EFFECTS OF PASSING FISH ON ACTIVITY OF LARVAL CHIRONOMUS TENTANS
(DIPTERA: CHIRONOMIDAE)

by

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A thesis submitted in conformity with the requirements
for the degree of Master of Science.
Graduate Department of Zoology
University of Toronto

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Effects of passing fish on activity of larval *Chironomus tentans* (Diptera: Chironomidae)

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Master of Science, 1997  
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Comparing behaviour and development of prey under constant presence versus complete absence of predators may underestimate or overestimate effects of predator presence, nevertheless, a number of studies have examined prey behaviour and subsequent fitness parameters under these conditions. I designed and implemented a system that allowed exposure of larval chironomid larvae (*Chironomus tentans*) to a single fish pass, because underwater video tapes in various lakes across Ontario showed that fish swam through any given area quickly, rarely hovering over an area. The race-way proved successful in controlling fish passes most of the times. Overall, I was confident that the design of the raceway allowed me to study the effects of a fish pass, similar to those seen in the field, on the activity of chironomid larvae in the laboratory.

Using brown bullheads (*Ictalurus nebulosus*) and big and small pumpkinseed sunfish (*Lepomis gibbosus*), I found that larvae showed a definite decrease in activity immediately after a fish pass, however, larval responses were not affected by the size or the species of fish. Larval activity and the median activity lost was highest at the high larval hunger-level. This suggests that while hungry larvae may compensate for low food availability by foraging more when no predators are detected, their growth and development may suffer more at high levels of fish presence than do well fed larvae.
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Introduction

Two scenarios of possible changes in prey activity (time) as exposure time (%) to predator stress increases. (a) Costs may be underestimated due to habituation, or (b) costs are overestimated due to overexposure of prey to predator.

Methods and Materials

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c) Mean larval activity (min/hour per larva) recorded over time (min) after exposure to a passing small pumpkinseed sunfish. Larvae were maintained at low, medium and high hunger-levels.

Descriptive: Larval activity (min/hour per larva) versus time (min). Adjusted and non-adjusted regressed control lines have been shown with respect to a fish effect plot.

Descriptive: Larval activity (min/hour per larva) versus time (min). Fish effects caused either reduced larval activity or increased larval activity, determining how event duration and activity time lost were eventually calculated.
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a) All events have an impact on activity lost
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   (ii) 40 minutes of a previous event (max. event duration calculated)
c) All events have a proportional impact on activity lost; activity lost from the end of the first event to the end of the second event is significant for events within:
   (i) 23 minutes of a previous event (min. event duration calculated)
   (ii) 40 minutes of a previous event (max. event duration calculated)
Evaluating costs and benefits associated with antipredator behaviour is a complex problem. Many studies have examined effects of antipredator behaviour on activity and fitness parameters of aquatic invertebrates and vertebrates (invertebrates: Dill 1987, Sih 1987; vertebrates: Sih 1991, Skelly 1992, Blake & Hart 1993, Burrows & Gibson 1995), but virtually all these studies have been carried out in small containers in the laboratory under conditions of complete absence of a predator versus constant presence of a predator in the immediate vicinity of prey (damselflies: Heads 1986, Dixon & Baker 1987, 1988; tadpoles: Lawler 1989; *Daphnia*: Stibor 1992; mayflies: Culp & Scrimgeour 1993). However, because predator movement patterns in the field are subject to continuous fluctuations depending on habitat use, foraging opportunities, ontogeny and season (Keast & Welsh 1968, Scott & Crossman 1973, Mittelbach 1981, Mittelbach & Chesson 1987, Butler 1988) it is unlikely that prey are ever exposed to either continuous presence or complete absence of predators in the field. Because of the unrealistic nature of some of these studies it is currently difficult to extrapolate laboratory findings to field situations.

Comparing behaviour and development of prey under constant presence or complete absence of predators may underestimate or overestimate effects of predator presence. Macchiusi & Baker (1992) suggested that experiments involving continuous exposure of prey to predator presence (e.g. Skelly 1992, Peckarsky *et al.* 1993, Ball & Baker 1995) could result in prey habituation and, therefore, an underestimation of the developmental costs incurred by prey. For example, consider a scenario where prey habituate to constant predator presence (Fig. 1a). Because prey habituate to constant high levels of predator presence (100% exposure in Fig. 1a) there may be no difference in foraging activity of prey exposed to constant predator presence and
Figure 1:

Two scenarios of possible changes in prey foraging activity (time) as exposure time (%) to predator stress increases. Typically studies examine costs incurred by prey at 0% and 100% predator presence, which may not represent what happens in natural settings. See text for details.

(a) Costs may be underestimated if prey habituate to predator presence.
(b) Costs may be overestimated if prey are overexposed to predator presence.
Fig. 1
relatively low, but more realistic, level of exposure to predators. Thus, given the scenario in Fig. 1a, a laboratory experiment using a predator versus no predator design would badly underestimate the impact of predator presence. Alternatively, many documented examples of predator-induced changes in prey behaviour (and ultimately development) may reflect an exaggeration of costs due to unrealistic, constant exposure of prey to predator. For example, in the scenario outlined in Fig. 1b, prey foraging activity may drop with an increase in the exposure to predators and a laboratory experiment comparing 0 vs 100% exposure may suggest a dramatic effect. However, if prey in the field are exposed to much lower levels of predator presence (e.g. 20%) the effect on prey foraging will be greatly reduced.

In addition to the laboratory studies described above, there are many field studies designed to test the effects of predators on prey behaviour and feeding rates (Sih et al. 1985, Feltmate & Williams 1991, Harvey 1991, Clark 1993, Peckarsky et al. 1993, review by Strauss 1991). However, like the laboratory studies, using enclosures in field settings limits natural interactions between predator and prey. Even if it is possible to maintain realistic field levels of mean or median densities, the spatial and temporal movements of predator and prey will almost inevitably be affected. For example, a habitat may have a mean density of 1 predator per square metre and thus it may seem relevant to use 1 m² enclosures with a single predator present. However, having a single predator present for extended periods of time may impose far greater costs than a natural field situation where, because of predator movement and aggregation (e.g. fish schooling), an area may experience periods of many predators per square metre balanced by periods with no predators. These windows of time with no predation may allow prey to forage actively and
During the past decade considerable work has been done on the antipredator behaviour of tubiculous chironomid larvae. Many chironomid larvae are benthic and construct tubes using substrate particles held together with a salivary secretion (Oliver 1971, Leuchs & Neumann 1990). Tubiculous larvae feed by extending the anterior section of their bodies out of their tubes and scraping algae and detritus from sediments in the area surrounding the tube. Hershey (1985) found chironomid species with tubes had fewer encounters with predators such as sculpins and that vulnerability to fish predation increased with time out of tubes (Hershey 1987). Macchiusi and Baker (1991) found tubes of larval Chironomus tentans reduced encounters with pumpkinseed sunfish (Lepomis gibbosus) and that larval activity outside of their tubes dropped as the time predatory fish were present increased (Macchiusi & Baker 1992).

Laboratory studies have also shown that larval C. tentans larvae pay a developmental price for decreasing activity in the presence of fish (Macchiusi & Baker 1992; Ball & Baker 1995). Ball & Baker's (1995) study showed that in the continuous, non-lethal presence of pumpkinseed sunfish, chironomid larvae exhibited reduced growth rates, smaller adult size, slower development rate and lower fecundity. Subsequent studies (Ball & Baker 1996) indicated these changes could be interpreted as costs of larval antipredator behaviour rather than adaptive life history shifts.
with laboratory experiments described earlier, i.e. they studied development of chironomids under conditions of predator present versus predator absent. In their treatments they maintained chironomids in small tanks (26 x 15 x 10 cm deep) with a pumpkinseed sunfish continuously present for many weeks. While their results indicated fish presence altered prey development, the design of their study does not allow any estimate of the impact of varying fish presence for larvae in the field. For example, Collins' (1989) work with underwater time-lapse video recordings in the field indicates that fish activity varies greatly between lakes, sites within a lake, across seasons or within a day at one site. Also, video playbacks of fish activity in the field confirm that fish generally swim through the benthos quite rapidly, rarely hovering over a given area (Collins & Hinch 1993), as frequently mimicked in a number of laboratory studies, including those of Ball & Baker (1995, 1996).

The purpose of this study is to design and implement a system for estimating how much potential foraging time is lost by chironomid larvae when exposed to a predatory fish simply swimming over them (fish pass). I experiment with brown bullheads (*Ictalurus nebulosus*) and pumpkinseed sunfish (*Lepomis gibbosus*), two of the most prominent fish in Collins' (1989) study, and 2 size classes of pumpkinseed sunfish, in an effort to determine if differences in modes of swimming and size, respectively, affect chironomid behaviour. I also attempt to determine both the horizontal and vertical distance over which chironomids detect fish predators.

