Detection of conspecific alarm cues by juvenile salmonids under neutral and weakly acidic conditions: laboratory and field tests

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A Thesis

In

The Department

of

Biology

Presented in Partial Fulfilment of the Requirements
for the degree of Master in Science at
Concordia University
Montreal, Quebec, Canada

August 2003

Your file Votre référence
ISBN: 0-612-83849-8
Our file Notre référence
ISBN: 0-612-83849-8

ABSTRACT

Detection of conspecific alarm cues by juvenile salmonids under neutral and weakly acidic conditions: laboratory and field tests

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A variety of fishes possess damage-released chemical alarm signals, that play a critical role in the detection and avoidance of potential predation threats. Recently, it has been demonstrated that the ability of fathead minnows *(Pimephales promelas)* and finescale dace (*Phoxinus neogaeus*) to detect and respond to conspecific alarm signals is significantly reduced under weakly acidic conditions (pH 6.0). Rainbow trout (*Onconrhynchus mykiss*) and brook charr (*Salvelinus fontinalis*) possess an analogous alarm signaling system. It is unknown, however if the trout alarm signaling system is likewise affected by relatively small changes in pH. I conducted laboratory and field trials to examine the potential effects of acute exposure to weakly acidic (pH 6.0) conditions on the detection and response of conspecific alarm signals by juvenile trout. My laboratory results demonstrate that while juvenile rainbow trout exhibit significant increases in antipredator behaviour under normal pH conditions (pH 7.0-7.2), they do not respond to the presence of conspecific chemical alarm signals (i.e. response is not different from controls) under weakly acidic conditions. Similarly, natural population of brook charr failed to detect conspecific alarm cues in a weakly acidic stream (mean pH 6.11) while they responded to these cues in a neutral stream (mean pH of 6.88). These data suggest significant, sub-lethal effects of acid precipitation on the ability of prey fishes to detect and respond to conspecific alarm cues in natural waterways.

ACKNOWLEDGEMENTS

I would like to thanks my supervisor Dr. Grant Brown for his generosity, and assistance throughout the course of my degree, John Gunn and Peter Keller for their help on field sites, Dr. James Grant and Dr. S. Dayanandan for their comments on the manuscript and finally Jocelyn Kelly for her assistance with the field trials. All work reported herein was conducted in accordance with Concordia University Animal Care Committee protocol # AC-2002-BROW. Financial support was provided by NSERC of Canada and Concordia University to G.E.B.

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Introduction

Sulphur and nitrogen oxides, released as a result of fossil fuel combustion, are major anthropogenic sources of surface water acidification worldwide (Rodhe et al., 1995). The severity of such impacts varies depending upon the rate and form of acid deposition (Rodhe et al., 1995) resulting in water bodies ranging from heavily (pH < 5.0) to weakly (pH 6.0-7.0) acidified. Over the past several decades, the impacts of such acidification on aquatic ecosystems have received considerable attention (i.e. Gagen et al., 1994; Barry et al., 2000; Hesthagen and Jonsson, 2002). Given their commercial and recreational value (Moyle and Cech, 1996), the effects of acid precipitations on the physiology and ecology of salmonid fishes has received much attention (Barry et al., 2000; Hesthagen and Jonsson, 2002; Van Sickle et al., 1996).

Heavily acidic conditions (pH < 5.0) can induce a decrease in fish abundance and recruitment and an increase in physiological stress and mortality (Environment Canada, 1997). Exposure of juvenile salmonids to intermediate pHs (~ 5.0 to 6.0) induces reduced swimming performance, lower growth rates, impaired sensory mechanisms, and reduced immunity (Lorz and McPherson, 1976; Wilson et al., 1994). While there is substantial information on heavily and intermediate acidified conditions on the physiological and ecological impacts on salmonid populations (Wilson et al., 1994), there is surprisingly little known regarding the effects of weakly acidic conditions (i.e., pH range 6.0 – 7.0) on the behaviour and fitness of juvenile aquatic vertebrates, especially salmonid fishes (Åtland, 1998).

Growing evidence suggests a potentially important impact of weakly acidic conditions might be the loss of chemical alarm signaling within aquatic communities. A

wide range of taxonomically diverse fishes possesses damage-released chemical alarm cues, which play a major role in predator-prey dynamics (Chivers and Smith, 1998; Brown, 2003).

