

Active space of chemical alarm cue in natural fish populations

Brian D. Wisenden¹⁾

(Biosciences Department, Minnesota State University Moorhead, 1104 7th Ave S,
Moorhead, MN 56563, USA)

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Summary

Chemical cues released from injured fish skin during a predator attack provide reliable information about the presence of predation risk. Here, I report estimates of the area avoided by littoral fishes after experimental release of chemical alarm cues in two small lakes in northern Minnesota. Minnow traps were labeled chemically with either water (control) or skin extract (chemical alarm cue) made from 2 cm² of cyprinid skin (redbelly dace in experiment 1, fathead minnows in experiment 2). Traps labeled with water were placed 1, 2, or 8 m from traps labeled with alarm cue. After 2 h, water-traps that were either 1 or 2 m distant from an alarm-trap caught significantly fewer fish than water-traps 8 m distant from alarm-traps. Conspecific and heterospecific skin extract produced similar area avoidance by fathead minnows. Redbelly dace showed a larger active space in response to conspecific than heterospecific alarm cues. Brook stickleback showed reduced catches within 2 m of skin extract of fathead minnows. Overall, the radius of active space was between 2 and 8 m under lake conditions with average subsurface currents of 0.82 cm/s. These data are the first field estimates of active space of ostariophysan chemical alarm cues.

Keywords: active space, chemical alarm cues, schreckstoff, predator-prey interactions, ostariophysy.

Introduction

The evolution of integrated and sophisticated sensory systems is compelling testimony to the selective value of information (Danchin et al., 2004). Public information guides and tunes the evolution of sensory receptors (proxi-

¹⁾ Corresponding author's e-mail address: wisenden@mnstate.edu

mately) and behavioral decision-making (ultimately). Water is the most ancient medium of life and an excellent solvent for the solution and dispersal of chemical information. Not surprisingly, chemical cues have shaped the elaboration of chemoreceptors and behavioral responses of aquatic organisms (Wisenden, 2003; Wisenden & Stacey, 2005; Wisenden & Chivers, 2006). Predation risk can be detected by the chemical signature of predators (Kats & Dill, 1998), chemical alarm cues released passively by damaged tissue of attacked prey (Chivers & Smith, 1998), and injury-released chemical cues of digested prey seeping from the digestive tract of predators (Chivers & Mirza, 2001). Alarm cues are released only in the context of a predation event and provide reliable information about (1) the presence of risk, (2) the species of prey attacked and (3) the size or age of the prey species (Kusch et al., 2004; Mirza & Chivers, 2004).

Information networks comprise multiple sources and receivers (Wisenden & Stacey, 2005). Chemical cues resulting from predatory attacks on prey are released into the public domain where they are available to receivers of multiple species. These cues indicate predation risk to members of the attacked (prey) species, and also to other species within range of the chemical cues that share predators with the attacked species. Collectively these ecologically-similar species form a 'prey guild' that respond to each others' injury-released alarm cues (Chivers & Smith, 1998). While alarm reactions to conspecific cues seem to be innate in fish, alarm reactions to heterospecific cues are acquired through experience (e.g., Chivers et al., 1995; Pollock et al., 2003, but see Schutz, 1956; Mirza & Chivers, 2001).

Active space, the volume of habitat in which a chemical cue affects the behavior of receivers, is an important variable because it determines the slope of the selection gradient shaping the evolution of receptors, behavioral responses to cues, and the ecology of intra- and inter-specific interactions of information networks (Wisenden & Stacey, 2005). In aquatic ecosystems, most work on active space has concentrated on orientation toward attractive odorants such as food (amino acids) or mates (sex pheromones) (see Weisburg, 2000, for a review). In an oft-quoted laboratory experiment, alarm cue derived from 1 cm² of fathead minnow skin induced an overt alarm reaction in minnows when diluted to a volume of 58 000 l (Lawrence & Smith, 1989). This is a volume equivalent to a sphere with a radius of 2.4 m, or a cylinder 1 m deep with a radius of 4.3 m. Subsequent work has shown that concentrations of alarm cue below the threshold for an overt behavioral response can

affect future receiver behavior in adaptive ways that reduce predation risk (Mirza & Chivers, 2003); thus, lab data suggest active space of alarm cue may be very large indeed.