Knowing the foraging time lost as a result of a single fish pass will facilitate estimates of foraging time lost to predators by prey in the field and will aid in the creation of experimental techniques and designs to quantify the developmental costs of field levels of predator presence.
same changes in larval activity as fish in the field. A series of artificial fish can be used to create a system that will mimic the exposure frequency and exposure time of benthic invertebrates to fish predators in the field. Such a system can be implemented in a study of the effects of field levels of fish presence on growth and development of prey.
All experiments were performed using a laboratory culture of *Chironomus tentans*. The culture was established in summer 1993 using egg masses supplied by Donna Bedard, Ministry of the Environment, Ontario, Canada. Larvae were reared in 3.0 L polypropylene tanks (26 x 15 x 10 cm deep) with a 1 cm layer of silica sand (particle diameter 250 - 500 μm) which larvae used to construct tubes. Tanks were filled with 2.0 L of 1 part distilled water to 3 parts dechlorinated water, and maintained at 22°C under a 14 L:10 D photoperiod. Aeration was provided using surgical tubing taped to the inside of each tub and inserted into a main air line. Larvae were fed by dispensing a food suspension using an inverted 5¼" pipette filled twice, every second day. The suspension was made of 2 g of finely ground "Tetra Conditioning Food, Vegetable Diet for Tropical Fish" in 50 mLs of distilled water. Tub sides were scrubbed and a 50% water change was performed once a week.

Pumpkinseed sunfish (*Lepomis gibbosus*) and brown bullheads (*Ictalurus nebulosus*) were collected from the pond on the Erindale College Campus, University of Toronto (43° 33' N, 79° 40' W). Sunfish were kept in 40 L glass aquaria in the laboratory at 22°C under a 16L:8D photoperiod, and fed *ad libitum* with "Tetra Bits Bite Size Food bits for Discus, mid-water and bottom feeding tropical and marine fish", *C. tentans* larvae and egg masses, and *Daphnia*. Bullheads were kept at 15°C in a flow through tank under an 11 L:13 D photoperiod, and were fed once every two days with "Big Al's Dry Perch Food", chopped frozen fish (sunfish and smelt) and "Big Al's Frozen Bloodworm Cubes".
Apparatus design: Race-way

To determine the effects of a single fish pass on activity of larval *Chironomus tentans* I designed a "race-way" (Fig. 2) to control the release and capture of fish. The raceway was a 235 cm x 60 cm x 26 cm high wooden trough, triple lined with construction-grade vapour barrier. Two 233 cm long x 0.5 cm x 20 cm high PVC dividers were placed along the length and 20 cm from each side of the trough, creating three tracks 233 cm long by 20 cm wide. Each track was divided into 3 zones (pre-treatment, treatment, post-treatment) by installing a gate 15 cm from one end of the trough and another gate 45 cm from the opposite end.

Gates were designed to move as smoothly as possible and to stay perpendicular to the water surface when raised and lowered; they were held in place using grooved PVC sheets attached perpendicular to each divider. Gates were raised using a length of string that passed through a series of pulleys and extended out of the environmental chamber, in which the race-way was placed, and into an adjacent room. This allowed me to control gate movement from outside the chamber.

Each track of the race-way was filled to a depth of 16 cm with dechlorinated water over a 4 cm layer of silica sand. Using air forced through an air stone in each track the water was aerated every night but not during trials as aeration may have disturbed the chironomids and would have made it difficult to view the chironomids through agitated water. The race-way was kept in an environmental chamber maintained at 21°C. I set the lights in the environmental
Figure 2:

Race-way design.
Note that gates are manipulated using strings and pulleys suspended over the race-way.
Fig. 2
(Not to scale)
at 8 a.m., acclimated under light in the laboratory till 4 p.m., after which they were transferred into the dark environmental chamber. The chamber remained dark till midnight followed by 7 hours of light. At 7 a.m. the lights were turned off in the environmental chamber allowing me to film under dark conditions from 7 a.m. to midnight.

Trials began with the pre-treatment gate closed and the post-treatment gate open. After a fish was placed into the pre-treatment zone, the pre-treatment gate was opened allowing the fish to move down the trough and subsequently be captured in the post-treatment zone by closing the post-treatment gate. Pilot studies suggested that once fish were placed into the pre-treatment zone they tended to move continuously, hence, to minimize any pre-treatment fish stimulation to chironomids I added PVC mesh (1 cm x 1 cm) into the pre-treatment zone to reduce the area from 300 cm$^2$ to 100 cm$^2$ (Fig. 3).

Creation of chironomid patches

Pilot studies showed that the best way to perform experiments in the race-way was to create "patches" of *C. tentans* larvae on petri-dishes (Fig. 4), and then transport the patches into the tracks of the race-way. I left the petri-dishes in the track while running each experiment as removing petri-dishes or pipetting larvae on to a track's sand layer disturbed larvae enough to force them out of their tubes. Also, leaving the petri-dishes in the track allowed larvae to maintain the positions they had established during acclimation and, hence, minimize larval interaction during any given experiment.
Figure 3:

Top view of race-way: camera and patch positions shown.
Fig. 3
**Figure 4:**

Chironomid acclimation chamber used to create chironomid "patch". (See text for dimensions).
PVC sleeve

Water

Chironomids placed here
Silica sand

Petri-dish cover

Vapour barrier
Rubber band

Fig. 4
consisted of an 8.3 cm diameter x 8 cm PVC cylinder (Fig. 4). One end was closed using an 8.0 cm diameter x 1 cm high petri-dish cover centred over a 25 cm x 25 cm sheet of double-lined plastic vapour barrier. The barrier was drawn up around the sleeve and held in place using an elastic band placed 2 cm from the top of the cylinder. The vapour barrier prevented leaks. Each chamber was lined with a 0.5 cm depth of silica sand and filled to a 5.5 cm depth with dechlorinated water.

A patch of larvae consisted of eight, IVth instar larvae randomly picked from my stock culture at 8 a.m. and placed into an acclimation chamber. For preliminary work, larvae were immediately fed 4 drops of 0.5 g Tetra Conditioning Food/ 25 mLs distilled water, dispensed from an inverted 5¼" pipette. For experiments involving different larval hunger levels, degree of hunger was induced by staggering the feeding times. Details on feeding regimes have been included under a description of the effect hunger level on larval activity. Preliminary observations indicated that 24 hours was sufficient time for larvae to settle and build tubes, provided food was available. Patches were left in the laboratory till 4 p.m. and then placed in the environmental chamber for use the next day. Once used in a patch a larva was never used again.

Patches of C. tentans were placed directly under cameras suspended over the race-way, at a distance of 20 cm from the post-treatment gate (Fig. 3). After they were in place I removed the elastic band from each acclimation chamber, slid the vapour barrier out from under each chamber and removed the PVC sleeves. I then gently pushed each petri dish into its respective track’s sand layer until the sand was level with that in its track. Any larva that left its tube was removed.
Five video cameras were set up around the race-way. Two Panasonic CCTV WV-CP410 cameras (each with a Panasonic WV-LA908C3 9 mm lens) and a Panasonic CCTV WV-BL200 camera (with a Panasonic WV-LA12B2 lens) were attached to a metal frame laid across the width of the trough, above the larval patches (Fig. 3). The cameras were 20 cm away from the post-treatment gate and each lens was approximately 8 cm above the surface of the water and focused on the patch directly below it. A Panasonic WV-1850 camera (with a Canon Zoom V6x16 16-100mm lens) was set up at the post-treatment end of the trough, approximately 1 metre above the ground, to view the length of the trough. All four cameras were attached to a Panasonic Quad System WJ-420 which was attached to a Panasonic AG-6730 Time Lapse VCR and a Panasonic WV-5410 monitor, in the environmental chamber. The quad system allowed signals from all four cameras to be viewed simultaneously on one screen.

The fifth camera, a Panasonic TV camera WV-1850 (with a Bell and Howell-Angrnieux 3 inch #1074189 Lens) was placed immediately above, and focused on, the post-treatment end of the race-way. This camera was attached to a Panasonic WV-5410 monitor outside the environmental chamber. By viewing the monitor after releasing fish from the pre-treatment zone I could see when fish swam past the post-treatment gate and then knew when to shut the gates from my position outside the chamber.
Larval activity was defined as the amount of time (min) per hour a larva had at least its head, thorax and three abdominal segments extended out of its tube. Hershey (1987) suggests that extension of one abdominal segment is sufficient, but due to light conditions in the environmental chamber and video resolution, I used three abdominal segments to be sure a larva was extended outside its tube. Activity was recorded using an NEC PC-8300 Portable event recorder and analyzed using the S&K Data Reception and Analysis programme. Further analysis was performed using T-shell programmes in UNIX, Systat for Windows, Quattro-Pro for Windows and Sigma-Plot for Windows.

Preliminary studies

(a) Effect of light on larval *C. tentans* activity.

Macchiusi & Baker (1992) observed chironomid larvae were more active under red light compared to white light, therefore, I ran all experiments under red light to maximize activity. However, a minimum of eight 40W SYLVANIA solid red bulbs were needed in the environmental chamber for adequate video resolution and I was concerned that this level of lighting may have been bright enough to significantly inhibit larval activity (Macchiusi, 1992).

I designed an experiment to examine larval activity under red-light and white-light (16 fluorescent tubes mounted approximately 2 m above the race-way). Chironomid patches were set up and fed 24 hours before the experiment, after which one patch was placed in each track of the
Red and white light trials were alternated until a total of four trials had been completed for each treatment.

