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Effects of Un-Ionized Ammonia on Hydropsyche Morosa Hagen Larava Net and Retreat Construction

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EFFECTS OF UN-IONIZED AMMONIA ON *HYDROPSYCHE MOROSA* HAGEN LARVA NET AND RETREAT CONSTRUCTION

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ABSTRACT

The objectives of this study were to (1) describe natural variability in retreat and net mesh dimensions and retreat and net tending behavior for the hydropsychid caddisfly *Hydropsyche morosa* Hagen and (2) perform laboratory experiments to determine the influence of un-ionized ammonia on *H. morosa* retreat and net construction. *H. morosa* were collected from fifteen streams in eastern South Dakota and head capsules, nets, and retreats were photographed and measured. Caddis were exposed to 0.00, 0.025, 0.05 and 0.1 mg/L un-ionized ammonia for 48 and 96 hours within experimental stream chambers. Data indicate that exposing caddis to un-ionized ammonia caused them to change their net/retreat construction and tending behavior. Net mesh variability increased 123% in 0.025mg/L and 227% in 0.05 mg/L treatments relative to controls. Net and retreat dimensions in experimental streams also demonstrated significantly greater variability when compared to nets collected from reference streams, indicating a container effect. Behavior in treated chambers was slower and more lethargic when compared to control chambers. Results suggest that un-ionized ammonia influences *H. morosa's* construction abilities and behavior, most notably in net mesh and retreat dimension variability.

INTRODUCTION

Water quality issues are a global concern and vary regionally depending on rainfall, climate, geography, and local human activities (Hynes 1970). Traditional water quality assessment methods have focused on chemical and physical characteristics of water. However, many degraded lakes and streams have eluded detection due to a lack of spatial and temporal integration of abiotic monitoring data. Biological monitoring offers an additional, integrative suite of tools for detecting degraded aquatic systems (Rosenberg and Resh 1993). Most current biological monitoring methods focus on community structure rather than individual organisms. New methods are needed to address whole organism effects from habitat alteration and water pollution (Barbour et al. 1999). Petersen and Petersen (1983) suggest "changes at the level of individual species can be more useful than community changes since (1) a species response oc-

curs before a community response and thus provides a form of environmental early warning and (2) the toxicant can be determined by bioassays on the responding species." These new methodologies would allow detection of disturbance effects prior to major shifts in community structure.

Net building members of the caddisfly family Hydropsychidae are very widespread and abundant (Wiggins 1977). Net spinning caddis larvae are classified as filter feeders and employ many different strategies to capture seston particles within their nets. Net mesh size is often influenced by water velocity and diatom concentration (Alstad 1987). Disturbances also influence net mesh size and construction. Petersen and Petersen (1983) found that heavy metals caused higher numbers of anomalies, specifically crosslinks, in nets built by Hydropsychids.

Un-ionized ammonia (NH_3) is listed as a pollutant of concern in 10 of 14 major South Dakota watersheds, including the Big Sioux watershed, which contains streams sampled for this study (South Dakota Department of Environment and Natural Resources 2000). South Dakota has approximately 9,937 river miles, of which 546 (of the monitored miles) have experienced minor to moderate degradation from un-ionized ammonia. Lakes have also been impacted. In South Dakota 800 lakes (<5000 acres in size) have experienced minor to moderate impact from un-ionized ammonia (South Dakota Department of Environment and Natural Resources 1994).

The objectives of this study were to (1) describe the natural variability in retreat dimensions, net mesh dimensions, and retreat and net tending behavior for the hydropsychid caddisfly *Hydropsyche morosa* Hagen and (2) determine the influence of un-ionized ammonia on *H. morosa* retreat and net mesh dimensions and behaviors. We hypothesized that treatments with elevated un-ionized ammonia would result in altered retreat/net dimensions and impaired retreat/net-tending behavior relative to controls.

MATERIALS AND METHODS

Over 150 stream sites were screened for caddisflies between the dates of April 24, 2000 and July 4, 2000. All stream sites were located in the Northern Glaciated Plains ecoregion. Caddis larvae were hand collected, identified and recorded. Those 15 sites containing the most *H. morosa* were used as collection points for laboratory experiments and those ten sites containing the most *H. morosa* were also sampled as reference streams to provide baseline measurements of head capsule, retreat length and width and net mesh sizes (Table 1).

