Predator-Induced Morphological Defenses: Costs, Life History Shifts, and Maternal Effects in Daphnia Pulex

Ralpoh Tollrian

Ecology, Volume 76, Issue 6 (Sep., 1995), 1691-1705.

Ecology, 76(6), 1995, pp. 1691–1705

PREDATOR-INDUCED MORPHOLOGICAL DEFENSES: COSTS, LIFE HISTORY SHIFTS, AND MATERNAL EFFECTS IN *DAPHNIA PULEX*[1]

RALPH TOLLRIAN
Max-Planck-Institute for Limnology, Postfach 165, 24302 Plön, Germany

Abstract. This study was designed to measure and separate the physiological costs of inducible defenses from life history trade-offs and maternal effects in the waterflea *Daphnia pulex*. Juveniles of *D. pulex* produce morphological changes ("neckteeth") and undergo life history shifts as defenses against predatory *Chaoborus* (phantom midge) larvae. These traits are induced by a chemical cue (kairomone) released by the predator. I performed life history experiments with and without *Chaoborus* kairomones at different food levels to quantify the induced changes and their potential physiological costs. The *Daphnia* clone used in this study also increased its body depth in response to the predator substance. Life history shifted toward a larger body size (both length and depth) and higher fecundity, which was balanced by an increased time to reach maturity and by increased adult instar durations. Reproductive effort was higher in the typical morph in the first adult instar, indicating resource allocation shifts towards growth in the protected morph. However, even in the absence of predation the chemically induced protected morph tended to show an increased intrinsic rate of population growth (r).

The longer time to reach maturity was not a direct physiological cost of neckteeth production, but a trade-off for larger body size. The life history shifts are independent of neckteeth formation. Developmental mechanisms leading to life history changes occurred after neckteeth were induced and could thus be uncoupled from neckteeth formation and its direct costs. In this study no direct costs were found. Carbon incorporation rates for the two morphs, at high and low food, were not different.

As a maternal effect, the larger females of the induced morph produced larger neonates which, in turn, matured at a larger size. Morphological changes, life history shifts, and maternal effects acted in concert to form defenses against *Chaoborus*.

This study shows that the often assumed high physiological costs resulting from the formation or maintenance of the defenses are not necessary to explain the evolution of inducible defenses. As morphological changes increase the visibility of *Daphnia pulex*, a fitness disadvantage can be caused by a changing predator regime (e.g., fish). The results of this study suggest that environments with changing predator selectivities favor the evolution of inducible defenses.

Key words: *antipredator; body size;* Chaoborus; *chemical ecology; chemical induction;* Daphnia pulex; *defense; kairomones; life history; phenotypic plasticity; predation; resource allocation.*

INTRODUCTION

Defense against predation is generally assumed to impose costs (Maynard Smith 1982). Costs can result, for example, from synthesis of extra body tissue or defense chemicals, or from escape to suboptimal environments. While costs are difficult to measure when the protection is formed permanently, the occurrence of inducible defenses offers the possibility of quantifying these costs in comparing protected and unprotected individuals of the same species or even the same clone. The key idea for the explanation of the evolution of inducible defenses is that costs are saved during times where the protection is not needed (for reviews see Schultz 1988, Harvell 1990).

However, theoretical models predict that life history shifts can also be adaptive responses to size-selective predation, by achieving earlier maturation at a smaller size by reduced adult survival and the opposite by reduced juvenile survival (e.g., Gadgil and Bossert 1970, Stearns 1976, 1992, Law 1979, Charlesworth 1980, Lynch 1980, Michod 1980, Taylor and Gabriel 1992). These theoretical considerations have been supported by results of experimental field studies. For example Vanni (1987) and Brett (1992) found larger primiparous *Daphnia* under *Chaoborus* predation (*Chaoborus* preying on small daphnids) and smaller primipara under fish predation (fish preying on large individuals) and Reznick et al. (1990) reported the same results for guppy populations.

Therefore, it has to be clarified whether, or to which degree, observed demographic changes in animals with inducible defenses represent "costs" for the formation of the defenses, trade-offs for the adaptive changes of

[1] Manuscript received 28 April 1994; revised 5 November 1994; accepted 9 December 1994; final version received 9 January 1995.

life history parameters, or further adaptations against predation.

Morphological defenses in animals are especially suitable for cost analyses, because the degree of protection can be judged visually. Chemically inducible morphological defenses are reported from several groups of aquatic organisms, e.g., algae (Hessen and van Donk 1993), protozoans (Kuhlmann and Heckmann 1985, Wicklow 1988, Kusch 1993), snails (Appleton and Palmer 1988, Crowl and Covich 1990), bryozoans (Harvell 1984), barnacles (Lively 1986), rotifers (Gilbert 1966, Stemberger 1988), and fish (Brönmark and Miner 1992). Especially numerous are reports from cladocerans, where several species respond morphologically to phantom midge larvae (Krueger and Dodson 1981, Hebert and Grewe 1985, Dodson 1988*a*, Stenson 1987, Tollrian 1990, Hanazato 1991), notonectids (Grant and Bayly 1981, Schwartz 1991), or fish (Tollrian 1994).

The relationship between *Daphnia pulex* and *Chaoborus* is a model system for the study of inducible morphological defenses. Juveniles of *D. pulex,* a large, pond-dwelling cladoceran, develop small protuberances in the neck region that are termed "Nackenzähne" or neckteeth. These morphological changes are induced by chemicals released by predatory *Chaoborus* larvae (Krueger and Dodson 1981), and are effective in reducing vulnerability to *Chaoborus* predation (Krueger and Dodson 1981, Havel and Dodson 1984, Tollrian 1995).

In *Daphnia pulex* there are many studies (listed in Table 8) reporting demographic disadvantages for the induced morph in the absence of the predator that can be interpreted as costs (e.g., longer development time, reduced survival, reduced clutch size). However, neckteeth are small structures and high energy costs resulting from their formation or maintenance therefore seem unlikely. Moreover, evolution should select for the formation of effective protections without large co-occurring disadvantages within the developmental constraints of the animal. In a recent study Spitze (1992) reported clones that formed neckteeth in the presence of the *Chaoborus* kairomone without showing measurable costs.

In the studies reporting demographic costs of neckteeth formation, usually neither has the attempt been made to separate costs for neckteeth formation and trade-offs for life history shifts, nor has the possibility of overestimation of costs been ruled out. Overestimations could result from co-occurring deleterious effects (e.g., resulting from enrichment of excretory products of the larvae or bacteria). Moreover, behavioral studies show vertical segregations of induced and typical *D. pulex* even in small vessels (Dodson 1988*b*, Ramcharan et al. 1992), which would lead in batch experiments to reduced food availability for the induced morph. Thus, it is likely that the "direct" physiological costs (e.g., energy requirements for neckteeth production or maintenance) have been overestimated in some studies.

Alternatively "indirect" costs could explain the lack of neckteeth formation in the absence of *Chaoborus.* For example, an effective and low-cost strategy against one predator might cause increased risk from other predators. It is well established that increased visibility is disadvantageous in the presence of visually hunting predators (e.g., fish; Confer and Blades 1975). Additionally *Chaoborus* larvae are excluded or reduced in the presence of fish by selective predation (Stenson 1980). *D. pulex* might often experience seasonal environments with changing predators.

Hence, I measured life history changes and costs and tried to separate them to test the hypothesis that energy disadvantages due to neckteeth formation are rather small, that the reported changes in demographic parameters, in the presence of *Chaoborus* kairomones, are not only costs for the formation of morphological defenses but trade-offs for protective life history shifts, and that the induced changes lead to an increase in visibility that would be disadvantageous in the presence of other predators (e.g., fish).

I performed life history experiments with and without *Chaoborus* kairomone at different food levels to quantify the induced changes and their potential physiological costs. I eliminated possible overestimations of the costs by using a flow-through system (Lampert et al. 1988) that provided homogeneous food and kairomone conditions, and by introducing a practical method to produce and purify the *Chaoborus* kairomone without deleterious effects to *Daphnia.* I separated life history shifts and their trade-offs, and costs for the production and maintenance of neckteeth, in a set of induction experiments. I additionally tested whether maternal effects might amplify the *Chaoborus* effects.

Methods

I used a single clone of *Daphnia pulex* for my experiments. The *D. pulex* clone is an obligate parthenogenetic clone from Canada (L. Weider, *personal communication*). I chose this clone because it forms comparatively strong neckteeth, which makes the detection of costs more likely. This clone forms neckteeth in the first three to four instars. Small neckteeth in the first instar are even formed without induction (details in Tollrian 1993).

Daphnids were cultured under constant dim artificial light in a temperature-controlled room at 20 ± 1°C in 1.5-L beakers at from five to ten individuals per beaker. *Scenedesmus acutus* from chemostats was used as food for both cultures and experiments. The food carbon concentration for cultures was high (1.5 mg/L) and well above the incipient limiting level (Lampert 1987). The food concentration was maintained constant by adding algae on a daily basis. Food levels for cultures and experiments were adjusted by measuring the optical

density of the food suspension photometrically at 800 nm, converting it to carbon concentration, and diluting it to the concentration needed. Aged lake water (2–3 d) from the mesotrophic Lake Schöhsee (northern Germany) was membrane-filtered (0.45 μm mesh) immediately before use in cultures or experiments.

