Costs and benefits of Daphnia antipredator behavior and consequences on community stability

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COSTS AND BENEFITS OF *DAPHNIA* ANTIPREDATOR BEHAVIOR AND CONSEQUENCES ON COMMUNITY STABILITY

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Biological Sciences

by

Wiebke J. Boeing
B.S., University of Potsdam, 1993
M.S., University of Technology Dresden, 1996
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To
Hanna
Renate and Wulf
Till and Lena
Anne and Jakob
Kristie

Thinking is more interesting than knowing, but less interesting than looking
– Goethe (1749-1832)
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ABSTRACT

Predator-prey interactions are an important factor in energy transfer through food webs. To estimate effects of altered energy flow caused by costs and benefits of antipredator defenses and to study their implications for community stability, I used the planktivorous fish – Chaoborus – Daphnia system. In the laboratory, I found that clonal variance of Daphnia antipredator defenses was high, and that behavioral, morphological, and life-history defenses were uncoupled. To estimate the costs and benefits of antipredator defenses in the presence of predators, I conducted lake-enclosure (1-m diameter x 7-m deep) experiments in the field, taking advantage of two features of the antipredator defense: (1) Daphnia exposed to only the predator-chemical should incur the cost of the defense, but not the cost of predation, and (2) antipredator response varies among Daphnia clones. Controls had no predators, ‘real’ predation exposed the clones to predators and in ‘ghost’ predation, predators were sequestered in a mesh tube away from Daphnia. I exposed a Daphnia clone responsive and a clone non-responsive to each predator, and I measured population growth (r) of Daphnia populations. Cost of predation was calculated as [responsive clone (r) in control treatments – responsive clone (r) in the ghost predation treatments]. Benefit was calculated as [responsive clone (r) – non-responsive clone (r) in the real predation treatments]. I found that, due to the antipredator defenses, Daphnia population growth was reduced by 32 % in the presence of Chaoborus predators. The benefit of the defense, however, was 68 % increased population growth.

Defenses to fish predators caused a cost of 16 % and benefits of 35 %. In both cases, benefit of antipredator defense did exceed cost, but cost was nevertheless substantial. I also found the fish predator to benefit from the antipredator behavior of its prey. Planktivorous fish lost weight (-0.375 g) when preying on the non-responsive clone and gained weight (+0.225 g) in
presence of the responsive clone, which was able to sustain a higher population density. Prey stability was highest in predation treatments with the responsive clone. In control treatments, *Daphnia* showed typical boom and bust cycles. A population model yielded similar results.
CHAPTER 1

GENERAL INTRODUCTION
Fitness is a compromise between ecological requirements that often place conflicting demands on the physiology, morphology, life history and behavior of organisms. These requirements may involve avoidance of parasites, including bacterial or viral infections, exposure to xenobiotics, reducing inter- and intra-specific competition, ameliorating potential effects of toxins associated with food or environmental agents, food gathering, and the timing of, and investment in, reproduction. One of the most intensively studied ecological requirements is predator avoidance. Over evolutionary time, various antipredator defenses have evolved in prey to escape predation. These defensive adaptations can be divided into primary (or passive) defenses meant to avoid capture, and secondary (or active) defenses that are used to escape once captured, thereby avoiding mortality. Primary defenses can be (1) morphological such as crypticism (Kettlewell 1955; Pietrewicz and Kamil 1977), aposematic coloration (Curio 1976; Guilford 1988), and mimicry (Brower and Brower 1972; Huey and Pianka 1977; Rowe et al. 1986; Mather and Roitberg 1987), (2) modifications to life-history such as reproduction earlier in life (Gadgil and Bossert 1970; Dodson 1989a), and (3) behavioral such as migration (Mech 1977; Bollens and Frost 1989) and reduced activity (Koperski 1998) that lowers the encounter rate with predators. Some morphological defenses are secondary, for example, toxic secretions (Eisner 1970; Brodie 1977; DeMott et al. 1991; Bolser and Hay 1996), and armament (Gilbert 1967; Porter 1973; Myers and Bazely 1991; Hessen and van Donk 1993). Behaviors like flight (Howland 1974), schooling, swarming (Milinski 1977), and fighting back (Brodie 1978), also represent secondary defenses. The variety of primary and secondary defense responses are evidence of the strength and pervasiveness of the evolutionary pressures to reduce predation.
Predators not only have direct, lethal effects but can, by their presence, also trigger indirect, non-lethal effects that can substantially influence their prey. In many cases, these non-lethal effects are costs that prey bear for antipredator defenses. The maintenance of many primary and secondary defenses is probably accompanied by trade-offs such as reduced growth and reproductive rates. In particular, the evolution of inducible defenses suggests that many defenses may be too costly to maintain in the absence of predators. By only expressing the defenses when needed, the prey can avoid costs during times of low predation threat.

Defenses are inducible if they require external stimuli for activation. They are thought to be favored over constitutive defenses, when (1) the cues associated with the predator are not fatal, (2) the fitness costs balance the benefits of the defense, (3) the probability of encountering the predator is high but unpredictable (Harvell 1990), (4) the presence of the predator in time and space is intermittent, and (5) prey have reliable cues for detecting the presence of a predator (Lively 1986; Riessen 1992).

Chemically-inducible defenses have thus far been found in most eukaryotic groups including protists, plants, and animals and are also found in the immune system of vertebrates (review by Tollrian and Harvell 1999) and in response to exposure to toxins (Guengerich 1993). Freshwater organisms have become model organisms for the study of those defenses, especially after the discovery that highly distinctive morphotypes once believed to be separate species belong to the same species. Those morphotypes are an expression of morphological defenses against predators. The seasonal alternation of different morphological phenotypes had been described for dinoflagellates, rotifers, cladocerans, and larval crab by Wesenberg-Lund (1908) and Hartmann (1915) and is termed cyclomorphosis (Hutchinson 1967). Today,
cyclomorphosis is widely accepted as an adaptive change in response to size-selective predation (Brooks 1965; Zaret 1973; Dodson 1974a; Kerfoot 1978; Gilbert 1980).

From a fitness (as measured by the intrinsic rate of population change, $r$) standpoint, the idea that the cost of any defense should be at least balanced by the benefit is very well supported by laboratory studies. However, the most convincing evidence that prey are adapted to balance risk of predation against cost of defense comes from field studies of inducible defenses. Inducible defenses are often ideal for such analyses because, by exposing prey only to the predator-avoidance cue and not real predators, the cost of the antipredator response can be quantified independently of the cost of the defense plus predation. As might be expected, the most important benefit seems to be reduced predation (Gilbert 1966; Halbach 1971; Havel and Dodson 1984, Berenbaum et al. 1986; Kuhlmann 1990; Brönmark and Miner 1992; Vicari and Bazely 1993; Kusch 1994; Lürling and van Donk 1996; Fyda and Wiackowski 1998; Otto 2000).

On the cost side, there is compelling evidence of a physiological toll for induced anti-herbivore defenses in plants (Coley et al. 1985; Gulmon and Mooney 1986; Fagerström 1989; Rausher 1996; Zangerl et al. 1997). Induced defenses in protozoans (Kusch and Kuhlmann 1994; Fyda and Wiackowski 1998), mollusks (Nakaoka 2000), crustaceans (Stich and Lampert 1984; Havel and Dodson 1987; Palmer 1990; Loose and Dawidowicz 1994), insect larvae (Peckarsky 1996; Otto 2000), and amphibians (Lardner 1998; Peacor and Werner 2001), as well as induced immune responses in chickens (Klasing et al. 1987) cause a lowering of growth, competitive ability, and reproduction. However, in some cases the costs of defense in terms of reduced fitness either could not be found, or fitness was actually enhanced in the induced phenotype. Such counter-intuitive results have been reported for
phytoplankton (Hessen and van Donk 1993; Lampert et al. 1994), rotifers (Gilbert 1980; Stemberger 1990), and for behavioral and morphological defenses in zooplankton (Dawidowicz and Loose 1992a; Spitze 1992; Tollrian 1995b). It is noteworthy that all of those studies that failed to detect a cost of the induced defense were conducted in the laboratory and involved planktonic organisms.

The costs and benefits have primarily been studied in the laboratory. However, costs and benefits really cannot be accurately measured in the laboratory, where natural conditions of light, temperature, oxygen, predators, etc. are difficult to reproduce. Comparatively little is known about whether the costs and benefits are even manifest in nature, let alone if they play an important role in the ecology of predator and prey, relative to other factors. I chose to compare the costs and benefits of antipredator defenses both in the laboratory and in nature.

The purposes of this dissertation are (1) to investigate if costs and benefits of an induced antipredator defense are detectable under near-natural conditions, (2) to determine if the costs are substantial relative to the impacts of predation, and (3) to estimate impacts of induced defenses on population and community stability.

My research focus was the planktivorous fish – Chaoborus – Daphnia trophic system. There are several advantages of working with this system. In nature, these three taxa form the central elements in the foodwebs of freshwater lakes. The outcomes of their predator-prey relationships largely determine how energy flows through lake communities (Ramcharan et al. 2001b). Thus, the fish – Chaoborus – Daphnia system provides an ideal case where the costs and benefits of antipredator behavior would have strong implications for the ecology of natural communities. This system also involves some of the most-studied inducible defenses (review by Tollrian and Dodson 1999).
Planktivorous fishes are the most diverse group of vertebrates and one of the most common predator for zooplankton (Nelson 1994). With high feeding rates and selection for the biggest, most visible prey items, they can decimate their prey populations within short time periods (Brooks and Dodson 1965; Zaret and Kerfoot 1975; Taylor 1980).

Phantom midge larvae *Chaoborus* spp. (Diptera, Insecta) are carnivorous, tactile ambush predators, which can drastically alter zooplankton composition and abundance by intense predation pressure (Dodson 1972; Kajak and Rybak 1979; von Ende 1979; Yan et al. 1991; Benndorf et al. 2000; Ramcharan et al. 2001b). As an adult, *Chaoborus* spp. are flying, non-biting midges that deposit eggs on the surface of standing water bodies. Larvae develop through four planktonic instars, increasing dramatically in size from a few mm to a 1-2 cm while undergoing an ontogenetic dietary shift from rotifers to larger zooplankton. The size of the zooplanktonic prey that *Chaoborus* spp. are able to ingest depends on the mouth gape of the larvae. Therefore, they typically prefer smaller prey items (Allan 1973) but the last two instars are even able to ingest juvenile *Daphnia* spp., some of the largest herbivorous zooplankton species commonly found in standing freshwater systems (Dodson 1974b). Once *Chaoborus* spp. larvae pupate, they stop feeding. They hatch as adult midges, mate, and lay their eggs. One or two generations are produced each year in the temperate zone (Hilsenhoff 1991).

In many temporary ponds, phantom midge larvae are the sole predator. As one of the largest zooplankton species in ponds and lakes, phantom midge larvae are thus subject to heavy fish predation. *Chaoborus* species composition changes from the large, pigmented, non-migrating *Chaoborus americanus* in fishless lakes, to medium-sized *C. trivittatus* and *C. flavicans*, to small *C. punctipennis* in lakes with high fish abundance (Stenson 1980; Wissel
However, even in the presence of high densities of fishes, in deep lakes that provide a dark, hypolimnetic refuge, migrating *Chaoborus* species can sustain high densities and thus large populations (Pope et al. 1973; Nilssen 1974; von Ende 1979; Carter et al. 1980; Yan et al. 1985; Ramcharan et al. 2001a).

Members of the genus *Daphnia* (Cladocera, Crustaceae) are cyclic parthenogens that are nearly ubiquitous in temperate freshwater habitats. Natural populations, especially in temporary ponds, hatch from diapausing resting eggs (ephippia) in early spring. Once grown, females undergo ameiotic reproduction and produce diploid eggs that do not need fertilization but develop directly. Offspring are released from the brood chamber as freely swimming neonates, about 600 µm long, after a few days of development. Depending on temperature and food conditions, sexual maturity can be reached within five days to several weeks. Sexual reproduction is triggered by unfavorable environmental conditions, when males are produced ameiotically and are able to fertilize meiotically-produced eggs of females. Maximum size for *Daphnia* is species dependent. The largest species can reach between 2 and 3 mm. With the use of their second antennae, they are able to conduct extensive vertical migrations in short periods of time (> 30 m in less than one hour) (Peters and de Bernardi 1987).

*Daphnia* species are popular study organisms for genetic, ecological, and evolutionary analyses. They are common and widespread, can easily be kept in the laboratory as clonal lineages, and have short generation times. They are also efficient filter feeders that are an important determinant of water quality (Uhlmann 1954). As they are highly vulnerable to predation by fish and *Chaoborus* spp., they have also developed complex behavioral, morphological, and life-history antipredator responses that vary among clones. These antipredator defenses are inducible and a chemical cue (kairomone) excreted by the predator
triggers deployment of the defense. By learning about how *Daphnia* species use their defenses, we can learn more about the energetic and life-history strategies behind other defense systems across the animal Kingdom as well as inducible resistance to disease and toxicants. Furthermore, we can gain insight on how inducible defenses impact population and community dynamics by comparing *Daphnia* clones that employ those defenses versus clones that are unresponsive to the predator chemical.

The following (second) chapter of this dissertation explores the clonal variability in the different defense mechanisms in response to *Daphnia*’s two main predators, fish and *Chaoborus*. I also investigated possible coupling between the behavioral, morphological, and life-history traits of multiple *Daphnia pulex* clones. The null hypotheses that there is no difference in strength and combination of defense mechanisms employed among *Daphnia* clones and that behavioral, morphological, and life-history traits are independent of each other were tested. From these laboratory experiments, two clones, one that exhibited several antipredator defenses towards *Chaoborus* chemical in the laboratory and one that did not, were chosen for further field experiments described in the third chapter.

In the research of the third chapter, I used in situ mesocosms to test the hypothesis that costs and benefits of *Daphnia* antipredator defenses in response to *Chaoborus* predation are measurable under near-natural conditions. Two clones, one with inducible defenses and the other without, were exposed to either (1) predation by *Chaoborus*, (2) only the kairomone (ghost predation), or (3) ordinary lake water (control). By comparing the difference in population growth in the real predation treatments between the clone that did defend itself and the undefended one, the benefits of the defense should become apparent. Similarly, by comparing differences in population growth between the control and the ghost predation
treatment of the defended clone, I should be able to calculate costs of the defense in a near-natural setting.

A similar experiment with fish as the predator was conducted and is presented in the fourth chapter. The fifth chapter analyzes the population dynamics of prey and predator in the field experiment with fish to investigate the hypothesis that prey defenses have a stabilizing effect on communities. A simple model was produced to explore the implications of prey defense on population and community stability.
CHAPTER 2

MULTIPLE SURVIVAL STRATEGIES OF *Daphnia* IN RESPONSE TO PREDATOR KAIROMONE
Introduction

In nature, organisms often have to balance the conflicting demands of food gathering, predator avoidance, and environmental constraints to maximize fitness. For example, plants are dependent on chemical and morphological defenses to thwart predation by herbivores. At the same time they must defend against pathogens and deal with temporal variation in nutrient and water availability (Agrawal and Karban 1999). Similarly, tadpoles of the common frog (*Rana temporaria*) use a refuge in the presence of fish predators. This behavior is enhanced at low food conditions, which leads to a smaller size at metamorphosis (Nicieza 2000). Smaller size can affect outcome of the competitive interactions in the tadpoles (Laurila 2000).

Although antipredator defenses are effective in reducing mortality, there are sometimes trade-offs associated with them. Antipredator defenses are often complex and can be conflicting, for example, a defense against one predator can make an organism more vulnerable to another predator (Tollrian and Dodson 1999). Furthermore, antipredator defenses are often coupled with a reduction in reproductive output (Kerfoot and Sih 1987).

In aquatic systems, the three-dimensionality of the environment adds to the complexity of prey defense. Zooplankton in these open-water environments must often balance conflicting requirements to obtain food and avoid predators. Food quality and quantity is usually highest in the epi- or meta-limnion, depending on lake type (Wetzel 2001). However, predators can make this favorable environment less desirable. The impacts of predators can be mitigated by altered behavioral, morphological, and life-history responses in the prey that are triggered by a chemical cue (kairomone) exuded by the predator (De Meester et. al. 1999; Tollrian and Dodson 1999). When visually-feeding fish are abundant, many zooplankton such as the herbivorous cladoceran, *Daphnia* spp., typically react during the
daytime by migrating downward into deeper, colder water-layers (Ringelberg 1991b; van Gool and Ringelberg 1995). This so-called diel vertical migration is one of the most-studied phenomena in ecology (De Meester et al. 1999). They also become smaller and more transparent (Vanni 1987; Dodson 1988a and 1989a; Brett 1992; Weber and Declerck 1997; Riessen 1999), with longer tail-spines (Dodson 1988a; Spitze and Sadler 1996; Kolar and Wahl 1998) and helmets (Tollrian 1994), and they produce more and smaller eggs and neonates (Dodson 1989a; Macháček 1991; Stibor 1992; Riessen 1999). These responses either reduce predation or allow high population growth to compensate for high predation (Zaret and Suffern 1976; Stich and Lampert 1981; Lampert 1989; 1993). Because water temperature is lower at greater depths, *Daphnia* eggs take longer to develop (Bottrell 1975) causing a decrease in growth rate (Dawidowicz and Loose 1992b); a principle cost of avoiding fish predation.

Conversely, the opposite response seems to be favored in the presence of invertebrate predators in the genus *Chaoborus* (Riessen 1999), which are mouth-gape limited and consume smaller prey (Pastorok 1981; Riessen et al. 1988; Tollrian 1995a). During the day, larger *Chaoborus* usually avoid fish in the hypolimnion or lake sediment (Luecke 1986; Dawidowicz et. al. 1990, Voss and Mumm 1999). To reduce predation by *Chaoborus*, *Daphnia* may exhibit positive phototaxis – the opposite response to fish, where the prey avoid lower waters during the daytime (Ohman et al. 1983; Dodson 1988b; Ramcharan et al. 1992; Nesbitt et al. 1996). *Daphnia* may also develop neck-spines on the dorsal margin of their heads in juvenile instars (Krueger and Dodson 1981), and some species produce helmets (Mort 1986; Dodson 1988a), and/or produce larger neonates (Lüning 1992; Spitze 1992; Weber and Declerck 1997; Walls and Ventelä 1998).
The strength of the antipredator behavior (vertical migration response to fish predators) in *Daphnia* spp. varies with genotype. It was once assumed that genotypes that did not react to the kairomone could not detect the substance (Sih 1987; Dodson 1989b; Dumont and De Meester 1990; De Meester 1993; Pijanowska et al. 1993). However, other findings suggest more complex interactions.

A negative coupling, in which zooplankton either express one antipredator defense or another (e.g., morphology or behavior), but not both, was found in numerous studies, mainly involving depth selection behavior and body size. Negative couplings might (1) reduce redundancy in protection from predators, (2) reduce multiple costs of defense, and (3) also provide for multiple successful defense strategies in response to predation. When comparing two *Daphnia* species in seven lakes, Leibold and Tessier (1991) found that *D. galeata* did not migrate vertically and remained in the epilimnion all day, but did express life-history changes, becoming smaller to avoid fish predation. Larger-bodied *D. pulicaria*, did the opposite – they did not shift towards a smaller body size, but migrated into the hypolimnion during daytime. At low predation pressure, both *Daphnia* species had a similar body size and remained in the epilimnion. This result is significant because the coupling between the two *Daphnia* species was opposite: one expressed behavioral, but no life-history responses and the other expressed only life-history changes but no behavioral changes. However, both were apparently successful strategies.