The estimate from Lawrence and Smith (1989) was calculated from a dilution series in the lab under ideal and yet artificial conditions where no other factors influenced behavioral response to alarm cue. Active space is potentially influenced by several factors. One factor is cue dilution (Lawrence & Smith, 1989) through molecular diffusion and advection (mass movement of water). A second factor is receptor sensitivity that varies among and within species (Stacey & Sorensen, 2002). A third factor is socially-mediated behaviors (Mathis et al., 1996; Stacey et al., 2001). In field studies using underwater video, unconstrained natural populations of conspecific and heterospecific fishes avoid areas where alarm cues have been released (Wisenden et al., 2004; Wisenden & Barbour, 2005; Friesen & Chivers, 2006; but see Magurran et al., 1996). In other field studies, minnows and ecologically similar heterospecifics consistently avoid minnow traps baited with sponge blocks soaked with skin extract relative to traps scented with water (Mathis & Smith, 1992; Chivers & Smith, 1998).

Active space of behavioral avoidance of minnow chemical alarm cues has never been tested in a field setting. Here, I report the results of simple field experiments to estimate active space of chemical alarm cues derived from skin extract of two fishes, northern redbelly dace (*Phoxinus eos*) and fathead minnows (*Pimephales promelas*). These fish are both cyprinids (superorder Ostariophysi). The Ostariophysi comprise 64% of all freshwater fish species (Nelson, 1994) and are well known for alarm responses to skin extract (Pfeiffer, 1977; Smith, 1992). Redbelly dace and fathead minnows commonly co-occur in the littoral zone of small lakes in mid-northern latitudes across North America, and respond to each others' alarm cues (e.g., Wisenden et al., 2003; Wisenden & Barbour, 2005). I placed control minnow traps (scented with blank water) along the shore line of two study lakes, spaced apart by 1, 2 or 8 m from traps scented with minnow skin extract. As a first attempt to quantify active space of cyprinid alarm cue I used inter-trap distances at the low end of active space estimated by the laboratory data of Lawrence & Smith (1989) because alarm reactions are more difficult to demonstrate in the field than in the lab (Wisenden et al., 2007). The risk-allocation hypothesis (Lima & Bednekoff, 1999; Sih et al., 2000; Mirza et al., 2006) explains how behavioral responses can be exaggerated in the context of laboratory aquaria

because, in contrast to omni-present predation risk experienced by fish in the wild, test subjects in captivity are maintained at low or zero perceived predation risk. When alarm cues are presented in the laboratory setting, the change in perceived risk over background is greater than what would be the case in the context of the natural habitat, producing a relatively more intense behavioral response in the laboratory.

Materials and methods

Field sites

This experiment was carried out on two separate occasions in two small lakes in Itasca State Park, MN, USA: Budd Lake (47°09'59.78"N, 95°10'28.90"W, elev. 485.5 m) in June 2003 and Deming Lake (47°10'13.14"N, 95°10'07.12"W, elev. 476.1 m) in June 2006. Budd Lake (ca. 5 ha in area) contains northern redbelly dace (*Phoxinus eos*) and fathead minnows (*Pimephales promelas*). Deming Lake (also ca. 5 ha in area) contains these two species, and in addition contains abundant brook stickleback (*Culaea inconstans*) and in past studies, incidental catches of blacknose shiner (*Notropis heterolepis*), golden shiner (*Notemigonus crysoleucas*), bullhead catfish (*Ameiurus melas*), yellow perch (*Perca flavescens*), pumpkinseed sunfish (*Lepomis gibbosus*) and Iowa darters (*Etheostoma exile*). Predators on fathead minnows and red belly dace in these lakes are primarily birds, great blue herons (*Ardea herodias*), common loons (*Gavia immer*) and banded kingfishers (*Ceryle halcyon*).

Stimulus preparation

In 2003, skin extract from redbelly dace (RBD cue) was the source of chemical alarm cue. Dace were collected from Budd Lake, transported to the laboratory facility at the Itasca Field Station, and killed by cervical dislocation. Skin fillets were carefully removed from each side of the body. Area of the skin fillets was estimated by measuring the length and mid-range width of each fillet. A total of 53.53 cm² of skin was collected from 14 dace (mean \pm SE total length = 52.0 \pm 0.82 mm). Skin fillets were placed in a beaker containing 100 ml of well water resting on a bed of crushed ice. Maintaining alarm cue at a cool temperature was a precaution to minimize the risk of