To minimize variability due to maternal effects, cultures of test animals were synchronized and raised under the same standardized conditions. Offspring of the third or fourth clutch were used for each new generation. The mothers were transferred to new media every 3 d after releasing their clutches.

Neckteeth formation in *D. pulex* is dependent on concentration of *Chaoborus* kairomone (Parejko and Dodson 1990, Tollrian 1993). It consists of a syndrome including discrete small "teeth" and a continuous change of the neck region. Based on this reaction, a scoring method was applied that converts the strength of neckteeth formation in individuals to ≈20 classes of percent induction values (Tollrian 1993).

Chaoborus *kairomone production and purification*

For life history experiments that require large amounts of kairomones with the same activity, the use of *Chaoborus* rearing water is inconvenient as large amounts of water need to be frozen. Daily production of *Chaoborus* rearing water includes the uncertainty that the excreted amount of the kairomone can be variable, even between days. The use of an extract derived from *Chaoborus* larvae offers the possibility to produce a large amount of the kairomone with identical activity that can be stored. Hebert and Grewe (1985) introduced a method for extracting kairomones from boiled larvae. In my experiments I prepared an extract from *Chaoborus* larvae. One gram of *Chaoborus* (wet mass) was finally concentrated in one mL of extract. First, a crude extract was prepared from fourth instar *Chaoborus flavicans* larvae. Preliminary experiments showed that it is important not to homogenize the larvae, as this results in undesirable enrichment of organic compounds. The kairomone is readily water soluble and can be extracted by boiling the uncrushed larvae for 10 min. The larvae were removed by a 30-μm mesh gauze. Extraneous particles were removed by stepwise filtration ending with 0.1-μm mesh filtration (cellulose acetate filters). Centrifugation for 15 min at 12 000m/s^2 prior to filtration reduced the time needed for filtration.

As this crude extract proved to be harmful to sensitive *Daphnia* species (R. Tollrian, *personal observation*) and thus might also influence the more robust *D. pulex,* I included a further step of purification. The kairomone can be quantitatively removed from water by solid phase extraction with a C18 bonded silica gel (Tollrian and von Elert 1994). I extended this method for use with extracts. Prior experiments showed that all activity is retained by these C18 cartridges (500 mg; Internationale Chemie Technik, Frankfurt-am-Main, Germany). Aliquots of the extract were applied to the cartridges, which were preconditioned by rinsing with 5 mL of methanol and ultrapure water (Nanopure) each. The aliquots, containing 1% (volume/volume) methanol, were passed through the cartridges, were washed with 5 mL of ultrapure water, and were desorbed with 10 mL of methanol. The methanol was collected, finally evaporated to dryness, and resuspended in ultrapure water in the original concentration. This purified extract was used in all life history experiments. The extract was frozen in 2-mL tubes (Eppendorf) at −60°C. The portion needed was thawed shortly before use in the experiment. In the experiments a concentration of 2 μL extract/mL medium was used, which was tested to be just high enough to induce strong neckteeth.

Life history experiment one: influence of food concentration

The life history experiments were designed to show the effect of the kairomone and possible costs for the formation of neckteeth. Three life history experiments (LH 1–3) were performed in a flow-through system (Lampert et al. 1988), which maintains constant food levels in time and space. The first LH experiment was designed to test for differences in growth or life history. Both high (1.0 mg/L) and low (0.1 mg/L) food carbon concentrations were used,as energy costs might best be seen at limiting food levels. Temperature was adjusted to 20°C. The daily fresh-prepared food suspension was pumped with peristaltic pumps from food reservoirs, where magnetic stirrers prevented settling of the algae, into flow-through chambers. Flow-through rate for each 250-mL vessel was adjusted to 60 mL/h.

The mothers of the experimental animals originated from the fourth clutch of one grandmother and were raised under 1.0 mg/L flow-through food-carbon conditions until they deposited their second clutch in the brood chamber. One day before hatching of the offspring the mothers were individually transferred to 100-mL beakers containing food carbon stocks of 1.0 or 0.1 mg/L. Half of the beakers of each concentration were additionally treated with *Chaoborus* extract at 2 μL/mL. Immediately after hatching two to three randomly selected neonates per mother were transferred to the flow-through vessels containing the different treatments. Initially ten neonates were raised per vessel, but by the third juvenile instar only five animals were raised per vessel. By using identical conditions for all mothers and offspring and by raising a few offspring each from many mothers, I tried to minimize variability from maternal effects.

The flow-through vessels were replaced daily to prevent growth of bacteria, which could degrade the kairomone. Daphnids were raised under these conditions until they deposited their second clutch into the brood chamber. The vessels were checked for exuviae to determine the instars. The daphnids of the typical morph (TM) and of the neckteeth morph (NM) were measured

daily. Body size was measured as length from the top of the head to the base of the tail spine. Size at maturity was determined as the size of the first instar with eggs. I measured body depth as the widest distance between dorsal and ventral carapace margins at right angles to the longitudinal body axis. Time to maturity was measured as the time interval from birth until they deposited their first clutch into the brood chamber.

Egg-bearing females could be recognized in the flow-through vessels visually. Usually the daphnids were checked every 2 h during the expected time of maturity. When the exact time of egg laying could not be observed, it was estimated from the egg stages. In the low food treatment some animals in both treatments reproduced with a delay of one instar. For these daphnids I used the size of the first abdominal process as a criterion for the onset of maturity (Stibor and Lampert 1993).

As a comparative measure of fitness, to evaluate the combined effects of clutch size and time to maturity I calculated the intrinsic rate of population increase r (per day) by the Euler equation:

$$1 = \Sigma\, e^{-rx} \cdot l_x \cdot m_x,$$

where x is the age (in days), l_x is the age-specific probability of survival, and m_x is the age specific fecundity. I calculated r only for the first two broods as later broods have only a weak influence (<10% increase, e.g., Riessen and Sprules 1990, Taylor and Gabriel 1992). Survival in experiments where animals are measured daily is likely to be influenced by handling. Mortality until maturity was <3% and differences between treatments were not observed in the absence of predation. Therefore I assumed no mortality ($l_x = 1.0$). In the flow-through system, groups of five animals were tracked instead of individuals. The timing and clutch size for the first brood was measured for every individual but it was not possible to identify the corresponding second brood for the individuals. Hence I calculated the differences of the means in time and clutch size between the first and the second clutch for the group and added for each individual these differences to the first clutch values. In the low food treatment the exact timing of the second brood was not measured. Therefore r was calculated only for the first brood to compare the two morphs.

Data were checked for normality with Wilk-Shapiro and differences between the treatments were tested with t tests after checking for equality of variances with Bartlett's test.

Life history experiment two: resource allocation

The second life history experiment was performed to test for differences in resource allocation. It was conducted in the flow-through system as described for the first life history experiment. This time only the high food concentration was used. Experimental animals were offspring of the fifth brood, which led to a larger neonate size compared to the first life history experiment. Thus it was possible to compare the effect of body size on r. A part of the experimental animals of the different stages were removed after measurements, dried at 60°C for 24 h, and weighed with a microbalance (Sartorius, Germany) to the nearest 0.1 μg. Ten animals of each treatment were measured and dissected in the first and in the second adult instar, ≈4–8 h after depositing the eggs. The eggs in this stage are still covered with the egg membrane and therefore relatively robust. The eggs were counted and weighed separately from the soma. As a measure of resource allocation I determined reproductive effort for each adult instar by dividing the egg mass by the total mass.

Data were analyzed and the fitness parameter r was calculated as described for life history experiment one. After linearization through log-log transformations, regressions were calculated for soma mass and total mass data, and slopes and intercepts were compared between treatments by ANCOVA.

Life history experiment three: maternal effects

LH 1 and LH 2 were designed to measure the individual trade-offs for the defense and therefore used offspring of uninduced mothers. However in nature this situation fits only for the first generation that encounters the predator. In later generations mothers themselves will have been exposed to *Chaoborus* kairomones. To look for kairomone-dependent maternal effects on life history, first-clutch F_1 offspring of the mothers of both morphs from the high-food treatment in LH 1 were raised in high food carbon (1.0 mg/L) in the flow-through system in either *Chaoborus* or control medium, until they reached the second adult instar. Body size in each instar, time to reach maturity, size at maturity, and clutch size were measured. Data were analyzed and population growth rate r was calculated as described for life history experiment one.

Separation of costs and life history shifts

In this experiment I tested whether life history shifts may be independent of neckteeth formation. Induction for neckteeth formation in the first two juvenile instars occurred in the experimental clone in the late embryo stage, while induction for neckteeth formation in the third instar occurred in the last half of the first juvenile instar (R. Tollrian, *unpublished data*). Therefore I exposed daphnids to the kairomone, starting in the late embryo stage. Transfer to control medium directly after the first juvenile instar led to the formation of neckteeth in three juvenile instars. To ensure that the juveniles had no further contact with the kairomone they were washed during transfer by successively placing them in five 1.5-L beakers containing control medium. In a parallel treatment daphnids were raised in control me-

dium and were transferred to *Chaoborus* medium after the first instar, which resulted in no neckteeth or very small neckteeth only in the fourth instar. For controls, daphnids were raised and maintained in either control medium, or *Chaoborus* conditioned medium.