The detection of chemical alarm cues by nearby conspecifics and some sympatric heterospecifics elicits a dramatic, short-term increase in species-typical antipredator responses (Chivers and Smith, 1998; Smith, 1999). These overt (Smith, 1999) responses have been well documented and include a variety of behaviour patterns such as increased shoal cohesion, area avoidance, dashing, freezing and reduced foraging and mating effort (Chivers and Smith, 1998). Chemical alarm cues can also elicit a variety of covert behavioural responses (Smith, 1999) including acquired recognition of novel predators, induced morphological and life history changes and the assessment of local predation risk through predator inspection behaviour (Chivers and Smith, 1998; Mirza et al., 2001; Brown, 2003). Individuals responding to conspecific and/or heterospecific alarm cues can increase their probabilities of surviving an encounter with a predator or avoid the predator altogether (Chivers and Smith, 1998; Smith, 1999). Recently, several authors have demonstrated that the detection of alarm cues and subsequent avoidance of predators, by signal receivers, translates into a direct survival benefits for the receivers (Mirza and Chivers, 2001a, 2003a, 2003b; Chivers et al., 2002). In addition, acquired recognition of novel predators (a covert response to alarm cues, Smith, 1999) likewise leads to increased survival during encounters with predators (Mirza and Chivers, 2000; 2001b; Gazdewich and Chivers, 2002).

Brown et al. (2002) demonstrated that the ability of two cyprinid species (fathead minnows, *Pimephales promelas* and finescale dace, *Phoxinus neogaeus*) to detect and

respond to conspecific and artificial alarm pheromones is significantly impaired under weakly acidic conditions (pH 6.0). When exposed to conspecific skin extract or hypoxanthine-3-*N*-oxide (H3NO), the putative Ostariophysan alarm pheromone, (Brown et al., 2000, 2001, 2003) under neutral (~ pH 7.6) conditions, both minnows and dace exhibited significant increases in antipredator behaviour. When acclimated to weakly acidic conditions (pH 6.0) over four days and retested, there was no significant response to natural or artificial alarm cues. Following reacclimation to natural pH conditions, the response to natural and artificial alarm cues returned to pre-acid exposure levels. Brown et al. (2002) went on to demonstrate that this loss of response by cyprinids is due to a non-reversible structural change in the alarm cue molecule itself, and not to olfactory receptor damage. These results suggest that prey fishes are at a significant disadvantage in natural waterways affected by acid precipitation (Brown et al., 2000, 2002).

A similar loss of chemical alarm signaling function has been demonstrated in a non-ostariophysan species, pumpkinseed sunfish (*Lepomis gibbosus*; Leduc et al., in press). Juvenile sunfish were exposed to the skin extracts of conspecifics, green sunfish (*Lepomis cyanellus*), an allopatric congener, and H3NO under neutral (pH 7.5) and weakly acidic (pH 6.0) conditions. Under neutral conditions, pumpkinseed sunfish exhibited strong antipredator behaviour in response to all three experimental stimuli (compared to distilled water controls). However, the response to conspecific and congener skin extracts was significantly reduced and completely absent for H3NO under weakly acidic conditions (Leduc et al., in press).

Juvenile salmonids, including rainbow trout (*Oncorhynchus mykiss*) and brook charr (*Salvelinus fontinalis*) possess damage-released chemical alarm cues (Brown and

Smith, 1997; Mirza and Chivers, 2000, 2001c). It remains unknown, however, if the ability of these juvenile salmonids to detect and respond to conspecific chemical alarm cues is similarly affected under weakly acidic conditions. In addition, previous studies have only been conducted under laboratory conditions. Field verification of the potential effect of weakly acidic conditions on the use of chemical alarm cues is required (sensu Magurran et al., 1996; Smith, 1997). I conducted this current study to: 1) assess the potential impact of weakly acidic conditions on the detection of conspecific alarm cues by juvenile salmonids and 2) verify that the loss of response to conspecific alarm cues occurs under natural conditions (i.e. is not a laboratory artifact).

Experiment 1: laboratory trials

Materials and methods

Test fish:

Juvenile rainbow trout were obtained from a local hatchery and kept in 300-L stream channels equipped with a single chiller unit set to 12°C, and filled with dechlorinated tap water. The water was continuously filtered and the trout were fed *ad libitium* daily with commercial trout chow. The photoperiod was adjusted to 14 hours light, 10 hours dark.