Activity was recorded only over the last 60 minutes of filming. For each trial, activity was pooled across the three tracks and averaged. Mean larval activity under white light was $4.11 \pm 1.1$ (S.E.) min/hour per larva, significantly lower than the $15.84 \pm 4.8$ (S.E.) min/hour per larva recorded under red-lights (two sample t-test, $n = 4$, $df = 6$, $p < 0.01$). This difference is similar to that in Macchiusi & Baker (1992) and suggested that I could perform all my experiments under high red-light conditions (maximum of eight 40W bulbs) and expect a marked increase in larval activity.

(b) Effect of gate movements on activity of larvae in neighbouring tracks.

As my main experiment was designed to include a control treatment where no gates would be moved in a particular track, it was necessary to determine if gate movements in adjacent tracks would affect larval activity in tracks where gates were not moving. Hence, the following experiment was designed to test for effect of gate movements on activity of larvae in neighbouring tracks.

Eight patches were set up and larvae were fed 24 hours before the trials. Before a patch was positioned in a track of the race-way, all pre-treatment gates were shut and all post-treatment gates were opened. A single patch was placed in a randomly chosen track, red lights were turned on, video recording was started and I left the chamber. Observations from the experiment on the
after I left the chamber. Larvae were, therefore, given a 15 minute acclimation period (Fig. 5a), followed by 15 minutes of pretreatment activity filming after which pre-treatment gates in the two tracks without patches were opened and left open for the remainder of the experiment. After 10 minutes I closed the post-treatment gates in the two tracks without patches. No gates were moved in the treatment track in order to isolate the effects of gate movements in other tracks on larval activity in the treatment track. Filming continued for another 20 minutes. I chose a period of 10 minutes between opening and closing gates because preliminary observations indicated fish took approximately 10 minutes to move from pre- to post-treatment zones (fish usually spent the first 8 to 9 minutes hovering in the pre-treatment zone). Each trial of a gate-movement treatment was alternated with a control trial, where no gates were moved. Fifteen replicates of each treatment were completed over a 4 day period.

To test for effects of pre-treatment gate movement on larval activity I compared the log transformed activity during the 5 minutes after pre-treatment gate movement in the treatment trials (Fig. 5b) with activity in the same 5 minute interval in the control treatment. Because the differences in log transformed activity were not significantly different from a normal distribution (Lilliefors, p > 0.3; Zar 1984) I used a two-sample t-test which indicated no significant difference (n = 15, df = 14, \( t = 0.005, \sigma = 0.234, p > 0.9 \)), suggesting movement of the pre-treatment gate did not affect larval activity. The same design was used for analysis of effects of the post-treatment gates. Again, the differences in log transformed activity were normally distributed (Lilliefors, p > 0.4) and a two sample t-test showed no significant difference (n = 15, df = 14, \( t = 0.085, \sigma = 0.238, p > 0.1 \)).
Figure 5:

Time-line used to film chironomids while testing for gate movement effects on larval activity in neighbouring tracks.
Fig. 5
Effects of passing fish and hunger level on larval activity.

I examined the effects of single passes by pumpkinseed sunfish (two size classes), and brown bullheads on larval activity. Pumpkinseeds and bullheads were chosen for their different foraging and swimming techniques. Observations showed that pumpkinseed sunfish tended to swim relatively slowly and fed by orienting to and striking at prey on the substrate. In contrast, bullheads were vigorous swimmers that frequently swam in contact with the substrate. Small pumpkinseeds averaged 7.2 cm (fork length), large pumpkinseeds averaged 14 cm and bullheads averaged 14.5 cm.

I used larval chironomids at three hunger levels to examine effects of hunger on larval
hours before experimentation, for the medium level I set up and fed the larvae 24 hours before the experiment, and for the high hunger level I set up and fed larvae 48 hours before experimentation. Because high hunger patches were set up 24 hours before low and medium hunger patches the effect of hunger is confounded by time since disturbance. I initially attempted to set up all patches 48 hours before the experiment and fed the low and medium hunger levels more often but extra feeding in the small containers fouled the water. It is unlikely that there was any effect of the different set-up schedules as all larvae were disturbed in the same way and at the same time when placed in the tracks of the race-way.

For each trial one track had a fish present and was used to determine the effects of a single pass by a fish on larval activity; the second track had no fish and was used to determine the effects of gate movements on larval activity within the same track; the third track was a control where no fish was used and gates were not moved. Information from all three tracks was used to calculate the amount of time larvae spent foraging before and after exposure to a passing fish.

Prior to every trial, all pre-treatment gates were closed and all post-treatment gates were opened. After a fish was placed into a pre-treatment zone, a chironomid patch was placed in each of the three tracks; all three patches were at the same hunger level. Red lights were turned on, filming was started and I left the environmental chamber. Both fish and chironomids were acclimated for 15 minutes followed by 15 minutes of pre-treatment activity filming before opening the pre-treatment gate in the fish- and gate-treatment tracks. In the fish-treatment track I shut the post-treatment gate immediately after the fish passed into the post-treatment zone. Fish
minutes the trial was aborted. In the gate-treatment track I shut the post-treatment gate 10 minutes after the pre-treatment gate was opened. Filming continued for another 60 minutes.

Failed trials included: fish not moving out of pre-treatment zone, fish never swimming into the post-treatment zone and fish taking longer than 10 minutes to cross the length of the trough. In these cases the experiment was stopped immediately and no data were analyzed. There were instances where fish passed over a chironomid patch more than once or fish struck at the patch. I continued filming in these instances in the hopes of reaching sizeable replication to examine how activity would change with multiple passes and strikes. However, there were so few of these cases that I could not statistically analyze them. Therefore, analysis was restricted to trials with single passes and no strikes.

There were a total of nine treatments in a set (3 fish types x 3 hunger levels). For each trial in each set, the tracks in which the fish, gate and control treatments were assigned were randomized. As filming of each trial took a total of 1 hour and 40 minutes, each set could theoretically be filmed in 1½ days. However, at least one trial failed in all sets. I re-ran the failed trial within a maximum of 48 hours. If the trial failed again, I aborted the entire set and no data recorded in this set were used in any statistical analyses. Mean activity was recorded in each five minute interval for 85 minutes of any trial and converted to minutes a larva spent out of its tube/hour per larva.
Video images recorded in the experiment described above allowed me to examine larval activity in response to the horizontal distance between a fish and a patch. Camera 4 captured the image of the passage of fish from pre- to post-treatment zones. Using the quad system images on a monitor, I attempted to determine the number of chironomids out of their tubes after every 5 cm of fish passage distance. Theoretically, fourteen sets of nine treatments were available for use. However, because a great deal of freeze-framing, which was required to determine the number of chironomids active over the short period of time it took fish to travel the length of the trough (generally 1 to 2 minutes), quality of the recordings was compromised and thus my useful sample size was quite small.

**Effects of vertical distance of fish on larval C. tentans activity**

Underwater time-lapse video imaging collected in the field by N. Collins, Erindale College, showed that fish swam at various distances above the bottom of the lake (personal communication, observation). To determine how larvae responded to potential predators at different vertical distances from the bottom of a lake, I measured larval activity when larvae were exposed to two types of predators (brown bullhead and pumpkinseed sunfish, mean fork length 14.2 and 13.8 cm, respectively) at three vertical distances in an aquarium.

To establish the boundaries of the three vertical distances I divided an aquarium (72.5 x 30 x 44 cm deep) into four zones by inserting three mesh dividers. Dividers (30 x 70 cm pieces of plastic mesh, mesh size 1 x 1 cm) were placed at depths of 4, 17, and 30 cm from the bottom.
I filled the aquarium with a 44 cm depth of dechlorinated water over a 1 cm depth of silica sand. A video camera with a macro-lens was set-up outside one side of the aquarium, and attached to a time lapse VCR. The camera was focused onto an 8.3 cm diameter zone at the bottom of the aquarium. A single SYLVANIA 40 W solid-red bulb provided all light.

I selected sixty-four, early IVth instar larvae at 8 a.m. each day and placed them into a 3.0 L opaque polypropylene tank (26 x 15 x 10 cm deep) lined with a 1 cm depth of silica sand and filled with 2.0 L of 2 parts dechlorinated water: 1 part distilled water. Larvae were fed 16 drops of 1 g Tetra conditioning Food / 20 mLs of distilled water. This concentration was equivalent to Ball's (1994) high food level which proved to be adequate to maintain larval condition. Once fed, larvae were acclimated under light for 10 hours. The tank was then covered to block light for 7 hours and then uncovered to allow exposure to another 7 hours of light. This effectively reversed the light cycle with relatively equal light and dark periods, allowing me to begin filming the next morning in a dark period.

Treatments consisted of one of two types of fish presented at one of three depths and a control treatment for a total of seven treatments (one set). Larvae were not acclimated or filmed on petri-dishes in the aquarium as the edge of the dish interfered with a clear view of the larvae during playback. Instead, eight larvae were pipetted out of the acclimation tub and dropped into the 8.3 cm diameter zone at the bottom of the aquarium. Occasionally larvae would abandon their tubes after being pipetted into the aquarium and I discarded trials with less than 5 larvae in a
the sand layer; all dividers were present in all treatments. In fish-treatments, dividers and fish were placed into the aquarium in the appropriate order. I began filming as soon as the final divider was positioned. For each set, treatments were randomly selected without replacement and each set was completed in one day, before starting on a new set. Fourteen sets were completed in 14 days. Larval activity was recorded from the last 45 minutes of 60 minutes of film using an NEC PC-8300 portable event recorder and analyzed using the S&K data receptor and analysis programme.
Effects of a single fish pass on larval C. tentans activity.

