

Predator diet cues and the assessment of predation risk by juvenile brook charr: do diet cues enhance survival?

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Abstract: Recent studies have shown that predator diet cues provide important information that prey animals can use to assess predation risk. Predator-naïve prey animals may even learn to recognize unknown predators when they detect conspecific cues in the predator's diet. We examined the importance of predator diet cues in the responses of juvenile brook charr (*Salvelinus fontinalis*) to chemical cues of predators. In our first experiment, we showed that charr respond to chemical cues of adult yellow perch (*Perca flavescens*) that were fed a diet of either brook charr or rainbow trout (*Oncorhynchus mykiss*), but not to perch fed a diet of brook stickleback (*Culaea inconstans*). Responses to diet cues from perch fed both charr and trout may indicate that charr are responding to evolutionarily conserved salmonid alarm cues in the predator's diet. In a second experiment, we exposed charr to chemical cues from predatory northern pike (*Esox lucius*) that were fed charr, trout, or stickleback. Live-predation trials revealed that charr exposed to diet cues from pike fed salmonids exhibited higher survival than charr exposed to diet cues from pike fed a non-salmonid. These results are the first to document a survival benefit for prey exhibiting behavioural responses to predator diet cues.

Résumé : Des études récentes ont démontré que des signaux provenant du régime alimentaire d'un prédateur procurent des informations importantes que peuvent utiliser les proies pour évaluer leurs risques de prédation. Les animaux qui n'ont jamais subi de prédation peuvent même apprendre à reconnaître des prédateurs inconnus lorsqu'ils détectent des indices de la présence d'animaux de leur propre espèce dans le régime alimentaire d'un prédateur. Nous avons tenté de déterminer l'importance de tels signaux dans les réactions d'ombles de fontaine juvéniles (*Salvelinus fontinalis*) aux stimulus chimiques émis par des prédateurs. Dans une première expérience, nous démontrons que les ombles réagissent aux stimulus chimiques de perchaudes (*Perca flavescens*) adultes nourries d'ombles de fontaine ou de truites arc-en-ciel (*Oncorhynchus mykiss*), mais pas à ceux des perchaudes nourries d'épinoches (*Culaea inconstans*). Les réactions des ombles aux signaux chimiques des perchaudes nourries à la fois d'ombles et de truites indiquent peut-être que les ombles réagissent aux signaux de détresse de salmonidés qui sont présents dans le régime des prédateurs et qui ont été conservés au cours de l'évolution. Dans une seconde expérience, nous avons mis les ombles en présence de signaux chimiques de grands brochets (*Esox lucius*) prédateurs nourris d'ombles, de truites ou d'épinoches. Les expériences de prédation d'animaux vivants ont révélé que des ombles exposés aux signaux chimiques de brochets nourris de salmonidés ont un meilleur taux de survie que les ombles exposés aux signaux chimiques de brochets nourris d'autres types de poissons. Nos résultats révèlent pour la première fois que des réactions comportementales au régime alimentaire de prédateurs peuvent favoriser la survie des proies.

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Introduction

Predation is a strong selective force in the evolution of behavioural, morphological, and life historical traits (Sih 1987; Lima and Dill 1990; Chivers and Smith 1998; Wisenden 2000). The ability of a prey animal to avoid capture often requires that it be able to accurately assess local predation risk. Prey animals commonly assess predation risk using chemical cues (Kats and Dill 1998).

One of the most interesting recent advancements in our understanding of the role of chemical cues in predation risk assessment comes from studies that have manipulated the predator's diet. Chivers and Mirza (2001a) recently reviewed some 40 papers that have assessed the influence of predator diet on the intensity of antipredator responses among aquatic vertebrates (e.g., see Mathis and Smith 1993a; Brönmark and Petterson 1994; Laurila et al. 1997; Stabell and Lwin 1997; Wildy et al. 1999; Chivers and Mirza 2001b). Generally, these studies indicate that the intensity of the prey response is reduced (or even absent) if the predator is fed a diet that does not contain the prey. The level of sophistication of chemosensory assessment of predation risk appears to be much greater than previously thought; all of the work examining predator diet effects in vertebrates has been published in the last decade (reviewed in Chivers and Mirza 2001a).