Ten *H. morosa*, along with their nets and retreats were collected from each of the ten reference streams. Visual identification by head capsule pattern (Fig. 1) was used to select *H. morosa* (Schefter and Wiggins 1986). The selected caddis, nets and retreats were collected from various parts of the stream riffle and placed into plastic containers for observations. Visual observations of net/retreat interactions and duration of interactions were made for fifteen minutes. Collected caddis larvae, nets and retreats were then transported live

Table 1. Original fifteen sample sites, corresponding counties, and geographic locations.

Sample Site	County	Lat & Long	Site Use
Bachelor Creek reach 1	Moody	N43 55.57 W96 43.70	Reference & Experiments
Bachelor Creek reach 4	Moody	N43 57.88 W96 50.91	Experiments
Brookfield Creek	Moody	N43 53.76 W96 38.87	Reference & Experiments
Big Sioux trib A	Brookings	N44 23.90 W96 47.17	Experiments
Deer Creek	Brookings	N44 20.13 W96 41.27	Reference & Experiments
Deer Creek trib A	Brookings	N44 21.83 W96 36.45	Reference & Experiments
Deer Creek trib B	Brookings	N44 23.02 W96 30.79	Reference & Experiments
Hidewood Creek A	Hamlin	N44 36.71 W96 54.34	Reference & Experiments
Hidewood Creek B	Deuel	N44 42.20 W96 45.25	Reference & Experiments
Medary Creek trib A	Brookings	N44 19.84 W96 36.41	Not used - dry
Medary Creek trib B	Brookings	N44 17.32 W96 35.26	Reference & Experiments
Medary Creek trib C	Brookings	N44 17.57 W96 34.04	Reference & Experiments
Six Mile Creek	Brookings	N44 25.66 W96 41.23	Reference & Experiments
Spring Creek	Moody	N44 5.99 W96 36.46	Experiments

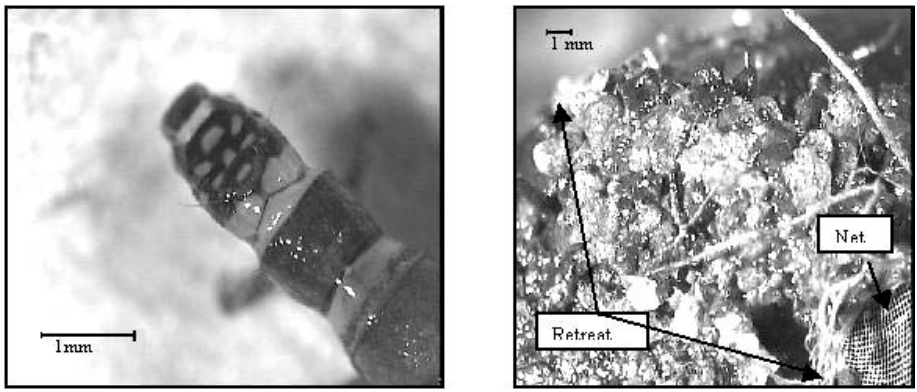

Figure 1. *H. morosa* head capsule (dorsal view) (a) and retreat with net (b) collected from Hidewood Creek, Hamlin County, South Dakota.

to the South Dakota State Environmental Biology laboratory for microscopic measurement.

Each head capsule, net and retreat were individually photographed and saved to disk with a code number linking every caddis with its net and retreat. Head capsules and nets were photographed at a power of 25x and retreats at a power of 7x. A stage micrometer was also photographed at 25x and 7x to facilitate measurement calibration. Head capsules were measured from eye to eye and retreats were measured at the widest and longest points. Net photograph threshold was adjusted to 200 to give a black and white appearance. Ten random cells were selected using a random numbers table; all other cells and background materials were erased. Measurements (height, width, and area) from the randomly selected cells were saved in text files by clicking the "filter": "measure": "features" option within Adobe Photoshop 5.5. All photos

were measured in pixels and data were entered into an Excel spreadsheet and converted into millimeters using the calibration from stage micrometers.