Effect of gate movement on larval activity within the same track

To determine if gate movement affected activity of larvae within the track where the gate was moved, I compared chironomid activity in the gate treatment recorded during the 5 minute intervals immediately before and after the pre-treatment gate was opened. Before testing for gate effects I first tested for effects of fish type and hunger level on the difference in activity between these two periods, to determine if I could pool data across treatments. Hurlbert (1984) and Zar (1984) both suggest log transformation of data for ANOVAs as preferable to correct for problems of multiplicative data and for instances where variances are heteroscedastic and standard deviations in the data are proportional to the means. Hence, I log transformed data where appropriate. To test for effects of fish and hunger on larval activity, I calculated the mean activity (log transformed) before the pre-treatment gate was opened and subtracted it from that recorded after the gate was opened. Of the nine treatments only data from the large pumpkinseed, high larval-hunger level was significantly different from a normal distribution (Lilliefors, p < 0.05); variances were homoscedastic (Fmax test, p > 0.05). A two-way ANOVA (N = 14, n =126) indicated neither type of fish (SS = 0.05, df = 2, p > 0.5) nor larval hunger-level (SS = 0.03, df = 2, p > 0.5) affected activity differences; there was no interaction (SS = 0.27, df = 4, p > 0.4). This suggested data could be pooled across treatments to test for within-track gate effects on larval activity.
after the pre-treatment gate was opened, the differences in log transformed activity were significantly different from a normal distribution (Lilliefors, p < 0.05). A Wilcoxin Signed Rank test indicated no significant difference, suggesting no change in larval activity as a result of pre-treatment gate movement (n = 126, Z = 0.466, p > 0.6).

The same calculations were performed on activity collected before and after the post-treatment gate was closed. Distribution of log transformed activity differences in the bullhead: low and medium hunger level, and the small pumpkinseed: high hunger level data were significantly different from normal (Lilliefors, 0.03 < p < 0.005), however, variances were homoscedastic for all nine treatments (Fmax, p > 0.05). A two-way ANOVA indicated no fish effects (SS = 0.167, df = 2, p > 0.3), no larval-hunger level effects (SS = 0.033, df = 2, p > 0.5) nor any interaction (SS = 0.351, df = 4, p > 0.25). Differences in log transformed activity collected before and after post-treatment gate movement were significantly different from a normal distribution (Lilliefors, p < 0.05). A Wilcoxin Signed Rank test indicated no significant difference in larval activity, again suggesting no change in larval activity as a result of post-treatment gate movement (n = 126, Z = 0.180, p > 0.8).

Comparison of control and fish treatments

As there was no significant effect of gate movement on larval activity, I used larval activity recorded in the fish and control treatment tracks to estimate effects of a passing fish on larval activity. Occasionally a fish hovered in the pre-treatment holding zone after the gate was opened but it normally took a minute or less to cross the length of the track and about
treatment activity in three 5 minute intervals, immediately before the fish passed over the chironomid patch, and post-treatment activity in twelve 5 minute intervals, immediately after the fish passed over the larvae patch. For each control treatment, I measured activity during the same time periods as in the corresponding fish treatment. Visual inspection of time plots of mean larval activity (min/ hour per larva) (Figs. 6a-6c) showed larval activity was highly variable, however, in all treatments activity dropped during the 5 minutes after the fish passed over the patch.

Inspection of time plots (Figs. 6a - 6c) suggested that control pre-treatment activity was higher than fish pre-treatment activity across all treatments except bullhead: low and medium hunger level, and small pumpkinseed: medium hunger level, where control pre-treatment activity fluctuated around fish pre-treatment activity. These observations suggested that larval chironomids in fish-treatment tracks were detecting the presence of fish before the pre-treatment gate was opened and I wanted to determine if pre-treatment activity in tracks with fish was significantly different from that in control tracks.

Before comparing pre-treatment activity measured in control and fish treatments I first determined if there were any fish or hunger-level effects. If not, data could be pooled to increase my sample size. I calculated mean activity over the 15 minute pre-treatment time for each fish and control treatment. All 18 samples were normally distributed (Lilliefors, 0.06 < p < 1.00) except the distribution in the control's bullhead: high hunger sample (p = 0.001); data were homoscedastic (Fmax test, p > 0.05). For control treatments, a 2-way ANOVA (N = 14, n = 126) indicated hunger had an effect on larval activity (SS = 539.1, df = 2, p <
Figure 6a:

Mean larval activity (min/hour per larva) recorded over time (min) after exposure to a passing bullhead. Larvae were maintained at low, medium and high hunger-levels. Arrow (1) indicates moment the fish passed over the patch.

Standard error bars have not been shown. n = 14 for each treatment.
**Figure 6b:**

Mean larval activity (min/hour per larva) recorded over time (min) after exposure to a passing big pumpkinseed sunfish. Larvae were maintained at low, medium and high hunger-levels. Arrow (1) indicates moment the fish passed over the patch.

Standard error bars have not been shown. $n = 14$ for each treatment.
Fig. 6b
**Figure 6c:**

Mean larval activity (min/hour per larva) recorded over time (min) after exposure to a passing small pumpkinseed sunfish. Larvae were maintained at low, medium and high hunger-levels. Arrow (↑) indicates moment the fish passed over the patch.

Standard error bars have not been shown. n = 14 per treatment.
Fig. 6c
the neighbouring track did not affect larval activity (SS = 7.2, df = 2, p > 0.8) and there was no interaction (SS = 83.9, df = 4, p > 0.8). In fish treatments, a 2-way ANOVA (N = 14, n = 126) indicated no fish effect (SS = 217.7, df = 2, p > 0.9), no hunger effect (SS = 10.9, df = 2, p > 0.2) and no interaction between the two (SS = 187.9, df = 4, p > 0.6). As control pre-treatment activity data could only be pooled within hunger levels, the same was done with corresponding mean pre-treatment activity in fish treatments before comparing the two.

Differences in pooled mean pre-treatment activity recorded in fish treatments (n = 42) and corresponding control treatments (n = 42), at the low hunger level, were not significantly different from a normal distribution (Lilliefors, p = 0.85); similar results were found for pooled activity under medium and high hunger levels (Lilliefors, p = 0.056 and 1.00, respectively). At low and medium hunger levels, larval pre-treatment activity in the control was not significantly different from activity in the fish treatment (paired t-test, n = 42, df = 41; low: $\bar{d} = 2.25$, $s_d = 10.02$, p > 0.15; med: $\bar{d} = 0.61$, $s_d = 9.3$ p > 0.67); however, under high hunger conditions pre-treatment activity was higher in the control than in the fish treatment (paired t-test, n = 42, df = 41, $\bar{d} = 4.1$, $s_d = 11.7$, p = 0.029). I was concerned that performing a number of comparisons on the data increased the type-I error rate (Rice, 1989). Therefore, I performed a sequential Bonferroni test which suggested there was no significant difference in the control and fish pre-treatment activity ($\alpha = 0.017$, k = 3). Larvae in fish treatment tracks were not expected to react to the presence of fish prior to fish being released; never-the-less I decided to remain conservative and calculated the amount of foraging time lost by larvae after being exposed to a passing fish, by both ignoring and controlling for the possible effect of fish on pre-treatment activity.
Table 1:

Mean larval pre-treatment activity (min/hour per larva ± S.E.) for fish treatments versus controls, across three fish types (bullheads, big and small pumpkinseed sunfish) and three larval hunger-levels (low, medium and high).

After a Bonferroni correction (Rice 1989, α = 0.017, k = 3, lowest p value = 0.047) no significant difference was found between fish treatments and controls. However, to remain conservative in calculating activity loss at a later point, I did assume larvae were responding early to fish presence and accounted for the activity lost in a series of alternative calculations.
Table 1:

Mean Larval Pre-treatment Activity

(min/hour per larva ± S.E.)

<table>
<thead>
<tr>
<th>FISH TYPE/ HUNGER LEVEL</th>
<th>CONTROL</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bullhead Low</td>
<td>16.36 ± 1.6</td>
<td>15.40 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>15.71 ± 2.1</td>
<td>15.26 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>22.16 ± 3.0</td>
<td>17.54 ± 1.4</td>
</tr>
<tr>
<td>Big Pumpkinseed Low</td>
<td>17.02 ± 2.5</td>
<td>13.79 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>16.07 ± 1.4</td>
<td>16.26 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>19.40 ± 2.7</td>
<td>16.42 ± 2.2</td>
</tr>
<tr>
<td>Small Pumpkinseed Low</td>
<td>15.56 ± 2.6</td>
<td>12.79 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>17.42 ± 1.8</td>
<td>19.51 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>20.68 ± 2.6</td>
<td>15.97 ± 2.7</td>
</tr>
</tbody>
</table>
To estimate activity lost as a result of a single fish pass I calculated the difference between "potential" activity (determined from the control treatment) and "observed" activity (determined from the fish treatment) for each trial. Within each trial I regressed control data against time; regression reduced the impact of unusual data points as well as giving a general direction of activity levels across time. Data used to calculate each regression were not independent but the regression itself was not used to test statistical hypotheses.

