Chemical Cue Suites in Lake Zones: Their Effects on the Horizontal Migration of *Daphnia*

Andy Haines, Romi Burks, David Lodge

Abstract: *Daphnia play an integral role in lake functioning, as they filter large amounts of algae and serve as an important food source for both fish and invertebrates. Daphnia have been shown to undergo diel horizontal migration into the littoral zone of shallow lakes in order to avoid predation. Water from four different lakes on the UNDERC property in the Upper Peninsula of Michigan was used as treatments, and daphnids were exposed to chemical cues from both the littoral zone and the pelagic zone. Daphnids tended to migrate away from pelagic water in all cases, and when both pelagic water and littoral water were presented together, daphnids always migrated towards the littoral water. However, when littoral zone water was presented alone, daphnids had varying responses, most likely due to differences in the biotic makeup of the various lakes. These results support the idea of daphnid horizontal migration in the absence of a vertical refuge, and also shed some light on the idea of daphnid response to multiple predator systems.*

Introduction:

Daphnia are important filterers of algae, which helps to deter eutrophication in freshwater ecosystems (Perrow et al 1997). Due to their abundance in lakes and ponds as well as their large size in relation to other planktonic organisms, they serve as an important food source for a wide variety of predatory organisms. The chemical cues emitted by these predators serve as important signals to daphnids, and they often adjust their life history, morphology, or movement in response to these cues (Dodson 1989, Black 1993). For example, when exposed to *Chaoborus* cues, daphnids delay their time until first reproduction and develop neck teeth in order to reduce the likelihood of predation (Black 1993). In deep lakes, daphnids respond to pelagic predatory chemical cues by undergoing verticle migration and hiding in the colder, darker waters of the hypolimnion (Lampert 1993). This migration allows daphnids to avoid visual predators during the daylight hours, while being able to filter algae at night (Lampert 1993). However, in shallow systems, this verticle refuge is not available, so daphnids alternatively may undergo diel horizontal migration (DHM) into littoral zones (Lauridsen and Buenk 1996) in order to seek refuge in macrophytes. The complex structure associated with floating and submerged macrophytes lowers the foraging efficiency of fishes (Diehl 1988, Moss et al. 1998), although many predacious macroinvertebrates are associated with those macrophyte structures. Odonates, macroinvertebrates associated with the littoral zone of lakes and ponds, have been shown to prey efficiently upon daphnids (Pritchard 1964, Blois 1985, Hirvonen 1999, Burks et al. 2001). However, no research has been done in order to investigate the effects of odonate chemical cues upon daphnid behavior.

In order to investigate this question, we carried out a series of experiments to investigate the effects of odonate chemical cues upon daphnid growth, reproduction, and behavior. It has been shown that daphnids respond in different ways to the chemical cues, both individually and in combination, of macrophytes, odonates, and fish. When presented macrophytes alone, daphnids tend to avoid macrophyte beds as refuge (Lauridsen and Lodge 1996), while the introduction of fish cues to the system leads to the migration of daphnids into the macrophyte bed for protection (Lauridsen and Lodge 1996). Both macrophyte and fish chemical cues have an effect on daphnid growth and reproduction as well (Machacek 1991, Burks et al. 2000). Overall, the effects of fish and macrophyte cues are not additive, but rather it seems that there is a hierarchy of responses to the various cues when they are presented in different combinations. My preliminary research has shown that odonate chemical cues have no direct effect upon daphnids morphologically or reproductively, but rather, the prey of the odonates seems to have a significant effect. For example, daphnids respond the same way to odonates fed *Chaoborus* as they do to *Chaoborus* alone. However, odonate cue effects upon daphnid behavior have not yet been investigated. Insight into these chemical relationships will serve to give us a better understanding of the complex nature of ecosystem interactions, and may allow for better experimental modeling or a better ability to manipulate the biota of a natural ecosystem for water quality management or other uses.

Methods:

Field: The field portion of this experiment will be used to investigate the effects of whole ecosystem cues upon daphnid behavior. Initially, four lakes, Brown Lake, Kickapoo Lake, Morris Lake, and Ward Lake, all found on the property of the University of Notre Dame Environmental Research Center in Land O' Lakes, Wisconsin, were investigated. Each of these lakes are relatively shallow and contain a good littoral zone. A reading of pH, temperature, and chlorophyll content was taken at each lake, and a survey of the plant, fish, and invertebrate populations was carried out as well. Water samples were then taken from both the littoral and pelagic zones of each lake and used as experimental treatments on daphnids. Four different treatments were run for each lake, including a control treatment (no lake water), a littoral treatment, a pelagic treatment, and a treatment containing both pelagic and littoral zone water. The experimental system consisted of two one-liter plastic bowls connected by clear plastic tubing three meters in length and seven centimeters in diameter. In each case, the central tube was filled with regular tap water, and the bowls contained the treatments. For the control, both bowls were filled with tap water, thus providing no cue into the system. For the individual treatment experiments (littoral and pelagic alone), the lake water was placed in one bowl, and tap water was placed in the other bowl. For the combination experiments containing both pelagic and littoral zone water, the pelagic water was placed in one bowl, and the littoral water was placed in the opposite bowl. In each case, fifteen daphnids were added to the system immediately, and were allowed to move freely about the system for thirty minutes. One control treatment was run at the same time as each experimental treatment. After the thirty minute period was completed, daphnid localization was measured quantitatively using two inch demarcated segments on the central tube in order to show migration relative to the various cues in the system.