Nine treatment chambers (38 L capacity) made of 6.35mm gray plastic and measuring 1.0 m long, 30 cm wide and 30 cm deep were used in experimental trials. Treatments were assigned at random within three blocks. This arrangement allowed for differentiation of treatment effects from potential laboratory gradients. Maxi-Jet PH Aquarium Pumps (model MP900, 0.242 L/sec.) were attached to each chamber. Ceramic floor tiles (3.5"x3.5") nestled in a minimal amount (approximately two handfuls) of gravel were used as substrate. Chambers were filled with 10 L of tap water and pumps were allowed to circulate at least 24 hours before introduction of the caddisflies.

Ten to twenty *H. morosa*, their nets, and retreats were collected from each reference stream site. Observations on net and retreat interactions (duration and frequency) were performed for fifteen minutes and recorded to the nearest second. Collected caddis were transported back to the lab in a glass aquarium. Larvae were randomly placed into each chamber and a slow drip of Kaytee Forti-Diet rabbit food (slurry concentration 5g/L) was introduced. Caddis were then left overnight and allowed to acclimate to the artificial stream channels.

Un-ionized ammonia treatment doses were set to bracket the South Dakota water quality standard streams supporting warmwater marginal fish life propagation (0.05 mg/L) (State of South Dakota 1999). Target treatment levels for experiment one were 0.050 mg/L (low) and 0.10 mg/L (high). Because of high mortality in experiment one, target concentrations for experiment two were reduced to 0.025 mg/L (low) and 0.050 mg/L (high). Chambers were dosed with NH_4CO_3 to reach target un-ionized ammonia concentrations. Dosage amounts were based upon water temperature and pH of the water source. Water temperature and pH were taken immediately after placing the caddis into chambers and the following day before treatments began.

All nets and retreats were destroyed prior to dosing. Pulse treatments were imposed by introducing the calculated amount of ammonium carbonate into the water stream of each channel. Water temperatures, pH and ammonia/un-ionized values were then taken immediately after dosing, one, two, and four hours after dosing. Morning and afternoon readings were taken for 72 hours following treatment. All ammonia measurements were made using a Hach field spectrophotometer following the Hach salicylate method #10031 (Hach 1997).

Chambers were observed once per day for fifteen minutes each. Net and retreat interactions and duration of interactions were recorded. Only those caddis, nets, and retreats that could be seen without disturbing the substrate or tiles were observed. No tiles were moved in order to keep all nets and retreats intact.

Half of the caddis, nets, and retreats within view in each chamber were removed forty-eight hours after treatment, representing an acute exposure. All were photographed, measured and recorded (as described above). If no retreats or nets were present half of the caddisflies were still removed and recorded as having no retreats/nets built. Following exposure for 96 hours

(chronic exposure), the remaining caddis were removed with all nets and retreats. Pumps were shut off and the numbers of live, dead and pupating caddis were recorded. Chambers and tiles were triple washed with tap water. Used gravel was discarded and new gravel was collected and triple rinsed. Pumps were disassembled, cleaned and allowed to cycle new water for 24 hours. Chambers were then replaced in the rack following the random assignments. Each chamber was designated the same treatment level (control, low and high) for both experiments.

All data were entered onto Excel spreadsheets and imported into Statistix (Analytical Software 1994). Box and whisker plots were generated for all reference stream data. One-way analysis of variance (ANOVA) was used to evaluate experimental treatment differences after examination of treatment data indicated no significant block effects. Bartlett's Test was used to evaluate equality of variance among treatments. Those data displaying unequal variance by treatment were analyzed using the non-parametric Kruskal Wallace test (KW ANOVA).

RESULTS

Stream Observations

Head capsule data collected from reference streams demonstrated that all caddis collected were roughly the same size. Head capsule measurements fell between the intervals of 1.03mm and 1.31mm with one measurement falling outside this range, 0.81mm. Closer visual inspection of this specimen confirmed that it was an early instar *H. morosa*. Those caddis larvae used in experimental chambers had smaller head capsules than those observed from reference streams (KW ANOVA $p<0.01$). Average head capsule width taken from stream caddis larvae was 1.15 mm while that from experiment larvae averaged 1.06 mm.

