Ecology, 84(3), 2003, pp. 641–651

TROPHIC SIZE POLYPHENISM IN *LEMBADION BULLINUM*: COSTS AND BENEFITS OF AN INDUCIBLE OFFENSE

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Abstract. Trophic polyphenisms are examples of phenotypic plasticity where two or more morphs within a species exploit different food niches. In this context, induced traits that enhance feeding ability on certain prey types have been termed inducible offenses. Here, we describe a prey-induced continuous size polyphenism in the predatory ciliate *Lembadion bullinum*.

Further to previous reports of ''giant cannibals'' in this species, we show that *Lembadion* is able to gradually adjust its size to the size of its prey. Large size acts as an inducible offense, since large morphs have an increased gape-size and can exploit a wider food range than small morphs. Despite these benefits, large morphs reduce their size whenever small prey is available. This suggests that their fitness is governed by a trade-off. We experimentally demonstrate this trade-off by showing that, when offered small prey, large morphs achieve lower volume-specific feeding rates and lower maximal population growth rates than small morphs. Both results highlight that large morphs suffer demographic costs that make them inferior in small-prey environments. Consequently, inducible predator offenses may evolve as adaptations to situations where important prey characteristics vary with space or time.

Key words: ciliates; cost–benefit analysis; demographic costs; feeding rate; gape-limited predator; inducible offense; Lembadion bullinum; *phenotypic plasticity; population growth rate; predator–prey interaction; trophic size polyphenism.*

INTRODUCTION

Trophic or resource polymorphisms within a species can be defined as the occurrence of two or more morphs or phenotypes that exploit different food niches. The morphs may differ in morphology, behavior, or life history, and the differences between them may be genetically based (see examples in Smith and Skúlason [1996]) or be an expression of diet-induced phenotypic plasticity. Polymorphisms based on phenotypic plasticity have been termed polyphenisms. Following Padilla (2001), we use this term for discrete as well as continuous variation, as the difference is not essential for our underlying concepts. Examples for trophic polyphenisms have been reported from fishes (variation of jaw morphology; e.g., Meyer 1987, Mittelbach et al. 1999), insects (variation of jaw and head morphology; e.g., Bernays 1986, Greene 1989), amphibians (typical and cannibalistic larval morphs; e.g., Collins and Cheek 1983), crabs (Smith and Palmer 1994), snails (different radula types; Padilla 2001), rotifers (trimorphisms in *Asplanchna*; e.g., Gilbert 1980), and protozoa (induction of ''giants'' or ''macrostomes,'' i.e., large-mouthed morphs; Williams 1961, Giese 1973).

With regard to trophic polyphenisms, Padilla (2001) recently coined the term ''inducible offenses,'' which she defined as induced traits that enhance feeding ability on certain types of prey. This formulation highlights the parallels to inducible defenses of prey, which have become a major study object during the past two decades (reviewed by Tollrian and Harvell [1999*a*]). The evolution of inducible (as opposed to permanent) defenses requires the following conditions (Tollrian and Harvell 1999*a*:5): (1) variation in predation risk, (2) a reliable cue indicating the presence of predators, and (3) a functional trade-off between the benefits and costs of the defense. In analogy, the evolution of inducible offenses should be promoted by (1) fluctuations in the quality or quantity of available prey, (2) reliable cues indicating the presence of certain prey types, and (3) a functional trade-off between the benefits and costs of the offense. Compared to inducible defenses, inducible offenses have received considerably less attention. In particular, the costs of inducible offenses have rarely been studied (e.g., Gilbert and Stemberger 1985, Hewett 1988, Meyer 1989, Goldman and Dennett 1990, Trowbridge 1991, Robinson et al. 1996, Hampton and Starkweather 1998). Here, we describe an inducible offense in the predatory ciliate *Lembadion bullinum* Perty 1849 and experimentally demonstrate the cost–benefit trade-off governing the fitness of the various phenotypes.

Lembadion is a primarily benthic inhabitant of lakes, ponds, and slow streams (Foissner et al. 1994). It is a raptorial-feeding predator of large protists and has its gape size limited by the dimensions of a huge but inflexible peristome (cell mouth; see Plate 1). Several years ago, Kuhlmann (1993) described ''giant cannibals,'' which are induced in dense *Lembadion* cultures

Manuscript received 14 January 2002; revised 13 June 2002; accepted 5 August 2002. Corresponding Editor: S. Nylin.

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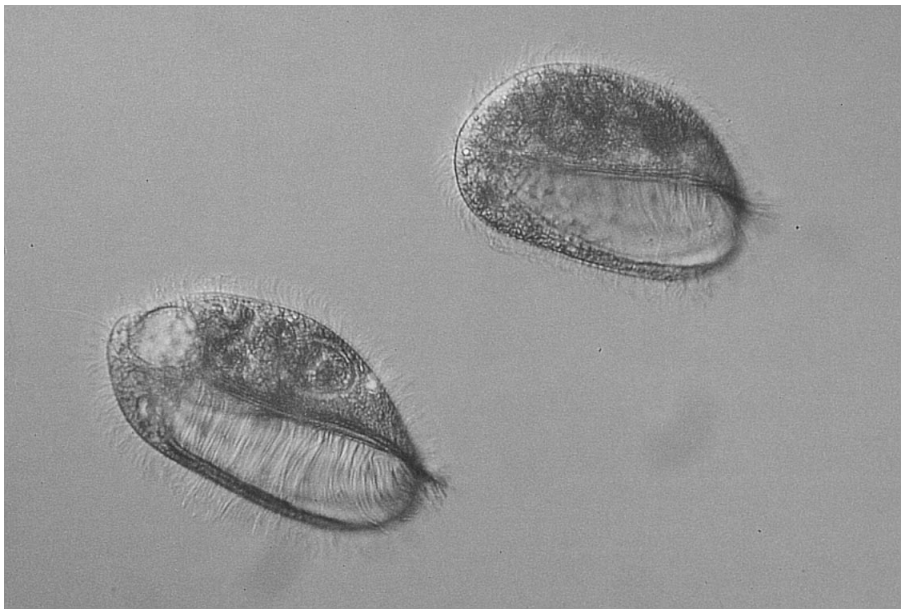