To contend with the problem of larvae detecting fish presence before the pre-treatment gates were opened I compared larval activity in fish treatments to the regressed control lines in two ways. First, I maintained the intercepts of the control lines; by doing so I essentially ignored any effect of fish presence on pre-treatment activity. The second method involved adjusting the intercepts of the control lines to match the mean pre-treatment activity value calculated in corresponding fish treatments (Fig. 7). The latter process maintained the original slope of the control line, but in effect "controlled" for the effect of fish presence on pre-treatment activity.

*Calculation of event duration and time lost for larval activity, after a single fish pass.*

Activity time-plots (Figs. 6a-6c) indicated larval activity dropped immediately after a fish pass but rose soon after. Before calculating the amount of activity lost by larvae after a fish pass I had to establish a criterion to recognize when a patch had recovered to a normal level of activity after responding to the fish pass. I defined "event duration" as the summed
Figure 7:

Descriptive: Larval activity (min/hour per larva) versus time (min).

Control lines used to compare larval activity in fish treatments to corresponding controls were regressed. Non-adjusted control lines essentially ignored the effect of fish presence in pre-treatment activity. Adjusted control lines were used to "control" for the effect of fish presence on pre-treatment activity; the slope of the original regressed control line was maintained and the intercepts were altered to match the mean pre-treatment activity values calculated in corresponding fish treatments.
Fig. 7
chironomid patch to, and including, the 5 minute interval in which larval activity in the fish trial returned to within at least 95% of larval activity in the corresponding control trial, or to a limit of 60 minutes after the fish pass, whichever came first (Fig. 8). In trials where larval activity in the fish trial was greater in the 5 minute interval after a fish pass than in the corresponding 5 minute interval in the control trial, I simply defined the event duration as I did above, except larval activity in the last 5 minute interval of the event was within at least 105% of larval activity in the corresponding control trial (Fig. 8).

For each event I calculated potential larval activity and observed larval activity. Potential activity was calculated using the regressed control line; activity (min/hour per larva) within each 5 minute interval of an event was divided by 12 to calculate activity (min/5 min interval per larva). Potential activity (min/5 min interval per larva) was then summed across all 5 minute intervals of the event to calculate potential activity (min/event per larva). The same calculations were used for observed larval activity in fish treatments. The amount of activity lost by chironomid larvae after a single fish pass was simply the difference between the potential activity and the observed activity. This procedure was performed using both the regressed control line (non-adjusted) and the regressed control line with altered intercepts (adjusted).
Figure 8:

Descriptive: Larval activity (min/hour per larva) versus time (min). Fish effects caused either reduced larval activity or increased larval activity which determined how event duration and activity time lost were calculated.

If larval activity decreased immediately after a fish pass:

Event duration = Summed time of each 5 minute interval, with the duration beginning when a fish passed over a chironomid patch to, and including, the 5 minute interval in which larval activity in the fish trial returned to within at least 95% of larval activity in the corresponding control trial, or to a limit of 60 minutes after the fish pass, whichever came first.

If larval activity increased immediately after a fish pass:

Event duration = Summed time of each 5 minute interval, with the duration beginning when a fish passed over a chironomid patch to, and including, the 5 minute interval in which larval activity if the fish trial returned to within least 105% of larval activity in the corresponding control trial.
Event duration

Activity = 105% \times \text{control activity}

Activity = 95\% \times \text{control activity}

Fig. 8
To test for effects of fish type and hunger level on duration of an event (Fig. 9), I used a 2-way ANOVA (N = 14, n = 126) on the event durations measured for all nine treatments. Six of the nine samples of event durations were significantly different from a normal distribution (Lilliefors, 0.00 < p < 0.04) and log transformation did not make them normal. However, variances were homoscedastic (Fmax, p > 0.05). Neither type of fish, nor fish size had an effect on event duration (SS = 136.1, df = 2, p > 0.8); there was no hunger effect (SS = 871.8, df = 2, p > 0.3) and no interaction (SS = 2672.2, df = 4, p > 0.2). Mean event duration ranged from 23 minutes to 40 minutes.

I used a 2-way extension of the Kruskal-Wallis (n = 126) test to determine effects of fish type and hunger-level on time lost for activity (Fig. 10); five samples were close to or significantly different from a normal distribution (Lilliefors, 0.01 < p < 0.08) and variances were heteroscedastic (Fmax, p < 0.05). There were no fish effects (p > 0.25), no hunger effects (p > 0.1) and no interaction (p > 0.25). These results suggested I could pool all the potential activity and test it against the observed activity to test if the change was significantly different from zero. Both pooled potential (n = 126) and pooled observed activity (n = 126) were significantly different from normal distributions (Lilliefors, p < 0.05); a Wilcoxin Signed Rank test indicated that time lost for activity was significantly different from zero (Z = 4.71, p < 0.001). Median activity lost was 1.16 ± 6.5 (S.E.) min/ event per larva, or 16% of the potential activity exhibited.
Figure 9:

Non-adjusted Control Line:

Mean event duration (min $\pm$ S.E.) of larvae at low, medium and high hunger-levels after a single fish pass by one of three fish types (bullhead, big and small pumpkinseed sunfish).
Figure 10:

Non-adjusted Control Line:

Median activity lost (min/event per larva) of larvae at low, medium and high hunger-levels after a single fish pass by one of three fish types (bullhead, big and small pumpkinseed sunfish). Box represents the 25th and 75th percentile. Whiskers represent the 5th and 95th percentile. Data outside this range are depicted by a dot (·).
Fig. 10
As described above, I also calculated event duration and activity lost by larvae after a single fish pass by comparing activity in the fish treatment with that in the control treatment where the intercepts of the control line were adjusted to the mean pre-treatment activity calculated in the corresponding fish treatment.

To test for effects of fish type and hunger level on duration of an event, I used a 2-way ANOVA (N = 14, n = 126) on event durations measured for all nine treatments. Seven of the nine samples were significantly different from normal distribution (Lilliefors, 0.00 < p < 0.02) and log transformation did not correct for normality. However, variances were homoscedastic (Fmax, p > 0.05). Type of fish (SS = 1600, df = 2, p > 0.1) and hunger level (SS = 776.2, df = 2, p > 0.3) had no effect on event duration; there was also no interaction (SS = 1030.9, df = 4, p > 0.6). Mean event duration ranged from 17 minutes to 36 minutes (Fig. 11).

To test effects of fish type and hunger level on the amount of time lost for activity, I performed a 2-way extension of a Kruskal Wallis (n = 126); data were significantly different from a normal distribution (Lilliefors, 0.00 < p < 0.02) and variances were heteroscedastic (Fmax, p < 0.01). There were no effects of fish type (p > 0.9) or hunger effects (p > 0.10) and there was no interaction (p > 0.25). These results suggested I could pool the potential activity (n = 126) and compare it to pooled observed activity (n = 126) to test if the change in activity was significantly different from zero. Both samples were significantly different from normal distributions (Lilliefors, p < 0.00); a Wilcoxin Signed Rank test indicated time
Figure 11:

Adjusted Control Line:

Mean event duration (min ± S.E.) of larvae at low, medium and high hunger-levels after a single fish pass by one of three fish types (bullhead, big and small pumpkinseed sunfish).
Fig. 11
Comparison of event duration and activity loss measured using the two different control lines.

To test if the event durations (pooled across treatments) calculated using the non-adjusted control lines were similar to the data calculated using the adjusted control lines (Fig. 13), I performed a Wilcoxin Signed Rank test because the differences between the two samples were significantly different from a normal distribution (Lilliefors, p < 0.001). The two samples (each n = 126) were significantly different from each other (Z = 3.23, p < 0.01). For lost activity, the differences between lost activity measured using the two techniques (Fig. 14) were significantly different from a normal distribution (Lilliefors, p < 0.0001); a Wilcoxin Signed Rank test indicated no difference (n = 126, Z = 1.63, p > 0.1) in the activity loss measured using the two techniques.

Effects of passing fish and hunger level on larval activity using only data from fish treatments

In addition to the analysis above it is possible to test for effects of fish type and larval hunger level on fish induced changes in activity by comparing the activity of larvae in tracks with fish, before and after the fish passes over. I log-transformed activity recorded in the two five minute periods directly before and after a fish passed over a patch and calculated the activity difference (5 minutes after - 5 minutes before); data were normally distributed in
Figure 12:

Adjusted Control Line:

Median activity lost (min/event per larva) of larvae at low, medium and high hunger-levels after a single fish pass by one of three fish types (bullhead, big and small pumpkinseed sunfish). Box represents the 25th and 75th percentile. Whiskers represent the 5th and 95th percentile. Data outside this range are depicted by a dot (•).
Fig. 12
Figure 13:

Median event duration (min) compared for adjusted and non-adjusted control lines (for three larval hunger-levels crossed with three fish types). Box represents the 25th and 75th percentile. Whiskers represent the 5th and 95th percentile. Data outside this range are depicted by a dot (·).
Fig. 13
Figure 14:

Median activity lost (min/event per larva) compared for adjusted and non-adjusted control lines (for three larval hunger-levels crossed with three fish types). Box represents the 25th and 75th percentile. Whiskers represent the 5th and 95th percentile. Data outside this range are depicted by a dot (•).
Fig. 14
to determine if the change in activity was affected by fish and hunger level. There was no effect of fish type \( (p > 0.7) \) nor any interaction \( (p > 0.7) \), but there was a hunger effect \( (p < 0.05) \). Activity decreased in all treatments (Fig. 15) with the greatest decreases occurring under high hunger conditions. This contrasts with the previous analysis where no hunger effect was found.