Results:

Daphnia showed significantly different responses to each of the three factors in the experiment. We found that daphnid distribution differed significantly between the four lake treatments (2-way ANOVA, lake $F_{3,36} = 5.85$, $p = .0023$), and as well between the various zones tested (2-way ANOVA, zone $F_{2,36} = 55.03$, $p < 0.0001$). In addition, there was a significant interaction between lakes and lake zones (2-way ANOVA, lake*zone $F_{6,36} = 8.85$, $p < 0.0001$).

Using multiple comparison tests (Tukey's), we identified specific distribution differences between whole lakes. Daphnia distribution when exposed to cues from Ward Lake significantly differed from daphnids exposed to all other lakes (Morris, $p = 0.0019$, Kickapoo, 0.0005, Brown, 0.0216), but distributions when exposed to Morris Lake did not differ from either Kickapoo or Brown Lakes ($p =$

0.6100, 0.3518, respectively). Distributions when exposed to Kickapoo cues did not differ significantly from daphnids exposed to water from Brown (p = 0.1536).

Next we looked at a comparison of daphnid distributions when exposed to different lake zones, followed by an investigation of interactions between whole lakes and lake zones. Distributions of daphnids exposed to chemical cues from different lake zones (littoral, pelagic or both) all differed significantly (p<0.0001). Within each whole lake, we also found differences in daphnid distribution when exposed to different lake zones. For example, in Ward Lake, daphnid distributions in the combination treatment differed significantly from distributions in both the littoral and pelagic treatments (p<0.0001 for both), while distributions in littoral and pelagic treatments did not differ from each other (p = 0.3788).

Results from Brown Lake mirrored Ward Lake. In Brown Lake, the distribution of daphnids exposed to both littoral and pelagic zones (i.e. combination) differed significantly from daphnids exposed to either littoral or pelagic zones alone (p<0.0001). As occurred in Ward, distributions did not differ significantly between the littoral and pelagic treatments. However, exposure to water from Kickapoo and Morris Lakes produced different daphnid distributions than occurred with chemical cues from Ward and Brown Lakes. In both Kickapoo and Morris, pelagic distributions of daphnids differed from both those in the littoral treatment and those in the combination treatment (Morris, p= <0.0001, 0.0016, respectively; Kickapoo, p= 0.0002, 0.0002, respectively).

Across lakes, we found a significant difference in daphnid distribution when exposed to water collected from the same zone (littoral, pelagic, or combo). For example, daphnids in the Ward Lake treatment preferentially migrated away from the littoral treatment when compared with all other treatments (Morris and Kickapoo, p= <0.0001, Brown, p = 0.0024). Daphnids exposed to Brown Lake chemical cues occurred further away from the littoral zone when compared to both Morris and Kickapoo (Kickapoo, p = 0.0024, Morris, 0.0016). No significant difference occurred between daphnids exposed to littoral water from either Morris or Kickapoo (p = 0.8828) (Table 1). In the pelagic treatments, no significant difference occurred between distributions in any of the lakes, as all daphnids preferentially migrated away from the pelagic water. (Table 2).

When a combination of treatments was used, only a significant difference occurred between the distribution of daphnids in Morris Lake and those in Ward and Brown (p= 0.0322; 0.0229 respectively). In this case, significantly more daphnids migrated towards the littoral zone in Ward and Brown than in Morris. In all other cases, the daphnids preferentially migrated away from the pelagic treatment and towards the littoral treatment without any significant difference (Table 3).

Table 1: Differences in Daphnid Distribution in the Littoral Zone of All Lakes (p-values)

	Ward	*Brown*	*Morris*	*Kickapoo*
Ward		0.0024	<0.0001	<0.0001
Brown	0.0024		0.0016	0.0024
Morris	<0.0001	0.0016		0.8828
Kickapoo	<0.0001	0.0024	0.8828	

Table 2: Differences in Daphnid Distribution in the Pelagic Zone of All Lakes (p-values)

	Ward	*Brown*	*Morris*	*Kickapoo*
Ward		0.4625	0.1897	0.1462
Brown	0.4625		0.5562	0.4625
Morris	0.1897	0.5562		0.8828
Kickapoo	0.1462	0.4625	0.8828	

Table 3: Differences in Daphnid Distribution in Combination Treatments (p-values)

	Ward	*Brown*	*Morris*	*Kickapoo*
Ward		0.8828	0.0322	0.1897
Brown	0.8828		0.0229	0.1462
Morris	0.0322	0.0229		0.3788
Kickapoo	0.1897	0.1462	0.3788	

Discussion:

Our results support the idea that daphnids undergo horizontal migration in shallow lakes to seek refuge from pelagic predators in the littoral zone (Timms and Moss 1884, Lauridsen and Buenk 1996, Lauridsen et al. 1998, Burks et al. 2001). They also raise interesting questions about the interactions between predatory cues within lake systems as a whole (Sih et al. 1998). Our experiment showed a significant difference both in daphnid responses to various zone chemical cues within one lake, as well as between cues from similar zones in different lakes. A discussion of the characteristics of these four lakes serves to shed some light on these differences in daphnid migration.