PLATE 1. Two individuals of the ciliate *Lembadion bullinum* (ventral view, anterior end to the right). These predators have a huge but inflexible cell mouth (the long ''gap'' in the lower half of each cell), which enables them to ingest prey of almost their own size. The length of the lower individual is ~140 μm. Photograph by M. Kopp.

when alternative food (in this case, *Colpidium campylum*) becomes scarce. Under such conditions, a few cells switch to cannibalism and delay their division until they are more than twice as large as the ''normal'' cells that they subsequently prey upon. This transformation is reversible: When *Colpidium* is offered again, the giants undergo several rapid divisions and regain the ''normal'' size.

The aim of the present study was twofold: First, the polyphenism of *Lembadion* should be characterized further. In particular, it is unknown so far whether giant induction requires starvation and cannibalism, or whether enlarged morphs can also be induced by the consumption of large non-conspecific prey, as is the case in other size-polyphenic ciliates (e.g., Giese 1973). Therefore, we performed induction experiments, where we tried to induce different morphs by raising *Lembadion* with prey of different size. Second, we investigated the benefits and costs for large morphs. Giants apparently are adapted to feeding on large prey. On the other hand, the quick reversal of giant formation suggests that large cells become disadvantaged once small prey is available. To test this hypothesis, we performed feeding experiments with various combinations of prey and predator size, and we estimated population growth rate of small and large *Lembadion* morphs in the presence of small prey. We will discuss our results in the light of the inducible offense concept.

MATERIAL AND METHODS

General methods

An initially clonal strain of *Lembadion* was obtained from K. Wiackowski (University of Krakow, Poland). *Lembadion* usually reproduces by binary fission at a maximum rate of about one division per day. Conjugation (sexual recombination) was infrequently observed in stock cultures, but never during experiments. Thus, while our *Lembadion* were not strictly clonal, genetic diversity was arguably very low.

Stock cultures of the different ciliate species were kept in 100-mL evaporation dishes or 1-L Fernbach flasks at 20°C in the dark. *Lembadion* were raised in artificial SMB medium ([Salt Medium for *Blepharisma*] 1.5 mmol/L NaCl, 0.05 mmol/L KCl, 0.4 mmol/L $CaCl_2$, 0.05 mmol/L $MgCl_2$, 0.05 mmol/L $MgSO_4$, 2.0 mmol/L phosphate buffer, pH 6.8 [Miyake 1981]) with *Euplotes octocarinatus*, *E. aediculatus*, or *Colpidium campylum* as food. *Euplotes* were kept in SMB and fed the unicellular green alga *Chlorogonium elongatum*. *Chlorogonium* was raised in SMC medium ([Salt Medium for *Chlorogonium*] = SMB + 1.25 mmol/L NH_4NO_3, 15 mmol/L $FeCl_3$, 0.8 mmol/L $MnCl_2$, slightly modified after Miyake [1981]) at 20°C under constant light and aeration. *Colpidium campylum* and *C. kleini* were cultured in a medium consisting of SMC + 300 mg yeast extract + 1 ''protozoan pellet'' (Carolina Biological Supply, Burlington, North Carolina, USA) per liter. This medium was inoculated with *Aerobacter aerogenes* and incubated on a shaker for 24 h. The resulting bacterial suspension was then inoculated with *Colpidium* and incubated for another two or three days. Finally, *Colpidium* were harvested by gentle centrifugation (200 g [g = 9.80665 m/s^2] using ''pear-shaped'' centrifuge tubes with cylindrical bottom) and resuspended in fresh SMB. *C. kleini* were kindly provided by K. Wiackowski (University of Kra-

kow, Poland). All other prey organisms were obtained from H.-W. Kuhlmann (University of Münster, Germany).

Experiments were generally conducted in six-well tissue culture plates (with 10-mL wells) at 20°C in the dark. Replicates of *Lembadion* were taken from independent stock cultures. A newly inoculated stock culture was assumed to be independent from its parent culture after 1 wk. In Experiments 2, 3, and 4, the following standardization procedure was applied to obtain cells with a well-defined nutritional state: An appropriate number of well-fed *Lembadion* with clearly visible food vacuoles were selected from a stock culture, transferred to fresh medium, and starved for 24 h.

Measurements of cell dimensions were performed on fixed samples using a computer-based image analysis system (AnalySIS, Soft Imaging Systems, Münster, Germany) connected to a Leitz Orthoplan microscope at 160× magnification. Volume of *Lembadion* was estimated as $\pi/6$ (length)(width^2), i.e., cells were assumed to be elongated spheroids. Fixation was achieved by addition of glutaraldehyde at a final concentration of 2% (Sherr et al. 1989).

For the feeding experiments (Experiments 3 and 4), prey were live-stained with DAPI (see Lessard et al. 1996, Pfister and Arndt 1998). This yields a brightly fluorescing nucleus, which can be easily detected inside the predator's food vacuoles. To obtain stained *Euplotes* or *Colpidium*, the cells were incubated with 1 μg/mL DAPI for 2 h. After the exposure, *Euplotes* were filtrated over a 15-μm gauze, whereas *Colpidium* were centrifuged three times and subsequently resuspended in fresh SMB. To allow the prey to recover from this procedure, experiments were started not earlier than 1 h after the removal of the stain.