Despite the widespread occurrence of responses to conspecifics in the diet of predators, only one study has ex-

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amined this phenomenon in salmonid fishes. Hirvonen et al. (2000) found that juvenile Arctic char (*Salvelinus alpinus*) would avoid entering the arm of a Y maze which contained diet cues from brown trout (*Salmo trutta*) fed Arctic char, but not the arm containing diet cues from brown trout fed commercial trout pellets.

The specific chemical cues that prey animals recognize in the predator's diet, which allow for recognition, are unknown in most cases. However, Mathis and Smith (1993a, 1993b) did observe that predator-naïve fathead minnows (*Pimephales promelas*) exhibit antipredator behaviour to pike fed minnows which contain alarm-substance cells, but not to diet cues from pike fed minnows which lack alarm-substance cells. The contents of the alarm-substance cells (Schreckstoff alarm cue) seems to be the cue to which minnows react.

Two studies have linked predator diet cues with the acquisition of predator recognition. Mathis and Smith (1993a) found that pike-naïve minnows exhibited an antipredator response to cues from pike fed fathead minnows but not to cues from pike fed swordtails (*Xiphophorus helleri*). When minnows, which were initially exposed to pike fed minnows, were subsequently tested for a response to cues from pike fed a different diet, they exhibited a strong response. This demonstrates learned recognition of the predator based on detecting conspecific cues in the diet of the predator. Similarly, Chivers et al. (1996a) demonstrated that pike-naïve damselfly larvae (*Enallagma boreale*) learn to recognize pike when they detect conspecifics in the diet of the predator.

In this study, we examine the importance of predator diet cues in interactions involving juvenile brook charr (*Salvelinus fontinalis*) and their predators. In our first experiment, we test whether predator-naïve brook charr respond to cues of predatory yellow perch (*Perca flavescens*) fed charr, perch fed rainbow trout (both charr and trout are salmonid fishes, Salmonidae), and perch fed brook stickleback (*Culaea inconstans*, Gasterosteidae). Brook stickleback are phylogenetically distant to brook charr, hence charr should not recognize stickleback cues as dangerous. Previous studies have demonstrated that brook charr recognize and respond to chemical alarm cues released by conspecifics and other salmonids. However, the intensity of the response to heterospecific alarm cues is lower than that to conspecific alarm cues. The alarm cues appear to be evolutionarily conserved within salmonid fishes (Mirza and Chivers 2001a). Consequently, we predict that brook charr should respond to chemical stimuli from perch fed charr and also to cues from perch fed trout, but not to cues from perch fed stickleback. We examine whether charr can still discriminate between conspecific and heterospecific salmonid alarm cues even after passage through the gut of a predator.

In our second experiment, we expose groups of predator-naïve brook charr to chemical cues of either pike fed a diet of charr, pike fed a diet of rainbow trout, or pike fed a diet of stickleback. Subsequently, we stage encounters between the charr and pike that have been fed a different diet. Previous studies have shown that prey chemically trained to recognize predators enhance their survival in the presence of predators in both aquatic (Berejikian et al. 1999; Mirza and Chivers 2000) and terrestrial systems (Downes 2002). Our study is the first to explicitly test whether prey animals that are exposed to predator diet cues will actually gain a sur-

vival benefit during an interaction with a predator. The reluctance of researchers to directly test survival of fishes may be in part a consequence of animal care concerns. Nevertheless, we believe that we must explicitly test the assumption that exposure to predator diet cues enhance survival. Such testing is critical to understand the ecological significance of predator diet effects.