Daphnids exposed to Ward Lake water avoided both the littoral and pelagic treatments, but preferentially migrated towards the littoral water when exposed to both. Ward Lake has a maximum depth of seven meters, and is surrounded by beds of sphagnum moss. The dominant invertebrates include Baetidae *Callibaetis*, Notonecta *Buenoa,* and Aeschnidae *Boyeria* (based on an unpublished independent survey). Based on the most recent surveys, there are minimal fish in Ward Lake, mostly minnows. Therefore, it is understandable that daphnids migrated away from both the pelagic and littoral treatments when they were presented alone. The presence of predatory chemicals in either zone could have influenced daphnid migration to safety in the tap water and away from the treatments. Daphnids have been shown to avoid notonectids in the vertical plane (Dodson 1988), and have been shown to avoid planktivorous fish in both the horizontal (Lauridsen and Lodge 1996) and vertical (von Elert and Pohnert 2000) planes. When presented with both cues in combination, daphnids strongly preferred to migrate towards the littoral zone, which could indicate that minnows are more threatening predators than the invertebrates present, and thus daphnids would rather contend with invertebrate predation than minnow predation. This result corresponds with the idea present by Sih et al. that in multiple predator systems, often risks are taken with one predator over the other due to a lesser negative impact by that predator (1998). However, little is known about the impacts of littoral invertebrates on daphnid mortality, so an investigation into that could lead to a better understanding of this system.

Daphnids exposed to Brown Lake water exhibited similar reactions as those exposed to Ward Lake water. The daphnids migrated away from both individual treatments, but migrated to the littoral treatment when a combination was presented. Brown Lake gets up to around 5.5 meters in depth, and is highly turbid. The littoral zone is dominated by *Elodea*, and it supports numerous species of invertebrates, including numerous species of odonata (Corduliidae *Epitheca,* Coenagrionidae *Nehalennia gracilis, and* Libellulidae *Leucorrhinia*), corixids, Neoplea *Striola*, and various limnephilidae (based on unpublished independent survey). Fish species in the lake include northern pike, walleye, black crappie, yellow perch, bluegill, pumpkinseed, golden shiner and white sucker. Again, the presence of possible predators in both the littoral and pelagic zones led to a daphnid preference away from both when they were presented alone. Daphnids have been shown to respond to both bluegill, pumpkinseed, and perch (Timms and Moss 1984, Dodson 1988, Lauridsen and Lodge 1996). Also, daphnid have been show to avoid *Elodea* in experimental systems (Burks et al. 2001). However, despite the presence of some piscivorous fish in the system, their densities were most likely not enough to deter the planktivores from entering the open water. This allowed the planktivores to illicit a stronger response from daphnids than the presence of invertebrates, and thus daphnids again migrated into the littoral zone when the two zones were presented in combination.

Daphnids in Kickapoo Lake water responded differently to littoral treatments than those exposed to Brown or Ward Lake water. Here, daphnids migrated towards the littoral zone water when it was presented alone. In Kickapoo Lake, which is relatively shallow (about 3.25 meters maximum), the dominant fish species include muskellunge, yellow perch, crappie, and northern pike. The shores are dominated by various species of lily pad and marsh grasses, and the dominant invertebrates include various species of odonata (Aeshnidae *Aeshna,* Aeshnidae *Boyeria,* and Libellulidae *Sympetrum*), as well ample gyrinids and notonectids. Perhaps the presence of lily pads or the dense marsh grasses in the littoral zone released cues which were attractive to the daphnids, indicating safe refuge in a relatively shallow and turbulent lake. Although no research has been done on the effects of lilypad chemical cues on daphnids, have been shown to aggregate under water lilies in shallow systems (Timms and Moss 1984), and the lilies have been shown to provide suitable refuge for daphnids from predators (Moss et al. 1998). Thus, despite the presence of invertebrate predators, daphnids may expect to find ample safe habitat in the littoral zone of Kickapoo Lake both when the treatment was presented alone, and when the treatment was presented in combination with the pelagic treatment.

The final lake, Morris Lake, reached a maximum depth of around 7 meters, and the shore line is dominated by *Elodea* and various lily pads. Fish species include stunted northern pike, yellow perch, and numerous minnows, and dominant invertebrates include numerous libellulid species, notonectids, gerrids, and an occasional lestid. Daphnids exposed to water from Morris Lake exhibited responses similar to those exposed to Kickapoo Lake water. Again, daphnids migrated away from the pelagic treatment, but towards the littoral in both the littoral alone treatment, and the combination treatment. The presence of lily pads as well as the dark color of the water again could have indicated to daphnids ample safe habitat in the littoral zone (Timms and Moss 1984, Moss et al. 1998). Thus, daphnids chose to contend with invertebrate predators in both the combination and littoral treatments.