biochemical degradation of the active ingredients in skin extract (Wisenden et al., unpubl. data). When all the skin fillets had been collected, they were homogenized for 1 min with a hand blender to rupture epidermal cells and release alarm cue. The resulting solution was filtered through a loose wad of polyester wool to remove connective tissue, and diluted to a total volume of 650 ml with well water. Diluted skin extract was dribbled in 25-ml aliquots onto 26 blocks of commercial cellulose sponge (3M[®] product MMMC31) of dimensions 38 × 36 × 42 mm. Thus, each block of sponge contained the equivalent of 2.06 cm² of redbelly dace skin. Arguably (although no data are available to support this assumption), 2 cm² seems to be a reasonable estimate of the amount of skin that might be typically injured during a predatory attack, and as such, each sponge block simulated the approximate amount of alarm cue that might be released from one attack on one dace. Sponge blocks were frozen at -20°C until needed. On the day of the field trials, only 24 alarm cue blocks were used. Concurrently, 39 blocks of sponge (only 36 of which were used) of the same dimensions were prepared, soaked in blank well water (control) and frozen at -20°C.

In 2006, skin extract from fathead minnows (FHM cue) was used. Fathead minnows were collected from Deming Lake and transported to the Itasca Biological Field Station. Skin extract was prepared using the same methods described above. A total of 11 minnows (63.18 ± 1.80 mm total length) were killed to produce 52.06 cm² of skin fillets. Twenty-four blocks of the same sponge material and dimensions were used, each infused with 25 ml of skin solution containing alarm cues equivalent to 2.17 cm² of fathead minnow skin. Alarm cue blocks, and blank water controls, were frozen at -20°C until needed.

Experimental protocol

Standard Gee[®] wire mesh minnow traps (23 cm in diameter, 44.5 cm long) were used in this experiment. A frozen sponge block was affixed with stainless steel wire to the center of each trap. There were three treatment combinations of water traps (i.e., traps with a sponge soaked with water). One third of the water traps were connected to an alarm cue trap by a pole of polyvinyl chloride plastic (PVC) 2.56 cm in outer diameter and 1 m in length (1mW and 1mA traps, respectively). A second third of the water traps were joined to an alarm cue trap by a PVC plastic pole 2 m in length (2mW and 2mA

traps, respectively). The final third of water traps were not joined to an alarm trap (8mW traps). Traps and trap-pairs were placed around the perimeter of each lake spaced about 10 m apart; thus, isolated water traps were about 8 m from the next nearest alarm cue trap. Trap depth was approximately 0.5–1 m and placed 1–2 m from shore. Wind conditions driving water movement (advection — see below) were calm on both occasions. Set duration for all traps was 2 h. After 2 h, a separate tally for each species for each trap was recorded. All captured fish were returned to the lake at the point of capture within 10 min of the end of the 2-h fishing interval. Twelve replicates of the treatments (i.e., 60 traps) were conducted in Budd Lake in 2003. Another 12 replicates (60 traps) were repeated in Deming Lake in 2006.

Dye tests to measure advection in the field

A 1.6 × 1.6 m sheet of negatively buoyant white plastic was held by lead weights to the substrate in the littoral zone of Deming Lake and Budd Lake on a calm sunny afternoon in June 2007. A series of concentric rings drawn on the sheet formed a target pattern with radii of 20, 40, 60 and 80 cm. A period of at least 15 min was allowed to pass so that any water currents created during set up could dissipate. Approximately 10 ml of food coloring pigment was carefully and gently released from a 60 ml syringe held just below the surface directly above the center of the target pattern. The times at which the center of the dye cloud passed each 20-cm radius was recorded and used to calculate speed of water movement. Direction of dye migration was approximated by estimating direction to the nearest 12° (30 min of the 12 cardinal directions of an analog clock). In all cases, the 6 o'clock position was the direction to shore, the 12 o'clock position was the direction toward open water. These directional data were later converted to degrees and combined with speed data to produce a velocity vector of water advection. Dye tests were repeated seven times at 3-min intervals at each of two locations in each lake. These data were collected to characterize general advection conditions in the littoral zones of the study sites.

Data analysis

Catch numbers are highly variable and were converted to ranks within species, and for subsequent ANOVAs for redbelly dace, within lake. For

initial exploration of the data, 2×5 factorial ANOVAs (2 levels of Lake-Year-Species Cue \times 5 levels of sponge cue-intertrap distance treatments) were performed on the number of fish caught per trap (statistical software used was SPSS 11.5). Separate ANOVAs were conducted for each species captured in the traps, namely, fathead minnows, northern redbelly dace, and brook stickleback. Tukey post-hoc pair-wise comparisons were conducted to determine significant differences among trap treatments (using SPSS 11.5). All tests are based on 2-tailed probability distributions.