The same pattern, where larger animals were more negatively phototactic was also apparent within a species in both a population of hybrid *D. hyalina x galeata* (De Meester et al. 1995) and in nine different *D. magna* clones (De Meester 1995). Among the nine *D. magna* clones used by De Meester (1995), those with a less negative phototactic behavior
matured at a smaller size and produced smaller neonates at a faster rate than did intermediately and negatively phototactic clones (De Meester 1994). Similarly, Reede and Ringelberg (1995) discovered that a non-migrating *D. galeata* x *hyalina* clone responded by having smaller neonates and a smaller size at maturity than did the migrating clone. Twelve *D. galeata* x *hyalina* clones exhibited a distinct pattern wherein the migrating clones produced more offspring for a given body size in the presence of fish kairomone, while the non-migrating clones changed their life-history towards smaller body size, earlier age at maturity, and the production of fewer eggs (Reede and Ringelberg 1998). Furthermore a negative relationship was reported between helmet development and migration behavior (Lysebo 1995). In one year, with co-occurring *Chaoborus flavicans* and three-spined stickleback (*Gasterosteus aculeatus*) as predators, one part of a *Daphnia galeata* population produced helmets and remained in the pelagial (open water zone), while the other part migrated into the littoral zone but did not develop helmets. The following year, when no *Chaoborus* was observed, *Daphnia* did not produce helmets. Prey seem to adapt the overall strategy that can be easiest achieved and optimizes fitness. Often larger species migrated vertically. Smaller species did not, but reproduced at smaller sizes or developed morphological defenses.

Conversely, several studies involving a larger number of response traits and clones suggest an uncoupling among defense mechanisms; genotypes express unique assorted combinations of antipredator defenses. Spitze (1992) could not detect a relationship between degree of neck-spine induction and changes in life-history traits, such as clutch size, in response to *Chaoborus* kairomone among 20 *D. pulex* clones from four lakes. Lüning (1994) also failed to find a relationship when she compared a morphological characteristic (neck-spines) and life-history traits such as age at maturity, size at first reproduction, and clutch size
among *D. pulex* clones. Three of her clones showed morphological and life-history changes in the presence of *Chaoborus* kairomones, while one exhibited only life-history responses.

Responses to fish kairomone also seem to be more variable than previously thought. Results of studies with two *D. magna* clones indicate that one reacted with behavioral and life-history defenses (phototactic behavior and size at first reproduction) while the other exhibited no behavioral responses but did vary life-history parameters such as size and age at first reproduction (Weider and Pijanowska 1993). Another study by Sakwińska (1998) supported these findings. Exposure of a *D. magna* clone to fish kairomone resulted in smaller, more numerous offspring, but caused no significant effect on size and age at maturity. De Meester and Weider (1999) isolated 22 different *D. hyalina x galeata* clones that all exhibited a significantly smaller size at maturity and produced smaller offspring when exposed to fish kairomone. However, only some of the epilimnetic clones exhibited a higher age at maturity and a lower population growth rate. Further evidence for unlinked predator defenses was also provided in a study (Repka et al. 1994) comparing life-history traits (age at maturity, clutch size, and growth) in one *D. pulex* clone that was exposed to different invertebrate predators (*Chaoborus, Mochlonyx, Notonecta, and Dytiscus*). Predator exposure typically affected one or more traits and even in the absence of neck-spine induction, life-history shifts were noticed.

In light of several studies that showed no association among the possible array of behavioral, morphological, and life history antipredator defenses, De Meester and Pijanowska (1996) introduced the uncoupling hypothesis, which states that predator responses are independent of each other with respect to genotype. They assayed six *D. magna* clones for their phototactic behavior and alertness (escape probability from a simulated predator) with
and without fish chemical. Two clones reacted in both ways (increased alertness and more negatively phototactic), three did not respond at all, and one clone was more alert but did not exhibit negative phototaxis. In a detailed study, Boersma et al. (1998) compared two behavioral (phototaxis and alertness), three morphological (spine length of juveniles and adults, eye diameter), and nine life-history parameters (age and size at maturity, growth, number of eggs for clutch 1-4, size of neonates, and percent of males) among 16 D. magna clones in response to fish chemical. The results supported the uncoupling hypothesis.

Evidence that different genetic information is responsible for morphological and life-history variation was gathered by Tollrian (1995b) and Repka and Pihlajamaa (1996), who demonstrated that neck-spine induction can be separated from life-history shifts by exposing D. pulex clones to Chaoborus kairomone during different stages of their development.

In this study, I contrasted combinations of behavioral, morphological, and life-history antipredator defenses of prey taken from different predation regime environments and analyze their adaptive significance. Furthermore, I studied prey response in relation to predation threat and prey vulnerability. I examined a large number of clones for their depth selection behavior in response to Chaoborus and fish kairomones. Selected clones were exposed to various concentrations of Chaoborus kairomone. I contrasted juvenile versus adult behavior, and patterns of co-occurrence among behavioral, morphological, and life-history responses to Chaoborus kairomone were compared.

The purpose of these experiments was to quantify clonal variability among several antipredator traits. I hypothesize that genotypes exhibit a high diversity of strength as well as combinations of expressed antipredator defenses. I also hypothesize that through local
adaptation, different clones should reflect effects of the selection pressures from the predatory regimes of their original habitats.

**Materials and Methods**

**Culture Conditions and Clonal Distinctions**

Fifty-nine *Daphnia pulex* females were collected in the early spring of 1997 and late spring to summer of 1998 from 18 distinct water bodies (lakes to temporary ponds) located in New York State (US) and south-central Ontario (Canada). Animals were collected with vertical hauls of a 130-µm zooplankton net and each female was maintained in the laboratory as a separate lineage (Table 2.1).

*Daphnia* were cultured in 1-L glass jars under controlled temperature conditions (20 ± 1 ºC) and fed *Chlamydomonas* above the limiting level (Lampert 1987). Culture water (aged tap water with added algae) was changed every 2-3 weeks before any female could release ephippial eggs, and five to ten individuals were retained to continue the lineage.

To confirm distinctness among the *Daphnia* lineages, I used a combination of a genetic approach and examination of behavioral differences. Some of the *Daphnia* lineages did not survive in the laboratory after I had conducted some of the experiments, and were not available for genetic analysis. For the genetic evidence I amplified microsatellites by Polymerase Chain Reactions (PCR), because they provide a high variability (Sunnucks 2000) and analyzed size difference of the microsatellite among clones on an acrylamide gel (difference detection limit of two base-pairs) (Rousseau et al. 1994), following the approach described by Noor et al. (2001). The DNA of the different *D. pulex* lineages was isolated by with a Puregene DNA isolation kit 400 (Gentra Systems, Minnesota), resulting in a 22 µl
Table 2.1: Origin and genotypes of *Daphnia pulex* clones

<table>
<thead>
<tr>
<th>Clone(s)</th>
<th>Location Collected</th>
<th>Name of Water Body</th>
<th>Habitat Description</th>
<th>Genotype</th>
</tr>
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<tbody>
<tr>
<td>ACP 3</td>
<td>NY State</td>
<td>Archery Course Pond</td>
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</tr>
<tr>
<td>ACP 5</td>
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<td></td>
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</tr>
<tr>
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<td>NY State</td>
<td>Alran Drive</td>
<td>small temporary pond</td>
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</tr>
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<td>ALR 5</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BRE 3</td>
<td>Ontario</td>
<td>Brewer Lake</td>
<td>brownwater lake</td>
<td>2</td>
</tr>
<tr>
<td>BRE 4</td>
<td>(Algonquin Park)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRY 3</td>
<td>NY State</td>
<td>Bryant Woods</td>
<td>small temporary pond</td>
<td>2</td>
</tr>
<tr>
<td>BRY 4</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
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<td>Ontario</td>
<td>Clarke Lake</td>
<td>brownwater lake</td>
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</tr>
<tr>
<td>CLA 5</td>
<td>(Algonquin Park)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COS 1</td>
<td>Ontario</td>
<td>Costello Lake</td>
<td>brownwater lake</td>
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</tr>
<tr>
<td>COS 2</td>
<td>(Algonquin Park)</td>
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<td>lake</td>
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<td>NY State</td>
<td>East</td>
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</tr>
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<td>pond</td>
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</tr>
<tr>
<td>FLC 3</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>clear, deep lake</td>
<td>3</td>
</tr>
<tr>
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<td>(Algonquin Park)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
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<td>NY State</td>
<td>Honeoye Lake</td>
<td>Finger Lake</td>
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</tr>
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<td></td>
<td>1</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>HOP 5</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>KN</td>
<td>Germany</td>
<td>Konstanz</td>
<td>eutrophic lake</td>
<td></td>
</tr>
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<td>Margaret Louise Park</td>
<td>small permanent pond</td>
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<td>pond</td>
<td>5</td>
</tr>
<tr>
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<td>NY State</td>
<td>Miller Pond</td>
<td>small permanent pond</td>
<td>3</td>
</tr>
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</table>

continue on next page
Table 2.1 continued:

<table>
<thead>
<tr>
<th>Clone(s)</th>
<th>Location Collected</th>
<th>Name of Water Body</th>
<th>Habitat Description</th>
<th>Genotype</th>
</tr>
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<td>Scott Lake</td>
<td>clear, deep lake</td>
<td>1 2</td>
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<tr>
<td>SCO 3</td>
<td>(Algonquin Park)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAL2</td>
<td>Ontario</td>
<td>Walker Lake</td>
<td>clear, deep lake</td>
<td>1</td>
</tr>
<tr>
<td>WAL3</td>
<td></td>
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<td></td>
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<td>WAL5</td>
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<tr>
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<td>2 1</td>
</tr>
<tr>
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<tr>
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<tr>
<td>WAL11</td>
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</tbody>
</table>
DNA preparation. Ten primers (five forward and five reverse) for microsatellite sequences of *D. pulex* provided by ‘GenBank’ (http://www.ncbi.nlm.nih.gov) were ordered (Colbourne unpublished). I was unable to amplify two microsatellites (Dpu46 and Dpu45) and therefore continued working with just three microsatellites. The first primers (Dpu122) amplified a segment of 132 basepairs, the second primers (Dpu40) a segment of 119 basepairs, and the third primers (Dpu7) a segment of 114 basepairs. One primer for each sequence was obtained with an M13 tail at the 5’ end. The PCRs were conducted in a 10-μl reaction volume with 0.5 picomoles of each primer, 0.4 fluorescent dye labeled M13, 200 μM dNTP’s, 1μl 10 X buffer (100 mM Tris pH 8.3, 500 mM KCl, 15 mM MgCl2), 1 U Taq polymerase, and 1 μl from the DNA prep of a single *D. pulex* lineage with a touchdown cycle (Palumbi 1996). Then, I added 3 μl of LiCor (Lincoln, NE) stopping buffer to the PCR products and 1 μl of each reaction was loaded onto an acrylamide gel (National Diagnostics Sequagel, Atlanta, GA) on a LiCor 4200 DNA sequencer for visualization.

To be conservative in terms of finding differences in genetic lines, I only distinguished between three genotypes for the Dpu122 and the Dpu40 microsatellites and seven genotypes for the Dpu7 microsatellite. Although many of the lineages that were assayed could be identified as different clones by the genetic approach, I also designated *D. pulex* lineages that showed significant differences in behavioral, morphological, or life-history traits as distinct clones. Also, individuals derived from different water bodies were assumed to be genetically distinct, as they had to hatch from different resting eggs (Lynch 1983). I was unable to assure a unique genetic make-up or different phenotypic traits for a total of twelve *Daphnia* lineages, which were ignored for the analyses.
Depth Selection Behavior (DSB) in Response to Fish Kairomone

Depth selection behavior (DSB) is defined as the mean vertical distribution of *Daphnia* in relation to vertical stratification (light) in the water column (De Meester et al. 1999). Mean vertical distribution (MVD) was calculated as followed:

\[
\text{MVD} = \frac{\sum (D_i \times i)}{\sum D_i}
\]

where \(D_i\) was the number of *Daphnia pulex* in each depth interval and \(i\) the median of the corresponding depth interval.

Phototactic behavior was evaluated in transparent acrylic tubes (2 m long, 6.9 cm inner diameter) that were externally marked in 10-cm intervals. The columns were stationed in a room where floor and walls were darkened with black cardboard to minimize light reflection. During an experiment the tubes were illuminated from the top by four, 40-W halogen lamps.

The control water consisted of aged tap water conditioned with artificial food pellets. To create fish-kairomone, 28 L of aged tap water was conditioned with six golden shiner (*Notemigonus crysoleucas*) individuals with a total weight of 20 g. The fish were fed to saturation starting three hours before the start of the experiment with artificial food pellets. The water was directly used for the experiments. Loose et al. (1993) and von Elert and Loose (1996) showed that the chemical signal is released by fish into the water within one hour and neither fish species nor nutritional state of the fish influence kairomone production.

I did an experiment on the behavioral response to fish kairomone of 41 clones. Each trial began by filling one tube with control water up to 155 cm (control tube) and the other with water including the fish kairomone (treatment tube). Fifty mL of *Chlamydomonas* algae suspension (from a continuous culture) was mixed into each tube. The algae concentration
had been determined photometrically at 470 nm and was diluted to a set value. Fifty adult (>1.3 mm) *Daphnia pulex* were enumerated from one clone were poured through a funnel and hose into the experimental tubes at 1 m depth. This procedure prevented the animals from either getting caught in the surface layer or capturing air under their carapace, and also assured that they all started out in the middle of the columns. All *D. pulex* in each 10-cm interval were then counted every 30 minutes for two hours. In every experimental session, I tested three haphazardly-chosen *D. pulex* clones for their DSB in response to fish kairomone by comparing the average depth of the control versus the treatment group (Δ DSB = control depth – treatment depth). Tests for each of 41 clones were replicated at least three times.

Preliminary experiments had indicated that type of food (either food pellets or *Daphnia*), amount and duration of feeding, as well as fish type (golden shiner, *Notemigonus crysoleucas* or pumpkinseed, *Lepomis gibbosus*) had no effect on the strength of reaction. Furthermore, comparison between tap water and tap water conditioned with food pellets did not result in any differences in mean vertical position of the *D. pulex* (Böing, unpublished data).

**Depth Selection Behavior (DSB) in Response to *Chaoborus* spp. Kairomone**

To obtain a large amount of *Chaoborus* spp. kairomone with identical activity, I extracted the chemical from the larvae and kept it frozen at –60 °C (Tollrian 1995 b). The kairomone was extracted following Hebert and Grewe (1985) by crudely cutting up 4th instar *Chaoborus trivitattus* and *C. flavicans*. The animals were then boiled for three minutes in water, and all particles were removed from the mixture by stepwise filtration down to 0.1 μm with cellulose acetate filters. I conducted the experiments within six months of kairomone extraction, with the filtrate kept frozen at –20 °C until usage.
The behavioral response of juvenile *Daphnia pulex* (< 1.1 mm) to *Chaoborus* spp. was tested for 33 *D. pulex* clones following the same experimental procedure as described above for fish. The control water was again dechlorinated tap water, while the treatment water was a mixture of freshly thawed kairomone and aged tap water. The added kairomone concentration was equal to the extract of ten animals L⁻¹.

The depth selection behavior experiments were originally conducted for the purpose of finding suitable clones to be used in field experiments. Therefore, not all of the clones tested for their reaction to *Chaoborus* spp. were used in the fish chemical treatments and vice versa. However, 28 clones were tested for exposure to both fish and *Chaoborus* spp. kairomones.

Seven *D. pulex* clones with differing strengths of responses (two reacted strongly to *Chaoborus* spp. kairomone (> 40 cm difference in DSB), four intermediate (10-30 cm difference), and one non-reactive clone) were then selected in order to examine the effect of varying levels of the kairomone on strength of response. I diluted the *Chaoborus* spp. kairomone to concentrations that equaled 5, 2.5, 1.25, and 0.625 extracted *Chaoborus* L⁻¹. For each clone I continued the dilution series until no kairomone effect could be detected. For the clone that did not react, I increased the kairomone concentration to 20 extracted *Chaoborus* spp. L⁻¹. Additionally, eight *D. pulex* clones (two with a strong, four with intermediate, and two with no response to *Chaoborus* spp. kairomone) were examined for the difference in DSB between adults (> 1.3 mm) and juveniles (< 1.1 mm) (Table 2.2).

**Chlorophyll a Evaluation**

I determined chlorophyll *a* levels as a measure of food concentrations at different depths. The experiments were set-up identically to the DSB behavior experiment but instead of counting *D. pulex*, I withdrew 200 ml of water from 30, 70, 110, and 150 cm in
Table 2.2: Depth selection behavior (DSB) of *Daphnia pulex* clones in controls and in response to predator kairomones, and experiments used in (Dilution = various concentrations of *Chaoborus* spp. kairomone, Ad-juv. = comparison between adult and juvenile *Daphnia*, Life table = morphological and life-history responses to *Chaoborus* kairomone). Numbers indicate mean values ± 1 S.E.; positive number indicates upward migration, negative number downward migration; numbers or X marked in bold indicate significant reaction (p < 0.05).

<table>
<thead>
<tr>
<th>Clone(s) Dominating Predators</th>
<th>DSB Adult <em>Daphnia</em> (cm)</th>
<th>Δ DSB of Adult <em>Daphnia</em> (cm)</th>
<th>DSB Juvenile <em>Daphnia</em> (cm)</th>
<th>Δ DSB of Juvenile <em>Daphnia</em> (cm)</th>
<th>Dilution</th>
<th>Ad-juv.</th>
<th>Life table</th>
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</thead>
<tbody>
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<td>ACP</td>
<td>25.14 ±2.96</td>
<td>2.26 ±0.32</td>
<td>24.62 ±2.21</td>
<td>2.87 ±2.70</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>ACP 3 <em>Chaoborus americanus</em></td>
<td>45.55 ±3.62</td>
<td>1.70 ±1.55</td>
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<td></td>
</tr>
<tr>
<td>ALR 3 <em>Chaoborus</em></td>
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<td>-14.82 ±1.29</td>
<td>20.23 ±0.87</td>
<td>16.32 ±1.21</td>
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<td>X</td>
<td></td>
</tr>
<tr>
<td>ALR 5</td>
<td></td>
<td>47.17 ±10.16</td>
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<td>22.20 ±6.67</td>
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</tr>
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<td>BRE 3 Fish</td>
<td>56.41 ±14.55</td>
<td>11.79 ±2.37</td>
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<td></td>
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</tr>
<tr>
<td>BRE 4 <em>Chaoborus</em></td>
<td>47.89 ±18.36</td>
<td>-6.93 ±5.16</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>BRY 3 <em>Chaoborus</em></td>
<td>29.06 ±4.80</td>
<td>1.63 ±0.67</td>
<td>67.69 ±2.64</td>
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<td>-2.33 ±0.48</td>
<td>43.02 ±1.36</td>
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<td>CLA 2 Fish</td>
<td>51.66 ±16.52</td>
<td>21.89 ±7.69</td>
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<td>57.31 ±15.11</td>
<td>6.61 ±3.32</td>
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<td>ESUM 3</td>
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<td>15.82 ±1.49</td>
<td>7.25 ±1.84</td>
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</tr>
<tr>
<td>FLC 1 <em>Chaoborus</em></td>
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<td>30.41 ±3.05</td>
<td>23.65 ±3.80</td>
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<tr>
<td>FLC 2</td>
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<td>37.07 ±2.10</td>
<td>20.45 ±1.77</td>
<td>13.64 ±2.83</td>
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<tr>
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<td>0.80 ±9.81</td>
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<td>HON 3 Fish</td>
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<td>-13.72 ±4.93</td>
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<tr>
<td>HOP 1 Neither</td>
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<td>22.95 ±2.49</td>
<td>8.65 ±2.14</td>
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<td>HOP 3</td>
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<td>25.15 ±1.13</td>
<td>5.37 ±1.38</td>
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<tr>
<td>HOP 4</td>
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<td>0.18 ±2.54</td>
<td>26.74 ±2.63</td>
<td>7.37 ±4.89</td>
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<td>HOP 5</td>
<td>11.07 ±1.98</td>
<td>-6.23 ±1.73</td>
<td>28.23 ±2.35</td>
<td>13.46 ±1.86</td>
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</table>

continue on next page
Table 2.2 continued:

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<tr>
<th>Clone(s) Dominating Predators&lt;sup&gt;1&lt;/sup&gt;</th>
<th>DSB Adult &lt;i&gt;Daphnia&lt;/i&gt; (cm)</th>
<th>∆ DSB of Adult &lt;i&gt;Daphnia&lt;/i&gt; (cm)</th>
<th>DSB Juvenile &lt;i&gt;Daphnia&lt;/i&gt; (cm)</th>
<th>∆ DSB of Juvenile &lt;i&gt;Daphnia&lt;/i&gt; (cm)</th>
<th>Dilution table Ad-juven. Life</th>
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<td>KN Fish, &lt;i&gt;Chaoborus&lt;/i&gt;</td>
<td>36.47 ±4.82</td>
<td>-6.26 ±2.04</td>
<td>75.82 ±4.50</td>
<td>50.56 ±3.27</td>
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<tr>
<td>MLP 3 &lt;i&gt;Chaoborus&lt;/i&gt;</td>
<td>37.58 ±3.26</td>
<td>32.17 ±4.38</td>
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<td>MLP 5</td>
<td>20.65 ±5.01</td>
<td>4.20 ±2.72</td>
<td>40.16 ±4.10</td>
<td>-2.27 ±2.24</td>
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<td>X</td>
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<td>MLR C. americanus</td>
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<td>-4.61 ±5.85</td>
<td>28.96 ±1.12</td>
<td>7.49 ±3.61</td>
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<td>MLR 4&lt;sup&gt;5&lt;/sup&gt; Notonecta</td>
<td>32.95 ±0.70</td>
<td>7.28 ±0.80</td>
<td>41.30 ±1.60</td>
<td>17.89 ±4.03</td>
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<td>SBL&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>14.67 ±7.26</td>
<td>41.68 ±1.61</td>
<td>16.19 ±3.21</td>
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<td>SCO 2 Fish, &lt;i&gt;Chaoborus&lt;/i&gt;</td>
<td>61.71 ±15.16</td>
<td>22.31 ±3.79</td>
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<td>SCO 3 &lt;i&gt;Chaoborus&lt;/i&gt;</td>
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<td>SCO 5</td>
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<td>6.81 ±4.06</td>
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<td>WAL2 Fish, &lt;i&gt;Chaoborus&lt;/i&gt;</td>
<td>66.30 ±14.09</td>
<td>-2.26 ±6.47</td>
<td>36.89 ±3.46</td>
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<td>WAL3 &lt;i&gt;Chaoborus&lt;/i&gt;</td>
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<td>-5.06 ±2.96</td>
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<td>WAL6</td>
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<td>WAL7</td>
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<td>WAL9</td>
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<td>WAL11</td>
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<td>F/C</td>
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<td>4.43 ±2.47</td>
<td>52.44 ±5.88</td>
<td>26.85 ±6.05</td>
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<td>Fish</td>
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<td>-13.72</td>
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<td>&lt;i&gt;Chaoborus&lt;/i&gt;</td>
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<td>34.71 ±3.89</td>
<td>18.59 ±3.47</td>
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<tr>
<td>Neither</td>
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<td>-4.53 ±2.05</td>
<td>30.32 ±4.64</td>
<td>11.91 ±3.47</td>
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Note:<sup>1</sup>Riessen and Böing pers. obs.
<sup>2</sup>see Table 2.5
<sup>3</sup>responsive clone used for experiment with <i>Chaoborus</i> as predator (Chapter 3)
<sup>4</sup>non-responsive clone used for experiment with <i>Chaoborus</i> as predator (Chapter 3)
and responsive clone used for experiment with fish as predator (Chapter 4)
<sup>5</sup>non-responsive clone used for experiment with fish as predator (Chapter 4)
<sup>6</sup>clone from Dodson (1988)
three replicate tubes. Sampling was repeated at 30, 60, 90, and 120 minutes. The 200 ml samples were kept in the dark and filtered through GF/C filters (1.2 µm pore size) within one hour. The filter was kept in the freezer for two days and then extracted overnight in 100% acetone. Chlorophyll $a$ concentrations were measured with a fluorometer (Turner Designs Inc. Sunnyvale, CA; model 10-005R) following standard methods (Greenberger et al. 1981).

**Life-Table Experiments**

Experiments were designed to quantify the variability in neck-spine expression, size of neonates, size at first reproduction (SFR), and intrinsic rate of population growth in the presence and absence of *Chaoborus* spp. kairomone. *Daphnia pulex* were incubated with the same kairomone that was produced for the depth selection behavior experiment. To reduce maternal effects (Tollrian 1995b), I used neonate (< 1 day old) *D. pulex* individuals that were derived from the third broods of mothers that were taken from the third brood of their mothers. Culture conditions were identical to the ones described above. I conducted ten replicates for each treatment and clone. Each *D. pulex* was placed in 100 mL of aged tap water with a high concentration of algae (50 µg Chl. $a$ L$^{-1}$). Water and algae were refreshed once daily. The *D. pulex* that were exposed to the kairomone received 2 mL of kairomone every 12 hours in a concentration that was equal to the extract of 10 *Chaoborus* spp. L$^{-1}$, the same as in the depth selection behavior experiment. Two mL of aged tap water were added to the control *D. pulex*. Five of the ten replicates were terminated as soon as eggs were observed in the individuals (to obtain SFR), while for the other replicates the original *D. pulex* was allowed to continue to grow and only the neonates were removed and fixed in 4% sugar formaldehyde (Haney and Hall 1973). The experiment was ended after all five *D. pulex* had three clutches.
Measurements and egg counts were completed with a dissecting microscope (Leica MZ8) that allowed magnification up to 500X. I measured the *D. pulex* using a computer-based image analysis (MORPHY) with calipers (Fowler S225) on digitized video images. Neck-spine induction was scored according to Tollrian (1993), where a morphologically “normal” neck = 0%, a “small” bump on the neck = 30%, and a “pedestal” = 50% induction. I differentiated the spines according to size, where a large spine scored 10% (the longest one was measured to be over 20 µm) and small spines that were barely visible 5%.

The intrinsic rate of population increase ($r$), a measure of individual fitness, was calculated with the Euler equation:

$$1 = \sum e^{rx} \cdot l_x \cdot m_x$$

where $x$ is the age in days, $l_x$ presents age-specific probability of survival, and $m_x$ is the age specific fecundity. It is sufficient to have only three broods, as later broods add little improvement in accuracy to the calculation of $r$ (Riessen and Sprules 1990).

I grouped the *D. pulex* clones into four classes according to the known predator regime of their native lakes and ponds (fish and *Chaoborus* (F/C), fish, *Chaoborus*, and neither; Tab. 2.2) (Riessen and Boeing, pers. observ.)

**Statistical Analysis**

Statistical analysis was conducted with SAS software version 8.2 (Copyright, SAS Institute Inc., Cary, NC, USA.) (SAS 2002). For the depth selection behavior, I applied repeated measures ANOVA with time as the repeated variable and clones and treatment as fixed effects (MIXED procedure) and p-values were Tukey adjusted. Life table parameters were compared with a MANOVA with clones and treatments as fixed effects. Correlation among various behavioral, morphological and life-history parameters were tested with simple
linear regressions. I estimated standard errors for the per capita rate of increase for the population \((r)\) with the Jackknife technique (Lenski and Service 1982), which is a better procedure than bootstrapping when juvenile mortality is below 25% (Meyer et al. 1986).

**Results**

The chlorophyll \(a\) concentration stayed above 50 \(\mu\)g L\(^{-1}\) at all depths throughout both experiments (Fig. 2.1). The distribution was fairly even throughout the water column except after 30 minutes where the concentration was higher towards the bottom of the tubes. However, the food concentration was always above limitation in all depths throughout the entire time of the experiment (Lampert 1987). Without a food or temperature gradient in the columns and an identical light gradient between control and treatment tubes, I can assume that the predator-incubated water was the primary influence on depth selection behavior.

**Behavioral Responses**

Different clones behaved differently in the absence of kairomone, producing a statistically significant clone effect in depth selection behavior (DSB) for adult *Daphnia* in control water (Table 2.2 and 2.3). The grouping of the *Daphnia* clones into four classes according to the known predator regime of their native lakes and ponds (fish and *Chaoborus* (F/C), fish, *Chaoborus*, and neither) revealed that *Daphnia* that co-occurred with fish predators in nature chose a deeper depth than clones found with either *Chaoborus* only or clones that did not occur with either predator (Table 2.2 and 2.4). Although only five out of 41 clones (12%) responded significantly to fish kairomone there was a significant clonal effect (Table 2.3), wherein only one clone exhibited a downward migration (-21 cm) while four clones migrated up (between 22 and 37 cm) (Table 2.2). The grouping of the *Daphnia*
Figure 2.1: Chlorophyll $a$ concentration in experimental tubes after 30, 60, 90, and 120 minutes with *Daphnia pulex* feeding in four different depths layers. Error bars show ± 1 standard error.
Table 2.3: ANOVA results of individual clones

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<th>F</th>
<th>p</th>
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<td>6994.07</td>
<td>10.08</td>
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<tr>
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<td>693.94</td>
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<td><strong>Δ DSB in response to fish kairomone</strong></td>
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<td><strong>Δ DSB in response to Chaoborus kairomone</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Clone</td>
<td>32</td>
<td>1682.85</td>
<td>3.57</td>
<td>&lt;0.0001</td>
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<tr>
<td>Error</td>
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<td>471.10</td>
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<td><strong>Dilution of Chaoborus kairomone</strong></td>
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Table 2.4: ANOVA results dividing *Daphnia* clones according to predator regime in their native habitats (Fish and *Chaoborus* (F/C), Fish, *Chaoborus*, or Neither). Grouping of lake types were analyzed with Tukey’s test.

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<td></td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td><strong>Life-table experiment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Neckspine induction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clone</td>
<td>3</td>
<td>5908.50</td>
<td>33.59</td>
<td>&lt;0.0001</td>
<td>F/C</td>
</tr>
<tr>
<td>Kairoxone</td>
<td>1</td>
<td>8772.89</td>
<td>49.87</td>
<td>&lt;0.0001</td>
<td>F</td>
</tr>
<tr>
<td>C*K</td>
<td>3</td>
<td>3120.65</td>
<td>17.74</td>
<td>&lt;0.0001</td>
<td>C</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>175.92</td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td><strong>Population growth (r)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clone</td>
<td>3</td>
<td>0.0002</td>
<td>0.06</td>
<td>0.9444</td>
<td>F/C</td>
</tr>
<tr>
<td>Kairoxone</td>
<td>1</td>
<td>0.0292</td>
<td>7.45</td>
<td>0.0077</td>
<td>F</td>
</tr>
<tr>
<td>C*K</td>
<td>3</td>
<td>0.0164</td>
<td>4.20</td>
<td>0.0182</td>
<td>C</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.0039</td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td><strong>Neonate size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clone</td>
<td>3</td>
<td>0.6707</td>
<td>351.71</td>
<td>&lt;0.0001</td>
<td>F/C</td>
</tr>
<tr>
<td>Kairoxone</td>
<td>1</td>
<td>0.0035</td>
<td>1.82</td>
<td>0.1787</td>
<td>F</td>
</tr>
<tr>
<td>C*K</td>
<td>3</td>
<td>0.0071</td>
<td>3.70</td>
<td>0.0121</td>
<td>C</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.0019</td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td><strong>Size at first reproduction (SFR)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clone</td>
<td>3</td>
<td>0.7975</td>
<td>38.61</td>
<td>&lt;0.0001</td>
<td>F/C</td>
</tr>
<tr>
<td>Kairoxone</td>
<td>1</td>
<td>0.0474</td>
<td>2.30</td>
<td>0.1317</td>
<td>F</td>
</tr>
<tr>
<td>C*K</td>
<td>3</td>
<td>0.0210</td>
<td>1.02</td>
<td>0.3875</td>
<td>C</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.0207</td>
<td></td>
<td></td>
<td>N</td>
</tr>
</tbody>
</table>
clones revealed that clones that came from Chaoborus habitats exhibited a slight, but not significant, upward migration, while only the clones with either fish or no predators in their original habitat showed (non-significant) downward migration in response to fish kairomone (Table 2.2 and 2.4). I picked nine clones to illustrate variability in DSB of adult Daphnia in the control (Fig. 2.2: white box plots) and fish-kairomone (Fig. 2.2: gray box plots) water.

For both predator kairomones, there was no significant decrease in strength of reaction over the two hour time period (p > 0.05) as tested by repeated measures ANOVA. However, for both predator kairomones, there was a significant effect of clones (p < 0.0001) (Table 2.3) Daphnia also showed a significant effect of clone in terms of DSB in controls (Table 2.2 and 2.3). The D. pulex clones that were taken from habitats with either only Chaoborus or no predators typically had a shallower DSB in controls than the clones that co-occurred with both fish and Chaoborus in nature (Table 2.4). The clone that was taken from a habitat dominated by fish predation was an exception and also chose also a shallow depth (Table 2.2). When exposed to Chaoborus kairomone, 19 out of 33 clones (58%) showed significant upward movement (between 14 and 54 cm) causing a significant clone effect (Table 2.2 and 2.3). Clones co-existing with Chaoborus exhibited the strongest response, while the individuals typically found with fish had a deeper DSB (Table 2.2 and 2.4). Again, for 9 clones I graphed the DSB of juvenile Daphnia for control (Fig. 2.3: white box plots) and Chaoborus conditioned (Fig. 2.3: gray box plots) water.

Diluting the Chaoborus kairomone concentration led to a decrease in strength of reaction for most clones that had significantly moved upward in response to the full strength kairomone (Table 2.2, Fig. 2.4).
Figure 2.2: Boxplots of depth selection behavior (DSB) of some selected clones in response to fish kairomone after 60 minutes. The control for each clone is on the left (white) and the treatment on the right (gray). Mean DSB is indicated by squares, while the median is the line dividing the box. The upper half of the box represents the 2nd quartile, the lower half the 3rd quartile of all data. The deviation bar includes 90% of the population, while X presents 99% and — shows maximum and minimum value.
Figure 2.3: Boxplots of depth selection behavior (DSB) of some selected clones in response to *Chaoborus* kairomone after 60 minutes. The control for each clone is on the left (white) and the treatment on the right (gray). Mean DSB is indicated by squares, while the median is the line dividing the box. The upper half of the box represents the 2\textsuperscript{nd} quartile, the lower half the 3\textsuperscript{rd} quartile of all data. The deviation bar includes 90\% of the population, while X presents 99\% and — shows maximum and minimum value.
Figure 2.4: Boxplots of depth selection behavior (DSB) of one selected clone (WAL 5) in response to various concentrations of Chaoborus kairomone after 60 minutes. Mean DSB is indicated by squares, while the median is the line dividing the box. The upper half of the box represents the 2nd quartile, the lower half the 3rd quartile of all data. The deviation bar includes 90% of the population, while X presents 99% and — shows maximum and minimum value.
Adult *Daphnia* showed either no or reduced upward migration when exposed to *Chaoborus* kairomone compared to juvenile *Daphnia* (Table 2.1, Fig. 2.5).

Comparison between differences in DSB to fish and *Chaoborus* kairomone revealed that all clones that were collected from a habitat with *Chaoborus* migrated upwards in response to *Chaoborus* kairomone while the only two clones with a downward movement were collected from *Chaoborus*-free habitats (Fig. 2.6). In contrast, the response to fish kairomone was more variable and only the clone taken from a habitat where fish was the sole predator clearly moved downward in the presence of fish kairomone.

**Comparing Behavioral, Morphological, and Life-History Responses**

The life-table experiment showed significant effects (MANOVA) of both clone and a clone by treatment (kairomone) interaction for all investigated morphological (neck-spine induction) and life-history (population growth rate (*r*), neonate size, and size at first reproduction (SFR)) characteristics, except neonate size and SFR (Table 2.3).

Grouping the *Daphnia* clones into their native predation regimes showed a slightly different pattern (Table 2.4). The two clones that co-occurred with fish predators were the only ones that did not produce neck-spines at all. Except for one clone that came from a predator-free habitat, all other clones had a significant neck-spine induction in the presence of *Chaoborus* kairomone. The main effects of clone, kairomone treatment, and their interactions were significant. The two clones from fish-inhabited lakes grouped together and clones from *Chaoborus*-dominated habitats showing the strongest induction of neck-spines (Table 2.4 and 2.5).
Figure 2.5: Boxplots of depth selection behavior (DSB) of one selected clone (FLC 2) comparing DSB of adults vs. juveniles in response to Chaoborus kairomone after 60 minutes. Mean DSB is indicated by squares, while the median is the line dividing the box. The upper half of the box represents the 2\textsuperscript{nd} quartile, the lower half the 3\textsuperscript{rd} quartile of all data. The deviation bar includes 90\% of the population, while X presents 99\% and — shows maximum and minimum value.
Figure 2.6: Strength of reaction in depth selection behavior (DSB) towards *Chaoborus* and fish kairomone of 28 different *Daphnia* clones. *Daphnia* clones were divided according to the predator regime in the habitat from which they were taken (Fish and *Chaoborus* (F/C), Fish, *Chaoborus*, or Neither).
Table 2.5: Differences in neck-spine induction, population growth ($r$), size of neonates, and size at first reproduction (SFR) in response to *Chaoborus* kairomone in the life-table experiments.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Dominant Predator</th>
<th>Neck-Spine Induction ($\Delta r$, Chaoborus Kairomone)</th>
<th>$\Delta r$ (Kairomone-Control)</th>
<th>$\Delta$ Neonate Daphnia Size (Kairomone-Control)</th>
<th>$\Delta$ SFR (Kairomone-Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACP</td>
<td>Chaoborus</td>
<td>25.56 ±5.03</td>
<td>-0.029</td>
<td>-0.010</td>
<td>-0.060</td>
</tr>
<tr>
<td>BRY 3</td>
<td>Chaoborus</td>
<td>46.15 ±4.60</td>
<td>0.042</td>
<td>0.050</td>
<td>-0.045</td>
</tr>
<tr>
<td>ESUM 2</td>
<td>Chaoborus</td>
<td>46.75 ±2.72</td>
<td>0.045</td>
<td>0.013</td>
<td>0.055</td>
</tr>
<tr>
<td>ESUM 3</td>
<td>Chaoborus</td>
<td>43.00 ±1.53</td>
<td>0.270</td>
<td>0.026</td>
<td>0.058</td>
</tr>
<tr>
<td>HON 3</td>
<td>Fish</td>
<td>0.00 ±0.00</td>
<td>0.003</td>
<td>-0.018</td>
<td>0.045</td>
</tr>
<tr>
<td>HOP 1</td>
<td>Neither</td>
<td>27.22 ±6.46</td>
<td>-0.018</td>
<td>-0.014</td>
<td>-0.069</td>
</tr>
<tr>
<td>HOP 2</td>
<td>Neither</td>
<td>12.73 ±4.49</td>
<td>0.111</td>
<td>0.052</td>
<td>0.231</td>
</tr>
<tr>
<td>HOP 3</td>
<td>Neither</td>
<td>9.00 ±0.95</td>
<td>-0.018</td>
<td>0.037</td>
<td>-0.129</td>
</tr>
<tr>
<td>HOP 4</td>
<td>Neither</td>
<td>48.18 ±2.93</td>
<td>0.001</td>
<td>0.028</td>
<td>-0.075</td>
</tr>
<tr>
<td>HOP 5</td>
<td>Neither</td>
<td>36.67 ±4.98</td>
<td>-0.135</td>
<td>-0.007</td>
<td>0.003</td>
</tr>
<tr>
<td>MLP 5</td>
<td>Chaoborus</td>
<td>41.92 ±3.94</td>
<td>0.171</td>
<td>-0.033</td>
<td>0.015</td>
</tr>
<tr>
<td>MLR 4</td>
<td>Chaoborus</td>
<td>17.5 ±8.54</td>
<td>-0.014</td>
<td>-0.013</td>
<td>-0.025</td>
</tr>
<tr>
<td>WAL6</td>
<td>F/C</td>
<td>0.00 ±0.00</td>
<td>0.095</td>
<td>-0.045</td>
<td>0.034</td>
</tr>
<tr>
<td>F/C</td>
<td></td>
<td>0.00</td>
<td>0.095</td>
<td>-0.045</td>
<td>0.034</td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td>0.00</td>
<td>0.003</td>
<td>-0.018</td>
<td>0.045</td>
</tr>
<tr>
<td>Chaob.</td>
<td></td>
<td>36.81 ±5.00</td>
<td>0.081</td>
<td>0.006</td>
<td>0.000</td>
</tr>
<tr>
<td>Neither</td>
<td></td>
<td>26.76 ±7.31</td>
<td>-0.012</td>
<td>0.019</td>
<td>-0.008</td>
</tr>
</tbody>
</table>

Note: Numbers indicate mean values ±1 S.E. Numbers marked in **bold** indicate significant reaction (p<0.05)
The difference in population growth rate \((r)\) between control and kairomone treatment was variable. I observed an increase in \(r\) for eight clones (six were significant, Jackknife technique) in response to predator-chemical, and five clones reduced population growth (three were significant) (Table 2.5). There was also a significant treatment effect and a clone by treatment interaction (Table 2.3). Those clones that co-occurred with *Chaoborus* had a significant tendency to increase \(r\) in the presence of kairomone (Table 2.5). Change in size of neonates and size at first reproduction were clearly clone-dependent (Table 2.3) and I found a significant clone by treatment interaction for change of neonate size. Five clones responded with a significant increase in neonate size and two with a significant decrease, one being from a habitat with fish. Surprisingly, an increase of neonate size can be accompanied by a decrease in SFR and vice versa (Tab. 2.5). *Daphnia* can react to predators by producing larger offspring while at a younger size. For both adults and juveniles, the clones from the *Chaoborus* and predator-free habitats grouped together (Tab. 2.4).