The experiment was conducted under high food conditions (carbon concentration 1.5 mg/L) with animals grown individually in 50-mL beakers. Beakers, food suspension, and medium were renewed every day. Size at maturity, time to reach maturity, and clutch size were measured. The occurrence of neckteeth was also recorded. Mothers were pretreated as described in life history experiment one. Twenty daphnids, randomly selected from five mothers, were used per treatment. Daphnids in treatments 1 and 3 and in treatments 2 and 4, were sisters from the same mothers. An ANOVA was calculated to test whether significant differences exist among the four treatments and a pairwise comparison of means (Bonferroni) was used to test where the differences occurred.

Carbon incorporation

Reduced ingestion rates have been reported for induced daphnids (Ramcharan et al. 1992), which could result from behavioral or morphological changes. I designed this experiment to look for differences in carbon incorporation that resulted from the changed morphology. Only juvenile *Daphnia* were used, as the direct costs for forming or carrying the protections can be expected to be highest in the size classes where they are formed or maintained. Daphnids were raised as described in life history experiment one in high food conditions in either *Chaoborus*-conditioned or control medium. Daphnids had been adapted to the experimental food conditions in control medium for 2 h prior to the experiment. The experiment was conducted in control medium.

I used a radioisotope technique to measure carbon incorporation without compensating for respiration losses (Lampert 1977). *Scenedesmus acutus* was incubated with ^{14}C-$NaHCO_3$ for 12 h and was offered to the daphnids at a high (1.0 mg/L) and a low (0.1 mg/L) carbon concentration.

Twenty-five daphnids of each morph were randomly selected for each treatment. The daphnids were incubated for 4 h in 2-L glass bottles, which were in an incubator at constant 20°C and mounted on a slowly rotating plankton roller to prevent settling of algae. Experimental daphnids of both morphs were enclosed together to eliminate the possibility of slightly different activities in different bottles. This was possible because the two morphs could be easily separated by the occurrence of neckteeth. At the end of the experiment daphnids were immediately killed in heated water. After measurement, they were dried for 24 h at 60°C and weighed the next day. Daphnids of equal length (± 10 μm) were pooled after measurements in groups of two to three for weighing.

Two replicates, each 50 mL, of both algal concentrations were filtered before and after the experiment and the activity was determined. Vials with the weighed daphnids were kept overnight in 0.3 mL of tissue solubilizer at 60°C, then cooled and filled with 5 mL of a scintillation cocktail (Packard). The samples were counted in a liquid scintillation counter. With the reference of the algal activity the activity of the animals could be converted into mass of carbon incorporated per hour.

I linearized the data by log transforming incorporation rate and body mass, calculated linear regressions, and compared slopes and intercepts between the two morphs with ANCOVA.

Measurements and statistics

All measurements were performed with a dissecting microscope that allowed magnification up to 160× (Leitz M3 Kombistereo). Handling time of the experimental animals throughout daily measurements was reduced by using a computer-based image analysis system that allowed measurements on digitized video images (Soft-Imaging Software, Münster, Germany). Statistical analysis was performed with the computer programs SYSTAT (V.: 5.01, SYSTAT, Evanston, Illinois, USA) and STATISTIX (V.: 4.1, Analytical Software, Saint Paul, Minnesota, USA).

Results

Life history experiment one: influence of food conditions

In the high food experiment (Table 1) means of body sizes were larger for the neckteeth morph (NM) only in the first mature instar. Body depth was larger in all instars for NM. The differences were significant in all but the third and fourth instars. The daphnids of both morphs matured in their fifth instar. The time to reach maturity was 3.5% longer for NM, and the time to reach the second adult instar was 2.6% longer for NM. Mean clutch sizes were larger for NM but the difference was not significant.

In the low food experiment (Table 1) body length was significantly greater for NM after the second instar. Body depth was significantly larger for NM in all instars. Most daphnids also matured in the fifth instar at low food. The time to reach maturity significantly increased (by 7 h or 5%) for NM. Clutch sizes were larger in both adult instars for NM but the difference was not significant.

In the low food experiment, growth was reduced for both morphs compared to the high food experiment (Table 1). Time to reach maturity increased at low food for TM by 17.5% and for NM by 19.4%. While clutch size was also reduced for both morphs, neckteeth formation was not affected by food resources in any instar (Fig. 1).

TABLE 1. Size and life history parameters for *Daphnia pulex* by instar at high and low food (experiment LH 1) for typical morph (TM) and neckteeth morph (NM). Data show means ± 1 SD. Significance levels for comparisons between treatments for each character were calculated with *t* tests.

Character	Instar	High food conditions			Low food conditions		
		TM	NM	*P*	TM	NM	*P*
Body length (mm)	1	0.66 ± 0.03	0.66 ± 0.03	0.894	0.66 ± 0.03	0.68 ± 0.04	0.108
	2	0.85 ± 0.06	0.87 ± 0.05	0.255	0.83 ± 0.03	0.86 ± 0.06	0.007
	3	1.12 ± 0.09	1.12 ± 0.07	0.852	1.03 ± 0.07	1.12 ± 0.06	<0.001
	4	1.45 ± 0.06	1.47 ± 0.08	0.325	1.28 ± 0.13	1.40 ± 0.06	<0.001
	5	1.81 ± 0.09	1.86 ± 0.06	0.035	1.48 ± 0.06	1.58 ± 0.04	<0.001
	6	2.05 ± 0.07	2.04 ± 0.07	0.756	1.53 ± 0.05	1.65 ± 0.06	<0.001
Body depth (mm)	1	0.37 ± 0.02	0.39 ± 0.02	0.014	0.37 ± 0.02	0.40 ± 0.06	0.009
	2	0.48 ± 0.04	0.50 ± 0.04	0.026	0.47 ± 0.02	0.51 ± 0.04	<0.001
	3	0.66 ± 0.06	0.68 ± 0.05	0.145	0.59 ± 0.05	0.67 ± 0.03	<0.001
	4	0.90 ± 0.04	0.93 ± 0.06	0.072	0.74 ± 0.08	0.86 ± 0.03	<0.001
	5	1.15 ± 0.06	1.21 ± 0.05	<0.001	0.91 ± 0.04	1.02 ± 0.04	<0.001
	6	1.33 ± 0.07	1.38 ± 0.05	0.032	0.96 ± 0.04	1.05 ± 0.03	<0.001
Time to maturity (h)	5	119.95 ± 1.27	124.15 ± 1.90	<0.001	141.00 ± 2.13	148.00 ± 2.05	<0.001
	6	180.39 ± 2.06	185.05 ± 1.88	<0.001	...	...	...
Clutch size	5	8.52 ± 2.43	9.95 ± 2.06	0.056	2.78 ± 0.71	3.15 ± 0.93	0.185
	6	15.11 ± 2.08	16.05 ± 1.73	0.138	3.00 ± 0.87	3.64 ± 0.93	0.111

Life history experiment two: resource allocation

In the second life history experiment (Table 2) again NM matured at a significantly greater body length. The body length difference remained significant also in the second adult instar. Soma mass, total egg mass, and total mass were significantly larger for NM in both adult instars. Clutch size was larger for NM in both adult instars. Time to reach maturity increased 7.5% for NM and the adult instar duration increased by 4%. The reproductive effort (RE) was significantly larger for TM in the first adult instar. Total egg mass equaled 70% of the soma mass for TM but only 50% for NM. In the second adult instar total egg mass equaled 90% for both morphs.

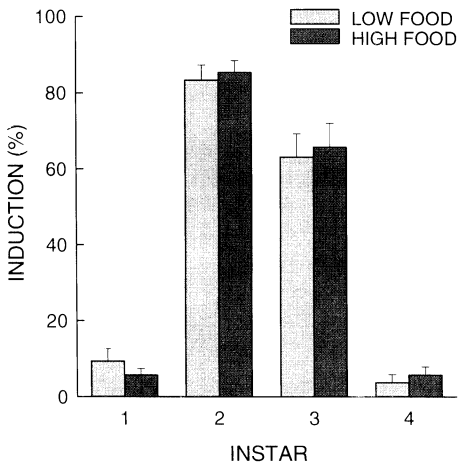

FIG. 1. Influence of resource availability on strength of neckteeth formation in juvenile instars at limited (0.1 mg/L) and unlimited (1.0 mg/L) food carbon conditions. (Error bars indicate ½ 95% CI).

Total mass, soma mass, and reproductive mass increased with body length (Table 2), but the higher total mass of NM was not only the result of a greater body length. The slopes of the regressions for the two morphs were not significantly different, but the difference in intercepts indicated a higher total mass per unit length for NM (Table 3). This was the same for soma mass, which showed in a regression with body length also a higher intercept for NM (Table 3).