Results

Fathead minnows

The number of fathead minnows caught per trap did not differ between Budd Lake in 2003, when alarm cue from redbelly dace was used, versus data collected in Deming Lake in 2006, when alarm cue from fathead minnows was used (ANOVA Lake-year-cue: $F_{1,110} = 1.38$, $p = 0.243$; trap type: $F_{4,110} = 15.00$, $p < 0.001$, Lake-year-cue*Trap type: $F_{4,110} = 0.996$, $p = 0.413$). Tukey post-hoc pair-wise comparisons tests (on pooled data for the two lakes) indicated that spatially isolated water-traps (8mW) caught more fathead minnows than alarm-labeled traps (2mA, 1mA; Figure 1). Water-labeled traps 1 or 2 m distant from an alarm-labeled trap (1mW, 2mW) caught intermediate numbers of fathead minnows. Therefore, the catch numbers in response to 2 cm² of conspecific or heterospecific skin extract was detectable in water traps 2 m from a source of alarm cue, relative to traps 8 m away. The 1mW-traps differed from the 1mA-traps whereas the 2mW-traps did not differ from the 2mA-traps. This suggests that intensity of area avoidance was greater for the 1mW-traps than for the 2mW-traps, i.e., that response intensity was graded in proportion to the distance from the alarm cue.

Northern redbelly dace

There was a significant difference in the overall number of redbelly dace caught in the two experiments (year-cue-lake effect) but there was no significant interaction; thus, trap treatment had similar effect in each lake (ANOVA Lake-year-cue: $F_{1,110} = 4.69$, $p = 0.032$; trap treatment: $F_{4,110} = 8.01$, $p < 0.001$, Lake-year-cue*Trap type: $F_{4,110} = 0.19$, $p = 0.946$). Separate ANOVAs performed for each lake separately showed significant overall

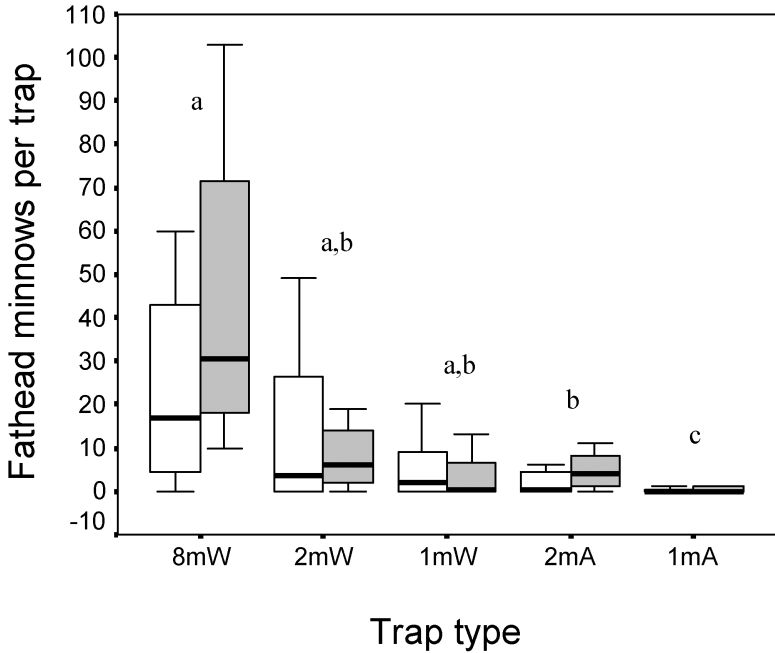


Figure 1. Medians (and quartiles, range) number of fathead minnows caught per trap in Budd Lake when alarm cue was derived from 2 cm² of redbelly dace skin (open fill) and in Deming Lake when alarm cue was derived from 2 cm² of fathead minnow skin (hatched fill). Trap treatments: 8mW, water-labeled traps at least 8 m from the nearest alarm-labeled trap; 2mW, water-labeled traps attached to an alarm-labeled trap 2 m away; 1mW, water-labeled traps attached to an alarm-labeled trap 1 m away; 2mA, alarm-labeled traps attached to a water-labeled trap 2 m away; 1mA, alarm-labeled traps attached to a water-labeled trap 1 m away. Shared letters above bars are not different by Tukey post-hoc pair-wise comparison tests ($p > 0.05$).