Plotting behavioral (control depth - treatment depth, \(\Delta \) DSB) versus morphological (neck-spine induction) and life-history (\(r\), control size - treatment size, \(\Delta \) neonate size and SFR) defense mechanisms revealed no correlations among these characteristics (Fig. 2.7). Interestingly, the only two clones that did not produce any neck-spines at all were the two co-existing with only fish in their natural habitat. And, only the clone that had to contend with *Chaoborus* in nature, showed an upward migration in combination with greatly reduced neonate size (Fig. 2.7, Tab. 2.2 and 2.5).

The plots of morphological and life-history parameters, as well as those of various life-history variables plotted against each other showed no pattern (Fig. 2.8).
Figure 2.7: Scatter graphs between behavioral (difference in depth selection behavior (Δ DSB) to Chaoborus kairomone) and (a) morphological (neckspine induction) or life-history (b: population growth (r), c: Δ size of neonates and d: first reproduction) variables. Daphnia clones were divided according to dominating predator in the habitat they were taken from (Fish and Chaoborus (F/C), Fish, Chaoborus, or Neither).
Figure 2.8: Scatter graphs between morphological (neckspine induction) and life-history (population growth ($r$), Δ size of neonates and first reproduction (SFR)) variables. *Daphnia* clones were divided according to dominating predator in the habitat they were taken from (Fish and *Chaoborus* (F/C), Fish, *Chaoborus* or Neither).
Discussion

The average depth selection behavior (DSB) varied greatly among the controls. When a *Daphnia* clone stays up higher in the water column in the absence of *Chaoborus* kairomones, it reduces the distance a clone can react the kairomone. In some cases, most of the individuals in the *Chaoborus* kairomone treatment were actually found within the top 5 cm of the water column. Other laboratory studies also found the mean vertical distribution to be close to the surface (Dodson 1988; Ramcharan et al. 1992).

Furthermore, the consequences of food concentration are still debated. Some studies found an increase in antipredator responses with higher food levels (Dodson 1988a), others show that defenses are expressed most significantly at lower food concentrations (Parejko and Dodson 1991).

Juveniles that are more vulnerable to invertebrate predation react stronger to the threat of predation, suggesting that *Daphnia* are able to evaluate advantages and disadvantages of the antipredator defense (Ramcharan et al. 1992). Similar findings with only the more vulnerable larger instars of *Daphnia schodleri* producing a cephalic expansion in response to notonectics (Schwartz 1991) and strongest neck-spine formation in the most vulnerable instars (Tollrian 1995a) support that statement. In a field study, Boronat and Miracle (1997) observed that under simultaneous predation pressure of fish and *Chaoborus*, juveniles of *Daphnia longispina* were found closer to the surface, while the adults dwelled predominantly in the rich, deep waters near the oxicline.

Although De Meester and Pijanowska (1996) suggested that antipredator reactions are not concentration dependent, my study does suggests that the difference in DSB is not likely to be an on-off mechanism but rather a scaled response. These results support the findings by
Ramcharan et al. (1992). This might be due to an ability of the *Daphnia* clones to evaluate the predator threat and act accordingly. My study showed this in the case for *Chaoborus* kairomone, and von Elert and Pohnert (2000) reported similar findings with crucian carp (*Carassius carassius*) kairomone. Studies examining morphological antipredator defenses have indicated that the proportion of *D. pulex* expressing neck-spines is dependent on *Chaoborus* density (Krueger and Dodson 1981; Brancelj et al. 1996), and the strength of induction is positively correlated to amount of *Chaoborus* chemical in the water following a Michelis-Menten saturation curve (Parejko and Dodson 1991; Tollrian 1993; Tollrian 1995b; Brancelj et al. 1996; Cernac and Brancelj 1996). Induced defenses in ciliates were also found to correlate positively with predator density (Wiackowski and Staronska 1999).

I was able to show that various behavioral, morphological, and life-history changes in response to *Chaoborus* kairomone were unrelated in a large number of *Daphnia pulex* clones. Boersma et al. (1998) support this finding studying fish mediated plasticity in *D. magna* clones. However, I do not claim that a coupling between certain traits does not exist. I do not feel that findings of a negative coupling among *Daphnia* clones that either express diel vertical migration or switch their life-history towards smaller body size in the presence of fish kairomone are contradictory to my results (Leibold and Tessier 1991; De Meester 1994; De Meester 1995; De Meester et al. 1995; Reede and Ringelberg 1995; Reede and Ringelberg 1998). *Daphnia* should aim for one of two strategies: Stay in the epilimnion and take advantage of high food concentrations and temperature but become less conspicuous (like my WAL 6 clone), or migrate on a diel cycle and produce larger, higher quality offspring that are more starvation resistant. To express the life history and behavioral defense simultaneously does not appear to be advantageous and might only occur under special conditions. A clear
lake with high fish predation, including fish species that even forage in parts of the hypolimnion, might be such an example. In that case, Daphnia might be forced to express behavioral and life-history responses at the same time, to escape high rates of fish predation. And although Boersma et al. (1998) showed an uncoupling of responses to predator kairomone, they still detected significant genetic correlations between 29% of eleven selected traits.

The general lack of a set of fixed antipredator defenses might explain some inconsistent, sometimes contradictory findings in the literature. Some studies even noticed behavioral, morphological, and life-history responses that are in disagreement to the common reaction norm. Reports by Stibor (1992), Weider and Pijanowska (1993) and Hanazato (1995) presented the commonly-accepted responses to fish kairomone of reduced maturation time and offspring size and increased brood size, while Hanazato et al. (2001) found the exact opposite. And, while the typical responses in presence of Chaoborus are increases in size at first reproduction and of neonates (Krueger and Dodson 1981; Havel and Dodson 1984; Riessen and Sprules 1990; Lüning 1992; Spitze 1992), some studies reported a decrease in size at maturity but similar egg number and size (Havel and Dodson 1987; Ketola and Vuorinen 1989). Furthermore, this study could not detect a correlation between size at first reproduction and neonate size, contradicting reports by Stibor (1992), Lampert (1993a) and Tollrian (1995b).

Surprisingly, the reaction towards fish kairomone was less dramatic and a significant downward migration was only found in one clone. The other four significantly reacting clones indicated upward movement. Other studies also found a significant upward movement in response to fish kairomone in one or more of their tested Daphnia clones (Dodson 1988b; De
Meester 1994; Boersma et al. 1998). Weber (1999) reported a significant upward orientation in some of her *Daphnia* clones when *Chaoborus* kairomone was present while no significant differences when *Daphnia* were exposed to fish chemical could be detected. She explained it in terms of the small scale of her experiments.

The comparison between the differences in DSB with *Chaoborus* and fish kairomone indicates that half of the clones showed an upward migration to both of the kairomones. *Chaoborus* as the main predator might primarily condition these clones. Most of the remaining clones demonstrated an expected response with an upward migration if confronted with *Chaoborus* chemical and a non-significant downward migration when fish kairomone is detected. Only one clone appeared to be fish kairomone conditioned and showed a downward swimming behavior for both predators.

These results might indicate that there is a possible confusion of the *Daphnia* about which predator kairomone is present or the reaction to different fish kairomones may vary. The possibility that a kairomone of an invertebrate predator resembles the fish kairomone closely cannot be excluded. Although other studies suggest that there are only slight, if any, differences in kairomone composition among different fish species (von Elert and Loose 1996), life-history traits of seven *D. galeata* clones in response to perch (*Perca fluviatilis*) and stickleback (*Gasterosteus aculeatus*) exudates varied in response to the different fish predators (Weber 1999). This implies that there might be (quantitatively or qualitatively) species-specific types of fish kairomones, which might also explain conflicting results found in the literature. Another explanation, other than differences in the fish kairomone itself, might be the near infinite clonal diversity resulting from various environmental backgrounds in *Daphnia*, which lead to various reactions to fish kairomone.
I also suggest that an upward migration in response to fish might in some cases be beneficial, especially in some clear lakes where the fish are still able to forage in deeper water layers. A possible scenario would be a lake with high densities of piscivorous species. To escape predation pressure themselves, planktivorous fish would have to seek protection in sub-optimal environments and stay close to the shoreline or deeper down in the water column, making the pelagic epilimnion of a lake the safest and most suitable place for large zooplankters (Werner et al. 1983). Although I have only little information on the density of piscivores in the lakes I investigated, Scott and Walker Lake both stood out for clarity (Secchi depth > 4 m), and three of the four clones that showed a significant upward migration in response to fish kairomone were from these lakes.

I also demonstrated that the original habitat from which the clones were taken influenced their antipredator defenses. The only two clones that did not produce neck-spines co-occurred with planktivorous fish in nature. Neck-spines probably increase conspicuousness, a disadvantage in presence of visual predators. The clone that is typically exposed to both predators (WAL 6), showed an upward migration in response to *Chaoborus* kairomone to reduce overlap with the invertebrate predator and a reduction in neonate size. This points toward a strategy of employment of behavioral defense mechanisms against *Chaoborus*, whereas the morphological and life-history alterations are against fish predators. Similarly, clones from *Chaoborus*-inhabited environments increased offspring size under *Chaoborus* kairomone exposure, while clones taken from environments without *Chaoborus* did not (Repka and Walls 1998).

However, effects between phenotypic adaptations and genotypic successions have yet to be differentiated. Instead of having few clones that express a general genotype adapted to a
large variety of environmental conditions, a study conducted by Stibor and Lampert (2000) suggests that a seasonal shift the clonal composition may be another successful mechanism to maintain high *Daphnia* densities in a lake. They found that a *Daphnia hyalina* population changed in clonal abundance through the season. When planktivorous fish biomass was high in summer, the predominant *Daphnia* clones responded by reducing their size at first reproduction and did not respond to *Chaoborus* kairomone, while in the fall months other clones only reacted to *Chaoborus* kairomone by increasing in size at first reproduction.

Furthermore, the applicability of laboratory results to the field is sometimes limited. While I found that many clones stayed close to the surface in the laboratory, this scenario would most likely not be found in a real lake situation, and field experiments have demonstrated indeed a much deeper mean vertical distribution (below 1 m) even in the presence of *Chaoborus* (Nesbitt et al. 1996). Two characteristics in nature prevent *Daphnia* from staying too close to the surface. First, UV light is known as an important factor to drive zooplankton deeper down in the water column (Leech and Williamson 2001; Rhode et al. 2001). In shallow ponds this might actually lead to a narrow habitat in which *Daphnia* are safe from both damaging radiation and invertebrate predation (Böing et al., in prep). Secondly, *Daphnia* might be more likely to be trapped at the surface because of wind and wave action and, therefore, avoid the top of the water column. However, the fact that 58% of the *Daphnia* clones had a significant behavioral change in the presence of *Chaoborus* kairomone indicates that *Chaoborus* predation is an important selective force for *Daphnia* (Dodson 1972; Benndorf et al. 2000).

I conclude that my laboratory experiments reflected some of the diversity of antipredator responses found in nature. Most *Daphnia* clones could be distinguished from
each other by their different reaction norms and by combinations of behavioral, morphological, and life-history responses towards predator-chemicals. I support my hypothesis of high genetic diversity leading to various combinations of behavioral, morphological, and life-history defenses, probably as an adaptation to local environmental conditions. Future studies should focus on changes in life-history traits over several generations. So far, life-history antipredator defenses in the literature were only observed for the first generation of individuals exposed to kairomones. However, in nature *Daphnia* often experience the effects of predator kairomones over several generations. Therefore, the question is raised: If a clone shows an alteration as a response to a predator kairomone, for how many generations can those effects persist in a population? For example, if an increase in population growth rate due to predator chemical were observed, would the next generations also respond in a similar way? Why can population growth rate be enhanced and why would the control not show maximum reproductive output? Is there a trade-off associated when population growth exceeds a certain level, like reduced quality of offspring with a lower survivorship rate? A plausible explanation is offered by Tessier et al. (2000) and Reznick et al. (2000), who suggest that an increase of population growth can only be observed at high food levels and the relationship is reversed at lower food availability. What about body size? It is known that larger individuals have the tendency to produce larger offspring (Green 1956; Brambilla 1982). Through how many generations can an increase in body size as a response to invertebrate predators be observed? Furthermore, the simultaneous exposure to fish and *Chaoborus* needs to be investigated. Weber and Declerck (1997) showed that those two kairomones could have neutralizing effects. Answers to those questions could enhance our understanding of possibilities and limitations of adaptations.
CHAPTER 3

COSTS AND BENEFITS OF DAPHNIA ANTIPREDATOR DEFENSE IN PRESENCE OF CHAOBORUS IN NATURE
Introduction

In a pelagic food web, energy flow can be modulated significantly by predator-prey interactions on various trophic levels, because prey organisms employ a variety of antipredator defenses (Carpenter et al. 1985; McQueen et al. 1989; Ramcharan et al. 1996; Böing et al. 1998; Benndorf et al. 2000; Ramcharan et al. 2001a and b). However, predator avoidance often places demands on prey behavior, morphology and life-history that compromises other goals such as feeding and reproduction, thereby entailing a cost in terms of fitness (Tollrian and Harvell 1999). Inducible defenses have a great advantage in that these costs are not fixed but remain flexible (Harvell 1990). They are incurred only when danger from predators is actual and the strength of the defense is often scaled to the risk of the predators (Ramcharan et al. 1992; Tollrian 1995b; Brancelj et al. 1996; von Elert and Pohnert 2000; Chapter 2). Theoretically, then, all inducible defenses must have an associated ecological cost, or they should have become evolutionarily fixed (Lively 1986).

Despite prey defenses, zooplankton composition and abundance in a freshwater lake can be drastically altered by predation from a voracious invertebrate predator, *Chaoborus* (Dodson 1972; Kajak and Rybak 1979; von Ende 1979; Yan et al. 1991; Benndorf et al. 2000; Ramcharan et al. 2001b). *Chaoborus* predation rate can be highly variable throughout the year, because predator biomass fluctuates and is also related to vertical overlap between predator and prey. *Chaoborus* showed daily food rations of about 20% of crustacean zooplankton production for most of the feeding period although shortly after hatching when intensive growth occurred this could increase up to 106% (Kajak and Rybak 1979). Consumption of crustaceans in the epilimnion could vary from 0 to over 100% of prey production in a eutrophic European lake. In June and September, consumption was found to
equal about 30-40% of prey production and even sometimes exceeded production (Kajak and Rybak 1979).

*Chaoborus* spp. are tactile, ambush predators that ingest their prey whole. A chemical substance exuded by larvae when feeding on zooplankton is used by *Daphnia* spp., large, filter-feeding cladocerans, as a clue to the predator’s presence, and *Daphnia* are able to express inducible defenses as a consequence. The *Chaoborus* – *Daphnia* system is perhaps the most studied example of inducible defense.

There are three types of defense that *Daphnia* use against *Chaoborus*; behavioral, morphological, and life-history. *Chaoborus* are mouth-gape limited and prefer to prey on smaller individuals (Pastorok 1981; Riessen et al. 1988; Tollrian 1995a), and the more vulnerable juvenile *Daphnia* express stronger defense mechanisms than adult *Daphnia*, which are protected by their large body size (Ramcharan et al. 1992; Tollrian 1995a; Chapter 2). A positive phototaxis resulting in an upward migration of *Daphnia* in daylight is the most rapid response and can be triggered within 30 minutes of introducing predator kairomone (Ohman et al. 1983; Dodson 1988b; Ramcharan et al. 1992; Nesbitt et al. 1996). This behavioral defense is effective in most cases, because *Chaoborus* prefers the dark hypolimnion or sediment of lakes to escape fish predation (Luecke 1986; Dawidowicz et. al. 1990, Voss and Mumm 1999). *Daphnia* that were exposed to the kairomone during egg development often react with the production of morphological structures, e.g., “neck-spines”, during juvenile instars that appear on the dorsal lower margin of their heads (Krueger and Dodson 1981). Neck-spines have been shown to decrease ingestion of captured prey by *Chaoborus* spp. by up to 60% (Krueger and Dodson 1981; Havel and Dodson 1984; Parejko 1991; Lüning 1995; Repka et al. 1995; Tollrian 1995a; Sell 2000). Moreover, life-history shifts towards larger size
at first reproduction and the release of larger neonates are other effective defenses (Brett 1992; Lüning 1992; Spitze 1992; Weber and Declerck 1997; Walls and Ventelä 1998; Riessen 1999; Barry 2000; Sell 2000).

The fact that those defense mechanisms are inducible and are only expressed when the predator is abundant suggests that they entail a cost (Dodson 1974b). However, this cost and the benefit of reduced predation as a result of the defenses have so far been ignored in models simulating energy flow through food webs. This might be due to contradictory results published in the literature. Some studies found that morphological alterations like spines, helmets, or crest production was coupled to a reduction in reproductive output in zooplankton species (Zaret 1969; Kerfoot 1977; O’Brien and Vinyard 1978; Grant and Bayly 1981; Dodson 1984; Riessen 1984; Havel and Dodson 1987; Ketola and Vuorinen 1989; Walls and Ketola 1989; Black and Dodson 1990; Riessen and Sprules 1990; Riessen 1992; Walls et al. 1991). However, those costs were ascribed to several different factors, including longer development time (Havel and Dodson 1987; Ketola and Vuorinen 1989; Walls and Ketola 1989; Black and Dodson 1990; Riessen and Sprules 1990), reduction in clutch size (Ketola and Vuorinen 1989; Black and Dodson 1990; Walls et al. 1991), and lowered survivorship (Ketola and Vuorinen 1989; Black and Dodson 1990). Other studies reported no reduction or even an increase in population growth in the presence of predator kairomone (Hebert 1978; Gilbert 1980; O’Brien et al. 1980; Stemberger 1990; Spitze 1991; Black 1993; Tollrian 1995b; Spitze 1996; Weber and Declerck 1997; Scheiner and Berrigan 1998; Barry 2000).