Life history experiment three: maternal effects

The offspring of mothers raised in the chemical presence of *Chaoborus* were born at a larger size than control daphnids (Table 4). The size difference remained significant throughout development. Also the body depth was significantly larger in all instars for NM. Thus, the maternal effects amplified the growth differences between both morphs. The larger NM also had significantly larger clutches, but matured 4 h (3.3%) later.

Fitness and effects of neonate size

In all experiments NM tended to have a higher mean intrinsic growth rate *r*, contrary to the "cost-of-defense hypothesis" (Fig 2). Differences in population growth rate between the two morphs were significant in life history experiment three, testing for maternal effects (F_1 generation) and in life history experiment two, where fifth clutch offspring have been used. The higher *r* was in both cases the result of a larger clutch size.

Body depth

I observed in all three life history experiments greater body depth for NM (Tables 1 and 4). As this could be the result of a larger body length, which co-occurred

TABLE 2. Body size and life history parameters for adult *Daphnia pulex* instars at high food (experiment LH 2) for typical morph (TM) and neckteeth morph (NM). Reproductive effort (RE) was calculated as the quotient of the reproductive mass divided by the total mass. Significacnce levels of comparisons between treatments for each character were with *t* tests. (Data are means ± 1 SD).

Character	Instar	TM	NM	df	*t*	*P*
Body length (mm)	5	1.98 ± 0.05	2.11 ± 0.07	46	−6.33	<0.001
	6	2.39 ± 0.07	2.48 ± 0.07	25	−2.77	<0.001
Soma mass (μg)	5	36.89 ± 4.96	60.63 ± 9.09	46	−8.90	<0.001
	6	61.77 ± 14.88	72.59 ± 11.97	25	−1.86	0.075
Total egg mass (μg)	5	26.06 ± 1.82	30.49 ± 4.62	46	−3.34	0.002
	6	55.45 ± 9.39	66.61 ± 11.43	25	−2.18	0.039
Total mass (μg)	5	62.95 ± 5.94	91.11 ± 8.70	46	−10.75	<0.001
	6	117.22 ± 7.27	139.20 ∓ 20.15	23.2	−4.14	<0.001
Clutch size	5	11.23 ± 0.73	14.71 ± 2.11	45.9	−8.51	<0.001
	6	23.83 ± 3.71	29.19 ± 4.85	25	−2.49	<0.001
Time ot maturity (h)	5	120.46 ± 1.85	129.43 ± 2.10	46	−13.52	<0.001
	6	180.67 ± 2.06	192.00 ± 2.47	25	−10.23	<0.001
RE	5	0.42 ± 0.03	0.34 ± 0.06	46	4.80	<0.001
	6	0.48 ± 0.10	0.48 ± 0.04	25	−0.05	0.963

in most experiments, I compared for the juveniles slopes and intercepts of length vs. depth regressions between the two morphs with ANCOVA, which revealed in all experiments significant differences (Table 5). While in LH 1 at high and low food treatments the intercepts were different, in LH 3 the slopes were different (Fig. 3). This shows that body depth was significantly larger for juveniles of the induced morph (NM) compared to the typical morph (TM) under both high and low food conditions. However, the magnitude of the difference was only 2–5%, not very large.

Separation of costs and life history shifts

Changes in life history parameters (e.g., size at maturity, age at first reproduction, clutch size) and morphological changes (neckteeth syndrome) can be separated. Morphological changes are induced in the late embryo stage or in the first instars, whereas life history changes require a longer or later exposure to the kairomone. A transfer of daphnids from *Chaoborus*-conditioned medium to control medium after the first instar (Table 6, treatment 2) led to regular neckteeth formation, but not to life history changes that were significantly different from the control (Table 6, treatment 1). Conversely, a transfer from control medium to *Chaoborus*-conditioned medium after the first instar (Table 6, treatment 3) led to no neckteeth during the first three juvenile instars, whereas life history changed significantly compared to treatment 1 and 2, but not compared to permanent exposure to the kairomone (Table 6, treatment 4). An ANOVA indicates significant differences for body size and time to maturity, and a pairwise comparison of means (Bonferroni) indicates that treatment 1 and 2 are significantly different from treatment 3 and 4.

Carbon incorporation

Carbon incorporation rates for juvenile *Daphnia* increased with body mass at both food concentrations (Fig. 4a, b). The regressions for carbon incorporated per hour vs. body mass, were not significantly different in slope or in intercept between TM and NM at low food levels, where morphological differences should have an influence (Table 7). At unlimited food levels slopes and intercepts were different and regressions

TABLE 3. Influence of *Chaoborus* kairomone presence vs. absence (treatment, *T*) and body length (*L*) on body mass. Regressions are of log soma mass and log total mass (both measured in μg) against log body length (measured in μm) (SE in parentheses below) for typical (TM) and neckteeth-induced (NM) morphs. Slopes and intercepts were compared between morphs with ANCOVA.

Experiment	Regressions					ANCOVAs				
	Morph	Constant	Slope	r^2	*N*	Character	MS	Error	*F*	*P**
Soma	TM	−14.14	2.35	0.96	37	Length	18.11	0.01	19.74×10^2	<0.001
		(0.633)	(0.086)	(0.221)		Treat	0.16	0.01	17.64	<0.001
	NM	−13.5	2.29	0.94	76	$L \times T$	<0.01		0.32	0.57
		(0.492)	(0.066)	(0.221)						
Total	TM	−17.98	2.92	0.98	37	Length	28.39		64.14×10^2	<0.001
		(0.372)	(0.051)	(0.164)		Treat	0.12	<0.01	27.15	<0.001
	NM	−17.58	2.88	0.98	76	$L \times T$	<0.01	<0.01	0.17	0.68
		(0.364)	(0.049)	(0.164)						

* Significant $L \times T$ interactions indicate non-homogeneous slopes. Significant treamtent effects indicate different intercepts.

TABLE 4. Test for maternal effects (experiment LH 3). Typical morph (TM) and neckteeth morph (NM) daphnids were first brood offspring of mothers born in the treatments. Size and life history parameters per instar at high food. Significant differences between treatments for each character were calculated with *t* tests. (Data are means ± 1 SD).

Character	Instar	TM	NM	df	*t*	*P*
Body length (mm)	1	0.61 ± 0.02	0.66 ± 0.03	38	−5.00	<0.001
	2	0.79 ± 0.03	0.84 ± 0.04	38	−5.26	<0.001
	3	1.03 ± 0.05	1.11 ± 0.07	38	−3.77	<0.001
	4	1.41 ± 0.04	1.49 ± 0.05	54	−6.54	<0.001
	5	1.82 ± 0.04	1.94 ± 0.05	35	−7.70	<0.001
Body depth (mm)	1	0.34 ± 0.01	0.37 ± 0.02	38	−4.88	<0.001
	2	0.43 ± 0.02	0.48 ± 0.02	38	−6.75	<0.001
	3	0.60 ± 0.03	0.68 ± 0.05	38	−5.71	<0.001
	4	0.86 ± 0.03	0.93 ± 0.04	54	−8.08	<0.001
	5	1.13 ± 0.04	1.24 ± 0.03	35	−9.35	<0.001
Time to maturity (h)	5	119.94 ± 1.31	123.90 ± 1.05	35	−10.18	<0.001
Clutch size	5	9.22 ± 0.73	11.32 ± 1.29	35	−6.01	<0.001

crossed; however, the difference was very small and most probably occurred due to outliers in the larger NM daphnids. Thus, there were no biologically meaningful differences in carbon incorporation between the two morphs under high vs. low food.

DISCUSSION

Although my study focused on a single clone of *Daphnia pulex,* the results reveal that high direct costs are not necessarily correlated with inducible defenses and that the observed demographic disadvantage (longer time to reach maturity) of the induced morph in the absence of the predator was a trade-off for the larger size at maturity.

Since the first experiments on demographic changes associated with neckteeth formation in *D. pulex,* a variety of controversial results has been reported. However, in contrast to this study and to Spitze (1992), most studies report fitness disadvantages for NM in the absence of predation (Table 8). Spitze (1992) reported clonal differences in body size, growth rate, fecundity, and age at reproduction. Although clonal differences might explain some of the variation among studies, some variation may also be explained by experimental conditions. Chemicals released by *Chaoborus,* in addition to the kairomone, or bacteria growing in the presence of *Chaoborus,* can be harmful to *Daphnia.* Bacteria can perhaps deplete oxygen or clog the filter meshes. Harmful effects are indicated by results from Ketola and Vuorinen (1989), who found growth in the robust species *D. magna* (which does not form neckteeth) reduced, when reared in *Chaoborus*-conditioned water. Survival was drastically reduced for NM in some studies (Table 8), which were possibly indications of deleterious effects.

