* (Aránguiz-Acuña *et al.*, 2010), in *K. slacki* (Gilbert and Stemberger, 1984), in *K. tropica* (Gilbert, 2009) and in *P. patulus macracanthus* (Sarma *et al.*, 2011b). Predators inducing spine development in *K. cochlearis* and *K. testudo* did not affect lorica length (Stemberger and Gilbert, 1984, 1987a).

Some types of costs may not always be associated with the induced defense and could be termed *variable costs* (Howard P. Riessen, personal communication). For example, the induced morph could be at a disadvantage only in certain biological interactions or environments; such a variable cost is often called an *environmental cost*. Environmental costs are unlikely to be detected in simple laboratory vessels but may be critical for understanding the evolution of predator-induced defenses, especially when laboratory experiments fail to detect a fixed cost.

EXPERIMENTAL DESIGN FOR DETECTING A COST TO ASPLANCHNA-INDUCED DEFENSE: METHODS, COMMENTS AND PROBLEMS

Rotifer species

Most studies have used species (*B. calyciflorus*, *K. testudo* and *K. tropica*) showing very large differences in spine

length, especially in the posterior spines, between the basic morph and the *Asplanchna*-induced morph (Tables I–III). Rotifers with less pronounced spine-development responses also have been tested. In *Platyonus patulus macracanthus*, one of the posterior spines of the induced morph was only slightly longer (80–85 μm) than that of the basic morph ($\sim 75 \mu\text{m}$) (Sarma *et al.*, 2011b), and in *Brachionus havanaensis* the longest posterior spine of the induced morph was moderately greater (140–160 μm) than that of the basic morph ($\sim 110 \mu\text{m}$) (Pavón-Meza *et al.*, 2007). While it seems reasonable that the cost of an *Asplanchna*-induced defense should be related to the magnitude of its morphological response, this may not be the case. Riessen (Riessen, 2012) cultured two clones of *Daphnia pulex* from the same pond with *Chaoborus* kairomone; the development of their neekteeth differed greatly, but the decrease in their reproductive rate was similar ($\sim 10\%$).

Reproductive rate

Life-table experiments have determined the survival and fecundity of individuals cultured from birth to death, and then used the Euler equation to calculate an intrinsic rate of natural increase (r_i). Other experiments have used renewed batch cultures to determine an instantaneous reproductive rate (r) from increases in population size over some time interval that includes multiple, overlapping generations. However, this interval has had to be short, probably similar to the lifespan of the rotifer, because increasing population density soon inhibits food availability and reproduction.

In all cultures determining the reproductive rate of induced morphs, both the initial females and all offspring produced during the experimental period should have maximally long spines. These guidelines seem obvious. However, they have rarely been completely followed, and the latter can lead to confusion regarding the interpretation of results. An *Asplanchna* stimulus must be continuously present for females to produce offspring of the induced morph, and methods for applying this stimulus may directly affect reproductive rate (see below).

Since both life-table and batch-culture experiments assessed exponential population growth over a short period of time, they may have failed to detect very small costs of an induced morphology. To extend the duration of renewed batch cultures, populations could be periodically harvested. Better yet, continuous cultures could be maintained using chemostats, similar to those successfully developed by many investigators for *Brachionus* (see Rothhaupt, 1985; Yoshida *et al.*, 2007).

Culture volumes have been very small (0.5–2.0 mL) in life-table experiments and larger (15–50 mL) in batch-culture experiments (Table III). While rotifers can reproduce well in very small volumes, possible allocation costs associated with swimming may be underestimated in such cultures. Another possible concern with all of these cultures is that some rotifers, such as *K. tropica*, occasionally adhere to the surface film (Gilbert, unpublished observations) and may be more likely to do this when they have long spines. Adding a few grains of hexadecanol to the surface of cultures can prevent this problem (Gilbert, unpublished observations).