effects of trap treatment (Budd Lake/RBD cue: $F_{4,55} = 4.16$, $p = 0.005$; Deming L/FHM cue: $F_{4,55} = 5.043$, $p = 0.002$, Figure 2). The overall trends observed for fathead minnows were largely mirrored in catches of redbelly dace in that significantly more redbelly dace were caught in 8mW-traps than in other trap types, the 1mW and 2mW traps were intermediate in catch rate, and traps with alarm cue caught the fewest fish. Catch rates in water traps indicate redbelly dace have an active space of at least 2 m in response to conspecific and heterospecific alarm cues. However, response to the heterospecific alarm cue from fathead minnows produced an intermediate response when a water-trap was 2 m away, but a strong avoidance response when a water-trap was only 1 m away. Although catch rates of redbelly dace

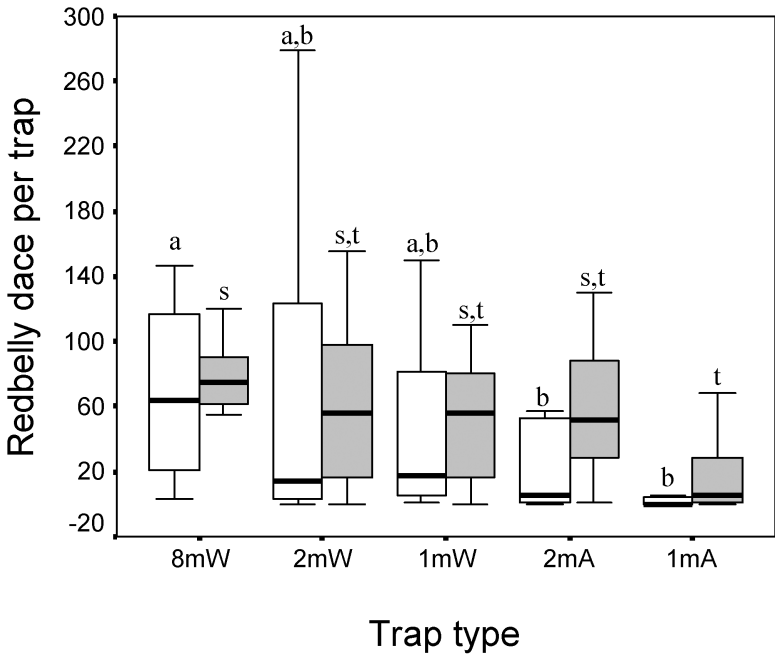


Figure 2. Medians (and quartiles, range) number of northern redbelly dace caught per trap in Budd Lake when alarm cue was derived from 2 cm² of redbelly dace skin (open fill) and in Deming Lake when alarm cue was derived from 2 cm² of fathead minnow skin (hatched fill). Trap treatment labels on the x-axis are described in the legend for Figure 1. Shared letters above bars are not different by Tukey post-hoc pair-wise comparison tests ($p > 0.05$). Tukey tests were performed on data from each lake separately.

were higher in Deming Lake (in response to fathead minnow alarm cue) than in Budd Lake (in response to redbelly dace cue), catch rates in 8mW traps between the two iterations of the experiment did not differ (Mann–Whitney $U = 61.0$, $p = 0.525$). This indicates that catch differences between iterations of the experiment was not due to differences in population density of redbelly dace in the two lakes. Instead, differences in catch rates are likely a result of a more intense avoidance of conspecific alarm cue than heterospecific alarm cue.

Brook stickleback

Stickleback occur in only one of the study sites, Deming Lake. In response to fathead minnow alarm cue, stickleback showed the same general avoidance radius as the two cyprinid fishes, but seemed to mimic best the avoidance