Experiment 1: exposure of brook charr to predator diet cues

The purpose of this experiment was to determine if predator-naïve brook charr could recognize predatory perch as a threat when the perch were fed a diet that contained conspecific charr or other salmonids (rainbow trout). Moreover, we examined whether charr responded to diet cues of perch fed brook charr more strongly than to perch fed rainbow trout.

Methods

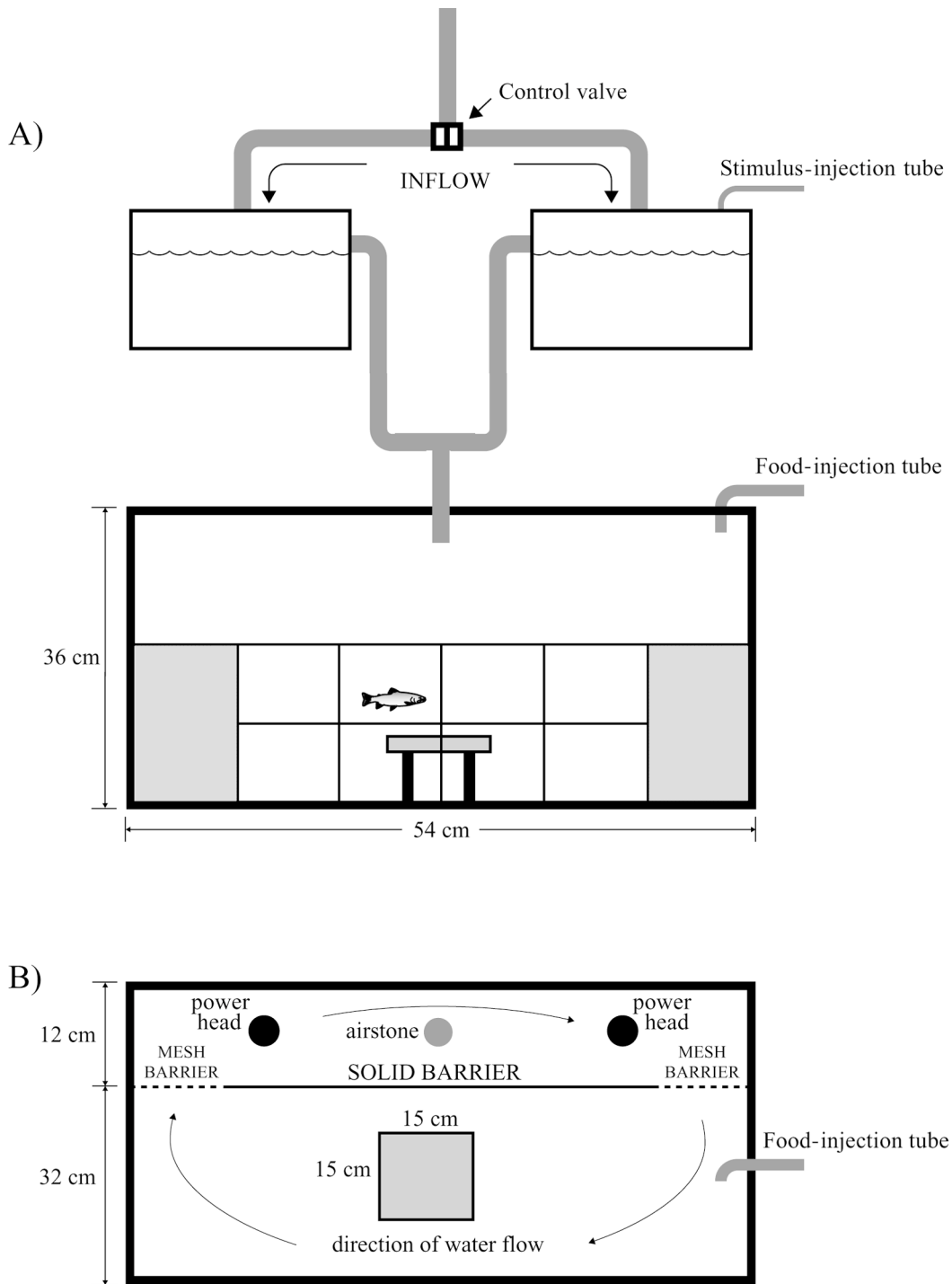
We obtained juvenile brook charr and rainbow trout from the provincial hatchery in Ft. Qu'Appelle, Saskatchewan, in May 2001. Adult perch were collected by seine net from Blackstrap Lake in central Saskatchewan and transported back to our laboratory at the University of Saskatchewan. Charr were kept in a 350-L artificial stream tank at 14°C. Fresh dechlorinated water was introduced into each stream tank at a rate of 1 L/min. Perch were held in 150-L tanks at 16°C under a 14 h light (L) : 10 h dark (D) photoperiod. Charr were fed daily a combination of commercial trout pellets and frozen brine shrimp, *Artemia* spp. Perch were maintained on a diet of fathead minnows.