While the average mesh area varied between reference streams, differences were not considerable. The majority of cells measured had an area within the range 0.05 to 0.20 mm^2 with the mean area of all sites being 0.11 mm^2. Only four of the 100 cells were determined to be outliers. The width of all retreats collected from reference streams fell between 5.50 mm and 11.50 mm. There were only two probable outliers measured from Deer Trib B and Hidewood B. These fell just over the 11mm mark. Average retreat width was very similar between all streams, ranging from about 7.54 mm to 9.25 mm. The overall average retreat width was 8.40 mm. In contrast, retreat length varied from 10.50 mm to 18.38 mm, with a mean of 14.62 mm. Average stream retreat lengths ranged from 13.15 mm to 15.40 mm.

Experiment Behavior Observations

Due to high mortality in the treatment chambers very few net/retreat interactions were observed (Table 2). Movements were observed to be very slow

Table 2. Observations from experimental chambers showing the number of nets/retreats and net/retreat interactions observed in each chamber and the number of *H. morosa* found dead and pupating after each experiment was completed.

Treatment	Total Number	#Alive	# Dead/ Pupating	#Net/ Retreat	#Net/Retreat Interactions
Control 1	30	8	17/5	1/7	0/0
Control 2	30	14	10/6	1/7	1/1
Control 3	30	7	17/6	5/7	2/2
(0.025mg/L)	20	5	4/11	1/5	0/0
(0.025mg/L)	20	6	6/8	3/6	0/1
(0.025mg/L)	20	4	7/9	1/5	0/0
(0.05 mg/L)	30	7	15/8	5/6	0/0
(0.05 mg/L)	30	9	12/9	1/6	1/1
(0.05 mg/L)	30	6	13/11	0/3	0/0
(0.1 mg/L)	10	1	5/4	0/1	0/0
(0.1 mg/L)	10	2	7/1	0/0	0/0
(0.1 mg/L)	10	1	8/1	0/0	0/0

and sluggish in the channels dosed with ammonium carbonate, but those in control channels seemed normal (compared to reference stream observations). A large number of caddis were observed trying to drift or move in treated stream channels, presumably to avoid the un-ionized ammonia. Thus, many larvae were found in the pumps, accounting for some of the mortality in the treatment chambers.

Morphometric Observations in Channels

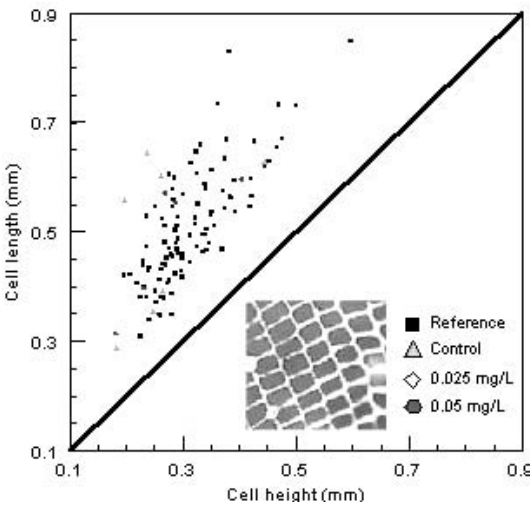

Figure 2. The relationship between cell length and height suggests that cells are not square in shape but more rectangular. No obvious treatment effects can be seen as the treatment measurements fall throughout the reference measurements.

Net mesh was found to be rectangular in shape (Fig. 2) and average mesh area did not appear to differ significantly among ammonia treatment groups (ANOVA, p=0.45). Mean mesh area for stream larvae and high treatment larvae were almost identical (0.11mm^2 and 0.12mm^2, respectively) while mean mesh area for control and low treatment nets was slightly lower (0.08mm^2 and 0.09mm^2 , respectively). The highest mesh area value was measured from reference stream larvae