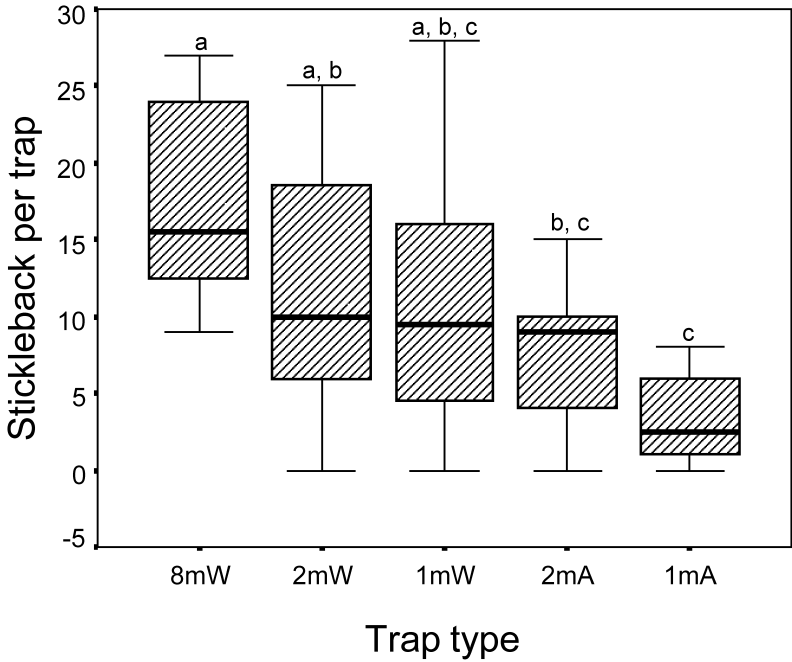


Figure 3. Median (\pm quartiles, range) number of brook stickleback caught per trap in Deming Lake when alarm cue was derived from 2 cm² of fathead minnow skin. Trap treatment labels on the x-axis are described in the legend for Figure 1. Shared letters above bars are not different by Tukey post-hoc pair-wise comparison tests ($p > 0.05$).

behavior of redbelly dace (Figure 3). There was a significant overall effect of trap treatment (ANOVA $F_{4,55} = 6.94$, $p < 0.001$). Post-hoc pair-wise Tukey tests indicated that 8mW-traps caught the most stickleback, 1mA- and 2mA-traps caught the least, and 2mW- and 1mW traps were intermediate. The radius of active space of stickleback in the field in response to fathead minnow alarm cue appears to be similar to cyprinid responses, with a radius of at least 2 m. As observed for redbelly dace, traps labeled with alarm cue caught more stickleback when a water trap was 2 m away than when a water trap was 1 m away.

Advection

Net subsurface advection averaged 0.82 ± 0.09 cm/s ($N = 28$; Figure 4). Direction of water movement shifted with each random gust of wind, often changing between each test run within a site (measured at 3-min intervals),