I pooled data within each hunger level to test whether the activity changes were different from zero. Data for the low and medium hunger level samples were significantly different from a normal distribution (Lilliefors, \( p < 0.01 \)) but that for the high hunger level sample was normally distributed (Lilliefors, \( p > 0.17 \)). I used the Wilcoxin Signed Rank test on the low and medium hunger level samples and a paired t-test on the high hunger-level sample. All changes in activity were significantly different or close to significantly different from zero (low: \( n = 42, Z = 3.22, p < 0.001 \); med: \( n = 42, Z = 1.76, p < 0.08 \); high: \( n = 42, df = 41, d = 0.27, s_d = 0.33, p < 0.0001 \)); a Bonferroni correction suggested "table-wide" significance \( (\alpha = 0.017, k = 3, n = 42) \) supporting the original analysis which suggested that a single fish pass can cause a decrease in activity.
Figure 15:

Median larval activity change (min/hour per larva) using only data recorded from fish treatments. Activity change was the difference of the log transformed activity before the fish pass from log transformed activity after the pass. Log transformation was necessary to compare only proportional changes in activity and not absolute values, as there was a great deal of variance in the data.
To determine the distance at which larvae first responded to fish movement as fish approached the chironomid patch, I attempted to record the number of larvae out of their tubes after every 5 cm of forward movement by the fish. However, fish rarely took longer than a minute to travel the length of the track, greatly reducing my observation time of larvae. With a considerable amount of freeze-framing required, film resolution was compromised and it was difficult to assess whether larvae were active or not; it proved impossible to generate any dependable quantity of data. In general, larvae rarely appeared to react to fish swimming towards the patch, and pulled back into their tubes only after the fish was about 1-2 cm from the patch or had passed over the patch.

Effects of vertical distance of fish on larval C. tentans activity.

Larval activity in the bullhead and pumpkinseed treatments was normally distributed across all treatments (Lilliefors, 0.4 < p < 1.00) except when fish were 4 cm from the bottom of the aquarium (Lilliefors, 0.002 < p < 0.04); data were homoscedastic (Fmax, p > 0.05). A one-way ANOVA (N = 14, n = 98) on larval activity (min/ hour per larva) recorded in the six treatments and the control indicated no significant difference (SS = 435.1, df = 6, p = 0.54) but the probability was clearly borderline (Fig. 16). A Tukey's post-hoc test (df = 91) indicated a significant difference in activity recorded in bullhead: 4 cm versus pumpkinseed: 4 cm treatments (p = 0.04) and borderline significance was detected between activity recorded in the bullhead: 4 cm versus control treatment (p = 0.056). Larval activity tended to increase in bullhead treatments as fish distance increased; in pumpkinseed treatments, larval activity
Figure 16:

Mean activity (min/hour per larva ± S.E.) recorded for larvae exposed to a bullhead or pumpkinseed sunfish presented at 4, 17 or 30 cm above the chironomid patch. Control trials were run with complete absence of fish. The bullhead:4 cm and pumpkinseed:4 cm as well as the bullhead:4 cm and control treatments were found to be borderline significant (Tukey's Test of Significance, p = 0.004 and 0.0056, respectively).

Means indicated by the same letter (a,b) were not significantly different at the p = 0.05 level (Tukey's Test of Significance).
Fig. 16
per larva when a pumpkinseed sunfish was present and 15.99 ± 1.4 min of activity/hour per larva under control conditions (water was infused with fish odour).
During the past 15 years many researchers have exposed various species of prey to predators under both laboratory and field conditions. These studies have reported decreases in prey foraging activity and increases in avoidance behaviour; some have indicated that predator presence inhibits growth and development (Table 2). Unfortunately, there are inherent problems in extrapolating results from most of these experiments to natural situations. Problems with these studies include: prey exposed to possibly unrealistic levels of predator density, predators in unnatural spatial conditions, predators present for extended periods of time, or combinations of the three scenarios. Despite these limitations, authors seem intent on extrapolating their results to field conditions and frequently making statements on the impact of antipredator behaviour on individual development, population growth or community structure (Table 2). Thus, while it seems likely that, in the field, predator presence will affect behaviour and development of prey, it is currently impossible to estimate the strength of these effects in relation to other environmental variables. The past 15 years of research has shown conclusively that most prey respond to the presence of predators but we have virtually no estimate as to the realistic impact of this change in behaviour to prey growth and development.

I focused on the problem of exposing prey to unrealistic predation stress using Chironomus tentans, because considerable work has been done on larval behaviour and development. Macchiusi & Baker (1992) were concerned with the problem that continuous
Table 2:

Examples of published research where prey (py) behaviour was documented after exposure to predators (pd) for possibly unrealistic periods of time or unnatural spatial conditions (including unrealistic predator and/or prey densities). Results have been summarized.

The limitations presented here are objective concerns and are retrieved directly from the authors' published works. Ball & Baker (1995, 1996) were used to a great extent as the base for my work.

<p>| Table 2: | Examples of published research where prey (py) behaviour was documented after exposure to predators (pd) for possibly unrealistic periods of time or unnatural spatial conditions (including unrealistic predator and/or prey densities). Results have been summarized. The limitations presented here are objective concerns and are retrieved directly from the authors' published works. Ball &amp; Baker (1995, 1996) were used to a great extent as the base for my work. |</p>
<table>
<thead>
<tr>
<th>YEAR</th>
<th>AUTHORS</th>
<th>ORGANISM(S)</th>
<th>LIMIT</th>
<th>CONCLUSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980</td>
<td>Peckarsky</td>
<td>mayflies (py)</td>
<td>3pd:6py for pd effects on py activity vs. 1pd:15py for contact expts; no field densities mentioned</td>
<td>+stonefly pd has considerable influence on distribution, behaviour, activity of mayfly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stoneflies (pd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1986</td>
<td>Sih</td>
<td>mosquito larvae (py)</td>
<td>small tubs used for expts; py continuously exposed to pd for over 4-6 hours</td>
<td>avoidance CAN have large effect on py’s life style, rather than just escaping when attacked</td>
</tr>
<tr>
<td></td>
<td></td>
<td>backswimmer (pd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1988</td>
<td>Rassmussen &amp; Downing</td>
<td>chironomid larvae (py)</td>
<td>although ambient leech density replicated, done so in small, fixed enclosures for 28 days</td>
<td>- leech pd ↑ spatial heterogeneity of chironomid, ↓ competition amongst larvae, affecting chironomid growth rates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>leech (pd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990</td>
<td>Rahel &amp; Kolar</td>
<td>mayflies (py)</td>
<td>py exposed to unrealistic near 0 mg O₂/L levels. Unlikely that py would normally be found at this level</td>
<td>py more susceptible to predation; ↓ growth/fecundity if avoid pd, ↑ death if remain in anoxic habitat</td>
</tr>
<tr>
<td></td>
<td></td>
<td>brown trout (pd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990</td>
<td>Skelly &amp; Werner</td>
<td>larval toad (py)</td>
<td>py exposed to pd +, in small containers (2.4 L of H₂O) for up to 58 days</td>
<td>41% ↓ py activity, 28% ↓ py growth rate. Py responses affect pop dynamics &amp; community level interactions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dragonfly larvae (pd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>Authors</td>
<td>Species</td>
<td>crayfish Exposure</td>
<td>Notes</td>
</tr>
<tr>
<td>-------</td>
<td>------------------</td>
<td>----------------------------------</td>
<td>-------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>1992</td>
<td>Skelly</td>
<td>tadpoles (py) salamander (pd)</td>
<td>extended exposure, small areas</td>
<td>field: heavier, faster development in - of pd; lab: ↓ activity in +</td>
</tr>
<tr>
<td>1993</td>
<td>Ode &amp; Wissinger</td>
<td>mayfly (py) stoneflies (pd)</td>
<td>small observation arenas; +/- chemical cues with pd contact</td>
<td>chem. stimuli act as 'early warning' signals; ↓ cost of premature escape (↓ foraging efficiency), ↓ vulnerability to fish predation</td>
</tr>
<tr>
<td>1993</td>
<td>Peckarsky et al.</td>
<td>mayfly (py) stoneflies (pd)</td>
<td>10 py individuals/15 cm diameter chamber exposed to pd + for up to 21 days</td>
<td>py mature at smaller size, suffer a loss in potential contribution of offspring to the next generation</td>
</tr>
<tr>
<td>1993</td>
<td>Stauffer &amp; Semlitsch</td>
<td>tadpoles (py) pike (pd)</td>
<td>py tested in a small chamber</td>
<td>py respond to pd differently, in a manner that may be adaptive for a palatable, cryptic species</td>
</tr>
<tr>
<td>1995, 1996</td>
<td>Ball &amp; Baker</td>
<td>chironomid larvae (py) pumpkinseed sunfish (pd)</td>
<td>py exposed to continuous + of pd for over 14 days (findings based in combination with reduced food level)</td>
<td>reduced emergence of adult py, reduced body size at emergence, greater age of emergence; influences on community structure</td>
</tr>
</tbody>
</table>
prolonged exposure to fish may result in inaccurate estimates of activity and growth under normal conditions. They exposed *Chironomus tentans* larvae to a variety of schedules of fish presence but did not find any direct evidence for habituation at high levels of predator presence. They did find evidence that larval foraging decreased as the time predatory fish were present increased and that the incremental reduction in foraging caused by fish presence was reduced at higher levels of predator presence. Macchiusi and Baker (1992) experimented with animals in small tanks and varied the time predators were present from 0% to 100%; after the 0% value the next lowest value was 10%. Presence time (%) was maintained by having fish present for a continuous 12 min period out of 2 hr (120 min) blocks of time. Thus, while Macchiusi and Baker (1992) realized the importance of varying the time predators were present, even their lowest level with a predator present now appears very different from what occurs under field situations.