Overall, daphnids generally avoided the pelagic treatment for all lakes. Although there was not a significant difference between daphnid responses to pelagic water from each lake, generally more daphnids migrated away from the pelagic in Ward and Brown treatments than in Morris and Kickapoo treatments. Overall, avoidance of pelagic water can most likely be attributed to the presence of planktivorous fish in each lake. In Brown and Ward, there were not enough piscivores to control planktivore position, and thus planktivores may have remained in the upper region of the pelagic zone on a more regular basis, leading to a stronger response from daphnids to those two treatments than to Kickapoo and Morris.

There was a significant difference in the responses of daphnids exposed to cues from the littoral zones of the four lakes. In both the Ward and Brown treatments, daphnids preferentially migrated away from the littoral zone, while in the Morris and Kickapoo treatments, daphnids preferentially migrated towards the littoral water. The most prevalent differences between the littoral zones of the four lakes is the presence of lily pads in Kickapoo and Morris, and their absence from Ward and Brown. While no lily pads were present, *Elodea*, a submerged macrophyte shown to ward away daphnia (Burks et al. 2001) was present, and thus could have enhanced the effect. Also, Kickapoo and Morris were much more turbid than Ward and Brown. Together, these factors led daphnids to prefer refuge in the littoral treatments of Kickapoo and Morris despite the presence of invertebrate predators, as well as a possibly higher density of planktivorous fish.

The combination treatment also produced interesting results. In all cases, daphnids preferred littoral water over pelagic water when they were presented together. This seems to solidify the idea of DHM, in that, when no vertical refuge is available, daphnids migrate horizontally into the littoral zone to avoid pelagic predators such as fish. Currently, tests are being run investigating the interactions of individual chemical cues (odonates, fish, and macrophytes) and their effects on daphnid horizontal migration. The results of these tests may be able to further explain the differences in migration patterns when daphnids were exposed to the various zones of the four UNDERC lakes.

Works Cited

Black, A.R. 1993. Predator-induce phenotypic plasticity in *Daphnia pulex*: Life history and morphological responses to *Notonecta* and *Chaoborus*. Limnol. Oceanogr. 38(5): 986-996.

Blois, C. 1985. The larval diet of three anisopteran (Odonata) species. Freshwater Biol. 15: 505-514.

Brönmark, C., and Hansson, L.A. 2000. Chemical communication in aquatic systems: an introduction. Oikos 88: 103-109.

Burks, R.L., Jeppesen, E., and Lodge, D.M. 2000. Chemicals from macrophytes and fishes suppress *Daphnia* growth and alter life history traits. Oikos 80: 139-147.

Burks, R.L., Jeppesen, E., and Lodge, D.M. 2001. Littoral zone structure as *Daphnia* refugia against fish predators. Limnol. Oceanogr. 46 (2): 230-237.

Diehl, S. 1988. Foraging efficiency of three freshwater fishes: effects of structural complexity and light. Oikos 53: 207-214.

Dodson, S.I. 1988. The ecological role of chemical stimuli for the zooplankton: Predator-avoidance behavior in *Daphnia*. Limnol. Oceanogr. 33(6): 1431-1439.

Dodson, S.I. 1989. Theecological role of chemical stimuli for the zooplankton: Predator-induced morphology in *Daphnia*. Oecologia 78: 361-367.

Hirvonen, H. 1999. Shifts in foraging tactics of larval damselflies: effects of prey density. Oikos 86: 443-452.

Johansson, F. 1993. Diel feeding behavior in larvae of four odonate species. Journal of Insect Behavior 6 (2): 253-264.

Lampert, W. 1993. Ultimate causes of diel vertical migration of zooplankton: new evidence for the predator-avoidance hypothesis. Arch Hydrobiol 39: 79-88.

Lauridsen, T.L., and I. Buenk. 1996. Diel changes in the horizontal distribution of zooplankton in the littoral zone of two shallow eutrophic lakes. Arch Hydrobiol. 137: 167-176.

Lauridsen, T.L., and Lodge, D.M. 1996. Avoidance by *Daphnia magna* of fish and macrophytes: Chemical cues and predator-mediated use of macrophyte habitat. Limnol. Oceanogr. 41 (4): 794-798.

Machacek, J. 1991. Indirect effect of planktivorous fish on the growth and reproduction of *Daphnia galeata*. Hydrobiol. 225: 193-197.

Moss, B., R. Kornijow, and G.J. Measey. 1998. The effects of nymphaeid (*Nuphar lutea*) density and predation by perch (*Perca fluviatilis*) on the zooplankton communities in a shallow lake. Freshwater Biol. 39: 689-697.

Perrow, M.R., Meijer, M.-L., Dawidowicz, P. and Coops, H. 1997. Biomanipulation in shallow lakes: state of the art. Hydrobiol. 342/343: 355-365.

Pritchard, G. 1964. The prey of dragonfly larvae (Odonata; Anisoptera) in ponds in northern Alberta. Can Jour of Zool. 42: 785-800.

Sih, A., G. Englund, and D. Wooster. Emergent impacts of multiple predators on prey. TREE 13 (9): 1998.

Timms, R.M., and B. Moss. 1984. Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. Limnol. Oceanogr. 29 (3): 472-486.

von Elert, E., and G. Pohnert. 2000. Predator specificity of kairomones in diel vertical migration of *Daphnia*: a chemical approach. Oikos 88: 119-128.