***Asplanchna* and the possibility of confounding effects**

An *Asplanchna* stimulus must be present in experimental cultures for parental females to produce long-spined offspring. However, *Asplanchna* treatments could affect reproduction of induced morphs in several different ways: directly by *Asplanchna* predation or waste products, and indirectly by induction of the morphological defense. Predation on test rotifers can be prevented by enclosing live *Asplanchna* in mesh containers (Pavón-Meza *et al.*, 2008; Sarma *et al.*, 2011b) or by using the medium in which *Asplanchna* has been cultured and then removed (Aránguiz-Acuña *et al.*, 2010). In both types of treatments, *Asplanchna* excretory products may inhibit, or promote, rotifer population growth, especially at high densities of *Asplanchna*. Use of *Asplanchna*-conditioned media also is problematical because the *Asplanchna* kairomone is very unstable (Gilbert, 1967; Halbach, 1970). Conditioned media rapidly lose activity and therefore may induce less than maximal spine development. The

half-life of activity in an *Asplanchna*-conditioned medium at 25°C was about 1.5 days (Halbach, 1970). Use of high *Asplanchna* densities to offset this loss of activity would lead to higher concentrations of *Asplanchna* excretory products.

Possible confounding effects of *Asplanchna* excretory products can be greatly reduced by using a low density of live *Asplanchna*. When not enclosed in mesh containers, which may reduce the diffusion of kairomone, the density of live *Asplanchna brightwelli* required to induce maximal spine development in *B. calyciflorus* and *K. tropica* is only ~ 8 individuals L^{-1} or $11 \mu g L^{-1}$ (Gilbert, 2011b). Predation by unconstrained *Asplanchna* on test rotifers can be eliminated if the species or individuals of this predator are too small to ingest the induced morph (Gilbert, 1980b, 2012a).

Only a few studies have examined the effect of predator excretory products on the reproduction of rotifers. For the first 12 days of an experiment, *Plationus patulus macracanthus* was strongly inhibited by media conditioned by two amphibians (Sarma *et al.*, 2011a). *Brachionus rubens*, which has no predator-induced defense, was inhibited by the medium conditioned by zebra fish but unaffected by those conditioned by *Asplanchna* or the copepod *Acanthocyclops* (Peña-Aguado *et al.*, 2008). In *B. havanaensis*, media conditioned by *Acanthocyclops* and an amphibian promoted reproduction and also modest spine development (García *et al.*, 2007).

Future experiments could consider an additional control treatment to help resolve the difficulty of confounding effects associated with an *Asplanchna* treatment. An inducible rotifer could be cultured with *Asplanchna* and also with a similar biomass density of another rotifer that induces no morphological response. Or, a rotifer with no inducible response could be cultured with *Asplanchna* or its conditioned medium.

The problem of confounding a defense cost with inhibitory or stimulatory factors associated with *Asplanchna* excretions probably could be greatly reduced or eliminated if the *Asplanchna* kairomone was identified. Threshold concentrations of pure kairomone required for maximal spine-development responses probably are extremely low and should have no direct effect on the reproductive rate of responding populations. The most ideal methodology for determining defense costs probably would involve the use of both purified kairomone and chemostat cultures.

Assessment of induced spine development

Spine elongation in response to *Asplanchna* kairomone increases continuously with kairomone concentration up to some maximal length (Gilbert, 2011b). It can be

quantified either by the spine length or by the ratio of spine length to body (lorica) length. In *B. calyciflorus*, and probably other rotifers that undergo considerable postnatal growth, *Asplanchna*-induced spines grow less rapidly than the body (Gilbert, 1967; Halbach, 1970). Thus, it is preferable to measure such rotifers at some given stage, e.g. neonates or adults. Halbach (Halbach, 1970) developed a spine to body length index that accounts for variation in body length. In *K. tropica*, there is negligible postnatal growth (Diéguez and Gilbert, 2011).