In replicated experimental treatments, measurements of individual cell properties, such as length, width, or number of food vacuoles, were generally done on samples of 10–30 cells per replicate. The means from these samples were used for statistical tests, in order to avoid pseudoreplication. However, numerical results will be presented as means ± 1 SD of the individual data, frequently pooled over all the replicates of a treatment. Statistics were calculated with STATISTICA for Windows 5.1 (StatSoft, Tulsa, Oklahoma, USA).

Experiment 1: size of Lembadion *raised with different prey*

In Experiment 1, we investigated the morphological reaction of *Lembadion* to four differently sized prey species: *Colpidium campylum*, *Colpidium kleini*, *Euplotes octocarinatus*, and *Euplotes aediculatus*. In the following, the *Lembadion* morphs induced with these prey will be referred to as the C-, K-, O-, and A-form, respectively.

Each of the four prey species (treatments) was used as food for four *Lembadion* cultures (replicates). Prior to the experiment, the *Lembadion* had been raised on *E. octocarinatus*. After at least 10 d of cultivation (a time span suggested by preliminary experiments) three samples were taken from each replicate at intervals of two days. From each sample, length and width of 30 cells were measured. Mean prey dimensions were determined from appropriate samples. The mean of prey length × width was computed as an index of prey size or ''bulkiness.''

Experiment 2: peristome size of Lembadion *raised with different prey*

Experiment 2 was designed to determine the influence of prey size on the anatomy of *Lembadion*'s peristome (cell mouth). We measured peristome length and width both absolutely and relative to cell length and width.

In this experiment, we applied three prey treatments. Using methods similar to Experiment 1, *Lembadion* were raised for at least 10 d with either *C. campylum* (C-form, 3 replicates), *E. octocarinatus* (O-form, 12 replicates), or *E. aediculatus* (A-form, 9 replicates). Before fixation, the cells were starved for 24 h as described in *General methods*. Sample size per replicate varied between 15 and 30 because the peristome can only be measured in cells with a proper orientation on the slide.

Experiment 3: feeding rate of small and large Lembadion *with large prey*

The results of the previous experiments indicated that large prey induce large-sized *Lembadion* morphs, which possess a large peristome. In the following, we investigated the benefits and costs experienced by these large morphs. The benefits were studied in Experiment 3, by estimating the feeding rate of small and large *Lembadion* feeding on large prey.

In Experiment 3a, the C-form (small) and the A-form (large) were fed *Euplotes aediculatus*. In Experiment 3b, the C-form (small) and the O-form (intermediate) were fed *E. octocarinatus*. In both experiments, treatments with each predator morph were replicated three times. Per replicate, around 100 standardized *Lembadion* of the respective morph were offered ~4000 stained prey in 1 mL of medium. After 1 h cells were fixed by addition of glutaraldehyde and the number of fluorescing food vacuoles per cell was determined immediately under an epifluorescence microscope at 160× magnification. In addition, length and width of 10 cells per replicate were measured for calculation of volume-specific feeding rates (i.e., absolute feeding rates divided by mean predator volume).

Experiment 4: feeding rate of small and large Lembadion *with small prey*

As a test for potential costs paid by large morphs, Experiment 4 was designed to study the influence of cell size on *Lembadion*'s success in feeding on small prey. In addition, we also aimed to study possible in-

TABLE 1. Results from Experiment 1: length and width of four *Lembadion* morphs and the prey they were induced with.

Lembadion				Prey			
Morph	Length (μm)†	Width (μm)	*n*‡	Prey	Length (μm)	Width (μm)	*n*
C-form	100.9 ± 6.48	66.9 ± 5.70	360	*C. campylum*	59.1 ± 7.71	25.0 ± 4.69	70
K-form	112.5 ± 6.71	73.6 ± 6.23	360	*C. kleini*	77.7 ± 14.85	37.4 ± 6.57	190
O-form	125.5 ± 12.07	77.4 ± 11.81	360	*E. octocarinatus*	90.1 ± 6.27	65.6 ± 6.83	90
A-form	143.1 ± 11.87	82.5 ± 14.77	360	*E. aediculatus*	124.4 ± 10.70	81.0 ± 10.22	260

† Means ± 1 SD.
‡ Total number of measured cells (30 cells per sample × 3 samples per replicate × 4 replicates).

teraction effects with prey density. We thus compared the feeding rates of the C- and the A-form at various densities of *C. campylum*.

Accordingly, the experiment had a 2 × 4 factorial design: Each of the two predator morphs was confronted with four prey densities, two low ones and two high ones (6.25, 12.5, 500, and 1250 individuals/mL). Each of the resulting eight treatments was replicated 11 times.

Preliminary experiments had shown that *Lembadion* needs some time to ''habituate'' to a new type of prey. Therefore, the usual standardization procedure was extended as follows: 48 h before the experiment, 200 well-fed cells were selected from each of 11 stock cultures of both morphs and transferred to 10 mL of fresh medium containing ~2000 *C. campylum*/mL (six-well tissue culture plates). For the last 24 h before the experiment, the cells were starved as usual. Each of the resulting 2 × 11 cultures of standardized predators (which had reached a final number of at least 400 cells) was then split into four aliquots and used for one block of replicates spanning the four prey densities.

Prey were live-stained as described in *General methods*, above. The experiments were carried out in 50-mL glass vessels. The vessels were placed horizontally into a slowly rotating ''plankton wheel'' (~35 rotations/h) to ensure homogenous mixing without turbulence. After 1 h, the *Lembadion* were filtered through a 15-μm gauze and fixed with glutaraldehyde, and the number of fluorescing food vacuoles per cell was determined immediately. In addition, length and width of 30 cells from the 6.25 prey/mL treatment of each block (see paragraph above) were measured for calculation of volume-specific feeding rates (i.e., absolute feeding rates divided by mean predator volume). The data were analyzed with nonparametric two-way ANOVAs (Scheirer-Ray-Hare extension of Kruskal-Wallis test, Sokal and Rohlf 1995:446).