Chemical Cue Suites in Lake Zones: Their Effects on the Horizontal Migration of *Daphnia*

Andy Haines, Romi Burks, David Lodge

Abstract: *Daphnia play an integral role in lake functioning, as they filter large amounts of algae and serve as an important food source for both fish and invertebrates. Daphnia have been shown to undergo diel horizontal migration into the littoral zone of shallow lakes in order to avoid predation. Water from four different lakes on the UNDERC property in the Upper Peninsula of Michigan was used as treatments, and daphnids were exposed to chemical cues from both the littoral zone and the pelagic zone. Daphnids tended to migrate away from pelagic water in all cases, and when both pelagic water and littoral water were presented together, daphnids always migrated towards the littoral water. However, when littoral zone water was presented alone, daphnids had varying responses, most likely due to differences in the biotic makeup of the various lakes. These results support the idea of daphnid horizontal migration in the absence of a vertical refuge, and also shed some light on the idea of daphnid response to multiple predator systems.*

Introduction:

Daphnia are important filterers of algae, which helps to deter eutrophication in freshwater ecosystems (Perrow et al 1997). Due to their abundance in lakes and ponds as well as their large size in relation to other planktonic organisms, they serve as an important food source for a wide variety of predatory organisms. The chemical cues emitted by these predators serve as important signals to daphnids, and they often adjust their life history, morphology, or movement in response to these cues (Dodson 1989, Black 1993). For example, when exposed to *Chaoborus* cues, daphnids delay their time until first reproduction and develop neck teeth in order to reduce the likelihood of predation (Black 1993). In deep lakes, daphnids respond to pelagic predatory chemical cues by undergoing verticle migration and hiding in the colder, darker waters of the hypolimnion (Lampert 1993). This migration allows daphnids to avoid visual predators during the daylight hours, while being able to filter algae at night (Lampert 1993). However, in shallow systems, this verticle refuge is not available, so daphnids alternatively may undergo diel horizontal migration (DHM) into littoral zones (Lauridsen and Buenk 1996) in order to seek refuge in macrophytes. The complex structure associated with floating and submerged macrophytes lowers the foraging efficiency of fishes (Diehl 1988, Moss et al. 1998), although many predacious macroinvertebrates are associated with those macrophyte structures. Odonates, macroinvertebrates associated with the littoral zone of lakes and ponds, have been shown to prey efficiently upon daphnids (Pritchard 1964, Blois 1985, Hirvonen 1999, Burks et al. 2001). However, no research has been done in order to investigate the effects of odonate chemical cues upon daphnid behavior.

In order to investigate this question, we carried out a series of experiments to investigate the effects of odonate chemical cues upon daphnid growth, reproduction, and behavior. It has been shown that daphnids respond in different ways to the chemical cues, both individually and in combination, of macrophytes, odonates, and fish. When presented macrophytes alone, daphnids tend to avoid macrophyte beds as refuge (Lauridsen and Lodge 1996), while the introduction of fish cues to the system leads to the migration of daphnids into the macrophyte bed for protection (Lauridsen and Lodge 1996). Both macrophyte and fish chemical cues have an effect on daphnid growth and reproduction as well (Machacek 1991, Burks et al. 2000). Overall, the effects of fish and macrophyte cues are not additive, but rather it seems that there is a hierarchy of responses to the various cues when they are presented in different combinations. My preliminary research has shown that odonate chemical cues have no direct effect upon daphnids morphologically or reproductively, but rather, the prey of the odonates seems to have a significant effect. For example, daphnids respond the same way to odonates fed *Chaoborus* as they do to *Chaoborus* alone. However, odonate cue effects upon daphnid behavior have not yet been investigated. Insight into these chemical relationships will serve to give us a better understanding of the complex nature of ecosystem interactions, and may allow for better experimental modeling or a better ability to manipulate the biota of a natural ecosystem for water quality management or other uses.

Methods:

Field: The field portion of this experiment will be used to investigate the effects of whole ecosystem cues upon daphnid behavior. Initially, four lakes, Brown Lake, Kickapoo Lake, Morris Lake, and Ward Lake, all found on the property of the University of Notre Dame Environmental Research Center in Land O' Lakes, Wisconsin, were investigated. Each of these lakes are relatively shallow and contain a good littoral zone. A reading of pH, temperature, and chlorophyll content was taken at each lake, and a survey of the plant, fish, and invertebrate populations was carried out as well. Water samples were then taken from both the littoral and pelagic zones of each lake and used as experimental treatments on daphnids. Four different treatments were run for each lake, including a control treatment (no lake water), a littoral treatment, a pelagic treatment, and a treatment containing both pelagic and littoral zone water. The experimental system consisted of two one-liter plastic bowls connected by clear plastic tubing three meters in length and seven centimeters in diameter. In each case, the central tube was filled with regular tap water, and the bowls contained the treatments. For the control, both bowls were filled with tap water, thus providing no cue into the system. For the individual treatment experiments (littoral and pelagic alone), the lake water was placed in one bowl, and tap water was placed in the other bowl. For the combination experiments containing both pelagic and littoral zone water, the pelagic water was placed in one bowl, and the littoral water was placed in the opposite bowl. In each case, fifteen daphnids were added to the system immediately, and were allowed to move freely about the system for thirty minutes. One control treatment was run at the same time as each experimental treatment. After the thirty minute period was completed, daphnid localization was measured quantitatively using two inch demarcated segments on the central tube in order to show migration relative to the various cues in the system.