It is important to indicate the degree of spine elongation occurring in populations of the induced morph, and not just whether spine lengths of basic and induced morphs are significantly different from one another. In most studies comparing the reproduction of basic and *Asplanchna*-induced morphs, the degree of spine development of the induced morph was reported and was maximal or nearly maximal. If it is not reported, valuable information may be lost.

ANALYSIS OF PUBLISHED STUDIES

Life-table experiments without *Asplanchna*

Several studies compared the survival and reproduction of basic and *Asplanchna*-induced females cultured singly without *Asplanchna* (Table III). In *B. calyciflorus*, the reproductive rate was similar for the induced and basic (very short spined) morphs at high and low concentrations of *Enterobacter* (Gilbert, 1980b), and significantly higher for the induced morph at all five concentrations of *Cryptomonas erosa* (Stemberger, 1990). In *K. tropica*, the reproductive rate of induced and basic morphs was similar at high and low concentrations of *Cryptomonas* (Gilbert, 2009). However, a cost may have been underestimated. Offspring were produced in the absence of *Asplanchna* and thus had less than maximal spine development.

Despite the similar methodology and possibility of underestimation, a major reproductive cost was found in *K. testudo*. The reproductive rate of the induced morph was ~50% that of the basic morph at the three highest concentrations of *Cryptomonas* but similar to the basic morph at the two lowest ones (Stemberger, 1988).

Life-table experiments with *Asplanchna*

In studies with four rotifer species, neonates of the basic morph were cultured with or without *Asplanchna* so that they would produce offspring of the induced or basic

morph, respectively (Table III). However, since parental females were of the basic morph, they were not subject to possible allocation costs related to bearing longer, induced spines during postnatal growth and throughout their lifetime. In addition, *Asplanchna* excretions could have affected reproduction of the parental females.

Gilbert (Gilbert, 1980b) cultured *B. calyciflorus* of the basic (short-spined) morph singly on *Enterobacter* with and without live, but not predatory, *Asplanchna girodi*. Production of long-spined offspring in the *Asplanchna* treatment did not affect the reproductive rate.

Pavón-Meza *et al.* (Pavón-Meza *et al.*, 2008) cultured *B. havanaensis* of the basic morph in cohorts of 20 individuals in vessels containing 50 μm mesh cages with or without live *Asplanchna*. They did not report the degree of spine development induced in the offspring, but a previous study (Pavón-Meza *et al.*, 2007) showed that the density of *Asplanchna* used should have been high enough to induce a 27–45% increase in the length of the longest posterior spine. At all four combinations of temperature and *Chlorella* concentrations, rotifers cultured with *Asplanchna* had a significantly lower reproductive rate. At 25°C, the decrease in reproductive rate was ~15% for both high and low algal densities.

Sarma *et al.* (Sarma *et al.*, 2011b) cultured *B. calyciflorus* and *P. patulus macracanthus* of the basic morph in cohorts of 20 females in vessels containing 50 μm mesh cages with four densities of *Asplanchna* (0, 1, 4, 16 individuals in 20 mL). Offspring spine development was not reported in these experiments, but was in separate population-growth experiments in the same study (see below); there, *Asplanchna* induced pronounced spine development in *B. calyciflorus* (especially at the two highest densities) and very slight spine elongation in *P. patulus macracanthus*. In the life-table experiments, *Asplanchna* did reduce reproduction at some densities, but this reduction was not clearly related to likely spine development in the offspring. In *B. calyciflorus*, spine elongation should have been near maximal with 4 *Asplanchna*, but reproductive rate at this density was not significantly different from that at either 0 or 1 *Asplanchna*. A lower reproductive rate occurred only with 16 *Asplanchna*. In *P. patulus macracanthus*, reproductive rate decreased significantly with *Asplanchna* density. However, the *Asplanchna* effect was associated with only minor spine elongation in the offspring; the longest posterior spine increased from ~75 μm at 0 *Asplanchna* to ~80 μm at 1 and 4 *Asplanchna* and to ~85 μm at 16 *Asplanchna*.