Still other studies have demonstrated that the relationship between neck-spine production and population growth is unrelated and varies among clones (Spitze 1992; Chapter 2). Most of those studies were conducted in the laboratory with only a single clone of
Daphnia. So far all experiments that have studied altered population growth rate in the presence and absence of Chaoborus kairomone have ignored multiple natural factors like UV light, food abundance, crowding, and competition, and have also failed to consider the simultaneous effects of behavioral, morphological, and life-history responses.

I conducted a mesocosm experiment designed to study combined effects of the various defense mechanisms and to estimate their costs and benefits in nature. Two characteristics of Daphnia make my approach possible: (1) Daphnia are cyclic parthenogens, and their antipredator defenses vary among clones, (2) Antipredator defenses are transmitted by a chemical cue exuded by Chaoborus larvae and, therefore, costs of antipredator defense can be uncoupled from the costs of real predation. I used two Daphnia clones, one with antipredator defenses to Chaoborus (responsive clone) and one without (non-responsive clone). Three different treatments were conducted for each clone: One treatment placed Daphnia together with predators (real predation), another placed Daphnia with predator kairomone (ghost predation), and a third placed Daphnia without any influence of predators (control). The difference in population growth (as expressed by \( r \), the intrinsic rate of natural increase) between control and ghost predation for the responsive clone should represent only the cost of the antipredator defense, independent of the cost of real predation (Table 3.1). By comparing population growth rates of the two clones in the real predation treatments, the clone defending itself is expected to have higher growth rates, which would be the benefit of the defense (Table 3.1).

The goal of this study is to estimate the magnitude of costs and benefits of the antipredator defenses in a representative lake. I wished to measure the costs and benefits of predator defenses under near-natural conditions and hypothesize that benefits to fitness
outweigh the costs of the defenses. In contrast to some laboratory studies that indicated no
cost of *Daphnia* antipredator defense (Hebert 1978; O’Brien et al. 1980; Spitze 1991; Black
1993; Tollrian 1995b; Spitze 1996; Weber and Declerck 1997; Scheiner and Berrigan 1998;
Barry 2000) I hypothesize that costs are detectable in nature.

The significance of this work is that, for the first time, costs and benefits in the
*Daphnia* – *Chaoborus* system considering all behavioral, morphological, and life-history
responses are estimated in the field. The results will indicate whether the costs of antipredator
defenses are substantial compared to the cost of real predation and estimate if, and by how
much, the benefits of expressing antipredator defenses outweigh the costs.

**Materials and Methods**

I chose two *Daphnia pulex* clones to conduct field experiments. One clone reacted
strongly to *Chaoborus* kairomone in previous laboratory experiments with upward migration,
neckteeth production, increase in population growth rate \( r \), and increase in neonate size (clone
BRY3, Chapter 2) and will be referred to as the responsive clone. Another clone did not show
any response to *Chaoborus* to the traits studied (clone HON3, Chapter 2), and was designated
the non-responsive clone. Both clones were grown to high densities in the laboratory before
the beginning of the experiment.

**Enclosure Set-Up**

I estimated the costs and benefits of antipredator defenses toward *Chaoborus* under
near-natural conditions with an enclosure study conducted during the summer of 1999. The
treatments consisted of two *Daphnia pulex* clones (responsive and non-responsive) and three
predator regimes (control, real predation, ghost predation) in a factorial design. Four
replicates were used in a total of 24 enclosures. Enclosures had a diameter of 1 m, were 9 m
long, and made of transparent, non-UV-coated polyethylene. The top half-meter was rolled around a PVC ring, which was used to secure the enclosures to a floating wooden frame.

Table 3.1: Logic of the experimental design and my expectations of the growth rates of *Daphnia pulex* clones according to different treatments. My approach to estimating costs and benefits is shown below.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Real Predation</th>
<th>Ghost Predation</th>
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<tbody>
<tr>
<td>responsive</td>
<td>A(^1)</td>
<td>B(^3)</td>
<td>C(^2)</td>
</tr>
<tr>
<td>non-responsive</td>
<td>D(^1)</td>
<td>E(^3)</td>
<td>F(^1)</td>
</tr>
</tbody>
</table>

responsive: Clone that exhibits antipredator behavior  
non-responsive: Clone that does not exhibit antipredator behavior  
\(^1\): Maximum population growth rate under given environmental conditions  
\(^2\): Growth rate affected by antipredator behavior  
\(^3\): Growth rate affected by both antipredator behavior and some real predation  
\(^4\): Minimum growth rate, full predation pressure, no costs of defense

Costs of real predation = E-D  
Costs of antipredator behavior = A-C  
Benefits of antipredator behavior = (B-E)-(A-D)
Empty, sealed plastic barrels provided floatation and kept the frame and the top of the enclosures about 0.5 m above the water surface. Each enclosure was sealed at the bottom and tied with a rope to a cinder block, that anchored the enclosure to the bottom of the lake. The enclosures were placed in Costello Lake (Algonquin Park, Ontario, Canada) at a lake depth between 9 and 10 m. Costello Lake is a medium-sized (33.5 ha), oligotrophic (total phosphorus = 11.1 µg L⁻¹), moderately stained, brown-water lake (dissolved organic carbon varies around 4 mg L⁻¹; color = 35 CoPt; Secchi depth about 3.5 m) of circumneutral pH (Arts and Sprules 1988). Its maximum depth of 18.9 m allowed high densities of planktivorous fish and Chaoborus spp. to coexist (Böing, pers. observ.).

The enclosures were filled with lake water drawn from 2.5 m depth with a fire pump and forced through a 202 µm mesh, which removed large zooplankton including Daphnia spp. and Chaoborus spp. while allowing the smaller natural zooplankton community to pass through. This resulted in an enclosed water column in each enclosure of approximately 7.5 m depth with a volume of 5800 L.

Afterward all enclosures were covered with a white 2-mm mesh (window screening) stretched over the top to prevent adult Chaoborus from laying their eggs in the enclosures. Two days after filling the enclosures, approximately 500 individuals from either D. pulex clone were added to 24 randomly chosen enclosures (one clone per enclosure). Densities were checked once a week with vertical hauls of a plankton net (30 cm diameter; 130 µm mesh size). On July 1st, fourteen days after initially adding D. pulex, densities were adjusted to 1 individual L⁻¹ by removing the appropriate amount of D. pulex with plankton nets. A second, smaller tube (30-cm diameter, 7 m long) made of 250-µm white mesh screening was then suspended in the middle of each enclosure. This mesh prevented movement of Daphnia and
Chaoborus between the tube and the enclosure, while allowing free flow of water, algae, and predator chemicals. The mesh tubes enclosed a water volume of 495 L.

On July 4th, Chaoborus trivittatus was added at a density of 1.1 L⁻¹ to the randomly assigned real predation treatments and the same total number of predators were sequestered in each of the mesh bags of the ghost predation treatments. No Chaoborus were put into the control enclosures. C. trivittatus was chosen because it is the species in North America that remains in the planktonic stage through the summer. It is also a large species able to feed on even large Daphnia (Pastorok 1981). On July 16th and again on July 26th, C. trivittatus densities were adjusted to 1.2 and 0.9 individuals L⁻¹, respectively. This assured that predator density stayed above 0.7 L⁻¹ throughout the experiment despite removal by sampling. This is a moderate density for Chaoborus, based on abundance commonly found in nature (Yan et al. 1985).

I also added nutrients (10 µg P L⁻¹ as P₂O₅; 11 µg N L⁻¹ as NH₄ and (NH₂)₂CO; 9 µg K L⁻¹ as K₂O; 0.015 µg B L⁻¹; 0.05 µg Cu L⁻¹; 0.11 µg Fe L⁻¹; 0.04 µg Mn L⁻¹; 3.6 x 10⁻⁴ µg Mo L⁻¹; and 0.04 µg Zn L⁻¹) to each enclosure at the beginning of the experiment. This was done to maintain productivity of algae and eliminate food limitation as a confounding factor during exponential growth of Daphnia.

Sampling Procedure

Sampling took place in 4-day intervals between July 1st and August 10th. Zooplankton was collected with a closing net (30 cm diameter; 130 µm mesh size) from four discrete depths (0-1 m; 1-3 m; 3-5 m; and 5 m – bottom). Samples were concentrated with an 80-µm mesh filter and fixed in 4% sugar-saturated formaldehyde (Haney and Hall 1973). A day before zooplankton samples were taken, depth profiles in 1-m intervals of temperature and
oxygen were obtained with a probe (Model 50B; YSI Incorporated) lowered in the mesocosms.

Additionally, I determined the day/night distribution of zooplankton (on sampling date 3). Day samples were typically taken around noon, while night sampling began three hours after sunset, usually around midnight. Chlorophyll \( a \) was determined three times (sampling date 2, 5, 8) for three randomly selected enclosures per predator treatment. Water samples for chlorophyll \( a \) measurements were obtained with a pump sampler. I collected 0.5 L from 0-1, 1-3, and 3-7 m and kept the samples in the dark. The samples were processed immediately in the laboratory and separated into the edible (< 30 \( \mu \)m) and inedible (> 30 \( \mu \)m) fraction with a Nitex mesh. Then both fractions were filtered onto a GF/C filter (1.2 \( \mu \)m pore size) and frozen at –20 °C for up to three months. The filters were sonicated and chlorophyll \( a \) extracted in 100 % acetone at 7 °C overnight. Chlorophyll \( a \) concentration was measured with a fluorometer (Turner Designs Inc. Sunnyvale, CA; model 10-005R).

**Sample Enumeration**

Enumeration took place with a dissecting microscope (Leica MZ8) that allowed magnification up to 500X. *Daphnia* and *Chaoborus* were counted for each sampling date. To estimate population densities, at least 100 *D. pulex* for each depth from each enclosure were counted. Before enumeration, formaldehyde was removed by washing the sample through an 80-\( \mu \)m net, then backwashing with distilled water into a counting chamber. All samples were brought to a known volume. If animal density was high, the sample was diluted by first stirring haphazardly to distribute the animals evenly, then withdrawing a 10 ml sub-sample with a large-bore mechanical pipette (10 mL Transferpettor), which was then diluted to a workable animal density. *D. pulex* were distinguished by eye between adults (approximately >
1.2 mm) and juveniles (approximately < 1.2 mm). *C. trivittatus* were counted as either 1<sup>st</sup> and 2<sup>nd</sup> instar (< 1 cm) or 3<sup>rd</sup> and 4<sup>th</sup> instar (> 1 cm).

*D. pulex* neck-spines were scored according to Tollrian (1993), where a morphologically “normal” neck was 0 %, a “little” bump on the neck was 30 %, and a “bump with convex sides” was 50 % induction. Each separate spine accounted for an additional 10 %.

Population growth (*r*) served as an estimate of fitness and was calculated with the equation:

\[
r = \frac{\ln(N_2) - \ln(N_1)}{\Delta t}
\]

where *N*₂ is the population density at time *t*₂ (later sampling date),

*N*₁ is the population density at time *t*₁ (earlier sampling date),

and \( \Delta t \) is the difference between time 1 and time 2 in days.

**Statistical Analysis**

On July 6<sup>th</sup>, a day after the first sampling, a heavy wind produced large waves, destroying some enclosures (two of responsive clone / control; one of responsive clone / real predation; and one of non-responsive clone / control). As a consequence, I lost some replicates, which led to an unbalanced design. Therefore, each treatment had between two and four replicates.

Statistical evaluation was conducted with SAS software (version 8). (Copyright, SAS Institute Inc., Cary, NC, USA.) (SAS 2002). To compare temperature, oxygen, and chlorophyll *a* among treatments, I performed a repeated measures ANOVA, with depth as the repeated variable. The effects of clone and predation regime on neck-spine induction, population growth rates and mean vertical distribution were analyzed with a two-way
ANOVA. P-values were Tukey adjusted. Normality of residuals was tested with Shapiro-Wilk’s.

**Results**

For most of the experiment, the epilimnion in both the lake and the enclosures was 3 m thick and temperature typically ranged between 20 and 25 °C (Fig. 3.1) except on July 24. On the last sampling date (August 9) the epilimnion extended down to 4 m. Usually, the temperature dropped quickly below 3 m and reached values of about 7 °C at the bottom of the enclosure bags. Temperature was consistent among all treatments and the lake (p > 0.05, repeated measures ANOVA).

Oxygen levels fluctuated around 7 and 8 mg L⁻¹ in the epilimnion (Fig. 3.2). Towards the end of the experiment, oxygen values decreased in the metalimnion, but always stayed well above 4 mg L⁻¹.

For chlorophyll $a$, only data from the beginning of the experiment (July 9th) are shown, as this is the period used for the calculation of costs and benefits of *Daphnia* antipredator defense. There was a significant effect of depth on chlorophyll $a$ (p = 0.001, repeated measures ANOVA). The chlorophyll $a$ concentration was highest in the deepest sample layer (3-7 m) for the control (p = 0.003) and the ghost predation treatment (p = 0.009) of the responsive clone (Fig. 3.3). Chlorophyll $a$ levels were similar for control and real predation treatments. For the ghost predation / responsive clone treatment the concentrations were significantly higher in 1-3 and 3-7 m compared to the control treatment (p < 0.0001). For the non-responsive clone, chlorophyll $a$ concentrations were lower than for the responsive clone (p = 0.0009). The real predation treatment of the non-responsive clone had significantly
Figure 3.1: Temperature profiles for the different enclosure treatments and the lake on each sampling date for control (C) (gray), ghost (G) (white), and real (R) (black) predation treatments of the responsive (+) (circles) and non-responsive (-) (squares) clone and the lake (diamond).
Figure 3.2: Oxygen profiles for beginning, middle and end of the experiment for control (C) (gray), ghost (G) (white), and real (R) (black) predation treatments of the responsive (+) (circles) and non-responsive (-) (squares) clone and the lake (diamond).
Figure 3.3: Chlorophyll a (< 30 µm) distribution on July 9th for responsive clone (top) and non-responsive clone (bottom); control – gray bars, ghost predation – white bars, real predation – black bars; error bars present ± 1 standard error.
higher chlorophyll \( a \) values in 0-1 m than the ghost predation treatment \((p = 0.049)\), and was the only treatment where chlorophyll \( a \) did not increase with depth.

Neck-spine induction (Fig. 3.4) was similar to that seen in the laboratory (Chapter 2). The responsive clone defended itself morphologically by producing neck-spines only in the ghost and real predation treatments, while the non-responsive clone failed to do so in all treatments \((p < 0.0001, 2\text{-way ANOVA})\).

Without predators, the responsive clone was much more evenly distributed throughout the water column than the non-responsive clone, which mainly stayed within the top first meter \((p = < 0.0001, 2\text{-way ANOVA})\) (Fig. 3.5). With either real predators \((p = 0.0002)\) or ghost predation \((p = 0.0077)\), the responsive clone stayed higher in the water column than for the controls. Although not significant, this upward migration during daytime was stronger for the real predation treatments than the ghost predation treatments \((p = 0.08)\). There was also a tendency for the non-responsive clone to have a shallower mean vertical distribution when exposed to \textit{Chaoborus} predation.

Analysis of population dynamics show that for both clones the controls had rapid growth rates from July 1\textsuperscript{st} until July 13\textsuperscript{th}, before the population size leveled-off for eight days and then decreased towards the end of the experiment (Fig. 3.6). Population size for the ghost predation enclosures with the responsive clone slowed down after addition of predators into the mesh bags but caught up with the population peak four days later, then followed the dynamics of the control bags closely. Similar to ghost predation, the \textit{Daphnia} population exposed to real predation also slowed down for eight days after predator addition, peaked on
Figure 3.4: Neckteeth induction for responsive and non-responsive clone. Error bars present ± 1 standard error.
Figure 3.5: Vertical day distribution of *Daphnia* on July 13th; top graphs represent responsive and bottom graphs non-responsive clone; control – gray bars, ghost predation – white bars, real predation – black bars, responsive clone (+), non-responsive clone (-); error bars present ± 1 standard error.
Figure 3.6: Population dynamics of *Daphnia pulex* clones; top graphs represent responsive and bottom graphs non-responsive clone; control – gray symbols, ghost predation – white symbols, real predation – black symbols. Note differences in Y-axes.
July 17th and then decreased to slightly lower levels than either control or ghost predation. The size of the *Daphnia* populations in the control and ghost predation treatments of the non-responsive clone had similar dynamics. The real predation treatment showed a strong decrease shortly after predator addition and then grew exponentially, reaching a maximum on July 17th, four days after the control and ghost predation enclosures. Again, similar to the real predation treatments of the responsive clone, the population of the non-responsive clone also decreased at a faster rate than either control or ghost predation. Real predation treatments of both *Daphnia* clones have a second, lower peak on August 2nd. In total, the non-responsive clone reached higher maximum values, with about 6 *Daphnia* L⁻¹, compared to the responsive clone, with about 4 *Daphnia* L⁻¹.

The first night sample, taken on July 13th, shows that the responsive clone had about the same vertical distribution as it did during day (Fig. 3.5), with the control populations broadly distributed, while populations in the ghost (p = 0.0375, 2-way ANOVA) and the real predation (p = 0.0047) treatments stayed predominantly in the epilimnion (Fig. 3.7). The non-responsive clone showed a clear downward migration at night, and its distribution was not significantly different from the responsive clone. The maximum densities were at depths of 3-5 m in the control and ghost predation and 1-3 m in the real predation. Real predation treatments of the non-responsive clone had a significantly shallower distribution than control (p = 0.004) or ghost predation (p = 0.01). During the daytime, *Chaoborus* stayed exclusively in the lowest strata sampled. Even at night, between 70 and 80 % of the predators remained there. Some animals migrated up a couple of meters and a few migrated all the way to the epilimnion.
Figure 3.7: Vertical night distribution of *Daphnia* (left graphs) and *Chaoborus* (right graphs) on July 13th; top graphs represent responsive and bottom graphs non-responsive clone; control – gray bars, ghost predation – white bars, real predation – black bars; error bars present ± 1 standard error.
The time period of positive population growth (July 1st – July 13th) of the control was used to calculate growth rate for *Daphnia* in both the control and ghost predation enclosures (Fig. 3.8). In the real predation treatments, there was only a predation effect \( p = 0.01 \), 2-way ANOVA) of *Chaoborus* right after addition of the predators (Fig. 3.6). Therefore, population growth estimates for real predation treatments of both clones from July 5th to July 9th were used as an indication of the magnitude of losses to the predator. With this index of population growth, the antipredator defense exhibited by the responsive clone caused a reduction in population growth of about 32 % (difference between control and ghost predation). However, shortly after predator introduction, the responsive clone only experienced a slight decrease in population growth, while the non-responsive clone had a heavy loss to predation in the short term. Under real predation, the responsive clone had 68 % higher population growth than the non-responsive clone. Since reduction in population growth for the responsive clone due to ghost predation was 32 %, the benefit of antipredator defense exceeded the cost by 36 %.

**Discussion**

Environmental conditions within the enclosures closely matched the lake on all sample dates, and were also well within the ranges of tolerance for both *Daphnia* and *Chaoborus*. Oxygen concentration was always above 4 mg L\(^{-1}\), which would not have been stressful for any of the species in my experiments (Davidson et al. 1998; Sell 1998). The temperature gradient in the metalimnion was steep. Egg development time is inversely related to temperature, and this can slow population growth substantially (Bottrell 1975).