Experiment 5: maximal population growth rate of small and large Lembadion *with small prey*

In Experiment 5, we investigated how cell size influences the maximal population growth rate *Lembadion* can attain with small prey. This was achieved by culturing both the C-form and the A-form with excess *C. campylum* as food.

The results of Experiment 1 showed that *Lembadion* changes its cell size in response to a new type of prey. Therefore, it is not possible to measure steady-state growth rates of the A-form with *C. campylum* as food. To correct for prey-induced changes in mean cell volume, we calculated population growth rates not only for cell number but also for total biovolume (i.e., for cell number times mean cell volume). These volume-corrected population growth rates are the best approximation for steady-state growth rates available. The volume correction (as applied here) should not be confused with the calculation of volume-specific feeding rates in Experiments 3 and 4.

We did five replicates for the A-form and six for the C-form. Each replicate was started with 100 well-fed *Lembadion* selected from independent stock cultures, which were placed into 10 mL of medium containing ~5000 *Colpidium campylum*/mL (day 0). After 24 h, 100 cells were transferred to fresh medium with the same amount of prey to continue the experiment (day 1). The rest were counted, fixed, and measured (length and width) in order to determine the daily population growth rate r and volume-corrected growth rate r_{vol}. This procedure was repeated for another seven days (days 2–8). In the period between day 0 and day 1, the *Lembadion* were supposed to habituate to the experimental conditions. Therefore, the data from day 1 were excluded from the analysis.

RESULTS

Experiment 1: size of Lembadion *raised with different prey*

The size of *Lembadion* remained constant over the three sampling dates and increased continuously with prey dimensions. Our four prey species induced four distinguishable size morphs of *Lembadion*, which we refer to as the C-, K-, O-, and A-form. Biometrical data for these morphs and their respective prey are given in Table 1.

Repeated-measures ANOVAs showed that prey species had a significant impact on mean length, width, and volume of *Lembadion*, whereas there was no significant influence of time (Table 2). Therefore, the data from the three sampling dates could be pooled to yield one mean value per replicate for each parameter. Using these values, there was a very close correlation between

TABLE 2. Results of repeated-measures ANOVAs for Experiment 1: the effect of prey species and time on mean length, width, and volume of *Lembadion*.

Factor	df effect	MS effect	df error	MS error	*F*	*P*
Mean cell length						
Prey species	3	3866.89	12	8.51	454.60	0.00000
Time	2	20.93	24	6.15	3.40	0.05004
Interaction	6	6.50	24	6.15	1.06	0.41507
Mean cell width						
Prey species	3	498.12	12	10.85	45.89	0.00000
Time	2	3.18	24	8.61	0.37	0.69547
Interaction	6	13.99	24	8.61	1.62	0.18368
Mean cell volume						
Prey species	3	1.87×10^{11}	12	2.14×10^{9}	87.57	0.00000
Time	2	1.66×10^{8}	24	1.77×10^{9}	0.09	0.91089
Interaction	6	1.30×10^{9}	24	1.77×10^{9}	0.73	0.62763

mean prey bulkiness (length × width), and mean length ($R^2 = 0.96$, $P < 0.0001$, $n = 16$ measurements), width ($R^2 = 0.92$, $P < 0.0001$), and volume ($R^2 = 0.96$, $P < 0.001$; Fig. 1) of the corresponding predator morph.

Experiment 2: peristome size of Lembadion *raised with different prey*

The dimensions of the peristome differed between the *Lembadion* cells from all three prey treatments (Fig. 2), with both length and width being largest in the A-form and smallest in the C-form (Table 3). Cell lengths and widths were similar to those recorded in Experiment 1. Relative peristome length (i.e., peristome length divided by cell length) was slightly higher in the O- and A-form than in the C-form (C-form: 0.74 ± 0.031, O-form: 0.80 ± 0.045, A-form: 0.82 ± 0.036; see Table 3), whereas relative peristome width did not differ significantly among the three morphs (C-form: 0.52 ± 0.046, O-form: 0.56 ± 0.073, A-form: 0.55 ± 0.059; see Table 3). Thus, the peristome changes almost isometrically with cell size.

Experiment 3: feeding rate of small and large Lembadion *with large prey*

In both experiments, *Lembadion* raised with one of the *Euplotes* species achieved significantly higher feeding rates than the smaller *Lembadion* raised with *C. campylum*. In particular, the C-form was almost completely unable to feed on *E. aediculatus*.

In Experiment 3a (*E. aediculatus* as food), the C-form reached a mean feeding rate of 0.006 ± 0.010 ingested prey per predator per hour, whereas the A-form ingested 0.60 ± 0.075 prey items per predator per hour (*t* test, $P < 0.001$). Data are means ± 1 SE (= standard deviation of the means from the three replicates). In Experiment 3b (*E. octocarinatus* as prey), mean feeding rates were 0.30 ± 0.120 prey individuals per predator per hour in the C-form and 1.37 ± 0.172 in the A-form (*t* test, $P < 0.001$). Calculating volume-specific feeding rates (number of prey consumed per hour and per 10^6 cubic micrometers of predator volume) yielded qualitatively similar results (Experiment 3a: C-form 0.047 ± 0.081, A-form 1.24 ± 0.309, $P = 0.003$; experiment 3b: C-form 1.98 ± 0.722, A-form 4.46 ± 0.829 prey·h^{-1}·(10^6 μm^3 of predator volume)$^{-1}$, $P = 0.018$).