Population-growth experiments

Four studies measured the rate of population growth in the presence and absence of an *Asplanchna* stimulus

(Table III). In three studies, populations were initiated with females (of unspecified or mixed ages) of the basic morph and cultured with or without live *Asplanchna* in mesh cages (Pavón-Meza *et al.*, 2008; Sarma *et al.*, 2011b) or in the medium conditioned by different densities of *Asplanchna* (Aránguiz-Acuña *et al.*, 2010). This design could underestimate a defense cost, since the proportion of induced morphs in the *Asplanchna* treatments was initially zero and then would have gradually increased over time as these females reproduced.

The population-growth trajectories presented by Pavón-Meza *et al.* (Pavón-Meza *et al.*, 2008) (their Fig. 3) show that population densities of *B. havanaensis* were lower in the treatments with *Asplanchna*, especially at the high *Chlorella* density at both 15 and 25°C. However, the population-growth rates they calculated from initial and final population sizes (their Fig. 4) show no significant effect of *Asplanchna* at 25°C, either at low or high algal densities. There was a significant effect of *Asplanchna* only at the high food level at 15°C (their Fig. 3).

Aránguiz-Acuña *et al.* (Aránguiz-Acuña *et al.*, 2010) cultured *B. calyciflorus* and *B. havanaensis* in the medium conditioned by different densities of *Asplanchna*. They found that media conditioned by high densities of *Asplanchna* decreased the rate of population growth and concluded that this suppression was due to a defense cost. However, some of their results do not support this interpretation. Compared with the medium not or weakly conditioned by *Asplanchna* (0 and 5 individuals L⁻¹), medium strongly conditioned by *Asplanchna* (100 and 500 individuals L⁻¹) greatly inhibited the population growth of *B. calyciflorus*—almost completely during the first 5 days of culture and then by about 50% during the next several days. Since populations in the strongly conditioned media failed to grow during the first several days, individuals would likely be the original ones of the basic morph and unlikely to have produced many offspring which could have developed *Asplanchna*-induced spines. Unfortunately, spine lengths were not reported in this experiment.

Spine-length data for *B. calyciflorus* in this experiment would have been useful for another reason: to determine how spine development varied with the density of *Asplanchna* in the conditioned medium and with the population growth rate. A likely result is not consistent with a defense cost. Spine lengths probably were longer in the medium conditioned by 100 or 500 *Asplanchna* L⁻¹ than in the medium conditioned by 10 *Asplanchna* L⁻¹, but population growth rates were similar in all of these media (their Supplementary data, Fig. S1a). Spine lengths were measured in another similar experiment designed to determine the time required for induced spine development, but the only

information provided was whether those in media conditioned by *Asplanchna* were significantly greater than those in the control medium (0 *Asplanchna* L⁻¹).

There is still another reason why this experiment would have benefited from information on spine lengths. While no inhibition of population growth occurred in the medium conditioned by the lowest density of *Asplanchna* (5 individuals L⁻¹), the induction-time experiment showed that this medium was just as likely to induce significant spine development after about 3 days as media conditioned by higher *Asplanchna* densities (their Fig. 4b). Furthermore, Gilbert (Gilbert, 2011b) showed that a density of 5 *Asplanchna* L⁻¹ was sufficient to induce near-maximal spine development in another strain of this rotifer. Thus, significant spine development may have occurred without inhibition of population growth.

Aránguiz-Acuña *et al.* (Aránguiz-Acuña *et al.*, 2010) conducted a similar population-growth experiment with *B. havanaensis*. Again, spine lengths were not given. The *Asplanchna*-conditioned medium reduced reproduction, but its effect was less pronounced than with *B. calyciflorus*. For example, one of the strongest conditioned media (250 *Asplanchna* L⁻¹) did not significantly reduce population-growth rate. As with *B. calyciflorus*, the population-growth rate did not differ among any of the five media conditioned by 10 or more *Asplanchna* L⁻¹. Thus, as discussed above for *B. calyciflorus*, likely increases in the spine-development response with *Asplanchna* density were not accompanied by decreases in reproduction.