Test stimuli

Eight juvenile trout donors (mean ± SE = 8.95 ± 0.3 cm F_L) were used to generate the alarm cue (from skin extract). Trout were killed with a blow to the head (in accordance with Concordia University Animal Care Committee protocol # AC-2002-BROW). Skin filets were collected from each side of their body and immediately placed into chilled distilled water, homogenized and filtered through glass wool to remove any larger particles. I collected a total of 79.61 cm^2 of skin and diluted the solution to a final volume of 890 ml with distilled water. The resulting concentration was similar to that used by Brown and Smith (1997; 1998) and was shown to elicit consistent antipredator responses in rainbow trout. Skin extract was frozen at –20°C, in 20 ml aliquots until needed. As a control, I also froze 20 ml samples of distilled water.

Experimental protocol

Experimental stream channels consisted of opaque Plexiglas ovals (2.5 x 0.66 m), divided with a watertight barrier down the centre line. This created two experimental channels per tank. Each channel was provided with individual water inflow and outflows, preventing mixing between the two channels. A weak laminar current (approximately 5 cm s^{-1}) was generated by continuously pumping water into the upstream end of the channel and allowing it to drain over a standpipe at the downstream end. Test fish were constrained to a 1.6 x 0.33 m section by mesh screens at the up-and down-stream ends of the channels. Temperature within the stream channels was maintained at 14° C throughout the experiment. Each channel contained a cover object, consisting of a

12.5 cm square ceramic tile supported by four cylinders (5.25 cm high), positioned at the mid point of the stream channel.

I placed two juvenile trout in each stream channel and allowed them to acclimate for 24 hours prior to testing. Pairs of trout were used, to reduce apparent stress (personal observations), however, all behavioural data was recorded on a single focal fish. Focal fish were randomly chosen prior to observations. At the time of testing, trout measured (mean ± S.E.) 9.37 ± 0.19 cm F_L.

Trials consisted of a 10-minute pre-stimulus and a 10-minute post-stimulus observation periods. All observations were videotaped using low light cameras, at approximately 10 lux. Following the pre-stimulus observation period, I injected either 20 ml of distilled water (DW; control) or 20 ml of conspecific skin extract (experimental) directly into the water inflow hoses. The post-stimulus observation period began as soon as the stimulus was fully injected. Test fish were exposed to distilled water or conspecific alarm cue, both of which had been buffered to pH 6.0 with the addition of approximately 0.1 ml of dilute H_2SO_4, or left untreated (pH 7.0-7.2). I buffered the stimulus directly (rather than the test tank water) as this allows minimize any physiological damage or stress to the test fish caused by weakly acidic conditions (Brown et al., 2002). Test fish were exposed to distilled water control and skin extract experimental cue on the same day but separated by at least one hour. Each fish was used in only one of the two treatments (either neutral or weakly acidic). The distilled water control trials were conducted before experimental trials, as any response to the experimental stimuli may have masked a response to the control stimulus (Hazlett, 1997; Lawrence and Smith, 1989; Brown et al., 2002). Test fish were used only once.

From the videotapes, I recorded three behavioural measures: time moving, time under shelter and time in the stimulus delivery area. Reduced activity level, freezing, increased shelter use, and dashing are typical antipredator behavioural responses for juvenile salmonids (Brown and Smith, 1997; Mirza & Chivers, 2001b). As a response to alarm cues in neutral conditions, I predicted that the amount of time spent moving and spent in the stimulus delivery area would decrease while the time spent under shelter would increase when compared to distilled-water control. However, in weakly acidified conditions, I predicted that the intensity of the response to the alarm cues would not be different compared to the distilled-water control. I conducted a total of 28 trials (14 paired control and experimental trials for both neutral and acidic conditions).

Statistical analysis

Given that my *a priori* prediction is that the level of acidity would have an effect on the intensity of the antipredator response, I used a paired t-test to conduct my statistical analysis. For each treatment (control and experimental), I calculated the difference between the pre- and the post-stimulus observation periods for each of the measured parameters (time spent moving, time under shelter, time in stimulus delivery area). I tested for any overall effect of pH on the behavioural response of trout to control versus experimental cues under neutral and weakly acidic conditions using paired t-tests. To control for increasing Type 1 error rates, alpha was set at 0.0082 according to the Dunn-Sidák method.