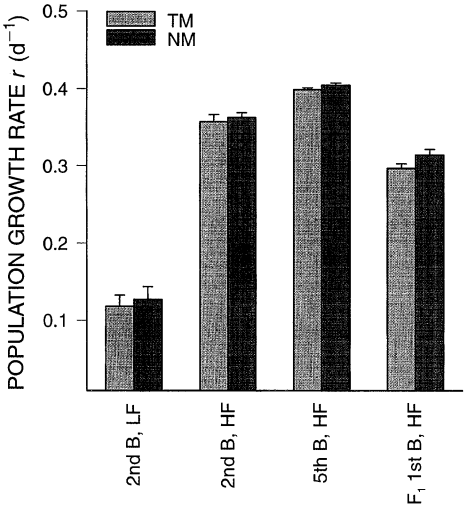

FIG. 2. Dependence of calculated intrinsic rate of population increase *r* on brood number of the mother (B), food condition (LF = low food, HF = high food), and treatment for daphnids of typical morph (TM) and neckteeth morph (NM). F_1 = mother generation and F_1 generation raised under the same conditions. (Error bars indicate ½ 95% CI).

Another reason for possible overestimations of costs was indicated by the results of Dodson (1988*b*) and Ramcharan et al. (1992), who reported upward migration directly to the surface in *Daphnia pulex* in the presence of the *Chaoborus* kairomone. If this is a typical behavior in many clones, it is possible that in most experiments with semicontinuous systems the induced morph could have suffered inferior food conditions compared to the typical morph, because most algae sink to the bottom of the vessels. Thus, the induced daphnids could show demographic disadvantages resulting from different food conditions. This possibility was excluded in my flow-through experiments.

A third possibility for cost overestimations is also indicated by Ramcharan et al. (1992), who reported reduced ingestion rates for NM under high *Chaoborus* densities. This effect could be a behavioral response to the predator or due to morphological differences (e.g., size of filtration screen). However, these effects probably have not been important in my study, as NM was not inferior at low food. In addition, my clone

TABLE 5. Influence of *Chaoborus* kairomone (treatment, *T*) and body length (*L*) on body depth (*D*) in juvenile *Daphnia pulex*. Regressions are of body depth (in μm) against body size (in μm) for high and low food treatments (1 SE in parentheses). Slopes and intercepts of regressions between typical (TM) and neckteeth-induced (NM) morphs were compared with ANCOVA.

Experiment	Food carbon level	Regressions					ANCOVAs				
		Morph	Constant	Slope	r^2	*N*	Character	MS	Error	*F*	*P**
LH 1	0.1 mg/L	TM	−40.677 (5.785)	0.611 (0.006)	0.990	123	*L*	6.33×10^6		1.05×10^4	<0.001
							T	5.38×10^4	602	89	<0.001
		NM	−31.606 (11.082)	0.632 (0.010)	0.969	120	*L* × *T*	1.67×10^3	597	2.80	0.096
LH 1	1.0 mg/L	TM	−82.240 (7.483)	0.670 (0.007)	0.989	100	*L*	7.12×10^6		2.44×10^4	<0.001
							T	1.12×10^4	291	38.67	<0.001
		NM	−80.501 (5.336)	0.683 (0.005)	0.993	115	*L* × *T*	6.28×10^2	290	2.27	0.143
LH 3	1.0 mg/L	TM	−76.229 (6.497)	0.661 (0.006)	0.993	93	*L*	7.97×10^6		2.74×10^4	<0.001
							T	3.54×10^2		1.22	0.271
		NM	−85.879 (5.690)	0.683 (0.005)	0.995	83	*L* × *T*	2.10×10^2	290	7.11	0.008

* Significant *L* × *T* interactions indicate non-homogeneous slopes. Significant treatment (*T*) effects indicate different intercepts.

showed no differences in carbon incorporation rates at low food condition, which excluded the possibility of morphological effects on food accumulation.

Separation of costs and life history changes

My results show that besides forming inducible morphological defenses, *Daphnia pulex* also responded demographically to the predator (e.g., larger size at maturity, longer time to reach maturity). Therefore trade-offs of the observed life history shifts have to be separated from costs resulting from the formation of morphological defenses. Most studies (Table 8) do not allow this differentiation. An exception is the study by Riessen and Sprules (1990), who exposed daphnids in the *Chaoborus* treatment only during the egg/embryo period and during a short juvenile period, similar to treatment one and two in my cost-separation experiment (Table 6). Thus, they tested only for direct costs. In agreement with my experiment, they found no differences between TM and NM in clutch size, body length, number of juvenile instars, egg volume, or survivorship (Table 8). However, they obtained a prolonged instar duration.

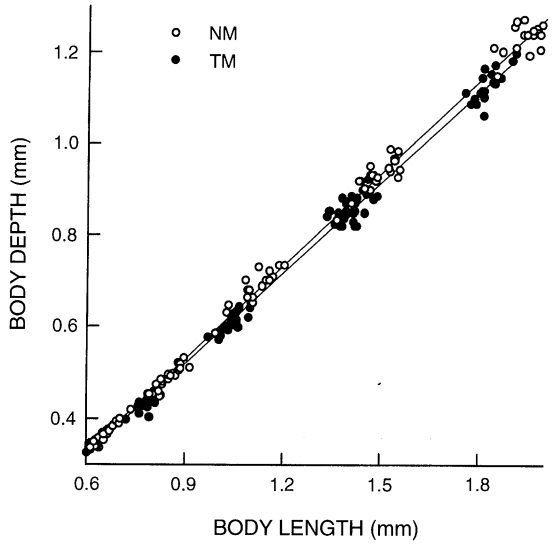

FIG. 3. Relation of body depth and body length for typical morph (TM) and neckteeth morph (NM) daphnids. (regression lines; data from the maternal effects experiment, LH 3).

The *D. pulex* clone in my study allowed a clear separation of direct physiological costs and trade-offs for life history shifts as neckteeth and life history were independently induced in this study. Life history shifts were induced after the induction of neckteeth formation and thus could be uncoupled from neckteeth formation. The observed changes, higher growth per instar, larger size at maturity, higher fecundity, and longer instar duration occurred without neckteeth formation, while neckteeth formation occurred without these life history shifts under the experimental conditions. Therefore the longer time to maturity was not the "cost" of neckteeth formation, but was a trade-off for the life history shifts.

Life history shifts

Taylor and Gabriel's (1992) model for optimal resource partitioning under various environments predicts that under predation by invertebrates selecting small prey sizes (e.g., *Chaoborus*), maturity will be delayed to attain a larger size. A higher portion of the energy will be used for growth, resulting in a lower *r* if this strategy would be performed in the absence of predation.

In accordance with the model, the NM in my study had a larger body size at maturity, at the cost of a longer time to maturity. A comparative measure for resource allocation is the reproductive effort (RE), which in my

TABLE 6. Separation of direct costs and life history shifts. Comparison of first adult instars. Significant differences between treatments were calculated with ANOVA; homogeneous groups, calculated with multicomparison of the means (Bonferroni, 5% error probability), are connected with horizontal lines. (Data are means ± 1 SD.)

	Treatment*			
	1	2	3	4
N	20	20	20	19
Body length (mm)	1.80 ± 0.07	1.81 ± 0.04	1.86 ± 0.04	1.87 ± 0.05
Clutch size	9.35 ± 1.56	9.35 ± 0.99	10.2 ± 1.24	10.42 ± 1.61
Time to maturity (h)	120 ± 2	121 ± 2	123 ± 2	124 ± 2

* Treatment 1: untreated control (minimal reaction); treatment 2: transfer from *Chaoborus* to control medium (with full neckteeth expression, no life history changes); treatment 3: transfer from untreated control to *Chaoborus* medium (no neckteeth formation, but life history changes); treatment 4: *Chaoborus* medium (maximal reaction).

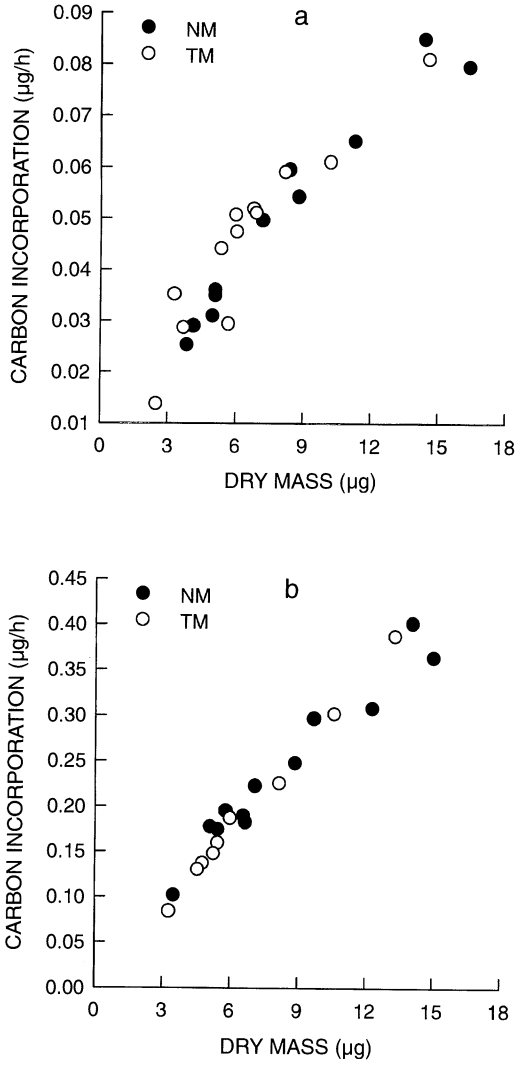

FIG. 4. Carbon incorporation rate in relation to body mass for typical morph (TM) and neckteeth morph (NM) daphnids at low food carbon (a; 0.1 mg/L) and high food carbon (b; 1.0 mg/L) conditions. Data for juveniles 0.6–1.1 mm body length.

study behaved as predicted by theory. RE was higher in the first adult instar for TM compared to NM, indicating that a higher proportion of the energy budget had been invested into reproduction, while no difference occurred in the second adult instar.