The population-growth experiments conducted by Sarma *et al.* (Sarma *et al.*, 2011b) with *B. calyciflorus* and *P. patulus macracanthus* were very similar in design to the life-table experiments they conducted in the same study (see above). In both species, populations cultured in 20 mL with 1, 4 or 16 *Asplanchna* in mesh enclosures had growth rates that were modestly but significantly lower than those cultured in 20 mL with mesh containers without *Asplanchna* (their Table 1). However, the reduced population-growth rates with *Asplanchna* were not strongly related to recorded spine lengths (their Figs 3 and 4). In *B. calyciflorus*, spines were much longer with 16 *Asplanchna* (~80 µm) than with only 1 *Asplanchna* (~45 µm), but growth rates at these predator densities were similar. In *P. patulus macracanthus*, the presence of *Asplanchna* induced very slight spine development, as noted above. When only 1 *Asplanchna* was present, spine length increased little, if at all, but population growth was reduced as much as it was with 4 and 16 *Asplanchna*.

Gilbert (Gilbert, 2012a) compared the population-growth rates of induced and basic morphs in both *B. calyciflorus* and *K. tropica*. In experimental treatments

for the induced morph, populations were initiated with individuals having long, *Asplanchna*-induced spines and cultured together with live *Asplanchna* to assure that all offspring developed long spines. Differences in spine development between the basic and induced morphs were about as great as possible throughout the 4- to 8-day growth periods. Mean posterolateral spine lengths for these *B. calyciflorus* morphs were 2 and 57 μm , and mean right posterior spine lengths for the *K. tropica* morphs were 25 and 94 μm , respectively. The potential of *Asplanchna* to decrease reproduction of the induced morph through either predation or inhibitory excretions was eliminated or reduced by initiating populations with well-defended induced morphs, and by using small individuals of the predator at low densities. In each of three experiments with *B. calyciflorus* and with *K. tropica*, there was no significant difference between the population-growth rate of the induced and basic morphs. These results permit a clear interpretation: reproduction of the *Asplanchna*-induced, long-spined morph was not reduced either by *Asplanchna* predation, *Asplanchna* waste products or any costs associated with the pronounced spine development.

SYNTHESIS

The experimental analysis of costs that may be associated with predator-induced defense in rotifers has involved comparisons of the reproductive potential of basic and predator-induced morphs of several species in laboratory culture. There are problems associated with experimental design and interpretation of results, and some methodological improvements could advance our understanding. Identification and purification of predator kairomones (especially the *Asplanchna* kairomone) and the use of chemostat culture may allow maintenance of induced morphs for longer periods of time without the possibility of predator excretory products affecting reproduction. Also, more attention should be given to separating effects of spine elongation and body size increase in some induced morphs on their reproduction at different food concentrations. Despite the limitations of laboratory studies conducted to date, some conclusions and patterns emerge.

The only strong evidence for an allocation or fixed cost associated with *Asplanchna*-induced defense in rotifers is Stemberger's (Stemberger's, 1988) life-table study with *K. testudo*. Reproductive rates of the induced morph with long posterior spines were much ($\sim 50\%$) lower than those of the basic morph at all food concentrations appreciably above the threshold food concentration (where $r = 0$). The reproductive cost may even have

been underestimated, because parental females were cultured in the absence of *Asplanchna* and thus would have produced offspring with less than maximal spine development. However, the absence of *Asplanchna* in the medium precluded the possibility that the reduced reproduction of the induced morph could be due to excretions from this predator.