Perch stimuli was collected from 6 adult perch (mean \pm SD, 13.50 \pm 1.08 cm standard length (SL)) fed a diet of either (i) brook charr, (ii) rainbow trout, or (iii) brook stickleback. Two perch were fed each of the three diets. Each perch was given 3–4 mL of whole fish, determined by volumetric displacement, every 3 days for three consecutive feedings. One hour after the final feeding, perch were removed and placed into 37-L aquaria with fresh dechlorinated water. After 24 h, perch were removed and the remaining water (perch stimuli) was pipetted into 50-mL aliquots and frozen at -20°C.

In this laboratory experiment, we exposed individual brook charr to chemical stimuli from adult perch fed a diet of either brook charr, rainbow trout, or stickleback. We tested 20 charr to each treatment and each charr received only one of the three treatments. The order of the treatments was randomized.

Each test chamber was set up as a gravitational flow-through system (Fig. 1 modified from Petranka et al. 1987). Water flowed into one of two 60-L header tanks, then spilled over into a 113-L test chamber (measuring 54 \times 44 \times 36 cm), and finally down the drain at a rate of 1 L/min. A single plastic tube ran into one of the two header tanks for introducing chemical stimuli. Each test chamber was subdivided into two compartments by an opaque barrier with a screen mesh at either end. The barrier was 12 cm from the long wall of the tank. This created an observation area of 54 \times 32 \times 36 cm. Behind the barrier, two small powerheads were used to create a circular flow in the tank. Each test chamber contained a single centrally located shelter object constructed from a ceramic tile (20 \times 10 cm) with three glass legs

Fig. 1. Schematic diagram representing the gravitational flow-through apparatus used in experiment 1 illustrating front (A) and top (B) views.



(7 cm). A single plastic tube was situated at the right-hand side of the chamber for introducing food. A grid of 9×9 cm squares was constructed on the front of the barrier to quantify movement of fish in the tank.

Fish were allowed to acclimate in the test chambers for 24 h prior to trials. Each trial was 18 min in length and consisted of a 8-min pre-stimulus periods and a 8-min post-stimulus period, with a 2-min stimulus-introduction period between the pre- and post-stimulus periods. We injected 50 mL

of perch stimulus into the header tank. Dye trials indicated that it took approximately 1.5 min for the stimulus to enter the test chamber from the header tank. At the beginning of both the pre- and post-stimulus periods, approximately 5 mL of previously frozen brine shrimp was introduced into the tank. The food was added to stimulate activity and would only remain in the water column for a few minutes. Charr were observed to forage predominantly in the water column, rather than pick food off the bottom. The water flow was

switched over to the second header tank after the pre-stimulus period to introduce the chemical stimulus into the test chamber.