Results:

Daphnia showed significantly different responses to each of the three factors in the experiment. We found that daphnid distribution differed significantly between the four lake treatments (2-way ANOVA, lake $F_{3,36} = 5.85$, $p = .0023$), and as well between the various zones tested (2-way ANOVA, zone $F_{2,36} = 55.03$, $p < 0.0001$). In addition, there was a significant interaction between lakes and lake zones (2-way ANOVA, lake*zone $F_{6,36} = 8.85$, $p < 0.0001$).

Using multiple comparison tests (Tukey's), we identified specific distribution differences between whole lakes. Daphnia distribution when exposed to cues from Ward Lake significantly differed from daphnids exposed to all other lakes (Morris, $p = 0.0019$, Kickapoo, 0.0005, Brown, 0.0216), but distributions when exposed to Morris Lake did not differ from either Kickapoo or Brown Lakes (p =

0.6100, 0.3518, respectively). Distributions when exposed to Kickapoo cues did not differ significantly from daphnids exposed to water from Brown ($p = 0.1536$).

Next we looked at a comparison of daphnid distributions when exposed to different lake zones, followed by an investigation of interactions between whole lakes and lake zones. Distributions of daphnids exposed to chemical cues from different lake zones (littoral, pelagic or both) all differed significantly ($p<0.0001$). Within each whole lake, we also found differences in daphnid distribution when exposed to different lake zones. For example, in Ward Lake, daphnid distributions in the combination treatment differed significantly from distributions in both the littoral and pelagic treatments ($p<0.0001$ for both), while distributions in littoral and pelagic treatments did not differ from each other ($p = 0.3788$).

Results from Brown Lake mirrored Ward Lake. In Brown Lake, the distribution of daphnids exposed to both littoral and pelagic zones (i.e. combination) differed significantly from daphnids exposed to either littoral or pelagic zones alone ($p<0.0001$). As occurred in Ward, distributions did not differ significantly between the littoral and pelagic treatments. However, exposure to water from Kickapoo and Morris Lakes produced different daphnid distributions than occurred with chemical cues from Ward and Brown Lakes. In both Kickapoo and Morris, pelagic distributions of daphnids differed from both those in the littoral treatment and those in the combination treatment (Morris, p= <0.0001, 0.0016, respectively; Kickapoo, p= 0.0002, 0.0002, respectively).

Across lakes, we found a significant difference in daphnid distribution when exposed to water collected from the same zone (littoral, pelagic, or combo). For example, daphnids in the Ward Lake treatment preferentially migrated away from the littoral treatment when compared with all other treatments (Morris and Kickapoo, p= <0.0001, Brown, $p = 0.0024$). Daphnids exposed to Brown Lake chemical cues occurred further away from the littoral zone when compared to both Morris and Kickapoo (Kickapoo, $p = 0.0024$, Morris, 0.0016). No significant difference occurred between daphnids exposed to littoral water from either Morris or Kickapoo ($p = 0.8828$) (Table 1). In the pelagic treatments, no significant difference occurred between distributions in any of the lakes, as all daphnids preferentially migrated away from the pelagic water. (Table 2).

When a combination of treatments was used, only a significant difference occurred between the distribution of daphnids in Morris Lake and those in Ward and Brown (p= 0.0322; 0.0229 respectively). In this case, significantly more daphnids migrated towards the littoral zone in Ward and Brown than in Morris. In all other cases, the daphnids preferentially migrated away from the pelagic treatment and towards the littoral treatment without any significant difference (Table 3).

Table 1: Differences in Daphnid Distribution in the Littoral Zone of All Lakes (p-values)

	Ward	*Brown*	*Morris*	*Kickapoo*
Ward		0.0024	<0.0001	<0.0001
Brown	0.0024		0.0016	0.0024
Morris	<0.0001	0.0016		0.8828
Kickapoo	<0.0001	0.0024	0.8828	

Table 2: Differences in Daphnid Distribution in the Pelagic Zone of All Lakes (p-values)

	Ward	*Brown*	*Morris*	*Kickapoo*
Ward		0.4625	0.1897	0.1462
Brown	0.4625		0.5562	0.4625
Morris	0.1897	0.5562		0.8828
Kickapoo	0.1462	0.4625	0.8828	

Table 3: Differences in Daphnid Distribution in Combination Treatments (p-values)

	Ward	*Brown*	*Morris*	*Kickapoo*
Ward		0.8828	0.0322	0.1897
Brown	0.8828		0.0229	0.1462
Morris	0.0322	0.0229		0.3788
Kickapoo	0.1897	0.1462	0.3788	

Discussion:

Our results support the idea that daphnids undergo horizontal migration in shallow lakes to seek refuge from pelagic predators in the littoral zone (Timms and Moss 1884, Lauridsen and Buenk 1996, Lauridsen et al. 1998, Burks et al. 2001). They also raise interesting questions about the interactions between predatory cues within lake systems as a whole (Sih et al. 1998). Our experiment showed a significant difference both in daphnid responses to various zone chemical cues within one lake, as well as between cues from similar zones in different lakes. A discussion of the characteristics of these four lakes serves to shed some light on these differences in daphnid migration.