Experiment 4: feeding rate of small and large Lembadion *with small prey*

Both predator type and prey density had a significant effect on absolute as well as volume-specific feeding rates, with no significant interactions between the two factors (absolute feeding rates: predator type $H = 6.95$, $P = 0.008$; prey density $H = 70.35$, $P < 0.0001$; interaction $H = 1.96$, $P = 0.58$; volume-specific feeding rates: predator type $H = 9.48$, $P = 0.002$; prey density $H = 99.20$, $P < 0.0001$; interaction $H = 0.97$, $P = 0.81$; see Fig. 3). At all prey densities, absolute feeding rates were higher in the A-form. Volume-specific feeding rates, however, were higher in the C-form. This is because mean feeding rates of the two morphs differed only by a factor of 1.38 (averaged over the four prey densities), whereas their mean volume differed by a factor of 2.38 (mean volume of the A-form: 554 ± 126 × 10^3 μm^3; mean volume of the C-form: 233 ± 47 × 10^3 μm^3). Both measures of feeding rate increased with prey density and nearly leveled off at 1250 prey/mL.

Experiment 5: maximal population growth rate of small and large Lembadion *with small prey*

Population growth rates for both cell number (r) and total biovolume (volume-corrected growth rates r_{vol}) were significantly higher in the C-form than in the A-form (Table 4, significant effects of predator morph). These differences remained constant over the course of the experiment (nonsignificant interactions between time and predator morph, reflecting the parallel graphs in Fig. 4a). Although growth rates varied significantly over time (significant time effects), there was no consistent (increasing or decreasing) trend, but merely fluctuations around some constant base level.

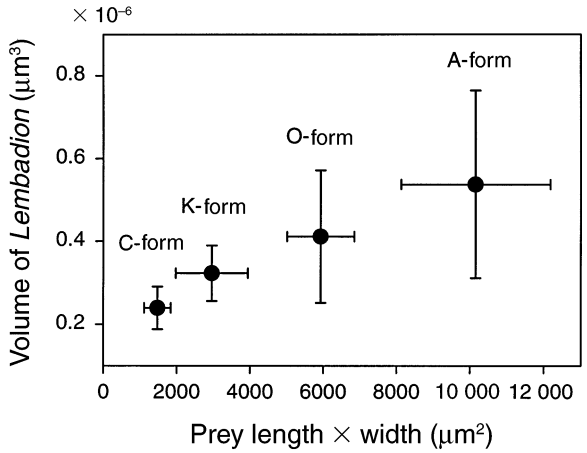

FIG. 1. Results from experiment 1. Mean volume of four *Lembadion* morphs as a function of mean prey ''bulkiness'' (= length × width), showing the close correlation between predator and prey size. Prey were *Colpidium campylum* for the C-form, *C. kleini* for the K-form, *Euplotes octocarinatus* for the O-form, and *E. aediculatus* for the A-form. Data were pooled over four replicates and three sampling dates for each prey species. Error bars represent ± 1 SD. For further biometrical data, see Table 1.

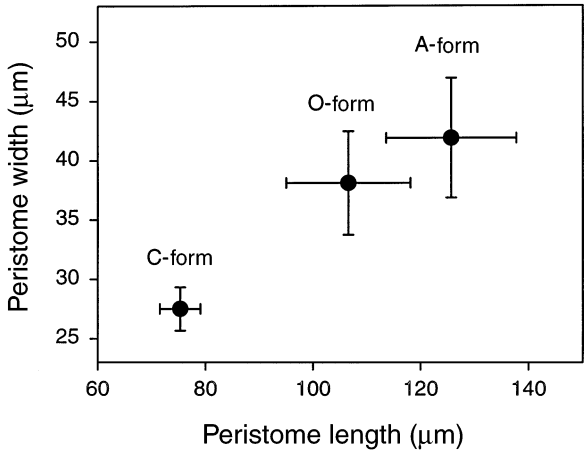

FIG. 2. Results from Experiment 2. Mean length and width of the peristome (cell mouth) in three *Lembadion* morphs (pooled over all replicates). Large prey induce predators with large peristomes and, therefore, a large gape size. The C-form was raised with *Colpidium campylum* (small prey), the O-form with *Euplotes octocarinatus* (intermediate-sized prey), and the A-form with *E. aediculatus* (large prey). Error bars represent ± 1 SD.

In the course of the experiment, the volume of the C-form remained more or less constant, whereas the volume of the A-form decreased considerably but did not reach the level of the C-form (Fig. 4b). This is reflected by a significant effect of the interaction between time and predator morph on mean predator volume (Table 4).

DISCUSSION

A prey-induced continuous size polyphenism

Our results show that *Lembadion bullinum* displays a prey-induced continuous size polyphenism. In other words, *Lembadion* is able to adjust its size to the size of its prey: the larger the prey, the larger the predator (Fig. 1). This adjustment involves an isometric change in the dimensions of the peristome (cell mouth) and, thus, of gape size (Fig. 2). By raising *Lembadion* with prey species of four different sizes, we obtained four distinguishable morphs, which we termed the C-, K-, O-, and A-form, respectively. The size distributions of these morphs overlap widely. Thus, the morphs are not qualitatively different, but merely differ in the average expression of a phenotypically plastic trait, that is size. Mean size of a morph is stable as long as the size of the dominant prey does not change (Table 2). Indeed, cultures of the various morphs can be maintained for months (M. Kopp, *personal observation*). Continuous polyphenisms similar to that of *Lembadion* have been reported from *Onychodromus indica* (Kamra and Sapra 1994), *Stylonychia mytilus* (Giese and Alden 1938), *Blepharisma americanum* (Giese 1973), and *Didinium nasutum* (Hewett 1980).

TABLE 3. Results of overall Kruskal-Wallis *H* tests and post hoc Mann-Whitney *U* tests with Bonferroni correction for Experiment 2: the effect of prey species on absolute and relative length and width of the peristome (cell mouth) of *Lembadion*.