Results

Under neutral conditions, trout significantly decreased time spent moving ($t = 3.56$, $df = 13$, $P = 0.0035$, Fig 1A), and time spent in the stimulus delivery area ($t = 2.84$, $df = 13$, $P = 0.0061$; Fig 1B) when exposed to conspecific skin extract versus a distilled water control. In response to conspecific skin extract, individuals increased their time under shelter, though this difference was not statistically significant ($t = -1.40$, $df = 13$, $P = 0.18$; Fig 1C). Under weakly acidic conditions, however, there was no significant effect of skin extract on time moving ($t = 0.49$, $df = 13$, $P = 0.63$; Fig 1A), stimulus delivery area use ($t = 0.13$, $df = 13$, $P = 0.89$; Fig 1B) or time under shelter ($t = 0.27$, $df = 13$, $P = 0.79$; Fig 1C). As an additional control for potential temporal effects associated with our experimental design, we compared the baseline (pre-stimulus) scores between distilled water and skin extract (alarm cue) stimuli for neutral and acidic conditions. We found no difference in baseline activity for either neutral ($F_{(3, 24)} = 0.56$, $P = 0.65$) or acidic ($F_{(3, 24)} = 0.20$, $P = 0.89$) treatments. Thus, these data demonstrate that under laboratory conditions, the ability of juvenile rainbow trout to detect and respond to conspecific skin extracts is significantly impaired under weakly acidic conditions. The mean times spent under shelter, in movement and in the stimulus delivery area for the neutral and weakly acidic treatment are listed in table 1.

Experiment 2: field trials

Materials and methods

Test sites

The data were collected between July 13th and 23rd 2002 in streams located near Sudbury area in Ontario, Canada. Sites in two streams of different pH were chosen to conduct the study: Little Sand Cherry Creek (49° 39.64 N, 081° 12.90 W) and Windy Creek (46° 39.66 N, 081° 26,50 W) located in Morgan and Dowling County respectively. Each site was within two meters from the shore of the streams. With the exception of pH, these streams were chosen for their physical similarities (Table 2). There was however, a significant difference in the mean current velocity and in the mean water temperature between both streams. The mean pHs for Sand Cherry Creek and Windy creek were 6.88 and 6.11 respectively.

Test fish

Test fish were located via snorkeling in each of the studied streams, 20 to 30 minutes prior to testing. I conducted trials starting downstream and moved upstream to avoid multiple exposures of test fish to SE. A distance of at least three meters separated each test fish from the next test fish. The observer (AL) was located approximately one meter upstream from focal fish in order for the injected stimuli to directly reach the test fish (see below). From visual observations, the test fish were of similar size to the fish captured for skin extract preparation (see below). Brook charr of ~4 cm to 7cm have a mean territory area of $0.1m^2$ to $1.0m^2$ (Grant and Kramer, 1990) which made direct visual observation easy to conduct.

Test stimuli

Skin extract was generated from 13 trout donors captured from Sand Cherry Creek (mean ± SE = 4.88 ± 0.033 cm F_L) and 7 donors from Windy Creek (mean ± SE = 6.68 ± 1.61 cm F_L). The procedure was similar as described above with the difference that the skin extract was not frozen in aliquots but immediately placed in a glass container in an ice-chilled cooler to be used in the following hours. To generate the test stimulus, we used an average of 6.05 cm^2 (± 0.20) of skin for an average volume of 29.97 ml (± 0.88) of stream water. The actual skin extract used in the field was the result of blending the skin of more than one donor (from two to three donors for a given preparation but always in the proportions mentioned). This concentration was similar in both the laboratory and field parts of the study.

Experimental protocol

At each trial site (see Table 2), the time of day, the river surface current speed, the river width and depth were recorded. I used a portable "Acumet" temperature and pH meter to record air and water temperature as well as the water pH. Each trial were videotaped using a "Sea Viewer" underwater camera held approximately one meter upstream from focal fish where stimulus injection took place (to ensure that the test fish was in the injection plume of our stimuli). I let the test fish acclimate to the observer and camera's presence for 5 minutes prior to testing. Twenty trials were conducted in Little Sand Cherry Creek and 10 in Windy Creek (since brook charr density of was much lower in the latter stream). As in the laboratory experiment, I compared the antipredator responses resulting from exposure to experimental and control stimuli (SE and stream

water respectively) in a neutral and a weakly acidified stream. The following behavioural parameters were recorded: number of feeding attempts (displacement of at least half a body length toward an object drifting or on the substrate) and the number of aggressive interactions (displacement of at least half a body length toward either a conspecific or an heterospecific with or without a biting attempt). I did not attempt to measure the time spent moving and the time spent under shelter since brook charr need to swim to maintain their position in the stream current and in many trials, no shelter was visible.