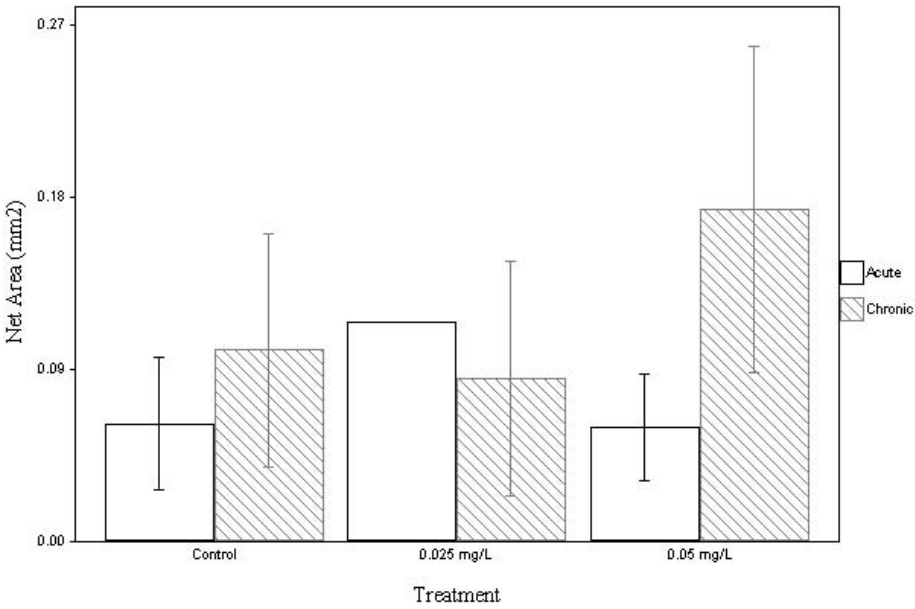

Figure 3. The average net mesh area from experimental streams broken out by exposure time to un-ionized ammonia. Acute exposure being 48 hours and chronic exposure equal to 96 hours in the treatment chamber. High mortality eliminated replicate larvae within the 0.025 mg/L treatment.

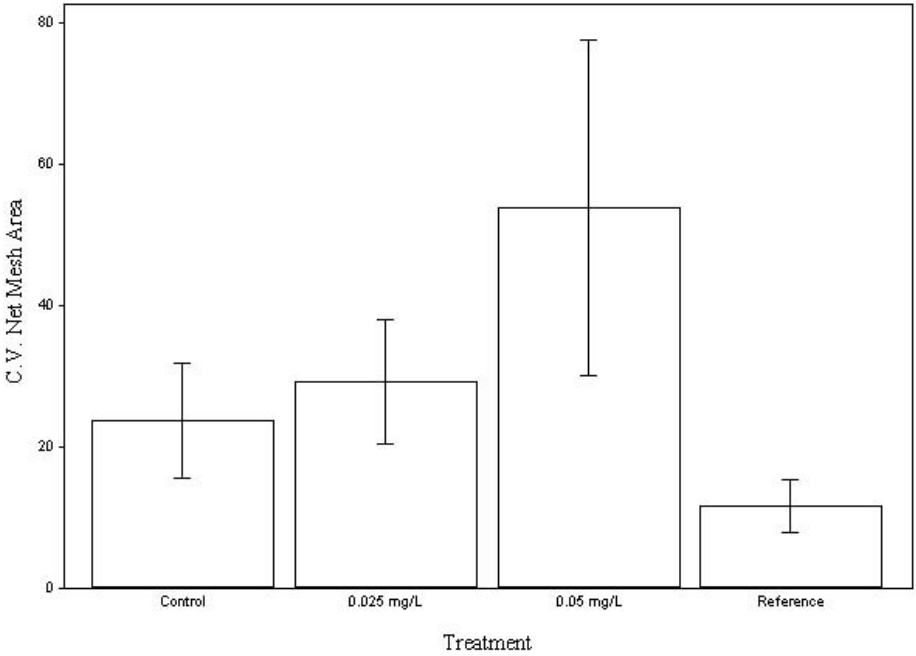

Figure 4. Coefficient of variability in net mesh area between reference sites and treatment streams.

while the lowest was taken from the low treatment (0.35mm^2 and 0.02mm^2, respectively).