Underwater video-recordings in the field indicate that fish activity varies greatly between lakes, sites within a lake, across seasons or within a day at one site (Collins 1989). Also, video playback of fish activity in the field shows extended periods of time when no fish are present. Most importantly, when a fish does swim through a given area of the benthos it does so quite rapidly, rarely hovering over a given area (Collins & Hinch 1993, personal observations). However, having a fish hovering over prey for extended periods is precisely the protocol used by Macchiusi and Baker (1992) and Ball and Baker (1995, 1996). Ball & Baker (1995, 1996) showed that in the continuous, non-lethal presence of pumpkinseed sunfish, chironomid larvae exhibited reduced growth rates, smaller adult size, slower development rate and lower fecundity. Unfortunately, their study involved exposing larval
larval habituation to pumpkinseed sunfish, the reductions in growth and development of larval chironomids in their study can best be viewed as extreme and exaggerated values compared to the effects of prey exposure to fish predators in the field.

**Design, implementation and limitations of the raceway**

As part of a larger study designed to measure the impact of realistic levels of fish presence on development of larval *C. tentans*, I designed a system to evaluate the effects of a single fish "pass" on the foraging activity of larvae. Video recordings by Collins (1989) and Collins and Hinch (1993) indicated that fish normally swam through an area quickly and only rarely hovered over an area. I termed the former swimming pattern a fish "pass" and designed a race-way (a series of tracks in a trough) that would allow me to study the effect of a controlled, yet realistic, fish pass on activity of chironomid larvae. In the race-way, fish were acclimated in a closed pre-treatment zone, after which they were released from the pre-treatment zone, allowed to pass (treatment) over a patch of chironomids and into a post-treatment zone that was immediately closed using a drop-gate; chironomid activity was filmed before, during and after the treatment.

Before starting experiments using fish I ran a series of experiments to test some possible limitations of the system. I found that red light conditions resulted in much higher levels of activity than did white light and, while this does not imply activity under red light is the same as in the dark, it suggests red lights can be used to simulate "dark" conditions.
in the treatment track or any neighbouring track. The lack of a "gate effect" is critical in allowing interpretation of my results for future studies on effects of realistic levels of fish presence; i.e. changes in behaviour described in this study should reflect changes seen in the field where there are no gates.

The race-way design, including camera placement, proved successful in controlling fish passes most of the time. It was difficult to control situations where the fish remained in the pre-treatment zone, double-backed, ate the chironomids or hovered over the chironomid patch; as described earlier, cases where these events occurred were excluded from analysis. However, these situations all reflect what can happen in nature and, given sufficient sample sizes, it should be possible to analyze the consequences of these events on chironomid activity. The difficulty here is that such events are virtually impossible to control or create and one would have to run a great number of replicates to ensure a sufficient number of such cases.

A second, and possibly more important problem with the race-way was that of the chironomids detecting the presence of a fish in the pre-treatment zone. While I tried to prevent fish from moving around in the pre-treatment zone by minimizing the area and restricting their movement, observation indicated larval activity in fish treatments was lower than that in control tracks, even before the pre-treatment gates were opened. I ran three comparisons between fish tracks and control tracks (one for each food level), activity at the high hunger-level was found to be significantly lower in the fish tracks compared to that in control tracks, but when I used a Bonferroni correction to modify the alpha level (Rice 1989)
tracks. Despite the analysis suggesting that chironomids were not reacting to the presence of a fish behind the gate, I chose to be conservative and analyzed the data in two ways: I assumed there was no effect of fish in the pre-treatment zone and I statistically controlled for the possible effect of fish in the pre-treatment zone.

If subsequent studies indicate chironomids can detect fish in the pre-treatment zone of the race-way it may be that the larvae detect fish through olfaction i.e. larvae may have detected fish odour (Macchiusi and Baker 1992, Ode & Wissinger 1993) that may have leaked from the pre-treatment zone into the treatment area. However this seems unlikely; *C. tentans* do respond to fish odour, but the difference in activity is small (Macchiusi and Baker 1992) and, more importantly, the control tracks were not free of fish odour as water in all tracks of the race way was infused with fish odour (this stemmed from randomizing treatments across tracks). Note, having water in all tracks of the race-way infused with fish odour is likely more realistic than having control tracks without fish odour since any lake or pond with fish will be infused with fish odour, even if a fish may not be in the immediate vicinity of a prey. If larvae did detect the presence of predators from behind the gate using cues other than odour (mechanical stimuli) it may be possible to alleviate this problem in the future by using longer tracks, i.e. the effect of predators behind gates should decrease with an increase in distance between the gates and the chironomids (R. Baker, personal communication). Overall, I was confident that the design of the raceway allowed me to study the effects of a fish pass, similar to those seen in the field, on the activity of chironomid larvae in the laboratory.
I examined the impact of passes by 2 species of fish (pumpkinseed sunfish and brown bullheads) on chironomid activity. Brown bullheads are benthic feeders that readily disturb the substrate when they swim; pumpkinseed sunfish feed on both benthic and planktonic prey and, while they often swim very close to the bottom, do not readily disturb the substrate.

My results indicated that there was a definite decrease in larval activity immediately after a fish passed over a chironomid patch but that the effect did not depend on the size or species of fish. Thus, it appears that larvae were responding to a disturbance but the degree of response was not affected by the type of disturbance. All 3 types of fish posed a real threat to the chironomids and, thus, retreating into tubes when fish had passed appeared to be an appropriate response. It may be that chironomids would not have responded to other species of fish (planktivores or piscivores) that do not pose a threat, however, this seems unlikely. Observations of chironomids in cultures indicated they responded to mechanical stimuli in general; i.e. if we accidently bumped the culture tanks chironomids that were active outside of their tubes would normally pull back immediately. This is similar to Williams and Moore (1985) finding that Gammarus responded to both predatory and non-predatory fish.

It should prove interesting to assess responses of chironomids to invertebrate predators. Because they are so small, invertebrate predators may be less likely to disturb the chironomids. Bohanan (1988) indicated Odonata larvae near tubes of chironomids were not detected by the chironomids. Of course the ultimate cost of not responding to an unidentified
to hide from harmless stimuli they will likely suffer reduced fitness in terms of growth and development. Being able to accurately evaluate the level of threat associated with any disturbance would obviously be advantageous to any prey but given the diversity of various predators and the array of cues from each, it may be difficult to assess the information in the limited time available. Sih (1986) suggested that even stimuli as specific as the smell of dying or macerated conspecifics must arrive quickly enough to be of any use in determining immediate predation risk.

Another aspect of prey activity that has been questioned in a number of studies is prey hunger. I incorporated larvae at one of three hunger levels in my experiments to assess how their activity would change with respect to a single fish pass. I found that activity was highest at the high hunger-level and that the median activity lost was also highest at the high hunger-level. Macchiusi and Baker (1992) also found that larvae supplied with a low diet were more active than those supplied with more food. These data suggest that while hungry larvae may compensate for low food availability by foraging more when no predators are detected, their growth and development may suffer more at high levels of fish presence than do well fed larvae.

Effects of horizontal distance of fish on activity of larval C. tentans.

As mentioned in the results, data were hard to collect due to video resolution, but it was clear that larvae rarely responded to a fish until it was very close (within 5 cm of the patch) or had already passed over the larvae patch. It may be that the streamlined form of the
head allowing chironomids to detect the turbulence from the sides and posterior end of the fish. For future studies it should be possible to collect better information on the distance over which chironomids can detect predators passing to the side of them by using wider tracks. My tracks were only 20 cm wide and even if a fish remained close to the wall it was within approximately 5 cm of the edge of the chironomid patch. By using wider tracks it will be possible to record the activity of chironomid larvae as fish pass at greater distances from the side of the patch. For the few situations where fish actually struck at larval patches, the time for larvae to resume foraging seemed to be longer than the foraging time observed after larvae had been exposed to a simple pass.