The trials were 20 minutes long and divided as follows: 5 minutes pre-control, 5 min post-control, 5 min pre-experimental and finally 5 minutes post-experimental period. The control stimulus (stream water) was injected after the first 5 minutes and the experimental stimulus (SE) after 15 minutes (five minutes before the end the trial). The control treatments were always carried out first to prevent any lasting response to the experimental stimuli from hiding a response to the control stimulus (Hazlett, 1997; Lawrence and Smith, 1989; Brown et al., 2002).

Statistical analysis

Like in the laboratory experiment, my *a priori* prediction is that the level of acidity would have an effect on the intensity of the antipredator response. I used a paired t-test to conduct statistical analysis. For both foraging attempts and aggressive interactions, I calculated the difference between pre-stimulus and post-stimulus observation periods. These difference scores were analyzed as described for Experiment 1. Alpha was set at 0.013 (Dunn-Sidák method) to control for increasing Type 1 error rates.

Results

In the neutral stream, I found significant decreases in both foraging rate ($t = 6.58$, $df = 19$, $P < 0.0001$; Fig 2A) and aggressive interactions ($t = 3.81$, $df = 19$, $P < 0.002$; Fig 2B) for individuals exposed to conspecific skin extract versus distilled water controls. However, there was no significant difference in behaviour between the stream water controls and the skin extract experimental stimuli in the weakly acidic stream (foraging: $t = 0.13$, $df = 9$, $P = 0.90$; Fig 2A; aggressive interactions: $t = 0.41$, $df = 9$, $P = 0.69$; Fig 2B). To ensure that the observed differences were due to ambient pH and not population differences in overall activity, I compared baseline values (i.e., pre-stimulus scores) for foraging and aggressive interactions. I found no significant difference between these baseline values for the two (MANOVA: $F_{(2, 25)} = 0.18$ P, = 0.85; Table 3).

There was an significant difference in mean current velocity of the two studied streams as well as the mean water temperature (Table 2). These differences are not however, likely to have significantly affected my results (see discussion).

Discussion

The data indicate that juvenile rainbow trout and brook charr are impaired in their ability to detect and/or respond to chemical alarm cues under weakly acidified conditions. Under laboratory conditions, juvenile rainbow trout exhibited a significant antipredator response following exposure to conspecific skin extract under neutral pH, but not under weakly acidic conditions. Similar results have been found for cyprinid (Brown et al., 2002) and centrarchid (Leduc et al., in press) fishes. During field trials, juvenile brook charr from a neutral stream exhibited significant antipredator responses to conspecific

skin extract. However, charr from a weakly acidic stream did not exhibit any change in behaviour, compared to stream water injection, when exposed to the same alarm cue. These results suggest that: 1) juvenile salmonids may be at a disadvantage in weakly acidified environment, as they are unable to detect and respond to chemical alarm cues, and 2) represent the first field verification of this phenomenon.

While the exact chemical degradation mechanism is not known, the results suggest that the loss of alarm signaling in salmonids fish is due to changes in the alarm cue itself, rather than physiological stress or olfactory receptors damage. In the laboratory trials, I buffered the skin extract itself and no experimental manipulation was done to the tank water. The volume of acid presented to the test tank (~ 0.8 ml in 300 L) would not have resulted in a perceptible difference in pH (Brown et al., 2002). For field trials, I found no difference in baseline activity for foraging and aggression between our study streams suggesting that individual charr were behaving "normally". Thus these data, along with that of Brown et al. (2002) and Leduc et al. (in press) suggest that under weakly acidic conditions, the chemical alarm cues are being partially or completely degraded and are rendered undetectable by prey individuals.