However, in my study NM had a larger body size, which led to a larger clutch size, and although it was countered by a longer time to reach maturity, to a higher mean fitness component *r*. This is not in accordance with the model, which expects stronger disadvantages, which are only offset when the predation pressure exceeds a certain level. A possible explanation would be a better energy budget for larger animals, although this may not generally hold true for comparisons between species (review in DeMott 1989). Thus, if the investment into growth leads to an additional energy bonus the fitness parameter *r* cannot be expected to be equal between the two life history strategies. Also, in the other studies that showed life history shifts in *D. pulex* in response to *Chaoborus,* the size at maturity tended to be positively associated with the clutch size (Table 8).

In my study life history shifts tended to lead to a higher mean fitness for NM even in the absence of predation. This result seems counterintuitive, but is not impossible. Higher fitness for induced morphotypes in the absence of predation has been previously reported for other species or predators by Gilbert (1980), O'Brien et al. (1980), Havel and Dodson (1988), Stemberger (1990),and Black (1993).

The results show that body size is a key character in the observed life history changes. In the presence of the *Chaoborus* kairomone direct and maternal effects led to increased body size and size at maturity. Size at maturity is an important parameter in the life history of many species (Lynch 1980, Stearns 1992). Lampert (1993) showed for a single clone of *Daphnia magna* that a rapid decrease in size at maturity can be achieved within two generations by elimination of large animals. Neonate size increased with size of the mother and

TABLE 7. Influence of *Chaoborus* kairomone (treatment, *T*) and body mass (*M*) on the carbon incorporation rate of *Daphnia pulex*. Regressions for log carbon incorporated (measured in μg/h) against log body mass (measured in μg) (1 SE in parentheses) at high and low food conditions. Regression slopes and intercepts were compared between typical (TM) and induced (NM) morphs using ANCOVA.

Food carbon level	Regressions					ANCOVAs				
	Morph	Constant	Slope	r^2	N	Character	MS	Error	F	P*
0.1 mg/L	TM	−1.870	10.870	0.920	12	M	0.270		287.610	<0.001
		(0.05)	(1.00)	(0.06)		T	<0.001	0.012	0.010	0.914
	NM	−1.770	8.720	0.970	12	$M \times T$	0.010	0.002	4.020	0.059
		(0.03)	(0.48)	(0.03)						
1.0 mg/L	TM	−2.580	1.050	0.990	9	M	0.610		712.160	<0.001
		(0.04)	(0.05)	(0.02)		T	0.010		14.500	0.001
	NM	−2.360	0.810	0.990	12	$M \times T$	0.010	0.001	11.960	0.003
		(0.04)	(0.05)	(0.03)						

* Significant treatment (*T*) effects indicate different intercepts. Significant $M \times T$ interactions indicate non-homogeneous slopes.

determined size at maturity, which in turn affected neonate size of the next generation.

In my study size at maturity would be shifted in the opposite direction, by elimination of small adults. Size at maturity of the F_1 generation increased with brood number (i.e., size) of the mothers (Tables 1, 3, and 4). Although in the field an elimination of small adults could increase the size at maturity in the following generations, this occurred even quicker as an induced effect of the *Chaoborus* kairomones. *D. pulex* in contact with the *Chaoborus* cues grew to a larger size during the juvenile instars (Tables 2 and 3). Additionally as a maternal effect, mothers grown in *Chaoborus*-conditioned water gave birth to larger offspring, which in turn developed to a larger size at maturity (Table 4). While first brood offspring of TM were considerably smaller at maturity than fifth brood offspring, the maternal effect made NM first brood offspring nearly as large at maturity as fifth brood TM (Tables 3 and 5). Whether this effect was maternally induced or the result of the larger size of the induced mothers cannot be clarified.

The effect of a larger *Daphnia* body size on vulnerability to *Chaoborus* predation is not straightforward. *Daphnia* vulnerability to *Chaoborus americanus* (Parejko 1991) and *C. crystallinus* (Tollrian 1995) shows a peak at the second and the third instar. This is due to the opposing effects of encounter probability, which increases with prey size, and strike efficiency, which in turn decreases with prey size (Pastorok 1981). However, a larger induced first instar juvenile is actually more vulnerable, as it has a higher encounter probability without a higher protection (Tollrian 1995). Additionally, the instar durations are longer for NM. Thus, before the vulnerability peak is reached a larger body size imposes the disadvantage of higher encounter rate, whereas later the advantage of the reduced strike efficiency is overwhelming.

The major advantage may be that mature *Daphnia* are safer due to their size. The mature instar has ap-

TABLE 8. Literature comparison of simplified *Chaoborus* effects on *Daphnia pulex* life history parameters. + = higher for induced morph; − = lower for induced morph; o = no difference; n = not reported. B_B = body length at birth; B_M = body length at maturity; C_1 = first clutch size; T_M = age at maturity (+ means longer time to mature); r = calculated fitness component (intrinsic population growth rate); S = survival.

Reference	B_B	B_M	C_1	T_M	r	S
Black and Dodson 1990	n	−	o	+	−	o*; −†
Black 1993	n	+	+	+	o	−
Havel and Dodson 1987	n	−	o	+	−‡	n
Ketola and Vuorinen 1989	o	−	−	+	−‡	−
Lüning 1992	+	o	−	+	−‡	n
Riessen and Sprules 1990	o	o	o	+	−*; o†	o
Spitze 1992	+	+	+	o	+	n
	+	+	o	o	+	n
	−	o	+	−	+	n
	o	o	o	+	−	n
Vuorinine et al. 1989	o	−	n	+	n	n
Walls and Ketola 1989	o	−	−	+	−	n
Walls et al. 1991	n	o*; −†	o	o	o*; −†	n
Tollrian (*this study*)	o	+	+	+	+	o

* High food conditions.
† Low food conditions.
‡ Estimated from the results.

proximately a threefold duration compared with juvenile instars and carries in addition the important first clutch. *Daphnia* of 1.8 mm can occasionally be captured or killed by *Chaoborus crystallinus* whereas slightly larger daphnids are safe (Tollrian 1995). Thus attaining a larger size at maturity may be highly advantageous.

The increase in body depth, which has not been reported previously, is another possibly protective change in morphology. Swift (1991) showed that prey depth is a better predictor for prey size range in *Chaoborus* than body length. *Chaoborus* usually swallows prey that cannot be deformed only if its diameter is not wider than the larvae's head capsule diameter (Swift 1991). The increase in body depth might additionally make prey handling for the larvae more difficult.

Possible costs of neckteeth formation

We have to assume disadvantages connected to the formation of neckteeth in *D. pulex.* Neckteeth or cooccurring features have been shown to be advantageous in reducing mortality in the presence of invertebrate predators (Krueger and Dodson 1981, Havel and Dodson 1984, Parejko 1991, Tollrian 1995). If there is no disadvantage, we would have to expect that the defense is expressed permanently (Riessen 1984).

Costs for morphological protections in *Daphnia* can be diverse. Higher energy expenditures for the synthesis of extra tissue are one possibility. Helmets in *Daphnia cucullata* or *D. galeata mendotae* are larger than neckteeth. Nevertheless, Jacobs (1967) estimated the mass of a fully developed helmet in *Daphnia galeata mendotae* to equal just one-seventh of a parthenogenetic egg, and neckteeth might even be less. Lynch (1989) calculated for small *D. pulex* that only 6% of the dry mass is lost as total molt and differences between morphs due to the neckteeth should be much smaller. In contrast, the huge crests produced by *Daphnia carinata* as defense against notonectids (Grant and Bayly 1981) might require a significant proportion of the energy budget. However, the energy costs for synthesis of a *Daphnia* carapace are unknown.

Morphological changes can alter the hydrodynamics of zooplankton and probably result in higher energy requirements for swimming. Jacobs (1967) reported that helmeted *D. galeata mendotae* sink faster, and Stenson (1987) proposed that a larger capsule size in *Holopedium gibberum* should increase the drag or lower food uptake. Both effects should have led to disadvantages in my study in the low food treatment. Since they did not (Table 1), they do not appear significant for *D. pulex.*

For helmeted *Daphnia* species a reduced maximum clutch size due to a reduced brood chamber volume caused by a more slender body has been proposed (Jacobs 1967). However, this has been excluded for *D. cucullata* (Tollrian 1991) and was also not observed in this study.