The reproductive cost associated with the *Asplanchna*-induced morph of *K. testudo* is related to its greater sinking rate (Stemberger, 1988). Long spines increase the proportion of relatively dense lorica material in the body, and thus the specific gravity of the body. Thus, the allocation cost may involve both the diversion of energy or material from reproduction to spines, and also the energetic cost of swimming to offset the greater sinking rate. The increased specific gravity of the induced morph likely is only related to its greater spine development, since lorica length of basic and *Asplanchna*-induced morphs of this species is similar (Stemberger and Gilbert, 1987a). It is also possible that the lorica of the *Asplanchna*-induced morph of *K. testudo* is thicker and denser than that of the basic morph. In *Daphnia*, one of the effects of the *Chaoborus* kairomone is to induce a change in the architecture of the carapace that increases its thickness and rigidity (Laforsch *et al.*, 2004).

In stark contrast, pronounced *Asplanchna*-induced spine development can occur in *B. calyciflorus* and *K. tropica* without reproductive cost. This was demonstrated in both life-table experiments (Gilbert, 1980b, 2009; Stemberger, 1990) and, more convincingly, in population-growth experiments (Gilbert, 2012a). The most likely explanation for the absence of any detectable defense cost in these two rotifers and a large defense cost in *K. testudo* is that the lorica of *K. testudo* is thicker and more electron dense (Stemberger, 1988). In *B. calyciflorus* and *K. tropica*, the lorica is thin; also, the long, *Asplanchna*-induced spines are hollow to the tip (Stemberger, 1988; Gilbert, unpublished observations).

The occurrence of a fixed defense cost in *K. testudo*, but not in *B. calyciflorus* and *K. tropica*, suggests the generalization that appreciable allocation costs associated with predator-induced spine development likely occur in rotifers with a dense lorica but not in those with a thin one, even though the magnitude of spine development in the latter may be greater than in the former (Table II). Some field observations suggesting that predator-induced spine development is associated with lower fecundity in *Keratella quadrata* (Conde-Porcuna and Declerck, 1998) may be consistent with the probably dense lorica of this species; experiments to examine this possibility should be conducted.

The presence of *Asplanchna* did inhibit reproduction in some studies with *B. calyciflorus*, *B. havanaensis* and

P. patulus macracanthus. However, this inhibition often was not clearly related to increased spine length, or could be attributed to the medium rather than to the induced defense. Furthermore, most of these experiments were not optimally designed to detect a potential cost, because the initial rotifers in the *Asplanchna* treatments were of the basic morph. This problem is especially important in the life-table experiments (Pavón-Meza *et al.*, 2008; Sarma *et al.*, 2011b) in which survival and reproduction is recorded only for the initial rotifers.

It is reasonable to think that an allocation cost of an induced defense should be most readily apparent at low food concentrations, where fecundity is strongly food limited and likely to be affected by a diversion of energy from reproduction. However, there is no evidence for this. In one experiment with *B. calyciflorus*, long-spined individuals actually had significantly higher reproductive rates than short-spined ones at five food concentrations, including two that were at or below the threshold food concentration (Stemberger, 1990). Also, in *K. testudo*, *Asplanchna*-induced spine development reduced reproduction at the three highest food concentrations but not at the three lowest ones (Stemberger, 1988).

Long-spined individuals of phenotypically plastic rotifers may occur in the absence of *Asplanchna*, and thus in conditions where the spines are of no apparent benefit. For example, following the disappearance of *Asplanchna*, there is a long developmental lag for the loss of *Asplanchna*-induced spines; all individuals with long spines will persist until their death, and adults will continue to produce long-spined offspring throughout the remainder of their reproductive period (Gilbert, 2012a). Also, in some strains of *B. calyciflorus*, pronounced spine development normally occurs in the absence of *Asplanchna*. With increasing maternal age, offspring have posterolateral spines that gradually increase to lengths comparable to the longest ones induced by *Asplanchna* (Schroder and Gilbert, 2009). This maternal effect may be a bet-hedging strategy to insure the presence at all times of some *Asplanchna*-resistant individuals, and it may be most likely to have evolved in strains occurring in communities where *Asplanchna* is regularly common. The lack of a fixed cost associated with *Asplanchna*-induced defense in *B. calyciflorus* and *K. tropica*, and probably in other species with a thin lorica, indicates that long-spined morphs of these species occurring in communities without *Asplanchna* should not have a reduced potential for reproduction.