There were differences in vertical distribution patterns between the responsive and the non-responsive clone in the control treatment, with the non-responsive clone having a shallower mean vertical distribution than the responsive clone. Despite that, the responsive
Figure 3.8: Population growth rate of *Daphnia pulex* clones. For control and ghost predation treatments, estimates were taken from presamples (July 1st) to 3rd sample date (July 13th); for real predation treatments, population growth was calculated between 1st (July 5th) and 2nd (July 9th) sampling date. Responsive clone is shown on left side, non-responsive clone on right side of graph. Control – gray bars, ghost predation – white bars, real predation – black bars. Error bars show ±1 standard error.
clone showed an upward migration and neck-spine production in the ghost predation
treatments, while the non-responsive did not. Therefore, the ghost predation treatments were
effective in reproducing the antipredator defense, and the defense differed between the two
clones tested. This indicates that the predator kairomone was transported out of the mesh
tubes effectively. In addition to Nesbitt et al. (1996), this is only the second time that the
behavioral response of *Daphnia* to *Chaoborus* has been detected in the field. The non-
responsive clone did not show any measurable defense mechanisms to the invertebrate
predator. There was no neck-spine induction, and the vertical distribution of *Daphnia* did not
differ between the control and the ghost predation treatments. The shallower mean vertical
depth in the real predation treatments of both clones can be explained by an actual predation
effect, which mainly occurred in the metalimnion. When individuals that stay deeper in the
water column are actively removed, the percentage of *Daphnia* in the upper sampled layers
must increase.

The costs of the antipredator defense in response to *Chaoborus* caused a reduction of
32 % in population growth, and I estimated the benefits to be 68 % enhanced population
growth, exceeding the costs by 36 %. This supports my hypothesis that costs and benefits are
detectable in nature, and benefits outweigh costs.

Benefits of antipredator defense were calculated by the difference of predation impact
between the responsive and the non-responsive clone. The problems with the data are
twofold: (1) The vertical distribution of the controls between the responsive and the non-
responsive clone, the maximum density reached for each clone, and chlorophyll *a*
concentrations all differed considerably between enclosures with the responsive and non-
responsive clone. This confounds the comparability between these two clones and the
shallower mean vertical distribution during daytime by the non-responsive clone obscures the
effects of the behavioral upward migration of the responsive clone. (2) There is a lack of
overlap between predator and prey, even at night when most *Chaoborus* typically migrate up
to the epilimnion (Berendonk and O’Brien 1996; Dawidowicz et al. 1990; Tyossem 1990;
McQueen et al. 1999). This might be due to a handling ‘shock-response’ by *Chaoborus*
throughout my experiments. Dawidowicz (1993) observed in his experiment that *Chaoborus*
introduced into 1-m experimental tubes failed to migrate at the beginning, and only after
about three weeks did approximately 50% of the population migrate up to the epilimnion.
Furthermore, *Chaoborus* in the earlier experiment reacted to water change by remaining in the
sediment to a larger extent. Similarly, *Chaoborus* in other enclosure experiments showed only
a very small upward migration during nighttime (Nesbitt et al. 1996; Wissel unpub. data,
Young unpub. data) and this was also observed for certain periods of time in the field (Kajak
and Rybak 1979). However, overlap between predator and prey is essential for predation
impact. Predation risk is the product of prey vulnerability and density risk, which is positively
correlated with predator density and overlap between predator and prey (Williamson and
Stoeckel 1990). Calculations of an overlap index between the responsive and non-responsive
clones in my experiments revealed negatively correlated prey and predator distributions in both
cases. Therefore, the benefits had to be calculated for the beginning of the experiment, right
after initial introduction of the *Chaoborus* predators.

The estimate of antipredator benefits to the responsive clone (a 68% higher
population growth rate compared to the non-responsive clone) might be due to a combined
effect of behavioral, morphological, and life-history defense. I believe that the benefit of the
antipredator defense for the responsive clone was mostly caused by the behavioral response
(upward migration). Although the mean vertical distribution was higher during daytime for
the non-responsive clone, this relationship was reversed during night for the ghost and real
predation treatments. A morphological benefit due to neck-spine production can also reduce
predation rate, but there were no observed neck-spines at the beginning of the experiment, and
they could only have started to appear a couple of days after Chaoborus introduction.
Similarly, life-history responses typically need a generation before they become effective as
the juveniles in the brood pouch need to be exposed to the cue to express these responses.
Other unknown beneficial factors like increased alertness, dead-man response, reduced
swimming or stronger escape behavior once captured might have also decreased predation on
the responsive clone. However, those responses were not quantified in my study.

This study is the first attempt to measure benefits of antipredator behavior in nature
and the experimental outcome should provide a first rough estimate of reduced Chaoborus
predation as a result of employing antipredator defenses. My estimate of a 68 % reduced
mortality rate due to antipredator defenses is higher than previous laboratory estimates. Neck-
spines alone have shown to increase escape rate from Chaoborus attacks by up to 60%
(Krueger and Dodson 1981; Havel and Dodson 1984; Parejko 1991; Lüning 1995; Repka et
al. 1995; Tollrian 1995a; Sell 2000). The high estimate in my study might be due to a
simultaneous benefit of behavioral responses, and maybe to a limited extent some
morphological defenses. However, as I chose the most responsive and the least responsive D.
pulex clone from previous laboratory experiments (Chapter 2) the estimate might be higher
than typically found between two Daphnia clones.

The calculation of the cost of antipredator defense was less ambiguous, as the
difference in population growth between control and ghost predation enclosures of the same
clone (responsive) were compared without confounding effects of predation. For the first time the costs of antipredator behavior have been measured in nature. So far, laboratory estimates have failed to combine the effects of behavioral, morphological, and life-history constraints on maximum population growth as a result of expressed antipredator defenses. I estimated the cost of antipredator defenses to be a 32 % reduction in population growth of the non-responsive clone compared to the responsive clone. Although in the controls the responsive clone generally stayed in deeper, colder water compared to Daphnia under ghost predation, they still had a higher population growth rate and reached maximum population size faster. On average, 20 % more Daphnia for the non-responsive clone than for the responsive clone stayed below the 3-m thermocline. The average temperature between 3 and 7 m was more than 10 °C below the epilimnion temperature. This should reduce somatic growth and increase egg development time, resulting in a decrease in population growth of around 50 % for the exposed individuals (Stich and Lampert 1984; Reede-Dekker 1998). Theoretically, this would lead to an overall reduction of population growth rate in the non-responsive clone by 10 % due to temperature effects. However, the opposite was observed. The non-responsive clone showed a higher population growth rate than the responsive clone.

The reduction of Daphnia population growth under ghost predation can only be explained by the antipredator defenses exhibited in response to Chaoborus kairomone. The shallower vertical distribution of the responsive Daphnia in the ghost predation treatments probably led to the significant decrease in algae concentration close to the surface (Fig. 3.3 and 3.5), while food sources in the metalimnion remained largely unused. In a laboratory experiment, ingestion rate of juvenile Daphnia was further reduced by an unexplained effect of the presence of Chaoborus at low food levels (Ramcharan et al. 1992), which might have
also played a role in my enclosure experiments. In combination with the upward migration in presence of *Chaoborus*, this might considerably add to the cost of the defense in nature. As a reduced portion of the water column is used, *Daphnia* are more crowded and have to compete more strongly for available food.

An additional factor that might have contributed to the cost of antipredator defense due to upward migration in response to *Chaoborus* was detrimental UV-radiation (Williamson 1996). Harmful radiation can penetrate down to about 1 m in lakes that have a dissolved organic carbon content comparable to Costello Lake (Leech and Williamson 2000). This can reduce *Daphnia* survival and reproduction (Grad et al. 2001). The neck-spine production itself probably did not contribute to lower population growth. This clone did not experience a reduced fertility rate in previous laboratory experiments due to *Chaoborus* kairomone exposure, in which neck-spine production exceeded the induction observed in the field (Chapter 2). The main reduction in population growth of the ghost predation treatment took place between 4 and 8 days after predator introduction. Other unmeasured changes in life-history parameters (e.g., larger but fewer neonates) could also have contributed to reduction in population growth. For example, the responsive clone did show a significant increase in neonate size as a response to *Chaoborus* kairomone in the laboratory (Chapter 2).

Xenobiotics have been shown to cause neck-spine production and other antipredator responses commonly found to *Chaoborus* kairomone. Therefore, xenobiotics might also contribute to a reduction in population growth even in the absence of *Chaoborus* predators or act synergistically with the kairomone (Dodson et al. 1995; Hanazato 1998; Hanazato 2001).

In summary, I speculate that the reduction in population growth caused by *Chaoborus* kairomone is a combined effect of increased body size, effects of UV radiation, crowding, and
competition for limited food resources in the upper water layer. Reduction in population growth by 32% as a consequence of antipredator defenses seems high. Out of 37 clones, this *Daphnia* clone showed the strongest reaction towards *Chaoborus* kairomone in the laboratory (Chapter 2) and perhaps the cost I estimated is above average. Since this is the first study attempting to measure the costs of *Daphnia* antipredator behavior under near-natural conditions, this value cannot be compared to any other estimates in the literature. Hopefully, further field studies will soon be able to put that value into perspective.

In the laboratory, reduction in population growth due to *Chaoborus* kairomone varied widely between 0 and 28% (Havel 1987; Black and Dodson 1990; Riessen and Sprules 1990; Walls et al. 1991). The variance can be partly explained with different food levels and exposure time to predator kairomone (Riessen 1999). Thus, differences in population growth between the responsive and the non-responsive clone would be expected to be larger at lower food concentrations (Black and Dodson 1990; Riessen and Sprules 1990; Parejko and Dodson 1991; Walls et al. 1991; Weber 2001). However, these laboratory estimates only incorporate morphological and life-history costs into their estimates. Therefore, the higher estimate in my study might be explained by costs due to the behavioral response (upward migration), resulting in enhanced competition for food and exposure to UV radiation.

Furthermore, instead of a direct cost to population growth rate, there could also be an indirect cost involved. Antipredator adaptations might increase the mortality rate to other predators (Havel and Dodson 1987; Tollrian 1995b). So the largest fitness advantage of employing inducible defenses might be the flexibility to adapt to predators that select for opposite traits (e.g., *Chaoborus* and fish). However, I only tested for costs and benefits in response to one predator (*Chaoborus*), and the cost of vulnerability to another predator was
not included in my analysis. Because effective defenses against *Chaoborus* and fish predators require opposite vertical migration patterns, this might be an additional evolutionary factor in favor of inducible defenses that allow flexibility. A similar experimental approach as presented in this study, with multiple *Daphnia* clones that respond to only fish, only *Chaoborus*, both predators, and neither one and that includes treatments with both predators at the same time might reveal the final answer to the nature of the costs of antipredator defenses against invertebrate predators.

The estimated benefits of the antipredator defense of 68 % outweigh the costs, which is the expected outcome (Sih 1982; Dill and Fraser 1984; Kohler and McPeek 1989). Over the course of a season an advantage of 36 % can have a substantial impact on competition between invulnerable and vulnerable *Daphnia* clones, even if the benefits are limited to short periods of time. Riessen (1992) predicts in a modeling approach that the invulnerable *Daphnia* clone is favored above a critical *Chaoborus* density, while the clone without neck-spine production has the advantage below that critical density. The critical *Chaoborus* density is influenced by food availability for the prey, which is related to population growth rate, and degree of spatial overlap between predator and prey.

To get an accurate and reliable estimate about the benefits of antipredator defenses in the field is a difficult task, as comparability between two genetically different clones will always be limited. In this study, differences other than antipredator defense mechanisms between the two *Daphnia* clones were obvious (mean vertical distribution, maximum densities), and other less conspicuous differences might add to this problem. The analysis is further complicated because there are clonal differences in the effects of temperature, food quality and quantity, and their interaction on life-history traits such as development time,

Lastly, *Chaoborus* predation is temperature dependent. In a laboratory study, *Chaoborus* selected *Daphnia* without neck-spines over spined phenotypes at 6 and 11 °C, while the relationship was reversed or obscured depending on clone at 22 °C (Dodson and Wagner 1996).
CHAPTER 4
COSTS AND BENEFITS OF *Daphnia* Antipredator Defense in Presence of Fish in Nature
Introduction

Brooks and Dodson (1965) noticed that large herbivorous zooplankton were absent from lakes with heavy fish predation, while they dominated most fishless lakes and ponds. They developed the size-efficiency hypothesis from this observation, which states that large zooplankton are superior competitors as they gather and assimilate their food more efficiently and, therefore, suppress smaller zooplankton species in the absence of fish predation.

Unquestionably, planktivorous fish play a major role in structuring the zooplankton community and influence their vertical (Zaret and Suffern 1976; Stich and Lampert 1981; Williamson and Magnien 1982; Gliwicz 1986) and horizontal (Malone and McQueen 1983; Pont and Amrani 1990) distribution. As visually guided predators, they prey on the most visible targets (Confer and Blades 1975); typically larger zooplankton that provide the most energy gain as dietary items for the fish (Brooks 1968). However, large zooplankton species have evolved a wide array of defense mechanisms to avoid fish predation by either reducing conspicuousness or decreasing accessibility. Defenses include downward migration into darker environments during daytime (Dodson 1988b; Neill 1990; Ringelberg 1991a; Bollens et al. 1992; van Gool and Ringelberg 1995), reducing body size and increasing transparency (Brooks 1965; Vanni 1987; Dodson 1988a and 1989a; Macháček 1991; Brett 1992; Stibor 1992; Engelmayr 1995; Weber and Declerck 1997) and increasing lengths of tail spines (Dodson 1988a; Spitze and Sadler 1996; Kolar and Wahl 1998) and size of helmets (Tollriean 1994). With these defenses, large zooplankton are sometimes able to maintain high biomass despite presence of planktivorous fish.

Defense mechanisms are of great benefit for zooplankton, as they considerably reduce mortality by fish predation. *Daphnia galeata mendota*, for example, can better escape guppy
predation when long helmets are expressed (Jacobs 1967). However, the benefits do not come cost free. Most studies measuring the cost of swimming in vertical migration agreed that swimming costs were negligible (about 0.1%), in both copepods (Vlymen 1970; Alcaraz and Strickler 1988) and *Daphnia* (Dawidowicz and Loose 1992a). The most substantive cost of antipredator defenses is that a downward migration into darker, colder water exposes zooplankton eggs to sub-optimal temperatures for most of the day. As a result, egg development is slowed, and population growth rate is decreased (Bottrell 1975; Orcutt and Porter 1983; Dawidowicz and Loose 1992b).

This study separates the costs of real fish predation on *Daphnia* from the costs of the defense mechanism only under near-natural conditions. This is the first study to attempt to quantify these costs and benefits in the field. While laboratory studies have certainly been instructive, at some point the importance of any natural phenomenon has to be evaluated in the field. Real-life tests place the costs and benefits of defense in the full context of other ecological constraints and subject to a normal range of environmental variability like UV light and natural food conditions.

Since the cost and benefit of defense may affect prey population growth and secondary production, as well as predator feeding rates, prey defense has the potential to alter the flow of energy through lake food webs. An estimate of costs and benefits under near-natural conditions has the potential to explain cases where the trophic cascade (Carpenter et al. 1985) cannot be observed in nature as predicted in theory. The trophic cascade hypothesizes that changes at the top of a food web will cause an unaltered chain reaction down to the bottom. However, under certain conditions prey defenses are effective enough to buffer and weaken
predation effects (McQueen et al. 1989; Ramcharan et al. 1996; Böing et al. 1998; Benndorf et al. 2000; Ramcharan et al. 2001a and b).

To estimate costs and benefits of Daphnia antipredator defenses in response to fish predators under near-natural conditions, I conducted an experiment using mesocosms. I chose two D. pulex clones, one D. pulex clone responded to fish-kairomone with antipredator defenses (responsive clone) and one did not (non-responsive clone). For each clone I conducted three treatments: (1) D. pulex were placed together with the fish predator (real predation), (2) D. pulex were only placed with fish kairomone (ghost predation), and (3) D. pulex grew without any influence of fish (control). I used the difference in population growth between control and ghost predation of the responsive clone as an estimate of the cost of the antipredator defense. The benefit of the antipredator defense was calculated by subtracting population growth rate of the non-responsive clone from the population growth rate value of the responsive clone in the real predation treatments.

This is the first time that costs and benefits in the Daphnia – fish system have been calculated from population growth values obtained in nature. Costs and benefits of antipredator defenses are urgently needed for improved predictions about energy flow through a food web.

I hypothesize that costs and benefits of antipredator defenses to fish predation are substantial and detectable in a field experiment. I also hypothesize, that the benefits will outweigh the costs of the antipredator defense.

Materials and Methods

I chose two Daphnia pulex clones to use in my field experiments with fish as predator. One clone reacted by migrating downward to kairomone of golden shiner (Notemigonus
*crysoleucas* in previous laboratory experiments (clone HON3, Chapter 2) and will be referred to as the responsive clone from now on. The other clone did not respond to the presence of golden shiner by a depth selection behavior (clone MLR4, Chapter 2), and will be called the non-responsive clone. Both clones were grown to high densities in the laboratory before the beginning of the experiment.

Golden shiner (*Notemigonus crysoleucas*) was chosen as the predator because *D. pulex* clones were previously tested to its kairomone in the laboratory (Chapter 2). They also seemed most suitable for enclosure experiments as they are planktivorous even as adults and had the highest survival rates in preliminary enclosure experiments when compared to other candidate predators, including northern redbelly dace (*Phoxinus eos*), pumpkinseeds (*Lepomis gibbosus*), and yellow perch (*Perca flavescens*) (Böing and Wissel, unpublished data). Golden shiner reaches a length of about 12 cm as adults and is widely distributed along the east coast of North America. They spawn when water temperature is about 20 °C between May and August, attaching their eggs to aquatic vegetation. Maturity is reached at a length of about 7 cm, typically during their second summer. Golden shiner is a typical lake species, an actively swimming fish that moves in schools in the pelagic zone. They are opportunistic midwater and surface feeders that prefer to feed on cladoceran zooplankton (Scott and Crossman 1973).

**Enclosure Set-Up**

My approach to evaluating under near-natural conditions the costs and benefits of antipredator defense employed against fish was similar to the one I described in detail in Chapter 3. The treatments were two clones (responsive and non-responsive) by three predator regimes (control, real predation, ghost predation) in a factorial design. Four replicates, resulting in a total of 24 enclosures were employed. The experiment was conducted during the
summer of 2000 in Costello Lake (Algonquin Park, Ontario, Canada). The polyethylene enclosures had a diameter of 1 m and were about 7.5 m long, resulting in a volume of 5,800 L. On June 24th, enclosures were filled with lake water from 2.5 m depth that was forced through a 100 µm mesh sock, made of thick polyethylene fleece fabric, to eliminate crustacean zooplankton. The next day, approximately 1,000 individuals from one or the other *Daphnia* clones were randomly added to each of the enclosures. Densities were checked regularly by vertical hauls of a plankton net (30 cm diameter, 130 µm mesh size) and adjusted to 1 *Daphnia* L⁻¹ on July 7th. A second, smaller tube (30 cm diameter, 7 m long, 495 L volume) made of 250 µm white mesh screening was suspended within each enclosure. Then, 2 golden shiner (*Notemigonus crysoleucas*) with an average weight of 2.2 g were added to randomly assigned real predation treatments. For the ghost predation treatments, the fish were contained within the mesh bags, while no fish were put into the control enclosures. Fish density was high, but within natural densities for lakes like Costello.

I also added nutrients (10 µg P L⁻¹ as K₂PO₄ and 300 µg N L⁻¹ as NH₄/NO₃) to each enclosure at 14-day intervals to keep the nutrient concentration fairly constant throughout the experiment.

**Sampling Procedure**

Sampling took place in 4-day intervals between July 8th and August 5th. The procedure of how the samples were obtained is described in detail in Chapter 3. Oxygen and temperature profiles were taken on the same day as zooplankton samples. Chlorophyll *a* was determined from the epilimnion (0-3) and metalimnion (3-7 m) on the 2nd, 5th, and 8th sampling date.
Sample Enumeration

Enumeration of zooplankton was the same as in Chapter 3. Measurements of *D. pulex* body size were taken for the first and third sampling date with electronic caliper software (MORPHY, Fowler S225 calipers). Images of zooplankton were projected on a video monitor for measuring. If available, at least 30 *Daphnia* from each depth were measured. Body length was measured from top of the head through the eye to the base of the tail-spine, and tail-spine length was estimated from its base to the tip.