	Peristome length ($H = 18.9$, $P = 0.0001$)		Relative peristome length ($H = 7.95$, $P = 0.0188$)		Peristome width ($H = 14.75$, $P = 0.0006$)	
Contrast†	*U*	*P*	*U*	*P*	*U*	*P*
C- vs. O-form	0	0.0044‡	0	0.0044	0	0.0044
C- vs. A-form	0	0.0091	0	0.0091	0	0.0091
O- vs. A-form	0	0.0000	44	0.5097	11	0.0013

Notes: No post hoc tests were conducted for relative peristome width, as the overall *H* test did not indicate any significant differences ($H = 1.96$, $P = 0.3746$). Relative peristome length = peristome length/cell length; relative peristome width = peristome width/cell width.

† The C-form was raised with *Colpidium campylum* (small prey), the O-form with *Euplotes octocarinatus* (intermediate-sized prey), and the A-form with *E. aediculatus* (large prey).

‡ Bonferroni correction means that differences are significant for $P < 0.0167$.

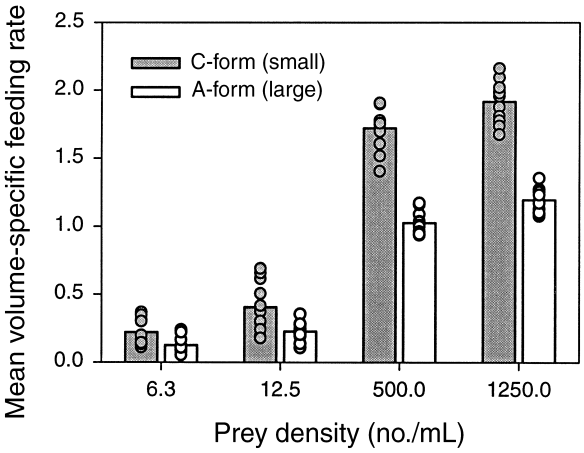

FIG. 3. Results from Experiment 4. Mean volume-specific feeding rate of two *Lembadion* morphs feeding on stained *Colpidium campylum* (i.e., small prey) for 1 h as a function of prey density. Volume-specific feeding rate is measured as the number of prey consumed per hour per 10^6 μm^3 of predator volume. Dots are means from the 11 replicates, whereas bars show the grand mean for all replicates. The large A-form always consumed significantly less prey per unit volume than the small C-form. This is evidence that the A-form suffers fitness costs that become apparent in the presence of small prey. Note, however, that absolute feeding rates (i.e., feeding rates not related to cell volume) are significantly higher in the A-form than in the C-form.

The ''giant cannibals'' described by Kuhlmann (1993, see *Introduction*) can be interpreted as part of *Lembadion*'s continuous polyphenism. We regularly found giants in our stock cultures, too. Generally, they were smaller than the A-form (M. Kopp, *personal observation*), which is in accordance with their feeding on smaller prey (starved C-form conspecifics are smaller than *E. aediculatus*). Thus, they fit neatly into the continuum shown in Fig. 1, and do not appear to be qualitatively different from other morphs. We conclude that ''giants'' are simply the morph adjusted to feeding on small conspecifics. Trophic polyphenisms are frequently coupled with cannibalism, in protozoa (reviewed in Giese 1973, Waddell 1992, Ricci and Banchetti 1993) and elsewhere (Gilbert 1980, Collins and Cheek 1983).

An inducible offense

Following Padilla (2001), trophic polyphenisms can be defined as the ability to react to certain types of food by expressing inducible offenses. Therefore, they should be discussed in analogy to inducible prey defenses, that is, in the context of benefits and costs, cues, and environmental variability (see *Introduction*). In the following, we will apply this framework to the size polyphenism of *Lembadion bullinum*. Thereby, we assume that the smallest *Lembadion* morph, the C-form, is ''noninduced,'' whereas all other morphs are ''induced'' to varying degrees.

Benefits and costs

According to the general theory of phenotypic plasticity (Tollrian and Harvell 1999*b*), the induced large cell size of *Lembadion* should have benefits as well as costs. Without benefits, it would not be adaptive. Without costs, it should be expressed permanently. This trade-off between benefits and costs has been investigated in Experiments 3–5.

The benefit for large morphs is the ability to consume large prey, which leads to an expansion of the utilized food range. The large A-form can feed on *Euplotes aediculatus*, which for the small C-form is virtually inaccessible (Experiment 3a). Similarly, the intermediate O-form is much more successful than the C-form in capturing *Euplotes octocarinatus* (Experiment 3b). These results are most easily explained as an effect of gape size (Experiment 2, Fig. 2). Similar ''gape size offenses'' have been reported from other polyphenic protozoa (Giese and Alden 1938, Williams 1961, Giese 1973, Hewett 1980, Wicklow 1988, Gomez-Saladin

TABLE 4. Results of repeated-measures ANOVAs for Experiment 5: the effect of *Lembadion* morph and time on population growth rate r, volume-corrected population growth rate r_{vol}, and mean cell volume.

Variable	df effect	MS effect	df error	MS error	F	P
Population growth rate r						
Lembadion morph	1	0.16	9	0.01	28.56	0.0005
Time	6	0.03	54	0.00	6.75	0.0000
Interaction	6	0.01	54	0.00	1.38	0.2403
Volume-corrected population growth rate r_{vol}						
Lembadion morph	1	0.55	9	0.01	105.31	0.0000
Time	6	0.11	54	0.01	15.28	0.0000
Interaction	6	0.01	54	0.01	1.46	0.2094
Mean cell volume†						
Lembadion morph	1	6.05	9	0.01	1011.87	0.0000
Time	7	0.04	63	0.00	13.97	0.0000
Interaction	7	0.11	63	0.00	38.30	0.0000

† Data have been log-transformed for the analysis.