Daphnids exposed to Ward Lake water avoided both the littoral and pelagic treatments, but preferentially migrated towards the littoral water when exposed to both. Ward Lake has a maximum depth of seven meters, and is surrounded by beds of sphagnum moss. The dominant invertebrates include Baetidae *Callibaetis*, Notonecta *Buenoa,* and Aeschnidae *Boyeria* (based on an unpublished independent survey). Based on the most recent surveys, there are minimal fish in Ward Lake, mostly minnows. Therefore, it is understandable that daphnids migrated away from both the pelagic and littoral treatments when they were presented alone. The presence of predatory chemicals in either zone could have influenced daphnid migration to safety in the tap water and away from the treatments. Daphnids have been shown to avoid notonectids in the vertical plane (Dodson 1988), and have been shown to avoid planktivorous fish in both the horizontal (Lauridsen and Lodge 1996) and vertical (von Elert and Pohnert 2000) planes. When presented with both cues in combination, daphnids strongly preferred to migrate towards the littoral zone, which could indicate that minnows are more threatening predators than the invertebrates present, and thus daphnids would rather contend with invertebrate predation than minnow predation. This result corresponds with the idea present by Sih et al. that in multiple predator systems, often risks are taken with one predator over the other due to a lesser negative impact by that predator (1998). However, little is known about the impacts of littoral invertebrates on daphnid mortality, so an investigation into that could lead to a better understanding of this system.

Daphnids exposed to Brown Lake water exhibited similar reactions as those exposed to Ward Lake water. The daphnids migrated away from both individual treatments, but migrated to the littoral treatment when a combination was presented. Brown Lake gets up to around 5.5 meters in depth, and is highly turbid. The littoral zone is dominated by *Elodea*, and it supports numerous species of invertebrates, including numerous species of odonata (Corduliidae *Epitheca,* Coenagrionidae *Nehalennia gracilis, and* Libellulidae *Leucorrhinia*), corixids, Neoplea *Striola*, and various limnephilidae (based on unpublished independent survey). Fish species in the lake include northern pike, walleye, black crappie, yellow perch, bluegill, pumpkinseed, golden shiner and white sucker. Again, the presence of possible predators in both the littoral and pelagic zones led to a daphnid preference away from both when they were presented alone. Daphnids have been shown to respond to both bluegill, pumpkinseed, and perch (Timms and Moss 1984, Dodson 1988, Lauridsen and Lodge 1996). Also, daphnid have been show to avoid *Elodea* in experimental systems (Burks et al. 2001). However, despite the presence of some piscivorous fish in the system, their densities were most likely not enough to deter the planktivores from entering the open water. This allowed the planktivores to illicit a stronger response from daphnids than the presence of invertebrates, and thus daphnids again migrated into the littoral zone when the two zones were presented in combination.

Daphnids in Kickapoo Lake water responded differently to littoral treatments than those exposed to Brown or Ward Lake water. Here, daphnids migrated towards the littoral zone water when it was presented alone. In Kickapoo Lake, which is relatively shallow (about 3.25 meters maximum), the dominant fish species include muskellunge, yellow perch, crappie, and northern pike. The shores are dominated by various species of lily pad and marsh grasses, and the dominant invertebrates include various species of odonata (Aeshnidae *Aeshna,* Aeshnidae *Boyeria,* and Libellulidae *Sympetrum*), as well ample gyrinids and notonectids. Perhaps the presence of lily pads or the dense marsh grasses in the littoral zone released cues which were attractive to the daphnids, indicating safe refuge in a relatively shallow and turbulent lake. Although no research has been done on the effects of lilypad chemical cues on daphnids, have been shown to aggregate under water lilies in shallow systems (Timms and Moss 1984), and the lilies have been shown to provide suitable refuge for daphnids from predators (Moss et al. 1998). Thus, despite the presence of invertebrate predators, daphnids may expect to find ample safe habitat in the littoral zone of Kickapoo Lake both when the treatment was presented alone, and when the treatment was presented in combination with the pelagic treatment.

The final lake, Morris Lake, reached a maximum depth of around 7 meters, and the shore line is dominated by *Elodea* and various lily pads. Fish species include stunted northern pike, yellow perch, and numerous minnows, and dominant invertebrates include numerous libellulid species, notonectids, gerrids, and an occasional lestid. Daphnids exposed to water from Morris Lake exhibited responses similar to those exposed to Kickapoo Lake water. Again, daphnids migrated away from the pelagic treatment, but towards the littoral in both the littoral alone treatment, and the combination treatment. The presence of lily pads as well as the dark color of the water again could have indicated to daphnids ample safe habitat in the littoral zone (Timms and Moss 1984, Moss et al. 1998). Thus, daphnids chose to contend with invertebrate predators in both the combination and littoral treatments.