Statistical Analysis

SAS software (version 8.2) (SAS 2002) was used for all statistical evaluations as explained in Chapter 3. Differences in the slope of body length to tail-spine length relationships were tested with regression analysis after both variables were log-transformed.

Results

Temperature conditions were identical between the enclosures and the lake (Fig. 4.1), while metalimnetic oxygen concentrations were lower in the lake during the first half of the experiment (repeated measures ANOVA) (Fig. 4.2). During the second half, oxygen concentrations of the enclosures were indistinguishable from the lake. The epilimnion in both the lake and the enclosures was 3 m deep and remained around 20 °C during the experiment (Fig. 4.2). Below 3 m, temperature dropped rapidly to values just above 7 °C. Oxygen concentration never dropped below 4 mg L\(^{-1}\) in either lake or enclosure, indicating that zooplankton was not oxygen limited at any depths (Davidson et al. 1998; Sell 1998). Chlorophyll *a* concentration is only presented for July 12\(^{th}\), as this is the time period during which *Daphnia* in the control enclosures showed positive population growth rates. The concentrations lay between 10 and 20 µg L\(^{-1}\) except in the epilimnion of the real predation
Figure 4.1: Temperature profiles for the different sampling dates.
Figure 4.2: Oxygen profiles for beginning, middle, and end of the experiment
treatment of the non-responsive clone, where chlorophyll $a$ levels significantly exceeded other treatments ($p = 0.01$, repeated measures ANOVA) with $24 \mu g$ L$^{-1}$. There was a tendency for chlorophyll $a$ values to be higher in the metalimnion than the epilimnion for the enclosures with the responsive clone (Fig. 4.3).

The tail-spines were longer for the responsive clone compared to the non-responsive clone and the slope of the body length to tail-spine length relationship was steeper for the responsive than for the non-responsive clone (Fig. 4.4).

The vertical distribution during daytime (when *Daphnia* were most vulnerable to predation by planktivorous fish) shows that most *D. pulex* were found only in the deepest water layer (5-7 m) in the real predation treatments (Fig. 4.5). The responsive and the non-responsive clone mean vertical positions were significantly deeper in the real predation treatments than either the controls ($p = 0.0002$ and 0.02, respectively, 2-way ANOVA) or ghost predation ($p = 0.002$ and 0.005) treatments. However, only the responsive clone seemed to migrate actively downward in the presence of fish kairomone (ghost predation) ($p = 0.03$), while no significant difference in mean vertical distribution could be found in the non-responsive clone. During nighttime, the difference in distribution between the control and ghost predation treatments of the responsive clone disappeared. Only in the real predation treatments did *Daphnia* of both the responsive and non-responsive clones remain significantly deeper in the water column than in either the controls ($p = 0.01$ and 0.0007, respectively) or ghost predation ($p = 0.03$ and 0.0006) treatments.

Costs of the antipredator defense were only detectable for the beginning of the experiment when populations were growing steadily. Therefore, population growth rate was
Figure 4.3: Chlorophyll a (< 30 µm) distribution on July 12th for responsive clone (top) and non-responsive clone bottom; control – gray bars; ghost predation – white bars; real predation – black bars
Figure 4.4: Body length to tail-spine relationship for responsive (+) and non-responsive (-) clone.
Figure 4.5: Vertical distribution of *Daphnia* on July 16th at day and night top graphs represent responsive (+) clone and bottom graphs non-responsive (-) clone; daytime distribution is shown at left, nighttime distribution at right control – gray bars, ghost predation – white bars, real predation – black bars
calculated for the time period between July 6th and July 12th. Population growth of *Daphnia* was reduced by 16 % in the responsive clone as a result of fish kairomone (control, responsive clone – ghost, responsive clone) \((p = 0.053, 2\text{-way ANOVA})\) (Fig. 4.6). This reduction is an estimate of the cost of predator avoidance. The responsive clone experienced 35 % less mortality due to fish predation than the non-responsive clone \((p = 0.02)\) (real, responsive clone – real, non-responsive clone), which represents the benefits of the antipredator defense.

**Discussion**

The temperature difference between the epi- and metalimnion in the enclosures was certainly large enough so that vertical position would strongly influence egg development time of *Daphnia* (Bottrell 1975). Oxygen concentration hardly differed between the surface and bottom of the enclosures and was similar across treatments. As values stayed above the limiting concentration for *Daphnia* at all times, oxygen levels should not have influenced vertical distribution.

Supporting my hypotheses, I estimated the cost of defenses against predators as a 16 % reduction in population growth rate and the benefits of lessened mortality loss to be 35 %. The cost can most likely be assigned to the downward migration of the responsive clone in the ghost predation treatment. A larger portion of that *Daphnia* clone remained in the hypolimnion where their eggs were exposed to considerably lower temperature, which causes a large increase in egg-development time (Bottrell 1975). Additionally, sub-optimal food conditions in the hypolimnion might further restrict maximum growth of those individuals as a consequence of the behavioral defense.

The benefits of defense probably resulted from decreased mortality due to fish predation of the responsive clone compared to the non-responsive one. Secchi depth was
Figure 4.6: Population growth rate of *Daphnia*. Values were calculated from July 6\textsuperscript{th} to July 12\textsuperscript{th}; responsive clone is shown on left side and non-responsive clone on right of graph; control – gray bars, ghost predation – white bars, real predation – black bars
typically below 3 m in the real predation treatments (Chapter 5), which should have limited
the foraging capability of golden shiner in deeper water layers. However, only the responsive
cloned reduced its mean vertical position and was able to take advantage of the low-light
refuge. As expected, the benefits of antipredator behavior did exceed the costs (Gabriel and
Thomas 1988). I found that the benefits of 35 % less population growth decrease due to
mortality caused by fish predation to exceed the costs of vertical migration by 19 %.

The body length to tail-spine length relationship showed that the responsive clone was
morphologically better defended against fish predation than the non-responsive clone. A
longer tail-spine of Daphnia is known to deter predation by fish that are smaller than 50 mm
(Kolar and Wahl 1998). Both initial tail-spine length and tail-spine growth in relation to body
size were higher in the responsive clone than the non-responsive clone. Similarly, only the
responsive clone showed a behavioral response to the threat of fish predation by downward
migration. This indicates that (1) the kairomone diffused through the mesh in the ghost
predation treatments, and (2) the non-responsive clone was indeed non-responsive as
indicated by previous laboratory experiments (Chapter 2).

Because the responsive clone exhibited both a morphological defense and a behavioral
defense, it would have incurred the costs and benefits of both defenses. I did not specifically
estimate the costs and benefits of the morphological defense alone and no literature values are
available. However, I believe that these costs would have been negligible or small, similar to
costs caused by neck-spine production (Chapter 3). In terms of benefits, the tail-spine would
be an effective defense only against juvenile fish, not the adult fish used in my experiments.

The cost estimate of 16 % caused by reduced population growth rate is low, compared
to the only other published values by Dawidowicz and Loose (1992b). They found a reduction
of 5% in population growth rate by exposing a *Daphnia magna* clone only to fish (*Leucaspius delineatus*) kairomone. However, when they simulated natural temperature stratification in 1-m tall columns in the laboratory, *Daphnia* responded with fish antipredator vertical migration when exposed to kairomone and population growth decreased by 63% relative to controls. Although their ‘epilimnion’ temperature of 21.7 °C is comparable to the epilimnion temperature of my experiments, the temperature drop to 9.6 °C at the bottom of their columns was not quite as large as the temperature of below 8 °C in the hypolimnion of my enclosures. Yet, the costs found by Dawidowicz and Loose (1992b) are four times larger than the costs I found in the field.

This enormous difference between my field results and the published laboratory results might be due to several factors. First, Dawidowicz and Loose (1992b) used a clone of *Daphnia magna*, which is the largest *Daphnia* species and the most vulnerable to fish predation. All individuals responded with an extreme diel vertical migration when exposed to fish kairomone, while the control animals always stayed above the thermocline. In contrast, in my experiments, the behavioral responses were not as extreme. Even in my controls, about 25% were exposed to temperatures sub-optimal for growth. In the ghost predation (kairomone) treatment that portion increased to 40% but this change from the controls was not nearly as dramatic as found in the experiment by Dawidowicz and Loose (1992b).

Besides temperature, food regime may also have reduced the cost of the defense relative to the laboratory estimates of Dawidowicz and Loose (1992b). In their experiment, a constant high food concentration of high quality, easily ingestible and digestible cells (2 mg C L⁻¹ of *Scenedesmus acutus*) was provided, guaranteeing maximum growth rate in the control and strong vertical migration in the fish kairomone treatments (Dini and Carpenter 1992).
Conversely, in my experiment, *Daphnia* were exposed to natural conditions of food quantity and quality that also differed between the epi- and the meta-limnion. Under low food conditions *Daphnia* are more willing to accept a higher predation risk and reduce migration amplitude (Dini and Carpenter 1992). In contrast, Riessen (1992) predicted that morphological defenses might be more advantageous when food is limiting. However, this might be different between behavioral and morphological defenses. With altered depth selection behavior, the water layer with the highest food concentration might be chosen, while morphological defenses would seem advantageous only if costs are small and mortality by predation exceeds or equals reproductive output.

The edible fraction of algae with values between 10 and 20 µg chlorophyll *a* L⁻¹ was fairly low, about the level typical of mesotrophic lakes (Wetzel 2001). In eutrophic lakes, chlorophyll *a* concentrations can be an order of magnitude higher. The chlorophyll *a* concentration was slightly increased in the metalimnion of enclosures with the responsive clone, which might have influenced depth selection behavior of the *Daphnia* (Johnsen and Jakobsen 1987; Pijanowska and Dawidowicz 1987). The increased chlorophyll *a* abundance in the epilimnion of the real predation treatment with the non-responsive clone is best explained by fast elimination of the largest *Daphnia* by fish predation. Loss of these efficient grazers relieved the edible fraction of algae from grazing pressure and allowed algae biomass to increase, while in the other predator treatments reduced algae biomass is most likely evidence of grazing by *Daphnia*.

In addition to food availability and temperature, several other factors might influence the strength of vertical migration and therefore the costs and benefits of avoidance of visually guided predators in natural lakes. One factor is seasonal variation of fish predation. Because
antipredator reactions are inducible and concentration dependent, *Daphnia* should only display the defense and incur the costs when needed. For example, in Lake Marsseveen, no migration pattern in *Daphnia* was observed in springtime, but did occur in early summer when large shoals of juvenile perch were feeding (Ringelberg et al. 1991). Competition and factors other than fish predation might dominate *Daphnia* population dynamics during parts of the year with low fish abundance.

Another factor that probably would also influence costs and benefits of antipredator defenses is the competitive outcome between responsive and non-responsive *Daphnia* clones and the strength of expressed antipredator defense. Strength of antipredator defense is subject to clonal variability (Chapter 2). In order to ensure detection in the field, my cost and benefit estimates were made with a clone that strongly reacted with the expected antipredator defenses and another that seemed the most unresponsive. Also prey density might impact perceived predation risk by the individual and strength of antipredator response (Tollrian, pers. comm.). At high prey density, there is a dilution effect and the individual animal is at a lower predation risk. In laboratory experiments, crowded *Daphnia* were shown to express morphological defenses to a lesser extent (Tollrian, unpub. data).

Furthermore, low dissolved oxygen concentrations could either eliminate a hypolimnetic refuge for *Daphnia* (Davidson and Kelso 1997; Ramos-Jiliberto and Zuniga 2001) or *Daphnia* might have to produce hemoglobin (Sell 1998), which could increase the cost of fish avoidance (Homer and Wallert 1983; Hanazato and Dodson 1995; Hanazato 1996; Kohl et al. 1997).

Predation by planktivorous fish is light dependent (Vinyard and O’Bien 1976; Confer et al 1978; Jacobs 1978). In some lake types, zooplankton might not migrate, if Secchi depth
is shallower than the epilimnion (Dodson 1990). Even large zooplankton like *Daphnia* might then be at an advantage, if they stay up in the warmer, food rich water layer (Pijanowska and Dawidowicz 1987). Therefore, lake clarity can influence the amplitude of diel vertical migration in large zooplankton. A direct positive correlation between Secchi depth and migration amplitude of *Daphnia* exists (Dodson 1990). In lakes with restricted visibility (high turbidity, color, or dissolved organic carbon) visually guided fish predators are at a disadvantage and decrease as a factor in structuring the zooplankton community (Wissel 2001). In lakes with a low-light regime, *Chaoborus* as the dominating predator should be a more important factor in structuring diel vertical migration patterns of zooplankton (Chapter 3).

Lastly, *Daphnia* susceptibility to fish predation changes over its lifetime. *Daphnia* are less vulnerable as juveniles and most vulnerable when carrying conspicuous eggs. For example, ovigerous copepods migrate less and stay at deeper depth than non-ovigerous females (Bollens and Frost 1991). Therefore, costs and benefits of antipredator behavior at the level of the whole population can vary with the ratio of juvenile to adult animals.

In summary, this study provides an estimate of the magnitude of costs and benefits of *Daphnia* antipredator behavior in nature. More studies that estimate costs and benefits are needed to fully understand the effects of factors like food abundance, clonal diversity of *Daphnia*, lake-clarity, and prey and predator densities.
CHAPTER 5

THE STABILIZING ROLE OF PREY DEFENSE IN A PELAGIC FOOD WEB
Introduction

The understanding of stability – the ability to predict how a system will change in the future – is a fundamental goal in ecology. General findings that are applicable to a wide range of ecosystems have been sought for a long time. For decades, stability was subjected to intense research that generated much controversy. This resulted in a wide range of definitions for stability. The terminology ‘stability’ has been used as a synonym for connectance (MacArthur 1955), domain of attraction (Holling 1973), resilience, variability, persistence, and resistance (Pimm 1991), constancy (Lampert and Sommer 1993), reliability (Naeem 1998), and temporal stability (Tilman 1999).

An overwhelming number of studies focused on the controversy of the hypothesis that a high species diversity increases the ecosystem stability (diversity-stability hypothesis) (MacArthur 1955; May 1972; Goodman 1975; McNaughton 1977; Pimm 1984; McNaughton 1993; Givnish 1994; Tilman 1996; Grime 1997; Ives et al. 1999; Tilman 1999; McCann 2000) or the resistance and resilience of an ecosystem to perturbations like nutrient pulses (Reinertsen and Langeland 1982; Cottingham and Schindler 2000) and changes in predator system (Hrbáček et al. 1961; Hurlbert et al. 1972; Stenson et al. 1978; Carpenter et al. 1992; Pérez-Fuentetaja et al. 1996).

Predators have a major influence on their prey population, sometimes leading to dramatic alternating cycles of peaking predator and prey population (Lotka 1925; Volterra 1926; Haukioja et al. 1983; Keith et al. 1984) even across large geographic areas (Dodd 1959; Lindström et al. 1994). Attempts to simulate these predator-prey cycles have proven difficult in both modeling and laboratory experiments, often resulting in extermination of prey and
predator populations (e.g., Hastings and Powell 1991; Begon et al. 1996; McCann et al. 1998). Stability is achieved only through one of three different techniques: (1) In mathematical simulations, predation functional response is modeled as an S-shaped function of prey density, so that consumption is disproportionally relaxed as prey decline. (2) Either the predator, the prey, or both are continually re-introduced in both mathematical models and laboratory chemostats, thereby preventing extermination. (3) Some fraction (up to 100 %) of the prey population is made invulnerable to the predator. With a fixed rate of conversion from the invulnerable to the vulnerable state, prey cannot be exterminated and the predator can never starve.

In nature, many different types of antipredator defenses confer invulnerability on prey. These include refuges, morphological defenses and life-history alterations, which are commonly found in presence of the predator (Tollrian and Harvell 1999). One particular type of defense, inducible defenses, should be best suited to stabilizing predator-prey dynamics. While inducible defenses confer protection, they also allow invulnerable prey to become vulnerable, once predation risk is reduced. Therefore, prey are most protected when predator populations are highest and are most able to exterminate the prey. This prevents elimination of prey followed by a crash in predator population.

Surprisingly, in only a few cases have the effects of invulnerable prey on population and community stability been investigated. So far, evidence for increased stability due to prey defenses was only raised in theoretical models (Abrams and Walters 1996; Chase 1999) but never actually confirmed in nature.

The paradox of inducible defenses is that prey production may be higher under predation pressure. Predators, by consuming prey, may actually benefit prey populations and
have stabilizing effects. Although predation is always a threat to the individual, production of
the prey population as a whole might be enhanced. Examples, where grazing of herbivores
increases the rate of nutrient turnover thereby enhancing plant productivity have been
described. This increased primary production was observed for algae (Drenner et al. 1984),
savannah grass (McNaughton 1979), or delta grasses (Zellmer et al. 1993). Grasslands can
also benefit in community composition from its predator the African elephant (Laws 1970).
By their feeding activities, the elephants destroy shrubs and small trees, opening up more
space for the grassland to extend.

I hypothesize that population stability of both prey and predator as well as energy flow
from the prey to the predator is enhanced, if prey employ inducible defense mechanisms.
Furthermore, I hypothesize that ‘the community in which herbivores are held down in
numbers and in which the producers are resource-limited will be most persistent’ (Hairston et
al. 1960).

To test the hypotheses, I used the well-studied Daphnia – planktivorous fish system.
Planktivorous fish are one of the main predators on Daphnia populations. Daphnia are able to
mitigate effects of fish predation by reducing visibility to optically-guided fish. They move
down into darker environments during daytime (Dodson 1988b; Neill 1990; Ringelberg 1991;
Bollens et al. 1992; van Gool and Ringelberg 1995), reduce body size and increase
transparency (Brooks 1965; Vanni 1987; Dodson 1988a and 1989a; Macháček 1991; Brett
1992; Stibor 1992; Engelmayer 1995; Weber and Declerck 1997). These antipredator
defenses are in fact very efficient at avoiding fish predation and do make responsive prey
organisms invulnerable to the predator. As there is a cost involved in expressing these
responses (Dawidowicz and Loose 1992b; Chapter 4), the strength of response is positively correlated with prey vulnerability and predator abundance (von Elert and Pohnert 2000).

The *Daphnia* – planktivorous fish system is well-suited to testing my stability hypothesis, because antipredator response among different *Daphnia* clones is variable (from no response to strong responses). By comparing a vulnerable clone versus a clone that is invulnerable to a certain degree, effects of antipredator defenses on community stability can be quantified. I compared temporal stability and change in predator (golden shiner, *Notemigonus crysoleucas*) biomass when enclosed with a *Daphnia pulex* clone that employed antipredator defenses (the ‘invulnerable’ or responsive clone) and a *Daphnia* clone that did not react to the presence of the predator (the ‘vulnerable’ or non-responsive clone). These data were then contrasted to treatments that contained only the *Daphnia* clones without predators.

**Materials and Methods**

For this analysis no new experiments were required. The data from the experiment conducted and described in Chapter 4 were used. A detailed description of the experimental set-up, sampling procedure, and population growth can be obtained in the previous chapter.

**Enclosure Set-Up and Sampling Procedure**

The treatments needed from the previously conducted experiment (Chapter 4) were two clones (responsive (‘invulnerable’) and non-responsive (‘vulnerable’)) x two predator regimes (control, predation) x four replicates. Therefore, only data from 16 (out of 24 total) enclosures were analyzed. Experiments were conducted in mesocosms of 1 m diameter and 7.5 m depth with a volume of 5,800 L. On June 24th, enclosures were filled with lake water and inoculated the following day with approximately 1,000 individuals from one or the other *Daphnia* clones, which were randomly added to each enclosure. When *Daphnia* density
reached 1 *Daphnia* L⁻¹ on July 7th, 2 golden shiner (*Notemigonus crysoleucas*) with an average weight of 2.2 g were added to randomly assigned predation treatments.