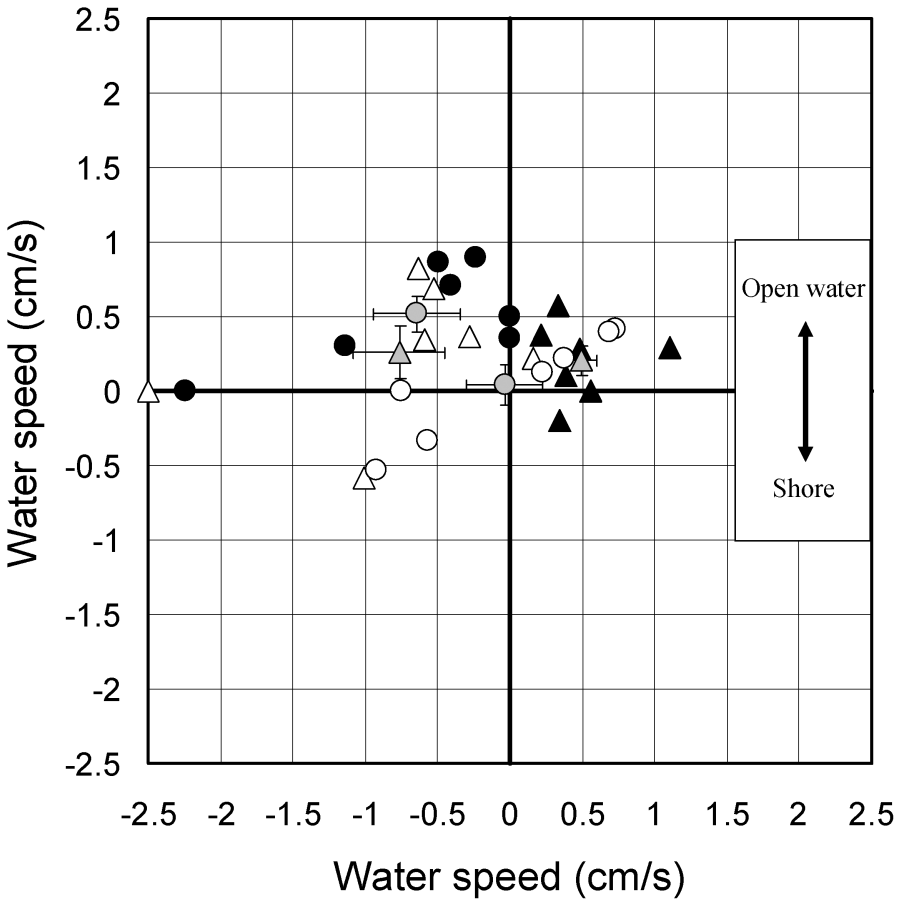


Figure 4. Speed and direction of migration of dye released in the littoral zone of the study sites. Black fill are for two locations (circles and triangles) in Budd Lake. Open fill are for two locations (circles and triangles) in Deming Lake. Data points at each location were recorded about 3 min apart. Gray shading indicates location mean.

and sometimes shifting direction and speed within a trial, causing the dye cloud to wander in an arc-shaped path. Net advection over 7 consecutive estimates at each location resulted in mean vector velocities with magnitudes of 0.81 and 0.53 cm/s in Budd Lake and 0.83 and 0.05 cm/s in Deming Lake.

Discussion

This is the first field measure of active space in an aquatic chemical alarm cue. Water-scented traps that were 1 or 2 m distant from alarm cue-scented

traps caught significantly fewer northern redbelly dace, fathead minnows and brook stickleback than water-scented traps 8 m distant from the nearest source of alarm cue. Alarm cues from fathead minnows and redbelly dace produced similar responses. Therefore, active space in the field created by 2 cm² of cyprinid skin is demonstrably greater than 2 m over a 2-h period. Moreover, intensity of trap avoidance was graded with distance from alarm cue source. Redbelly dace showed a less intense response to heterospecific alarm cue than to conspecific alarm cue. It is not clear why 1 m and 2 m alarm traps in 2006 caught different numbers of redbelly dace and stickleback. In 2006, 2-m alarm traps caught as many fish as water traps that were 1 and 2 m distant from alarm cues. One might speculate that the presence of fish in a water trap 2 m away (safety in numbers) gave the perception that an alarm trap was safe to approach. In another study, fathead minnows and redbelly dace preferred traps labeled with alarm cue when it also held a small shoal of conspecifics (Wisenden et al., 2003).

Interspecific reactions among prey guild members were clearly in evidence in this study. Behavioral responses to alarm cues derived from either redbelly dace or fathead minnows were broadly similar in both iterations of the study. Cross-species reactions are strong between these species because of shared predators and habitat overlap, and, in the case of the cyprinids, shared phylogeny (Schutz, 1956; Pfeiffer, 1977; Smith, 1992). Similar cross-species reactions between redbelly dace and fathead minnows were recorded in past studies in Budd Lake (Wisenden & Barbour, 2005) and Deming Lake (Wisenden et al., 2003). In this study, fathead minnows showed a consistent active space with a radius of more than 2 m in response to conspecific and heterospecific alarm cues. Redbelly dace showed a more intense response to the conspecific cue than to the heterospecific cue. An alternative explanation for higher catch rates of redbelly dace in Deming Lake might be that population density of redbelly dace was greater in Deming Lake than in Budd Lake. However, catch rates of redbelly dace in the 8mW control traps did not differ between iterations of the experiment; thus, redbelly dace did not respond to heterospecific cue as intensely as they did to conspecific alarm cue. Brook stickleback (superorder Acanthopterygii) are not closely related to cyprinids (superorder Ostariophysi), but responded to minnow alarm cue because they are in the same prey guild (Mathis & Smith, 1993; Wisenden et al., 1994, 2003; Friesen & Chivers, 2006).

There are three main determinants of active space: cue concentration, receptor sensitivity and social behavior. Cue concentration around scented traps is a function of diffusion and advection. Of these two phenomena, the effect of diffusion is negligible because it is very slow relative to subsurface advection. The role of subsurface advection in shaping active space in this study system is difficult to estimate because advection was capricious in magnitude, direction and linearity. The estimated net rate of water movement of 0.82 cm/s would carry alarm cue 57.6 m in 2 h if movement remained consistently in the same direction. This would be well beyond the estimate of active space in this study, and a distance that would leave the cue diluted below detection threshold (Lawrence & Smith, 1989). If the laboratory estimate of active space of fathead minnow skin extract (Lawrence & Smith, 1989) is applied to the field, then 2 cm² of skin should create an active space equivalent to a cylinder 1 m high with a radius of 6.07 m. The field estimate here of between 2 and 8 m is, therefore, consistent with the lab estimate based on receptor sensitivity.