The mean foraging attempt rates in our studied streams were 3.8 and 5.1 per minute in the pre-control treatments in Sand Cherry and Windy Creek respectively. Nislow et al., (1998) found similar mean foraging value for juvenile Atlantic salmon (*Salmo salar*) in Vermont streams during summer (from approx. 2.5 to 3.5 forays per minute). The mean rates of aggressive interactions were 0.39 and 0.20 Sand Cherry and Windy Creek respectively. Cutts et al. (1998) have found lower mean values of aggressive interaction rate in hatchery-reared juvenile Atlantic salmon (from ~0.02 to ~

0.07 aggressive act per minute). These differences can be attributed to the fact that unlike in my experiment, these fish were only exposed to conspecific and no interaction with heterospecifics occurred. It is also possible that the differences in the density of conspecifics could have further lowered the level of aggression of Cutts et al. (1998) results compared to my experiment.

While some of the physical characteristics of the studied streams differed (air and water temperature and current velocity), it is unlikely that these differences would have affected my results. The mean temperatures of both streams were well within the normal preferred summer ranges for brook charr (Scott and Crossman, 1973). Moreover, the antipredator response of brook charr to skin extract injection was significantly more accentuated in the neutral stream despite the current speed being faster than in the weakly acidic stream (Table 2). Mirza and Chivers (2000) have demonstrated that a single exposure to alarm cues was sufficient for the acquisition of anti-predator behaviour in brook charr. The baseline activity of charr was not significantly different between the studied streams suggesting that the observed lack of response to alarm cues in the acidified stream was likely due to changes in the alarm cue itself and not to population or habitat differences. However, experiments are ongoing to examine the potential influence of habitat and/or population variability on individual behavioural response under neutral and acidic streams.

Given the demonstrated survival benefit of chemical alarm cues for juvenile salmonids (Mirza and Chivers, 2001a), the loss of such information may result in significant direct and indirect fitness costs. The inability to gain information regarding local predation risk (Brown, 2003) may result in a direct increased mortality. Indirect

effect may also be present in the form of foraging-antipredator trade-offs. Individuals unable to assess local predation risk via chemical information may be forced to utilize a more risk aversive foraging strategies (i.e. increase vigilance). Such a foraging strategy may result in reduced foraging efficiencies and/or growth rates (G.E. Brown and P.E. Foam, unpublished data).

Throughout Europe and North America, the number of salmonids released from hatcheries for restocking purpose now matches or exceeds natural production (Brown and Laland, 2001). It is known that high levels of mortality can be attributed to naïve hatchery-reared individuals failing to recognize predator due to an inability to acquire knowledge prior to stocking (Suboski and Templeton, 1989). As a result, newly stocked individuals are at higher risk of predation compared to wild strains (Donnelly and Whoriskey, 1993; Shively et al., 1996; Brown and Laland, 2001). Juvenile salmonids rely on chemical alarm cues to learn the identity of novel predators (acquired predator recognition, Brown and Smith, 1998). Such learned recognition has been shown to significantly increase the survival of individuals during encounters with predators (Mirza and Chivers, 2000, 2003b). Our current results suggest that this critically important learning mechanism may be impaired under weakly acidic conditions, further reducing survival and fitness potentials. In fact, Leduc, Ferrari and Brown (unpublished data) demonstrated that juvenile rainbow trout are indeed unable to acquire the recognition of a novel predator under weakly acidic conditions. In addition, they found that the pH of the predator odour had no effect on learning.

Mirza and Chivers (2001a) have shown that within four species of salmonids, the chemical alarm signaling was conserved and elicited antipredator responses among the

species tested. It seems probable that the mechanism that impairs the detection of chemical alarm signaling is the same in the two species that we studied and could possibly be the same mechanism for all salmonids.

Therefore, despite the fact that pH of 6.0 can be non-lethal for certain species of salmonids, there could be potential fitness cost associated with these non-lethal pHs as failure to detect to alarm cues (and subsequent predation pressures) at this weak pH.

Table 1 Mean (± SE) baseline time spent moving, time in the stimulus delivery area and time under shelter for the pre-control and pre-experimental segments for each level of acidity.

Behavioural modalities	Neutral (pH 7.0)	Acidic (pH 6.0)
Time moving (s)		
control	159.6 ± 21.9	194.9 ± 55.7
experimental	160.6 ± 36.4	215.7 ± 56.5
Time under shelter (s)		
control	174.7 ± 53.8	196.5 ± 53.8
experimental	155.6 ± 48.3	157 ± 61.0
Time in stimulus delivery area (s)		
control	256.4 ± 58.7	197.0 ± 46.7
experimental	354.8 ± 66.1	251.9 ± 58.6

Table 2. Mean (± SE) values for the physical variables of Little Sand Cherry creek and Windy creek.