During both the pre- and post-stimulus periods we recorded the number of line crosses and the number of feeding bites. We also recorded the occurrence of motionlessness (the fish was stationary on the bottom or in the water column for a minimum of 30 s). A line cross was recorded when 75% of the fish's body crossed from one grid square to another. Changes between the pre- and post-stimulus periods for each response variable were calculated (post-stimulus minus pre-stimulus) and the difference in changes between pairs of treatments was analyzed using separate Wilcoxon–Mann–Whitney tests (Siegel and Castellan 1988). The family-wise error rate was assessed and controlled using the modified Bonferroni test (Keppel 1982). The modified Bonferroni test specifies that corrections to the family-wise error rate be introduced only when the number of comparisons exceeds $k - 1$, where k is the number of treatments (Keppel 1982). The comparisons must be based on a priori predictions. In this experiment there was a total of three treatments between which we made comparisons, thus the family-wise error rate was held to $P = 0.033$. We used a χ^2 test to analyze the occurrence of motionlessness among the three treatments. Although we had cells in which the frequency was <5 , the χ^2 test can still be used to make valid comparisons (Haldane 1943; Zar 1996). Based on predictions of reduced activity and reduced foraging of brook charr to conspecific and heterospecific alarm cues found in our previous studies (Mirza and Chivers 2000, 2001a, 2001b, 2002), we conducted one-tailed tests. All statistics were calculated using SPSS 9.0 (SPSS Inc. 1998).

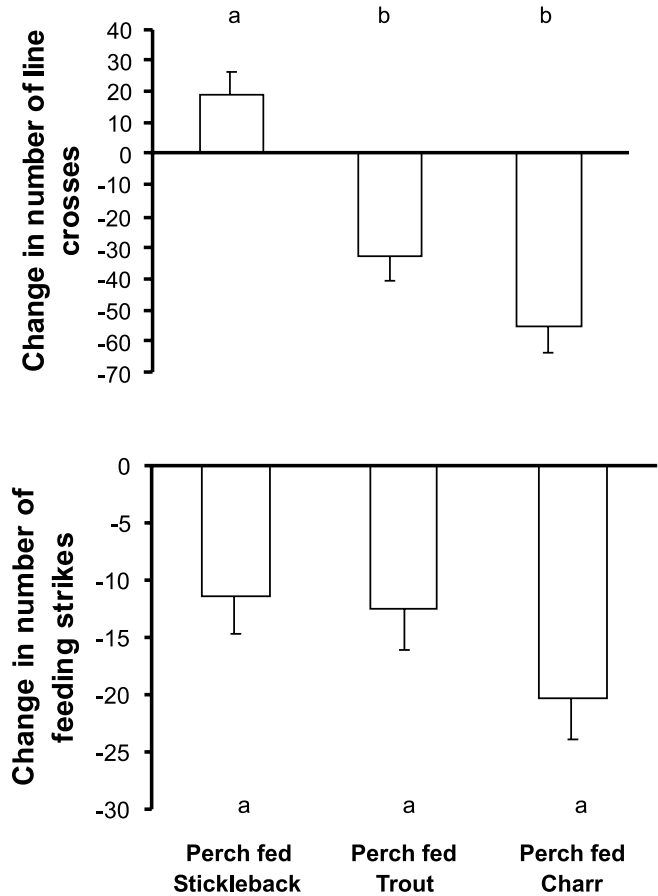
Results

Charr exposed to chemical stimuli from perch fed charr and perch fed a diet of rainbow trout both significantly decreased their number of line crosses when compared with charr exposed to chemical cues from perch fed a diet of brook stickleback ($Z = -4.73, p < 0.001$ and $Z = -3.98, p < 0.001$; Fig. 2). There was a trend for charr exposed to chemicals from perch fed charr to decrease their number of line crosses more than charr exposed to chemicals from perch fed trout ($Z = -1.60, p = 0.077$; Fig. 2).