There is no reduction in neckteeth expression under low food compared to high food (Fig. 1). This further suggests that the neckteeth formation may not have large direct costs. However, Parejko and Dodson (1991) found neckteeth expression to be reduced under high food and Riessen (1992) suggested that it might be advantageous to form stronger neckteeth when food is limiting. Thus, selection might favor neckteeth formation under limited food conditions despite costs. However, my results did not support these considerations.

Many clones of *D. pulex* form neckteeth in the first instar even without the *Chaoborus* kairomone (Vuorinen et al. 1989, Lüning 1992; R. Tollrian, *unpublished data*). The production of teeth without a direct induction may be an additional hint that neckteeth are not too costly.

This study cannot rule out that "possibility costs" exist, that is, the ability to respond to predator chemicals could include a permanent cost for providing and maintaining the facilities (e.g., chemosensors, genetic mechanisms, hormones; see Gabriel and Lynch 1992).

Thus, although direct costs for the formation of the small neckteeth should be expected, they seem to be relatively small, even in my clone that forms strong neckteeth. Probably larger disadvantages of the morphological protections are not shown directly in the absence of one predator, but might be in different environments. Generally fish (von Ende 1979, Stenson 1980, Elser et al. 1987) or salamander larvae (Spitze 1992) in ponds are reported to consume *Chaoborus* larvae as preferred diet and thus to reduce the importance of this predator. Moreover, the morphological protections against *Chaoborus* (larger body size per instar, increase in body depth, enlargement of the neck region) would lead to a higher visibility and therefore to a higher mortality in the presence of visually hunting predators. Thus, the presence of *Chaoborus* larvae may indicate to *Daphnia* that fish predation is not dominating, that morphological protections are advantageous, and that life history can be switched.

If predation on large prey exceeds a certain level, some *Daphnia* have the additional ability to respond with life history shifts to fish chemicals (Machácek 1991, Stibor 1992, Weider and Pijanowska 1993), or *Notonecta* chemicals (Dodson and Havel 1988, Lüning 1992, Black 1993). The actual threat by each predator indicated by the relative amount of chemical substances in the water may shift the hierarchy of reaction towards or close to optimal life history adaptation in a range of constraints and limitations evolved in the clonal history. When kairomones are absent life history might be switched to a "general purpose phenotype". On the other hand, we do not know whether low fish densities, which would be high enough to reduce *Chaoborus* and threaten the large *Daphnia pulex,* produce enough kai-

romones to provide a reliable fish-chemical cue. In this case an indirect signal (e.g., lack of *Chaoborus* kairomone) could be used instead. *D. obtusa* for example did not respond to fish chemicals in concentrations, whereas *D. galeata* responded (Machácek 1993).

The assumption that the disadvantage of morphological protections in *D. pulex* exists in different environments is supported by a theoretical study. Taylor and Gabriel (1992) calculated that the optimal strategy against invertebrate predation was the worst strategy under fish predation. In an expansion of their model to seasonal environments they concluded that the cost in fitness of adaptation to the wrong form of selective predation can be large (Taylor and Gabriel 1993).

Three factors are expected to favor the evolution of inducible defenses (review in Harvell 1990): (1) when the probability of contacts by biological agents is high but unpredictable, (2) when the cues associated with contact are reliable and not fatal, and (3) when the fitness costs offset some of the benefits of the defense. However, varying predator regimes with contrasting selectivity would be a different type of agent favoring inducible defenses. While under (3) the driving force would be a cost reduction in the absence of the predator, in the latter case increased mortality in the presence of another predator would be the driving force. Selection in the fluctuating presence of predators with contrasting selectivities should promote the evolution of inducible defenses.

Gabriel and Lynch (1992) calculated that the evolution of reaction norms (e.g., for life histories) would be favored over broadly adapted genotypes if the between-generation variation in the environment is large compared to the within-generation variation. The fixation of the life history late in ontogenesis, in my study, allows *D. pulex* to respond during ontogenesis to changing environments.

Morphological defenses, life history shifts, and maternal effects may work together to form effective protections against *Chaoborus* larvae. In my study physiological costs for neckteeth formation were not measurable. The observed demographic disadvantages were trade-offs for life history shifts. These results are not unexpected as selection should generally force the evolution of inducible defenses towards a high degree of protection at low costs, and neckteeth formation might be a defense close to optimality.

Acknowledgments

The manuscript benefited from discussion with W. Lampert, W. Gabriel, and two anonymous reviewers. W. R. DeMott improved spelling, grammar, and style of the manuscript.

Literature Cited

Appleton, R. D., and A. R. Palmer. 1988. Water-borne stimuli released by predatory crabs and damaged prey induce more predator-resistant shells in a marine gastropod. Proceedings of the National Academy of Sciences (USA) **85**:4387–4391.

Black, A. R. 1993. Predator-induced phenotypic plasticity in *Daphnia pulex*: life-history and morphological responses to *Notonecta* and *Chaoborus*. Limnology and Oceanography **38**:986–996.

Black, A. R., and S. I. Dodson. 1990. Demographic costs of *Chaoborus*-induced phenotypic plasticity in *Daphnia pulex*. Oecologia (Berlin) **83**:117–122.

Brett, M. T. 1992. *Chaoborus* and fish-mediated influences on *Daphnia longispina* population structure, dynamics and life-history strategies. Oecologia **89**:69–77.

Brönmark, C., and J. G. Miner. 1992. Predator-induced phenotypical change in body morphology in crucian carp. Science **258**:1348–1350.

Charlesworth, B. 1980. Evolution in age-structured populations. Cambridge University Press, New York, New York, USA.

Confer, J. L., and P. I. Blades. 1975. Omnivorous zooplankton and planktivorous fish. Limnology and Oceanography **20**:571–579.

Crowl, T. A., and A. P. Covich. 1990. Predator-induced life-history shifts in a freshwater snail. Science **247**:949–951.

DeMott, W. R. 1989. The role of competition in zooplankton succession. Pages 195–252 *in* U. Sommer, editor. Plankton ecology. Springer-Verlag, Berlin, Germany.

Dodson, S. I. 1988*a*. Cyclomorphosis in *Daphnia galeata mendotae* Birge and *Daphnia retrocurva* Forbes as a predator-induced response. Freshwater Biology **19**:109–114.

———. 1988*b*. The ecological role of chemical stimuli for zooplankton: predator-avoidance behavior in *Daphnia*. Limnology and Oceanography **33**:1431–1439.

———. 1989*a*. The ecological role of chemical stimuli for the zooplankton: predator induced morphology in *Daphnia*. Oecologia (Berlin) **78**:361–367.

———. 1989*b*. Predator-induced reaction norms—cyclic changes in shape and size can be protective. Bioscience **39**:447–452.

Dodson, S. I., and J. E. Havel. 1988. Indirect prey effects: some morphological and life history responses of *Daphnia pulex* exposed to *Notonecta undulata*. Limnology and Oceanography **33**:1274–1285.

Elser, M. M., C. E. von Ende, P. S. Sorrano, and S. R. Carpenter. 1987. *Chaoborus* populations: response to food web manipulations and potential effects on zooplankton communities. Canadian Journal of Zoology **65**:2846–2852.

Gabriel, W., and M. Lynch. 1992. The selective advantage of reaction norms for environmental tolerance. Journal of Evolutionary Biology **5**:41–59.

Gadgil, M., and W. M. Bossert. 1970. Life historical consequences of natural selection. American Naturalist **104**: 1–24.

Gilbert, J. J. 1966. Rotifer ecology and embryological induction. Science **151**:1234–1237.

———. 1980. Further observations on developmental polymorphism and its evolution in the rotifer *Brachionus calyciflorus*. Freshwater Biology **10**:281–294.

Grant, J. W., and I. A. Bayly. 1981. Predator induction of crests in morphs of the *Daphnia carinata* King complex. Limnology and Oceanography **26**:201–218.

Hanazato, T. 1991. Induction of development of high helmets by a *Chaoborus*-released chemical in *Daphnia galeata*. Archiv für Hydrobiologie **122**:167–175.

Harvell, C. D. 1986. The ecology and evolution of inducible defenses in a marine bryozoan: cues, costs, and consequences. American Naturalist **128**:810–823.

———. 1990. The ecology and evolution of inducible defenses. The Quarterly Review of Biology **65**:323–340.

———. 1992. Inducible defenses and allocation shifts in a marine bryozoan. Ecology **75**:1567–1576.

Havel, J. E., and S. I. Dodson. 1984. *Chaoborus* predation on typical and spined morphs of *Daphnia pulex*: behavioral observations. Limnology and Oceanography **29**:487–494.

Havel, J. E., and S. I. Dodson. 1987. Reproductive costs of *Chaoborus*-induced polymorphism in *Daphnia pulex*. Hydrobiologia **150**:273–281.

Hebert, P. D. N., and P. M. Grewe. 1985. *Chaoborus*-induced shifts in the morphology of *Daphnia ambigua*. Limnology and Oceanography **30**:1291–1297.

Hessen, D. O., and E. van Donk. 1993. Morphological changes in *Scenedesmus* induced by substances released from *Daphnia*. Archiv for Hydrobiologie **127**:129–140.