Variables	Little Sand Cherry Creek	Windy Creek
pH	6.88 ± 0.01	6.11 ± 0.01
Velocity (m/s)	0.57 ± 0.01	0.42 ± 0.01
Width (m)	5.31 ± 0.19	5.25 ± 0.20
Depth (m)	0.33 ± 0.01	0.30 ± 0.09
Cloud Cover (%)	0.50 ± 0.10	0.62 ± 0.09
T air (°C)	24.35 ± 0.24	22.70 ± 0.79
T water (°C)	23.64 ± 0.05	21.56 ± 0.43

Table 3 Mean (± SE) baseline rate of foraging attempts and aggressive interactions per minute for the pre-control and pre-experimental segments of the two studied streams

Behavioural modalities	Sand Cherry Creek	Windy Creek
Foraging attempts		
control	3.63 ± 0.39	5.20 ± 0.49
experimental	3.88 ± 0.44	5.06 ± 0.56
Aggressive interactions		
control	0.39 ± 0.11	0.20 ± 0.09
experimental	0.41 ± 0.05	0.25 ± 0.08

Figure 1. Mean (± SE) changes in time spent moving (A), in time under shelter (B) and time in stimulus delivery area use (C). Shaded bars represent experimental stimulus and open bars distilled-water control in both neutral and acidic conditions (pH 6.0).