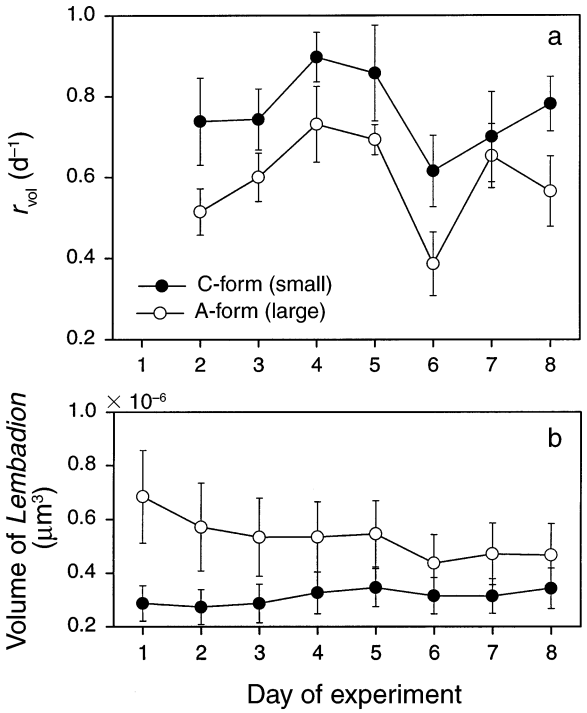

FIG. 4. Results from Experiment 5. (a) Daily volume-corrected growth rates r_{vol} (means ± 1 SD) for the C-form (solid circles) and the A-form (open circles) when fed with excess *Colpidium campylum* for a period of eight days. (b) Mean volume of the two morphs over time (means ± 1 SD of pooled individual data from all replicates). The A-form consistently grows slower than the C-form. This difference does not change over time, although the volume of the A-form steadily decreases. Our results indicate a volume-independent cost for large morphs. For statistical analysis, see Tables 2–4.

and Small 1993, Ricci and Banchetti 1993, Kamra and Sapra 1994) and the rotifer *Asplanchna* (Gilbert 1980).

Costs paid by large morphs should become apparent in the presence of small prey, since, under these conditions, large *Lembadion* regularly transform to small morphs. Furthermore, preliminary experiments indicate that with a mixture of two prey species, *Lembadion* always adjusts its size to the smaller one (M. Kopp, *personal observation*). Our discussion will focus on demographic costs; that is, we assume that *Lembadion*'s fitness can be measured in terms of population growth rate *r*. This assumption seems justified because protozoa generally live in variable environments (Taylor and Berger 1980, Fenchel 1982) that select for ''*r*-strategists.'' While *r* was determined directly in Experiment 5, it should also be closely linked to the volume-specific feeding rates measured in Experiment 4.

Experiment 4 was designed to investigate the influence of cell size on *Lembadion*'s success in capturing small *C. campylum*. At all prey densities, the A-form achieved higher absolute feeding rates than the C-form, but lower volume-specific ones (Fig. 3). In other words, the effect of their larger gape size did not fully compensate for their increased cell volume. Volume-specific feeding rates should be roughly proportional to population growth rate *r*, since gross growth efficiency (yield) in protozoa is generally found to be independent of volume (Finlay and Fenchel 1996). In contrast to absolute feeding rates, volume-specific feeding rates take into account that large cells generally need more food than small cells, due to their higher demands of energy for growth and reproduction. Certainly, any extrapolation from short-term feeding experiments to long-term fitness consequences must be applied with care. In particular, our estimate of cell volume is quite rough and we do not know how volume influences metabolic rates. Nevertheless, lacking more specific information, volume-specific feeding rates can serve as a useful first approximation to fitness (e.g., Goldman and Dennett 1990, Finlay and Fenchel 1996). Therefore, the results from Experiment 4 indicate that the A-form experiences costs in the presence of small prey.

The mechanism leading to these costs probably differs depending on prey density. At low prey densities, the predators did not become satiated, and their (absolute) feeding rates are proportional to ''success rate'' (i.e., the gradient at the origin of a typical Type II functional response curve [Jeschke et al. 2002]), which is a measure of their efficiency in attacking and capturing prey. Volume-specific success rate might be decreased in large cells because they have an unfavorable ratio of peristome area to volume. Costs via decreased foraging efficiency with alternative prey have also been reported for some other inducible offenses (Ehlinger and Wilson 1988, Hewett 1988, Meyer 1989, Ehlinger 1990, Goldman and Dennett 1990, Trowbridge 1991, Thompson 1992, Hampton and Starkweather 1998).

At high prey densities, almost all predators are ''digestion-limited'' (Jeschke et al. 2002); that is, their feeding rate is limited by the time needed to digest a single prey item and the number of prey items that can be digested simultaneously (''gut capacity''). Since the duration of the trials was too short for prey to become digested (prey items inside food vacuoles still looked almost intact; M. Kopp, *personal observation*), feeding rates in the high prey density treatments of Experiment 4 are basically a measure of gut capacity. Because the ''gut'' of a ciliate is simply its cytoplasm, our results show that, for some unknown reason, food vacuoles are packed more loosely into large *Lembadion* cells. Under the assumption that digestion time for one food vacuole is not smaller in large morphs than it is in small ones, this will lead to consistently lowered volume-specific feeding rates in large morphs also over longer time scales (i.e., when feeding rate is determined by an equilibrium of ingestion and digestion).