Overall, daphnids generally avoided the pelagic treatment for all lakes. Although there was not a significant difference between daphnid responses to pelagic water from each lake, generally more daphnids migrated away from the pelagic in Ward and Brown treatments than in Morris and Kickapoo treatments. Overall, avoidance of pelagic water can most likely be attributed to the presence of planktivorous fish in each lake. In Brown and Ward, there were not enough piscivores to control planktivore position, and thus planktivores may have remained in the upper region of the pelagic zone on a more regular basis, leading to a stronger response from daphnids to those two treatments than to Kickapoo and Morris.

There was a significant difference in the responses of daphnids exposed to cues from the littoral zones of the four lakes. In both the Ward and Brown treatments, daphnids preferentially migrated away from the littoral zone, while in the Morris and Kickapoo treatments, daphnids preferentially migrated towards the littoral water. The most prevalent differences between the littoral zones of the four lakes is the presence of lily pads in Kickapoo and Morris, and their absence from Ward and Brown. While no lily pads were present, *Elodea*, a submerged macrophyte shown to ward away daphnia (Burks et al. 2001) was present, and thus could have enhanced the effect. Also, Kickapoo and Morris were much more turbid than Ward and Brown. Together, these factors led daphnids to prefer refuge in the littoral treatments of Kickapoo and Morris despite the presence of invertebrate predators, as well as a possibly higher density of planktivorous fish.

The combination treatment also produced interesting results. In all cases, daphnids preferred littoral water over pelagic water when they were presented together. This seems to solidify the idea of DHM, in that, when no vertical refuge is available, daphnids migrate horizontally into the littoral zone to avoid pelagic predators such as fish. Currently, tests are being run investigating the interactions of individual chemical cues (odonates, fish, and macrophytes) and their effects on daphnid horizontal migration. The results of these tests may be able to further explain the differences in migration patterns when daphnids were exposed to the various zones of the four UNDERC lakes.

Works Cited

Black, A.R. 1993. Predator-induce phenotypic plasticity in *Daphnia pulex*: Life history and morphological responses to *Notonecta* and *Chaoborus*. Limnol. Oceanogr. 38(5): 986-996.

Blois, C. 1985. The larval diet of three anisopteran (Odonata) species. Freshwater Biol. 15: 505-514.

Brönmark, C., and Hansson, L.A. 2000. Chemical communication in aquatic systems: an introduction. Oikos 88: 103-109.

Burks, R.L., Jeppesen, E., and Lodge, D.M. 2000. Chemicals from macrophytes and fishes suppress *Daphnia* growth and alter life history traits. Oikos 80: 139-147.

Burks, R.L., Jeppesen, E., and Lodge, D.M. 2001. Littoral zone structure as *Daphnia* refugia against fish predators. Limnol. Oceanogr. 46 (2): 230-237.

Diehl, S. 1988. Foraging efficiency of three freshwater fishes: effects of structural complexity and light. Oikos 53: 207-214.

Dodson, S.I. 1988. The ecological role of chemical stimuli for the zooplankton: Predator-avoidance behavior in *Daphnia*. Limnol. Oceanogr. 33(6): 1431-1439.

Dodson, S.I. 1989. Theecological role of chemical stimuli for the zooplankton: Predator-induced morphology in *Daphnia*. Oecologia 78: 361-367.

Hirvonen, H. 1999. Shifts in foraging tactics of larval damselflies: effects of prey density. Oikos 86: 443-452.

Johansson, F. 1993. Diel feeding behavior in larvae of four odonate species. Journal of Insect Behavior 6 (2): 253-264.
Lampert, W. 1993. Ultimate causes of diel vertical migration of zooplankton: new evidence for the predator-avoidance hypothesis. Arch Hydrobiol 39: 79-88.
Lauridsen, T.L., and I. Buenk. 1996. Diel changes in the horizontal distribution of zooplankton in the littoral zone of two shallow eutrophic lakes. Arch Hydrobiol. 137: 167-176.
Lauridsen, T.L., and Lodge, D.M. 1996. Avoidance by *Daphnia magna* of fish and macrophytes: Chemical cues and predator-mediated use of macrophyte habitat. Limnol. Oceanogr. 41 (4): 794-798.
Machacek, J. 1991. Indirect effect of planktivorous fish on the growth and reproduction of *Daphnia galeata*. Hydrobiol. 225: 193-197.
Moss, B., R. Kornijow, and G.J. Measey. 1998. The effects of nymphaeid (*Nuphar lutea*) density and predation by perch (*Perca fluviatilis*) on the zooplankton communities in a shallow lake. Freshwater Biol. 39: 689-697.

Perrow, M.R., Meijer, M.-L., Dawidowicz, P. and Coops, H. 1997. Biomanipulation in shallow lakes: state of the art. Hydrobiol. 342/343: 355-365.
Pritchard, G. 1964. The prey of dragonfly larvae (Odonata; Anisoptera) in ponds in northern Alberta. Can Jour of Zool. 42: 785-800.
Sih, A., G. Englund, and D. Wooster. Emergent impacts of multiple predators on prey. TREE 13 (9): 1998.
Timms, R.M., and B. Moss. 1984. Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. Limnol. Oceanogr. 29 (3): 472-486.
von Elert, E., and G. Pohnert. 2000. Predator specificity of kairomones in diel vertical migration of *Daphnia*: a chemical approach. Oikos 88: 119-128.