Charr exposed to chemical cues from perch fed a diet of charr decreased their number of feeding strikes compared with perch fed stickleback cues. However, this result just failed to be significant ($Z = -1.81, p = 0.036$; Fig. 2). There was a strong trend for a reduction in foraging activity for charr exposed to perch fed charr diet cues compared with charr exposed to cues of perch fed a diet of trout ($Z = -1.67, p = 0.048$; Fig. 2). There was no difference in the change in foraging activity between charr exposed to chemicals from perch fed a diet of rainbow trout compared with perch fed a diet of stickleback ($Z = -0.135, p = 0.452$; Fig. 2).

Charr exhibited at least one period of motionlessness in 10 of 20 trials when exposed to chemical stimuli from perch fed a diet of brook charr compared with 4 of 20 trials when exposed to chemical stimuli from perch fed a diet of rainbow trout and 1 of 20 trials when exposed to chemical cues from perch fed a diet of brook stickleback ($\chi^2 = 11.2, df = 2, p = 0.002$).

Fig. 2. Mean (+ SE) changes in the number of line crosses and number of feeding strikes of brook charr (*Salvelinus fontinalis*) exposed to diet cues from yellow perch (*Perca flavescens*) fed either brook charr, rainbow trout (*Oncorhynchus mykiss*), or brook stickleback (*Culaea inconstans*). Different letters over bars denote significant differences at $p < 0.033$ (see text for details).



Experiment 2: staged encounters with live predators

The purpose of this laboratory experiment was to determine whether juvenile charr exposed to predator diet cues exhibit an increase in survival upon subsequently encountering the predator.

Methods

Juvenile brook charr and rainbow trout were obtained at the same time and held under the same conditions as in experiment 1. Juvenile pike were collected by seine net from Bright Water Reservoir in central Saskatchewan and transported back to our laboratory at the University of Saskatchewan. Pike were kept in separate 150-L tanks at 16°C. All fishes were held under a 14 L : 10 D photoperiod. Pike were maintained on a diet of fathead minnows until used in the experiment.

Pike stimuli were prepared as in experiment 1 using 6 juvenile pike (15.37 ± 1.37 cm SL) fed a diet of either brook charr, rainbow trout, or stickleback. The stimuli were frozen in 50-mL aliquots at -20°C until used.

Conditioning period

Brook charr were placed in 37-L aquaria and allowed to acclimate for 24 h. Tanks were wrapped with black plastic on three sides so that fish could not view fish in adjacent tanks. After this acclimation period, charr were exposed to one of three treatments: (1) chemical stimuli from pike fed brook charr, (2) chemical stimuli from pike fed rainbow trout, or (3) chemical stimuli from pike fed brook stickleback. We added 50 mL of the appropriate stimulus to each of the three tanks and allowed the charr to remain undisturbed for 2 h. Charr were conditioned in groups of 9.

Predation trials

Following the exposure period, we conducted live-predation trials where we staged encounters between pike and charr from each of the three treatment conditions. We conducted a total of 60 trials, 20 in each conditioning treatment (with charr previously conditioned to pike fed a diet of either charr, rainbow trout, or brook stickleback). Each predation trial consisted of an encounter between a predatory pike and 3 charr, thus a total of 180 juvenile charr (5.34 ± 0.67 cm) were used. Each group of charr was only used once and there was no difference in the sizes of the charr from each of the groups. We used a total of 20 different pike; each pike was tested 3 times, once in each of the treatments, with the order of the treatments randomized. Pike were tested 5 days apart. Prior to being used, pike were kept in 150-L glass aquaria and were fed minnows every 2 days. Between trials pike were fed minnows on day 3 after the initial trial to ensure that no charr remains were present in the digestive tract. Pike were fin-clipped to keep track of individuals.