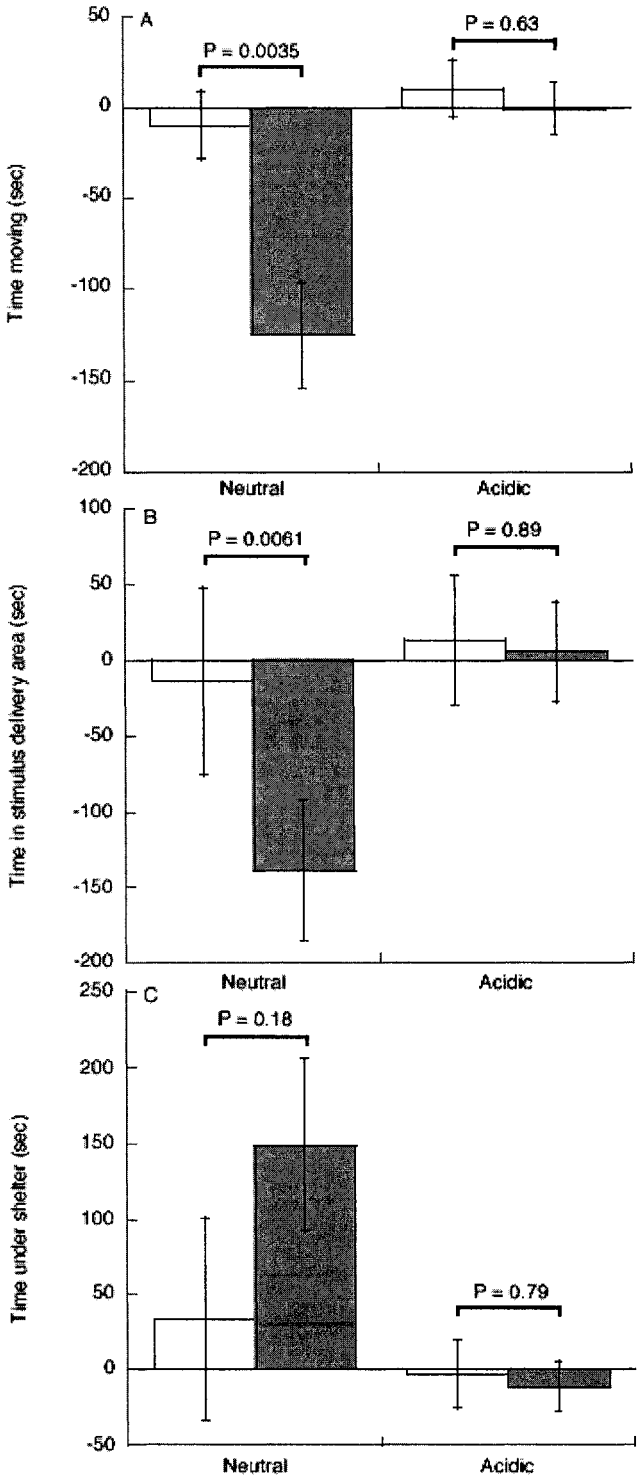
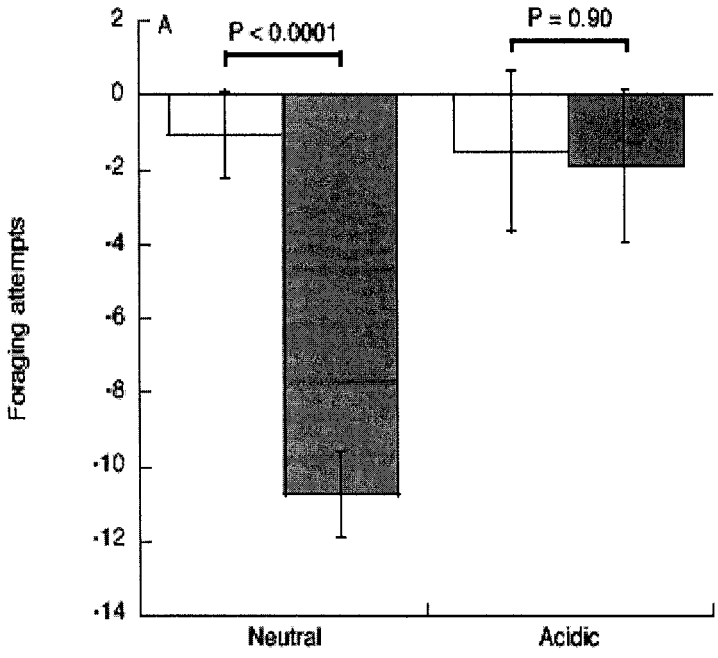
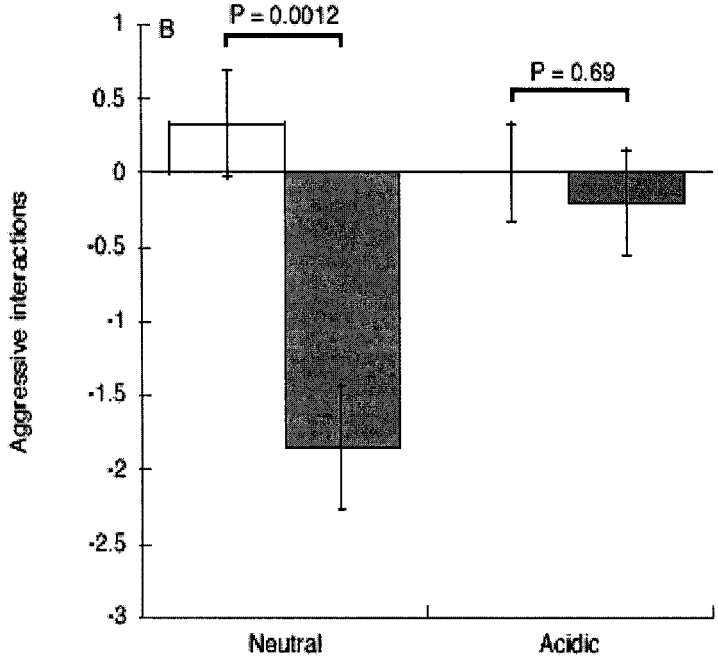

A
P = 0.0035
P = 0.63
Time moving (sec)
50
0
-50
-100
-150
-200
Neutral
Acidic
B
P = 0.0061
P = 0.89
Time in stimulus delivery area (sec)
100
50
0
-50
-100
-150
-200
Neutral
Acidic
C
P = 0.18
P = 0.79
Time under shelter (sec)
250
200
150
100
50
0
-50
Neutral
Acidic

Figure 2. Mean (± SE) foraging rate (A) and number of aggression (B). Shaded bars represent experimental stimulus and open bars stream water in both neutral and acidic conditions (pH ~6.11).


A
P < 0.0001
P = 0.90
Foraging attempts
2
0
-2
-4
-6
-8
-10
-12
-14
Neutral
Acidic


B
P = 0.0012
P = 0.69
Aggressive interactions
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
Neutral
Acidic

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