Receptor sensitivity thresholds for redbelly dace and stickleback are not known for minnow skin extract because a dilution series comparable to Lawrence & Smith (1989) has not been conducted for these species, or any other species to my knowledge. Intuitively, receptor sensitivity should be expected to vary by species, and by stimulus (species source of skin extract). Remarkably, fathead minnows, northern redbelly dace and brook stickleback all avoided areas of similar size around sources of either species-source of alarm cue. This suggests that a radius of active avoidance of 2–8 m is not strictly defined by a mechanical effect of cue dilution and receptor threshold sensitivity, but rather an integrated behavioral response honed by natural selection to an ecologically relevant distance for small fish to keep between themselves and the site of a recent predation event. That a similar radius of avoidance occurred in two study lakes in two different field seasons, and by several species of fish all add confidence to this conclusion. Trade-offs between area avoidance and foraging may be broadly similar for all littoral species. Moreover, because alarm reactions spread across free-swimming shoals by social facilitation (Mathis et al., 1996), active space in the field is influenced by more than the presence of detectable levels of chemical cue. The social dimension of active space is much more behaviorally dynamic than a simple stimulus-response dictated by receptor physiology. A more complete understanding of the ecological function of chemical alarm cues

awaits experiments under more controlled social contexts. Also, the experiments presented here were conducted under a limited range of ecological conditions. The role of common environmental parameters (i.e., lotic and lentic systems, freshwater and marine systems) await experimentation. Future studies should also test active space on other species of fishes, particularly non-ostariophysan fish species, and non-fish aquatic organisms.

The effect of wind energy in these particular study lakes was limited by a small surface area relative to lake depth. Both lakes are surrounded on all sides by steep embankments and tall riparian tree cover. As a result, the water column in Deming Lake does not undergo complete turnover during the period of thermal destratification that occurs each spring and fall (i.e., Deming Lake is meromictic). The limnology of Budd Lake has not yet been characterized, but it too is similar in area, relatively deep, and surrounded by steep hills on all sides supporting tall tree cover. Thus, the role of advection in these study sites may be less of a factor in determining size and symmetry of active space than might be the case in other water bodies with more exposure to wind and, consequently, more substantial and consistent subsurface advection. Advection may also play a role at the scale of microhabitat in that predatory attacks should cause changes in water currents in the immediate area of a predation event that may contribute to dispersal of chemical information and, thus, affect active space. The act of placing traps in the lake may have created similar water currents, thus simulating the water perturbations that might occur during a predation event.

Active space created by chemical indicators of predation risk should have a smaller radius than active spaces for semiochemicals associated with reproduction (e.g., lamprey: Vrieze & Sorensen, 2001; Li et al., 2002; goldfish: Sorensen et al., 2005) and foraging (e.g., decapod crustacea: Atema, 1996; Moore & Crimaldi, 2004) because the fitness reward for detection, orientation and location of a potential meal or mate has value even from a great distance. In contrast, information about predation risk in distant locations may hold little ecological relevance in trade-offs against other behavioral priorities. This argument implies that littoral fishes should olfactorally sample an area sufficiently large to assess concentration gradient and, thus, distance from the cue source. One field study indicates that fathead minnows and brook stickleback flee areas labeled with alarm cue and sample chemical information about predation risk from a safe distance (Wisenden et al., 1995). While area use in alarm cue-labeled areas returns to normal within 4 h of cue

release, individual brook stickleback and fathead minnows that directly experience alarm cue do not return to risky areas where alarm cue was released until 6 to 8 h after the source of the cue has been removed (Wisenden et al., 1995). Active space is, thus, also a function of active time. Active space and time are both anticipated to change dynamically over time as the predator either leaves the area and/or becomes sated, or the alarm cue becomes diluted and/or biochemically degraded. The active space demonstrated in this study was at least 2 m over a 2-h interval, without revealing any information about the dynamic changes in active space-time hypervolume that occurred within the 2 h or thereafter. Future work should be directed toward resolving the space-time integration of chemical indicators of predation risk.

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