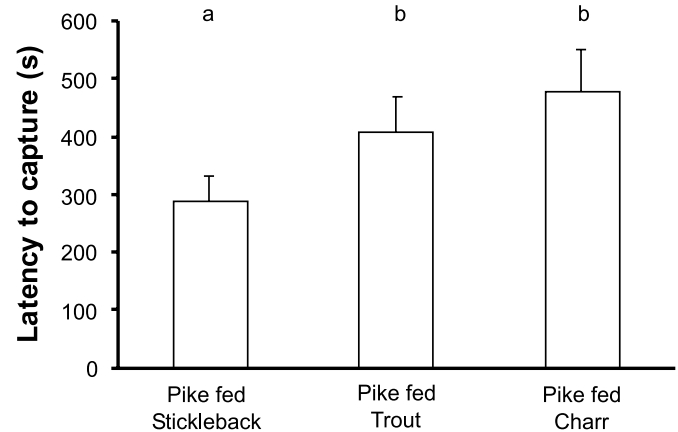
The test chambers were 189-L plastic tubs ($100 \times 47 \times 40$ cm) with 3 cm of granite chips for substrate. A single airstone was placed along the centre of the long wall of the tub for aeration. The three charr were placed at one end of the tub in a plastic bucket (diameter = 24 cm) that was open on both ends (no floor or lid) and perforated with dozens of holes. A single pike was placed in an open-ended perforated bucket (diameter = 29 cm) at the opposite end of the tub.

Trials began 4 h after fishes were placed in the perforated buckets. We removed the buckets and allowed the charr to interact with the pike. We recorded the identity, i.e., pike or charr, of the first fish to cross the centre of the tub to the opposite side and also the latency to first strike by the predator and latency to capture for each trial. Trials were conducted until the first fish was captured or for 15 min, whichever came first. We needed to end the trials when a charr was captured because the predation event would release chemical alarm cues and alert all the remaining prey that the predator was a threat (Mirza and Chivers 2000, 2001b). In those trials where a fish was not captured during the 15-min period, we assigned a latency to capture score of 900 s.

Statistical analysis

Wilcoxon's signed-ranks test was used to compare latency to first strike and latency to capture between pairs of treatments (Siegel and Castellan 1988). The family-wise error rate was corrected using the modified Bonferroni method and held at $P = 0.033$ as described in experiment 1 (Keppel 1982). We used a χ^2 test to examine whether the number of charr that crossed over to the opposite side of the tank be-

Fig. 3. Mean (+ SE) latency to capture during encounters with northern pike (*Esox lucius*) for brook charr previously exposed to chemical cues of northern pike fed a diet of either brook charr, rainbow trout, or brook stickleback. Different letters over bars denote significant differences at $p < 0.033$ (see text for details).



fore the pike differed among treatments. We predicted that charr exposed to predators fed charr or trout should be able to evade capture more than those that were exposed to predators fed another diet. Consequently we used one-tailed tests for our analysis. All analyses were performed using SPSS 9.0 (SPSS Inc. 1998).

Results

Two pike failed to attack charr in all three treatments and one pike died before being tested in all three treatment groups, hence all data from those three sets of trials were removed from the analysis.

Charr that had been previously exposed to chemical stimuli from pike fed a diet of brook charr crossed over to the opposite side of that tank before the pike did in 1 of 17 trials compared with 2 of 17 trials for charr previously exposed to chemical stimuli from pike fed a diet of rainbow trout and 14 of 17 trials for charr previously exposed to chemical stimuli from pike fed a diet of brook stickleback ($\chi^2 = 27.71$, $df = 2$, $p < 0.001$).

Pike that attacked charr were always successful, hence latency to first strike and latency to capture provided the same result. Charr previously exposed to chemical stimuli from pike fed a diet of brook charr or a diet of rainbow trout both had significantly greater latency to capture than charr previously exposed to chemical stimuli from pike fed a diet of stickleback ($Z = -2.39$, $p = 0.009$ and $Z = -2.30$, $p = 0.011$; Fig. 3). However, there was no significant difference in the latency to capture between charr previously exposed to chemical stimuli from pike fed charr compared with pike fed trout ($Z = -0.81$, $p = 0.21$; Fig. 3).