Sampling took place in 4-day intervals between July 8th and August 5th. Zooplankton was caught with a plankton net (30 cm diameter, 130 µm mesh) and oxygen and temperature profiles as well as Secchi depth were also taken. Chlorophyll *a* was determined from the epilimnion (0-3) and metalimnion (3-7 m) on the 2nd, 5th, and 8th sampling date.

**Stability Estimates**

For my purposes, I needed a measure to quantify population stability for the short duration of my experiment. The question to be answered was not related to reaction of an ecosystem after perturbation (resilience of resistance) but to estimate how much invulnerable versus vulnerable prey populations would vary over time. To answer that question, temporal stability (*Sₜ*) (Tilman 1999) seemed the most appropriate measure. Temporal stability (*Sₜ*) was calculated with the following formula modified from Lehman and Tilman (2000):

\[
Sₜ = \frac{\sum A_i}{\sqrt{\sum \text{Var}_i}}
\]

where each population (*Daphnia* populations in my case) was denominated by *i*, *A₁* is the normalized abundance (abundance for each sampling date divided by its respective mean abundance) of population *i* and *Varᵢ* represents the variation in abundance over time of the same population. This results in a dimensionless measure for temporal stability. Thus the population which fluctuates least in abundance over time will have the highest values for temporal stability. Without any variance, temporal stability would become maximal (infinite), while with a variance much larger than the mean, temporal stability would approach zero. The advantage of this measure is that it considers nonlinear population dynamics and is able to contrast populations that are or are not in an equilibrium state.
Although predator abundance cannot fluctuate in my experiments (due to the short

time period it can only decrease due to mortality), predator growth can. Therefore, predator

biomass, rather than abundance, was chosen as my index of predator density. Fish growth was

measured by taking the difference in weight at the beginning and end of the experiment of

each fish. At the beginning of the experiment a plastic container with water was tarred to zero

on an electronic balance. Previously, golden shiner had been caught from a nearby lake (Lake

Eos) with minnow traps and kept in a flow-through system in the laboratory for 1-2 weeks.

Each fish was caught with a hand net, added to the plastic container, and weight was recorded

in 0.1 g. I added a larger and a smaller fish to each enclosure so they could easily be identified

at the end of the experiment. When the experiment was terminated after five weeks on August

9th, all fish were collected from the enclosures, kept on ice and immediately after returning to

the laboratory I recorded their length and weight.

Modeling Approach

For theoretical exploration of my hypotheses on how antipredator defenses affect

community stability, I simulated the predator-prey interactions in my enclosures using

Ecopath with Ecosim (version 4) software (Christensen et al. 2000). Estimates for growth

rates of predator and prey were directly taken from my enclosure experiments (Table 5.1).

Fish were assumed to only be able to forage in the upper half of the enclosures that provided

enough light. A responsive (‘invulnerable’) *Daphnia* clone was assigned to be 90 %

invulnerable, while for the non-responsive (‘vulnerable’) clone 10 % of the population were

left invulnerable. Food conditions for both prey types were set to values that were well above

limiting levels, so that only predation effects could be isolated.
Table 5.1: Estimates of biomass, production and consumption used for the Ecopath/Ecosim model.

<table>
<thead>
<tr>
<th>Group Name</th>
<th>Trophic Level</th>
<th>Habitat Area</th>
<th>Biomass In Habitat Area (g m$^{-2}$)</th>
<th>P/B (y$^{-1}$)</th>
<th>C/B (y$^{-1}$)</th>
<th>P/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>3</td>
<td>0.5</td>
<td>6.67</td>
<td>0.13</td>
<td>7.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Invulnerable Daphnia</td>
<td>2</td>
<td>1.0</td>
<td>54.06</td>
<td>3.21</td>
<td>14.58</td>
<td>0.22</td>
</tr>
<tr>
<td>Vulnerable Daphnia</td>
<td>2</td>
<td>1.0</td>
<td>54.06</td>
<td>3.21</td>
<td>14.58</td>
<td>0.22</td>
</tr>
<tr>
<td>Edible Phytoplankton</td>
<td>1</td>
<td>1.0</td>
<td>0.90</td>
<td>393.44</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Detritus</td>
<td>1</td>
<td>1.0</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

P = Production  
B = Biomass  
C = Consumption
Statistical Analysis

For statistical analysis of change in chlorophyll a and population dynamics I conducted a repeated measures ANOVA with PROC MIXED using SAS software (version 8.2) (Copyright, SAS Institute Inc., Cary, NC, USA.). Values of temporal stability were analyzed with a two-way ANOVA (clones and treatments as fixed effects) and changes in fish weight with a t-test (SAS 2002).

Results

Measurements of chlorophyll a at the beginning, middle, and end of the experiment show that the values remained low (around 10 µg L⁻¹) in the control treatments (Fig. 5.1). The chlorophyll a concentration increased rapidly in both predation treatments (p < 0.0001, repeated measures ANOVA) but at a faster rate in the enclosures with the non-responsive clone. The total portion of the inedible fraction (> 30 µm) changed little, while the main increase can be attributed to enhanced biomass of the edible fraction (< 30 µm).

Secchi depth were inversely related to chlorophyll a concentrations (Fig. 5.1). Secchi depth was significantly lower in the predation treatments than the controls for both the responsive and non-responsive clone (p = 0.02 and 0.04, respectively, 2-way ANOVA).

Population dynamics reveal that controls for both clones reached their maximum 10 days after the experiments were started (July 16th) at a similar total Daphnia density of 7 L⁻¹ (Fig. 5.2). While the population density of the non-responsive clone crashed immediately after the peak, the responsive clone remained at high population densities for eight more days until July 24th before their population also declined. In the predation treatments, the population of the responsive clone still showed an increase the day after fish addition (July 8th), then decreased for eight days (July 16th) and remained at a constant level of about 0.5
Figure 5.1: Chlorophyll $a$ distribution (left Y-axis) and Secchi depth (right Y-axis) at beginning, middle and end of experiment. Error bars represent ± 1 standard error. C = control and P = predation enclosures; (+) = responsive and (-) = non-responsive clone; solid bars = chlorophyll $a$ fraction $< 30$ $\mu$m, striped bars = chlorophyll $a$ fraction $> 30$ $\mu$m.
Figure 5.2: *Daphnia* population dynamics for control and predation treatments for responsive clone (top) and non-responsive clone (bottom). Error bars show ± 1 standard error.
Daphnia L\textsuperscript{1} throughout the experiment. The biomass of the non-responsive clone continually decreased in the fish predation treatment until it reached values that approached zero 18 days into the experiments (July 24\textsuperscript{th}). Predation treatments varied significantly from controls (p < 0.0001, repeated measures ANOVA). Calculations of temporal stability revealed that the responsive clone under fish predation was the most stable population (ST = 13), while in control enclosures stability was strongly reduced (ST = 6) (p < 0.0001, 2-way ANOVA). The non-responsive clone under fish predation had the lowest stability index (ST = 2) (p < 0.0001, 2-way ANOVA).

Fish that were enclosed with the responsive clone gained about 0.2 g weight on average over the course of the experiment (Fig. 5.3). Fish that were only able to feed on the non-responsive Daphnia clone all lost considerable weight (p = 0.003, t-test).

The trends in fish growth were reproduced with the Ecopath with Ecosim model (Christensen et al. 2000) (Fig. 5.4). The responsive clone was able to sustain a large biomass and the fish first gained weight and then maintained their biomass. The non-responsive clone crashed at the beginning of the simulation, quickly approaching biomass values of zero. In this theoretical model, Daphnia biomass increased slightly after the crash and stayed at low biomass values. The fish biomass constantly decreased over the simulation period of one year.

**Discussion**

In the controls, the edible chlorophyll a fraction of the non-responsive clone stayed fairly constant, while the responsive clone significantly decreased chlorophyll a before the chlorophyll a concentration increased again. This result might be due to different abilities of the Daphnia clones to use low algae concentrations as a food source and to transfer energy into growth and reproduction (Tessier et al. 2000). Support for this theory is found in the
Figure 5.3: Weight difference of fish from beginning compared to end of experiment. Error bars show ± 1 standard error.
Figure 5.4: Ecopath with Ecosim model, simulating change in *Daphnia* biomass (left Y-axis, gray line) and fish biomass (right Y-axis, black line) over the duration of one year for ecosystems with responsive *Daphnia* clone (top) and non-responsive *Daphnia* clone (bottom).
population dynamics data. Despite lower food levels, the responsive clone sustained high biomass for a much longer period of time than the non-responsive clone. In the predation treatments, the chlorophyll $a$ concentration (especially the edible fraction) constantly increased over the period of the experiment. This increase was even stronger for the non-responsive than for the responsive clone. This result was most likely due to the decimation of the large herbivorous filter-feeders by fish predation. The predation impact was strongest for the non-responsive clone.

Both control enclosures showed a boom-and-bust cycle. Both $Daphnia$ populations increased in biomass, apparently overexploited their food source, and their populations crashed. The non-responsive $Daphnia$ population, that was completely vulnerable to fish, was basically eliminated by the end of the experiment. The highest stability was found for the responsive clone in the presence of fish predation. The fish predators kept the $Daphnia$ in check and prevented them from overexploiting their own food source, thereby stabilizing their population. The prey also stabilized predator growth because the antipredator behavior of the responsive $Daphnia$ clone made it impossible for the fish predators to extinguish their prey. As the invulnerable portion of the responsive clone could continue to reproduce and provide the fish predators with food, this outcome led to an increase in fish biomass.

Another factor that might enhance stabilizing effects between invulnerable $Daphnia$ and fish is an indirect relationship between fish and chlorophyll $a$ levels and the effects on Secchi depth. As fish reduce $Daphnia$, the zooplankton community grazing rate on phytoplankton drops (Carpenter et al. 1985). Effects of increased algae levels are twofold: (1) They reduce visibility (as measured by Secchi depth) in the water column. This would negatively affect predation rate of fish (Vinyard and O’Brien 1976; Confer et al 1978; Jacobs
1978; Dodson 1990; Wissel 2001), thereby increasing the refuge area and perhaps indirectly decreasing danger for *Daphnia*. (2) High food concentrations enhance productivity of *Daphnia*. Therefore, at low *Daphnia* populations they should show highest production rates because of both low predation and abundant food.

The fish that were in the enclosures with the non-responsive clone probably decimated their prey to levels below a minimum threshold for positive fish growth near the beginning of the experiment. That left them without food for the latter part of the experiment. They lost weight, likely as a result of insufficient food.

I found evidence that prey defenses do enhance energy flow through a food web. In the treatment that paired fish with the non-responsive clone, the enhanced algae biomass was not transferred to the highest trophic level (fish) as the link between phytoplankton and fish – herbivorous zooplankton – was eliminated. In contrast, the responsive clone managed to maintain high biomass throughout the experiment. It was able to use the high algae biomass with high reproductive output and the energy was transferred through the *Daphnia* to the planktivores. In the control enclosures very unstable boom and bust cycles were observed.

The effects of antipredator defenses at increasing fish biomass and sustaining a *Daphnia* population, could be closely simulated with an Ecopath with Ecosim model (Christensen et al. 2000), although the time frame differed considerably from the observations made in nature. The model predicted a long increase in *Daphnia* biomass of the responsive clone followed by oscillations, while in nature no oscillations were observed. However, the lack of oscillations might be due to the short observation time. Oscillations would be expected as a common predator-prey phenomenon over time.
In addition to protection from predation, inducible defenses have another possible stabilizing effect. These defenses have costs in terms of population growth. As the predator population increases, prey availability decreases because of both increased use of the defense and reduced prey population growth caused by the associated cost of the defense.

Other factors are expected to contribute to stability of prey populations. Functional response in combination with prey defenses can result in an important coupling mechanism. In a sigmoidal functional response predation decreases disproportionally once the prey density falls below a saturation limit (Murdoch and Oaten 1975). Strength of inducible prey defenses varies with predator abundance, with the strongest antipredator defenses being expressed at high predation risk (von Elert and Pohnert 2000). When prey biomass is high and predator biomass is low, predators should be efficient at catching prey and increase in biomass, resulting in a reduction of the prey. As prey diminish, their own food source will recover, while predators have difficulty finding and ingesting them. In turn, predators should either switch to alternative prey or their own population size should decline because of sub-optimal food conditions. This should give the prey population a chance to recover as a combined effect of increased growth rate (no food or competition and space limitation) and reduced mortality due to predation.

A similar effect may be found in regard to light intensity (Townsend and Risebrow 1982). Less visibility due to turbidity or color probably increases search time for fish (Vinyard and O’Brien 1976; Confer et al 1978; Jacobs 1978). This low light refuge should prevent prey extermination thereby supporting a higher and more stable biomass of large zooplankton. Some field evidence supports this hypothesis (Wissel 2001). However, I would only support this theory when the prey employs defense mechanisms at the same time.
Predators might also enhance community stability by enhancing species richness in a lake. By their presence alone they can create two or more different ecological niches that can support prey with a range of vulnerabilities. Without predation, competition is typically the main force structuring a zooplankton community and just few species of superior competitors are able to co-exist (Paine 1980). If fish predators are present, several different predation-mediated habitats might be created. The epilimnion with highest risk of fish predation might only be occupied by small or very transparent organisms. Other zooplankton that can migrate on a daily basis, can avoid the upper water layer during daytime. Larger species and individuals might have to stay in the hypolimnion all the time. In nature, this scenario does seem to exist. Smaller *Daphnia* species that migrate in the presence of fish can co-exist with larger, superior competitors as long as fish are present (Leibold 1991). The water column is not as homogenous as previously believed. The manifold species diversity found in lakes, once described as the paradox of the plankton (Hutchinson 1961), can be explained with the many microenvironments found, each providing different niches. Each layer in a stratified lake, the epi-, meta-, and hypo-limnion, can be subdivided into various habitats. Depending on lake clarity, UV light can penetrate the parts of the epilimnion and even influence the hypolimnion in some cases (Williamson et al. 1996). Food, competition, predators, and other physical chemical factors not only vary vertically throughout the water column but also horizontally. The littoral zone cannot be compared with the pelagic zone. And on top of all, most factors fluctuate on a diel cycle (Dumont 1968) and over time. All of these factors combined turn a lake into an environment with lots of different habitats that change over time.

In summary, I found that inducible prey defenses allow prey to switch between vulnerable and invulnerable modes. Inducible defenses stabilized a prey population as well as
the predator population relative to vulnerable prey. Furthermore, inducible antipredator
defenses might potentially prevent a prey from over-exploiting its resource base by reducing
prey population growth rate. They also prevent a predator from over-exploiting its resource
base and increase, rather than decrease the total energy flow through a food web.
CHAPTER 6
SUMMARY AND CONCLUSIONS
This dissertation covers a wide range of experimental scale from vials in a growth chamber, to long tubes in a laboratory, to enclosures in real lakes. The importance of my findings also covers a wide range from details about the variation in antipredator defenses among *Daphnia* clones to implications for the functioning of lake food webs. Below, I recap my important findings, explain their significance, and outline what I believe are the important studies that should follow from my work.

In Chapter 2, I explored the reaction norm of *Daphnia* antipredator behavior to both *Chaoborus* and fish predators in the laboratory. Furthermore, I investigated linkages among behavioral, morphological and life-history responses of different *Daphnia pulex* clones to *Chaoborus* predators. I found that variation among clones in behavioral, morphological, and life-history defenses was high, and that there was no correlation among these defenses.

My data suggested that clones possessed a suite of defenses perhaps as a result of local adaptation to prey vulnerability, particular lake habitats, predator regime and density. Expression of different combinations of behavioral, morphological, and life-history responses imply the existence of multiple fitness optima.

In Chapter 3, taking advantage of the variation of antipredator defenses among *Daphnia* clones and the fact that antipredator defenses are mediated by predator chemicals, I chose two *Daphnia* clones from previous laboratory experiments to estimate the costs and benefits of defenses against *Chaoborus* in the field. I conducted enclosure (1-m diameter x 7-m deep) experiments using the two *Daphnia* clones (responsive and non-responsive to predator chemical), three predation treatments (control, ghost predation, and real predation) and four replicates, resulting in a total of 24 enclosures. Controls had no predators, ‘real’ predation exposed each *Daphnia* clone with *Chaoborus* and in ‘ghost’ predation, *Chaoborus*
were sequestered in a mesh tube away from *Daphnia*. The mesh allowed predator-chemical to mix with the *Daphnia* but restricted predation by *Chaoborus*. I measured population growth \( (r) \) of both *Daphnia* populations. Cost of predation was calculated as responsive clone \( (r) \) in control treatments – responsive clone \( (r) \) in the ghost predation treatments. Benefit was calculated as responsive clone \( (r) \) – non-responsive clone \( (r) \) in the real predation treatments. I found that, *Daphnia* population growth of the responsive clone was reduced by 32 % when the antipredator defenses were employed. However, I observed an increase in population growth of 68 % of the responsive clone compared to the non-responsive clone in the presence of the predator. This growth indicates the benefit of the defense. Undefended *Daphnia* experienced 68 % less population growth due to greater predation.

Behavioral defense against fish predators is opposite to *Chaoborus*. *Daphnia* must migrate downward migration in response to fish, which exposes them to colder water and increases egg-development time, thereby reducing fitness \( (r) \). To determine the costs and benefits of antipredator defenses against fish, I conducted enclosure experiments with an identical set-up described above, only using fish instead of *Chaoborus* as predators. In this case I found the cost of the antipredator defense to be 16 % and the benefits 35 %.

For both predators, the benefit of antipredator defense did exceed the cost, but cost was still substantial. Therefore, it makes ecological sense for *Daphnia* to employ antipredator defenses only when the predator is present and a reliable signal is given off to the prey. This is likely why antipredator behavior is an inducible rather than a fixed defense.

Intriguingly, I also found that the fish predator (golden shiner, *Notemigonus crysoleucas*) had benefited from the antipredator behavior of its prey. In my experiments of five weeks duration, the planktivorous fish lost weight (-0.375 g) when preying on the non-
responsive clone. The fish ate most of the prey within the first few days and then were left with nothing to eat. With the missing zooplankton link, the energy from the algae could not be transferred to the level of the highest predator anymore. In the treatments with the responsive clone, behaviorally-protected *Daphnia* were able to sustain a higher population density providing fish with a stable food supply, so the fish tended to gain weight (0.225 g). Despite the substantial associated costs on growth rate of the prey population in near-natural environments, inducible prey defenses were shown to benefit not only the prey but also the predator population. These findings could be supported using a small model.

I also found the corollary - that fish predation stabilized the prey population. In predation treatments with the responsive clone stability was highest, while the fish eliminated the non-responsive clone. In control treatments, both responsive and non-responsive *Daphnia* overexploited their food resources and showed typical boom and bust cycles. These findings are very different from the trophic cascade hypothesis (Carpenter et al. 1985), or indeed, most ideas about the effects of predators (Sih 1985).

Future research should explore changes in life-history traits over several generations to understand possible maternal effects and the limits to prey response. Furthermore, focus on a multi-predator environment (planktivorous fish, invertebrate predators) is essential as well as the study on *Daphnia* deal with conflicting demands of avoiding these different predators. The importance of environmental factors (temperature, UV-light, sub-optimal food conditions) that contribute to the cost of the antipredator defense should also be quantified. Identification of the kairomone and the influence of water current (Finelli et. al. 2000) in its distribution need to be investigated.
My results show that it is essential for ecologists to fully understand inducible defenses and their impacts on energy flow through food webs, so that more accurate models can predict productivity, population dynamics, and community stability. This will eventually give us better insight in general population and community regulating factors and facilitate lake management for a better environment.
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