Jacobs, J. 1967. Untersuchungen zur Funktion und Evolution der Zyklomorphose bei *Daphnia*, mit besonderer Berücksichtigung der Selektion durch Fische. Archiv für Hydrobiologie **62**:467–541.

Ketola, M., and I. Vuorinen. 1989. Modification of life history parameters of *Daphnia pulex* Leydig and *Daphnia magna* Straus by the presence of *Chaoborus* sp. Hydrobiologia **179**:149–155.

Krueger, D. A., and S. I. Dodson. 1981. Embryological induction and predation ecology in *Daphnia pulex*. Limnology and Oceanography **26**:219–223.

Kuhlmann, H. W., and K. Heckmann. 1985. Interspecific morphogens regulating prey–predator relationships in *Protozoa*. Science **227**:1347–1349.

Kusch, J. 1993. Induction of defensive morphological changes in ciliates. Oecologia **94**:571–575.

Lampert, W. 1977. Studies on the carbon balance of *Daphnia pulex* as related to environmental conditions. I. Methodological problems of the use of ^{14}C for the measurement of carbon assimilation. Archiv für Hydrobiologie. Supplement **48**:287–309.

———. 1987. Feeding and nutrition in *Daphnia*. Memorie dell'Istituto Italiano di Idrobiologia Dott Marco de Marchi **45**:143–192.

———. 1993. Phenotypic plasticity of the size at first reproduction in *Daphnia*: the importance of maternal size. Ecology **74**:1455–1466.

Lampert, W., R. D. Schmitt, and P. Muck. 1988. Vertical migration of freshwater zooplankton: test of some hypotheses predicting a metabolic advantage. Bulletin of Marine Science **43**:620–640.

Law, R. 1979. Optimal life histories under age-specific predation. American Naturalist **114**:399–417.

Lively, C. M. 1986. Competition, comparative life histories, and maintenance of shell dimorphism in a barnacle. Ecology **67**:858–864.

Lüning, J. 1992. Phenotypic plasticity of *Daphnia pulex* in the presence of invertebrate predators: morphological and life history responses. Oecologia **92**:383–390.

Lynch, M. 1980. The evolution of cladoceran life histories. Quarterly Review of Biology **55**:23–42.

———. 1989. The life history consequences of resource depression in *Daphnia pulex*. Ecology **70**:246–256.

Machácek, J. 1991. Indirect effects of planktivorous fish on the growth and reproduction of *Daphnia galeata*. Hydrobiologia **225**:193–197.

———. 1993. Comparison of the response of *Daphnia galeata* and *Daphnia obtusa* to fish-produced chemical substance. Limnology and Oceanography **38**:1544–1550.

Maynard Smith, J. 1982. Evolution and the theory of games. Cambridge University Press, New York, New York, USA.

Michod, R. E. 1979. Evolution of life histories in response to age-specific mortality factors. American Naturalist **113**: 531–550.

O'Brien, W. J., D. Kettle, H. Riessen, D. Schmidt, and D. Wright. 1980. Dimorphic *Daphnia longiremis*: predation and competitive interactions between the two morphs. Pages 497–505 *in* W. C. Kerfoot, editor. Evolution and ecology of zooplankton communities. University Press of New England, Hanover, New Hampshire, USA.

Parejko, K. 1991. Predation by chaoborids on typical and spined *Daphnia pulex*. Freshwater Biology **25**:211–217.

Parejko, K., and S. I. Dodson. 1990. Progress towards characterization of a predator/prey kairomone: *Daphnia pulex* and *Chaoborus americanus*. Hydrobiologia **198**:51–59.

Parejko, K., and S. I. Dodson. 1991. The evolutionary ecology of an antipredator reaction norm: *Daphnia pulex* and *Chaoborus americanus*. Evolution **45**:1665–1674.

Pastorok, R. A. 1981. Prey vulnerability and size selection by *Chaoborus* larvae. Ecology **62**:1311–1324.

Ramcharan, C. W., S. I. Dodson, and J. Lee. 1992. Predation risk, prey behavior, and feeding rate in *Daphnia pulex*. Canadian Journal of Fisheries and Aquatic Sciences **49**: 159–165.

Reznick, D. A., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. Nature **346**:357–359.

Riessen, H. P. 1984. The other side of cyclomorphosis: why *Daphnia* lose their helmets. Limnology and Oceanography **29**:1123–1127.

———. 1992. Cost–benefit model for the induction of an antipredator defense. American Naturalist **140**:349–362.

Riessen, H. P., and W. G. Sprules. 1990. Demographic costs of antipredator defenses in *Daphnia pulex*. Ecology **71**: 1536–1546.

Schultz, J. C. 1988. Plant responses induced by herbivores. Trends in Ecology and Evolution **3**:45–49.

Schwartz, S. S. 1991. Predator-induced alterations in *Daphnia* morphology. Journal of Plankton Research **13**:1151–1161.

Spitze, K. 1992. Predator-mediated plasticity of prey life-history and morphology: *Chaoborus americanus* predation on *Daphnia pulex*. American Naturalist **139**:229–247.

Stearns, S. C. 1976. Life-history tactics: a review of the ideas. Quarterly Review of Biology **51**:3–47.

———. 1992. The evolution of life histories. Oxford University Press, New York, New York, USA.

Stemberger, R. S. 1988. Reproductive costs and hydrodynamic benefits of chemically induced defenses in *Keratella testudo*. Limnology and Oceanography **33**:593–606.

———. 1990. Food limitation, spination, and reproduction in *Brachionus calyciflorus*. Limnology and Oceanography **35**:33–44.

Stenson, J. A. E. 1980. Predation pressure from fish on two *Chaoborus* species as related to their visibility. Pages 618–622 *in* W. C. Kerfoot, editor. Evolution and ecology of zooplankton communities. University Press of New England, Hanover, New Hampshire, USA.

Stenson, J. A. E. 1987. Variation in the capsule size of *Holopedium gibberum* (Zaddach): a response to invertebrate predation. Ecology **68**:928–934.

Stibor, H. 1992. Predator induced life-history shifts in a freshwater cladoceran. Oecologia **92**:162–165.

Stibor, H., and W. Lampert. 1993. Estimating the size at maturity in field populations of *Daphnia* (Cladocera). Freshwater Biology **30**:433–438.

Swift, M. C. 1992. Prey capture by the 4 larval instars of *Chaoborus crystallinus*. Limnology and Oceanography **37**: 14–24.

Taylor, B. E., and W. Gabriel. 1992. To grow or not to grow: optimal resource allocation for *Daphnia*. American Naturalist **139**:248–266.

Taylor, B. E., and W. Gabriel. 1993. Optimal adult growth of *Daphnia* in a seasonal environment. Functional Ecology **7**:513–521.

Tollrian, R. 1990. Predator-induced helmet formation in *Daphnia cucullata* (Sars). Archiv für Hydrobiologie **119**: 191–196.

———. 1991. Some aspects in the costs of cyclomorphosis in *Daphnia cucullata*. Internationale Vereinigung für theo-

retische und angewandte Limnologie, Verhandlungen **24**: 2802–2803.

———. 1993. Neckteeth formation in *Daphnia pulex* as an example of continuous phenotypic plasticity: morphological effects of *Chaoborus* kairomone concentration and their quantification. Journal of Plankton Research **15**:1309–1318.

———. 1994. Fish-kairomone induced morphological changes in *Daphnia lumholtzi* (Sars). Archiv für Hydrobiologie **120**:69–75.

———. 1995. *Chaoborus crystallinus* predation on *Daphnia pulex*: can induced morphological changes balance effects of body size on vulnerability? Oecologia **101**:151–155.

Tollrian, R., and E. von Elert. 1994. Enrichment and purification of *Chaoborus* kairomone from water: further steps towards its chemical characterization. Limnology and Oceanography **39**:788–796.

Vanni, M. J. 1987. Effects of food availability and fish predation on a zooplankton community. Ecological Monographs **57**:61–88.

von Ende, C. N. 1979. Fish predation, interspecific predation, and the distribution of two *Chaoborus* species. Ecology **60**: 119–128.

Vuorinen, I., M. Ketola, and M. Walls. 1989. Defensive spine formation in *Daphnia pulex* Leydig and induction by *Chaoborus crystallinus* DeGeer. Limnology and Oceanography **34**:245–248.

Walls, M., and M. Ketola. 1989. Effects of predator-induced spines on individual fitness in *Daphnia pulex*. Limnology and Oceanography **34**:390–396.

Walls, M., H. Caswell, and M. Ketola. 1991. Demographic costs of *Chaoborus*-induced defenses in *Daphnia pulex*, a sensitivity analysis. Oecologia **87**:43–50.

Weider, L. J., and J. Pijanowska. 1993. Plasticity of *Daphnia* life histories in response to chemical cues from predators. Oikos **67**:385–392.

Wicklow, B. J. 1988. Developmental polymorphism induced by intraspecific predation in the ciliated protozoan *Onychodromus quadricornutus*. Journal of Protozoology **35**: 137–141.