Discussion

Our results clearly demonstrate that predator-naïve juvenile brook charr are able to recognize predators that are fed charr and trout, but not those that have been fed another fish diet. Moreover, we saw that predator diet cues enhance survival during subsequent encounters with the predator. Similar studies have shown that prey animals enhance their survival during

encounters with predators when they are warned by damage-released alarm cues (Mathis and Smith 1993c; Hews 1998; Wisenden et al. 1999; Mirza and Chivers 2001b) or exposed to predator odours (Berejikian et al. 1999; Mirza and Chivers 2000; Downes 2002). Ours is the first study to provide evidence of a direct survival effect for prey exhibiting a behavioural response to predator diet cues.

Mathis and Smith (1993a) and Brown et al. (1995) concluded that minnow alarm cue, or some active component thereof, survives passage through the gut of the predator and provides the diet cue to which minnows respond. We do not know the identity of the chemicals that brook charr recognize in the diet of the predator; however, we speculate that they are cueing in on salmonid chemical alarm cues in the predator's diet. Mirza and Chivers (2001a) found that juvenile brook charr exhibit antipredator responses to alarm cues of conspecifics, as well as those released by brown trout and rainbow trout. The responses are specific to members of the Salmonidae and not to any injured fish cue. The fact that we observed responses to both charr and trout in the diet of the predators in both experiments supports the conclusion that charr are responding to salmonid alarm cues that are evolutionarily conserved. In our current study, we found that juvenile charr showed a strong tendency ($P = 0.036$) to respond with a greater intensity to perch fed charr than to perch fed trout. Interestingly, Mirza and Chivers (2001a) found that brook charr responded with a greater intensity to conspecific alarm cues than to alarm cues of rainbow trout and brown trout. This provides additional support for the conclusions that salmonid alarm cues are responsible for the diet effect.

Our study shows that brook charr respond to conspecific and heterospecific salmonid cues from the diet of an unknown predator. Charr previously exposed to charr and trout diet cues exhibited a significantly greater latency to capture than charr previously exposed to stickleback diet cues. We ended trials when the first charr was captured because the predation event would cause the release of chemical alarm cues in the water, which would alert all of the prey that the pike was a threat. However, if the encounters had occurred outside the confines of the tank, charr may have fled the area and escaped predation altogether (Chivers et al. 1996b).

In our experiment the difference in survival time may result from a long-lasting behavioural effect of exposure to predator diet cues. We conducted the survival trials 6 h after the initial exposure to the diet cues (4 h after the charr were conditioned). It is possible that the charr could still be responding to the diet cues after this period of time, i.e., heightened alert in response to alarm chemicals. However, another possibility that seems more likely is that the charr had learned to recognize the predator's identity when it was exposed to the diet cues of the predator. Previous studies with minnows and insects have documented learning of the predator's identity based on a single exposure to diet cues (Mathis and Smith 1993a; Chivers et al. 1996a). Moreover, recent studies have documented that salmonid fishes can learn to recognize predators through single conditioning trials (Brown and Smith 1998; Berejikian et al. 1999; Mirza and Chivers 2000). Additional work is needed to differentiate between these two possibilities.

The level of sophistication in the use of chemical cues to assess predation risk is more complex than previously thought

(reviewed in Chivers and Mirza 2001a). Because predators that are feeding on conspecifics of the prey are often more dangerous than predators eating other prey (Chivers and Mirza 2001a; Mirza and Chivers 2001c), prey animals should have an advantage if they can use information from the last meal consumed by the predator to mediate the intensity of their response. By being able to differentiate between predators that are fed different diets, prey animals will not waste time and energy responding to predators that do not pose an imminent threat. Moreover, diet cues may facilitate learning and this learning will translate into increased survival for the prey. Our study is the first to document a survival benefit for prey exhibiting behavioural responses to diet cues in a predator-prey system.

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