Effects of vertical distance of fish on larval C. tentans activity

Results provided no evidence that chironomids were detecting or responding to pumpkinseeds 4 cm or more above the substrate. Chironomids responded to the presence of bullheads at the 4 cm depth and this may reflect the more vigorous swimming of this species compared to the pumpkinseed sunfish. Overall the results from this experiment suggest fish must be close (within 4 cm) of the substrate to affect the foraging of the chironomids. This may first appear to indicate that, in the field, fish will rarely affect foraging of chironomid larvae; however, observations of tapes by Collins (1989) and Collins & Hinch (1993) suggest fish usually swim very close to the substrate. In particular, bullheads normally passed so close to the substrate that they raised a plume of sediment.
In an effort to estimate the time for activity that may be lost in the field, I used the time and frequency of fish passes recorded from underwater tapes of nocturnal fish-movement at Mouse Lake (43 deg 11 min N, 78 deg 50 min W) and Ranger Lakes (45 deg 9 min N, 78 deg 50 min W), Ontario (N. Collins, personal communication). Collins set up time-lapse video cameras with infrared illumination at two sites in each lake; these lakes were chosen because they were different in terms of fish species and densities. Each site was further classified as "open" or "complex". "Open" sites were > 5 m away from objects providing vertical relief off the bottom, and "complex" sites were small opening in artificial weedbeds. I chose to use data recorded from Mouse Lake's open site 1 (17-18 July 1992) and Ranger Lake's open site 2 (14 July 1992) because preliminary calculations using fish passes from these two sites presented the minimum and the maximum values of time available to forage. At each site, a fish pass was defined as a fish that passed within a 15 cm radius from a focus spot (R. Baker, personal communication). Collins & Hinch's (1993, personal communication) indicated that fish fed mostly at the sediment level during nocturnal hours at Mouse and Ranger Lakes. Since I have established that the type of fish creating a disturbance may have no effect on the intensity of response exhibited by larval chironomids, I did not feel it necessary to distinguish the type of fish making a pass at any given time.

To calculate activity lost in the field, I considered three possible techniques (Fig. 17):

A) The simplest method is to multiply the number of fish passes by the maximum time lost for activity. I used the maximum activity time lost to ensure that I was being as conservative as possible; the maximum activity time lost was set at 7.66 min/event per larva, which was
Figure 17:

Methods used to calculate activity lost as a result of a series of single fish passes in the field. Minimum and maximum event durations were set at 23 minutes and 40 minutes respectively. The maximum activity lost across all calculations was 7.66 mins/event/larva and this was used to remain conservative in all calculations.

A) All events have an impact on the activity lost. Activity lost is the sum of all shaded areas. Double or triple hatched areas have been included as additional activity lost. This calculation perhaps best represents the traditional method of calculating activity lost.

B) Any event within 23 minutes of a previous event is disregarded. Activity lost is the sum of the hatched areas and double hatched areas are included twice. The same procedure is used for the maximum event duration. Notice how the double hatched areas are considerably smaller than those in method A.

C) All events are proportionally significant, relative to the event immediately preceding it. For events that occur within the event duration cycle of a previous event, additional activity loss is the area hatched from the end of the first event to the end of the second event. Again, the hatched area is considerably less than that in A and no time is accounted for more than once.
Fig. 17

B

Activity (min/hour per larva)

Time (min)

Fish event 1
Fish event 2
Fish event 3

C

Activity (min/hour per larva)

Time (min)
time lost would be counted twice and, therefore, exaggerate the effect of fish passing. However, if chironomids increased their time in tubes with each subsequent pass, then under conditions involving multiple passes, the exaggeration may not be serious.

For the remaining two methods of activity loss calculation, activity lost was calculated for both the minimum and maximum event durations (23 and 40 minutes, respectively) using the non-adjusted control line: B) For the second method of calculation I first disregarded any event within 23 minutes of a previous event. Activity lost was simply the product of the number of fish passes at least 23 minutes apart and activity lost per event (7.66 min/event per larva). The same procedure was followed for the maximum event duration of 40 minutes. C) All events were considered significant with varying impacts on activity lost depending on when an event occurred relative to an immediately preceding event. I made the assumption that activity lost as a result of a fish pass was spread out evenly over the entire event duration. Hence, for example, using the minimum event duration of 23 minutes, any event that did not overlap with any other event elicited an activity loss of 7.66 mins/event/larva. If a second event occurred 13 minutes after the first event, only the remaining 10 minutes of the second event's duration elicited the proportional amount of activity lost, i.e. $((7.66 \times 10)/23) = 3.33$ min/event per larva. Hence, the total activity lost now is $[(\text{number of fish passes beginning a new 23 minute cycle} \times 7.66 \text{ min/event per larva}) + (\text{proportional residual activity lost for every additional event within every 23 minute cycle})]$. The same calculations were performed using the maximum event duration of 40 minutes.
detects a fish pass while holding, to calculate activity time lost, is extreme relative to the other methods. The alternative methods (methods B and C) indicate that there may in fact be anywhere from 50 to 85% less activity time lost when compared to method A. These numbers should undoubtedly affect how we perceive classical studies on the effects of predators on prey behaviour and development. As well, it seems irresponsible to continue to use numbers that may reflect exaggerated ecological consequences, specifically when examining changes in prey development as a consequence of anti-predator behaviour.
Table 3:

Summarized results of three methods (A, B and C) used to calculate activity time lost (min; % of total potential time available) as a result of a single fish "pass", and corresponding time available to forage (min; % of total potential time available). Fish passes were recorded using underwater cameras set at two sites at Mouse and Ranger Lakes (N. Collins, personal communication). Methods used to calculate lost activity have been described below and in Fig. 17. The activity lost value used was 7.66 min/event per larva; where, applicable, minimum and maximum event durations used were 23 and 40 minutes, respectively.

A: Time lost = # of fish events x 7.66 min/event per larva

B40: Using a 40 minute event duration, only fish events at least 40 minutes apart are used. Time lost = (# of fish events at least 40 minutes apart) * (7.66 min/event per larva)

B23: Using a 23 minute event duration, only fish events at least 23 minutes apart are used. Time lost = (# of fish events at least 23 minutes apart) * (7.66 min/event per larva)

C: All events are significant and are accounted for proportionally. For example, the impact of a second event is only accounted for in the time immediately after the first event ends to the end of the second event. I assumed that activity was lost over the entire duration of an event.

C40: Events at least 40 minutes apart started each new event cycle. Events within each 40 minute cycle were accounted for proportionally. For example, if a second event occurs 30 minutes after the first, the time lost as a result of the second event is

\[
\frac{7.66 \text{ min lost/event per larva} \times 10 \text{ min}}{40 \text{ min}}
\]

Total activity lost = [(# of fish events at least 40 minutes apart) * (7.66 min/event per larva)] + [proportional time lost in each 40 minute cycle]

C23: The same calculations were performed as for C1, however, the event duration was set at 23 minutes.
<table>
<thead>
<tr>
<th>Predominant Fish Type</th>
<th>MOUSE LAKE</th>
<th>RANGER LAKE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yellow Perch, Brown Bullhead,</td>
<td>Pumpkinseed Sunfish, White Sucker,</td>
</tr>
<tr>
<td></td>
<td>Pumpkinseed Sunfish</td>
<td>Smallmouth Bass</td>
</tr>
<tr>
<td>Total Fish (fish/ha littoral area)</td>
<td>~ 6800</td>
<td>~ 500</td>
</tr>
<tr>
<td>Total Time Available (min)</td>
<td>250</td>
<td>240</td>
</tr>
<tr>
<td>Time Lost for Activity (%); (min)</td>
<td>A 64.34 (160.86)</td>
<td>51.07 (122.56)</td>
</tr>
<tr>
<td></td>
<td>B40 15.85 (39.64)</td>
<td>8.22 (19.72)</td>
</tr>
<tr>
<td></td>
<td>C40 19.60 (48.98)</td>
<td>11.65 (27.96)</td>
</tr>
<tr>
<td></td>
<td>B23 21.45 (53.62)</td>
<td>12.77 (30.64)</td>
</tr>
<tr>
<td></td>
<td>C23 32.06 (80.16)</td>
<td>14.88 (35.72)</td>
</tr>
<tr>
<td>Time For Foraging (%);(min)</td>
<td>A 35.65 (89.14)</td>
<td>48.93 (117.44)</td>
</tr>
<tr>
<td></td>
<td>B40 84.14 (210.36)</td>
<td>91.78 (220.28)</td>
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<td></td>
<td>C40 80.40 (201.02)</td>
<td>88.35 (212.04)</td>
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<tr>
<td></td>
<td>B23 78.55 (196.38)</td>
<td>87.23 (209.36)</td>
</tr>
<tr>
<td></td>
<td>C23 67.94 (169.84)</td>
<td>85.12 (204.25)</td>
</tr>
</tbody>
</table>
Results of my study, Macchiusi & Baker's (1992) work on larval activity, Ball & Baker's (1995, 1996) work on larval development at constant presence and complete absence of a fish predator and Collins & Hinch's (1993) examination of fish movement in natural conditions, can be combined to begin to estimate the costs of anti-predator behaviour on development of *C. tentans*. To study the effects of natural levels of predator presence on *C. tentans* in the laboratory, it should be possible to develop a device that mimics the presence of a fish and alters larval activity to the same extent as recorded in this study; i.e. results from my study could be used as a bench mark in the development of a device that would mimic the effects of real fish. Once this "artificial fish" is successful in causing the required activity loss, a programme could be developed to simulate the frequency and timing of fish passes already recorded at different times of day and season (Collins & Hinch 1993). With the use of such a system, natural fish movement patterns documented in field studies could be incorporated into the strict control of laboratory systems and used to better estimate the costs of anti-predator behaviour of larval *C. tentans*. 