Finally, Experiment 5 yielded direct evidence that large morphs suffer demographic fitness costs. When both the C- and the A-form were cultured with excess

C. campylum, the A-form attained significantly lower population growth rates *r*. This result also holds true for volume-corrected population growth rates r_{vol}, which take into account that the mean size of the A-form decreased over the course of the experiment. The mechanism behind these costs may be found in the looser packing of food vacuoles indicated by Experiment 4. Again, however, this extrapolation can only be tentative. In any case, the mechanism does not seem to be directly linked to cell volume, but rather to some aspect of physiology: Although, over the course of the experiment, the difference in cell volume between the two morphs decreased roughly by a factor of three (Table 4 and Fig. 4b), the difference in r_{vol} remained constant (nonsignificant interaction between predator type and time; see Tables 2, 3, and Fig. 4a). This indicates that readjusting the cell physiology to a new prey species requires more time than the mere change in cell size. Costs in terms of lowered population growth rate have also been reported for large morphs of *Didinium nasutum* (Hewett 1988), and theoretically predicted for the ''campanulate'' morph of *Asplanchna silvestrii* (Gilbert and Stemberger 1985).

In summary, expressing its inducible offense by increasing in cell size is advantageous for *Lembadion* when only large prey is present. Due to their increased gape size, large morphs can exploit resources that are inaccessible to small morphs. With small prey, in contrast, large morphs suffer costs, as they attain lower volume-specific feeding rates (though higher absolute ones) and a lower maximal population growth rate. These costs can be characterized as environmental costs (Tollrian and Harvell 1999*b*) because they only act in a specific environment (i.e., when the large morph faces small prey). However, it cannot be ruled out that there are additional allocation costs (Tollrian and Harvell 1999*b*) for the production and operation of large cells.

Cues

The induction of offenses requires cues that indicate various types of prey. It is not clear how *Lembadion* ''measures'' prey size. To our knowledge, this question has not yet fully been answered for any other protozoan predator with a continuous size polyphenism, either. Since *Lembadion* reacts to a physical property of prey (i.e., size), this reaction need not be species-specific. Therefore, the identification of prey via chemical cues (see, e.g., Buhse 1967, Lennartz and Bovee 1980, Lennartz 1986, Gomez-Saladin and Small 1993, Smith-Somerville et al. 2000) appears rather unlikely. Much more parsimonious would be the use of mechanical cues. This hypothesis is in accordance with Kuhlmann's (1993) finding that the induction of giants relies on direct cell-to-cell contacts. In *Oxytricha bifaria*, giant formation is triggered by the energy of collisions with potential prey (Ricci et al. 1991). Yet Kuhlmann did not find evidence for a similar mechanism in *Lembadion*. Thus, it seems most plausible to us that *Lembadion* ''measures'' prey size using a mechanical cue that is directly linked to the feeding process. This hypothetical detection mechanism must allow the predator to distinguish a few large prey items from many small ones.

Once a change in prey size has been determined, transformation is initiated and predator size readjusted. Although we did not explicitly measure the rate (speed) of transformation, conclusions from our results combined with the findings of Kuhlmann (1993) give rise to some interesting speculations, which might warrant further investigation. The formation of large morphs appears to be a one-step process. According to Kuhlmann, giant cannibals appear spontaneously in starving cultures and gain their final size within one generation (though only a few cells are lucky enough to swallow a large prey item in the first place). In contrast, the transformation from large to small morphs is effectuated via multiple cell divisions, and thus takes several generations. In Experiment 5, transformation of the A-form fed *C. campylum* was not fully completed after 8 d ($\sim$7.6 generations). This appears very slow, and may in part be explained by the ad libitum food conditions applied in this experiment. In many protozoans, including *Lembadion* (M. Kopp, *personal observation*), cell size is positively correlated with food concentration (see references given in Zalkinder 1979). In Experiment 1, all transformations seem to have been completed within 10 d. Kuhlmann reports that most ''giants,'' when fed *C. campylum*, regain the size of ''normal'' cells (C-form) within 2 or 3 d, but for some of them, the transformation may last 5–10 d. Taken together, these findings suggest that the formation of large morphs might be faster than that of small ones. The rate of transformation might also depend on environmental conditions such as food concentration. A slow, ''prudent'' reduction of cell size might be adaptive, as the risk from having the wrong morphology is greater for small cells (starvation) than for large ones (nonlethal demographic costs).

Environmental variability

Like other examples of phenotypic plasticity (Stearns 1989, Tollrian and Harvell 1999*a*), inducible offenses can be discussed as adaptations to a variable environment, in particular with fluctuating food supply. While the microenvironment of *Lembadion* has not yet been the subject of any detailed field study, protozoa are generally found to live a ''feast and famine'' existence (Fenchel 1982), to which they have evolved numerous adaptations (apart from trophic polyphenisms, e.g., high starvation resistance [Fenchel 1982, Lynn et al. 1987], swarmer phenotypes [Nelsen and Debault 1978, Salt 1979], or encystation [see De Puytorac 1984]). An essential adaptation to fluctuating food supply is the ability to rapidly and efficiently exploit ephemeral

food patches. This might be the reason why the highly efficient and rapidly growing small morphs are preferred once small prey is available in sufficient concentration. In the absence of small prey, transformation to a large morph enables *Lembadion* to switch to alternative food sources. A special case of this strategy is the use of cannibalism as a ''lifeboat'' mechanism. Conspecifics are likely to be abundant after a rich food patch has been depleted. In summary, its continuous polyphenism allows *Lembadion* to fine-tune its morphology to the prevailing environmental conditions. The evolution of inducible predator offenses can be expected in situations where important prey characteristics vary with time or space and might be more common than generally expected.

ACKNOWLEDGMENTS

We thank Hans-Werner Kuhlmann for giving valuable advice on the cultivation of ciliates. S. Diehl, J. Jeschke, K. Jürgens, K. Kessler, W. Lampert, and two anonymous reviewers provided helpful comments on the manuscript. J. Grey helped to improve the language.

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