While the basic and *Asplanchna*-induced morphs of *K. tropica* reproduced at the same rate in two separate studies (Gilbert, 2009, 2012a), therefore indicating no fixed cost to induced spine elongation, Zagarese and

Marinone (Zagarese and Marinone, 1992) reported some preliminary observations with non-induced, short-spined individuals of different clones suggesting an inverse relationship in the basic morph between spine length and reproductive potential. From one pond, they isolated three clones in which the basic morph varied in the length of its right posterior spine—from $\sim 25 \mu\text{m}$ in one clone to $\sim 38 \mu\text{m}$ in the other two clones. When 10 replicate populations of each clone were allowed to grow without a predator for 5 days in the laboratory, the final number of individuals in the clone with the shortest spine was significantly greater ($N = \sim 85$) than that in the clones with the longer spine ($N = \sim 50$; $P < 0.05$). The spine lengths of predator-induced individuals of these clones were not reported. Further research with a variety of species comparing the reproductive potential of different clones varying in the extent of spine development in both basic and predator-induced morphs would supplement studies on these morphs within given clones and could examine the possibility of a plasticity cost associated with the evolution of predator-induced defense.

Future research should consider the possibility of environmental costs associated with predator-induced morphs. Such costs probably would not be evident in simple laboratory experiments and might occur only in more complex, and highly variable, natural environments with many biological interactions. Predator-induced spine elongation likely affects many environmental interactions besides providing defense against the inducing predator. Long spines induced by one predator may increase susceptibility to another predator. There is some evidence for this in *K. tropica*. The longer spines induced by copepods and other crustaceans can reduce copepod predation, but appear to increase vulnerability to notonectid predators (Marinone and Zagarese, 1991; Zagarese and Marinone, 1992). Spine elongation also could affect swimming behavior, sinking rate and entrainment in turbulent flow. As an example, since the induced morph of *K. testudo* has a higher specific gravity than the basic morph (Stemberger, 1988), it may be more likely to sink into deeper layers of the water column that are colder and have less oxygen and food.

The predator-induced defenses discussed in this review all involve cases where the predator releases a kairomone that stimulates spine development. The only partial exception to this was the effect of *Asplanchna* on the left spine of *K. tropica*; while the most exuberant individuals of the induced morph have a much longer right posterior spine and longer anterolateral and anterosubmedian spines, they lose the left posterior spine (Gilbert, 2009). Loss of the left spine may permit

greater development of the right spine, and hence greater protection from *Asplanchna* predation.

However, two predators are known to inhibit spine growth in *K. tropica*. Both posterior spines are reduced or lost in the presence of the notonectid *Buenoa* (Zagarese and Marinone, 1992) or the ostracod *Cypris pubera* (Gilbert, 2012b). Shorter spines appear to decrease this rotifer's susceptibility to notonectid predation, but are of unknown significance regarding the ostracod. The possible costs of predator-induced spine reduction are unknown. An allocation cost might involve a greater sinking rate or less efficient swimming, and a likely environmental cost would be greater susceptibility to some predatory copepods and *Asplanchna*.

In conclusion, further research in several areas is needed to advance our understanding of predator-induced defense in rotifers. Purification and identification of predator kairomones, especially the *Asplanchna* kairomone, would help to reveal the molecular pathways involved in the expression of the defense. Also, it would eliminate the need for live *Asplanchna*, or its conditioned medium, in experiments comparing the reproductive potential of basic and induced morphs. Electron microscope studies of these morphs in different species could determine if induced morphological defense involves changes in the thickness, density or architecture of the lorica, and if species with a heavy lorica are more likely to have an allocation cost associated with the induced defense. Finally, the cost of predator-induced defense may involve types of responses and costs not yet, or only barely, investigated. Behavioral responses may occur, as in *Daphnia*, and plasticity and environmental costs may be more likely than allocation costs to explain the evolution of induced defenses.

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