The effect of treatments on mesh area did vary by exposure time (significant treatment by time interaction; 2-way ANOVA p=0.039). The largest difference was seen in the 0.05 mg/L treatment (Fig. 3). Larvae exposed for 96 hours constructed nets with larger and more variable net mesh area than those exposed to acute exposure (48 hours). Control treatments demonstrated a similar pattern.

Mesh area variability was found to be lowest in control channels and highest in high treatment channels (Fig. 4; KW ANOVA, p<0.01). Control mesh area coefficients of variation averaged 23.7% with a minimum of 15.3% and maximum of 36.5% while those of low treatment channels ranged from 17.1% to 39.5% (mean = 29.2%) and those of high treatments ranged from 19.4% to 78.1% (mean = 58.9%). Much of the added variability in mesh area of treated nets appeared to be due to high numbers of anomalies and cross-links not observed in control channel nets (Fig. 5). Net mesh area coefficients of variation were low among reference stream samples by comparison to experimental channels, ranging from 3.5% to 22.1% (mean = 11.6%).

Experimental retreat widths fell well within the "normal" (reference stream) range (KW ANOVA, p= 0.43). All average retreat length measurements were approximately the same, but there was a wider range of measurements measured from experimental channels (KW ANOVA p= 0.03). Comparison of means revealed the difference was between the reference streams and 0.05 mg/L treatment (p = 0.15). Variability in retreat length and width was higher among measurements from experimental channels, with highest variability observed in 0.025 mg/L treatments.

CONCLUSIONS

H. morosa collected from reference streams in eastern South Dakota typically had similar net mesh size and retreat dimensions among all sampled sites. Larval hydropsychids construct nets with rectangular mesh of regular dimension (Loudon and Alstad 1990). Mesh size has been shown to vary by species and larval instar. Retreat lengths were slightly more variable than widths. This may be due to the size of substrates used to construct the retreats. Larger or smaller substrates may be located in different parts of the riffle, which would lead to the caddis in that particular part of the riffle building larger or smaller retreats.

Un-ionized ammonia seemed to inhibit *H. morosa* activities and impair retreat and net building behavior in low and high treatment channels. *H. morosa* observed in the control chambers moved freely and behaved in a manner similar to those in reference streams. In contrast, caddis in treated channels displayed slow, lethargic movements and some were observed attempting to drift. Many times there was no movement at all. The slow movements seen in the treatment chambers could be interpreted as the outward signs of physiological stress due to high un-ionized ammonia concentrations.

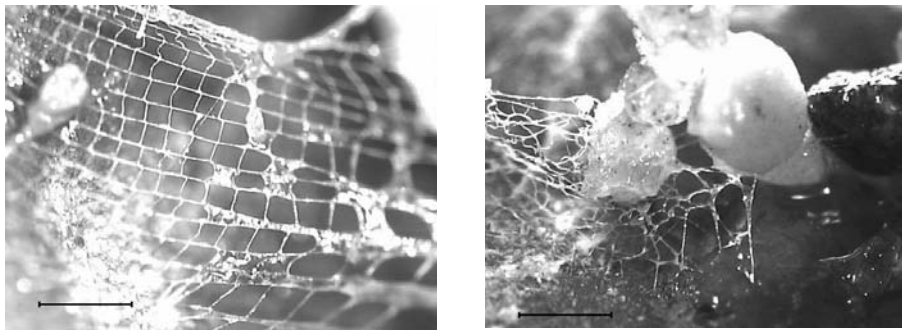

Figure 5. Nets collected from experiment 2, control treatment, chronic exposure (left) experiment 2 high treatment chamber (0.05 mg/L), chronic exposure (right).

The effects of un-ionized ammonia appeared to impair caddis net building leading to net mesh of irregular shape and with greater mesh area variability. Because of the varying net mesh sizes in the different treatments it can be inferred that the un-ionized ammonia had an effect on net construction abilities. Petersen and Petersen (1983) made similar observations, noting that exposure to heavy metals caused Hydropsychids to construct nets with more crosslinks between individual cells.

Low net and retreat building success in Experiment 1 was probably due to high mortality in treated channels, particularly in high treatment channels. This is cause for concern because the low treatment target concentration was established at the South Dakota water quality standard (0.05 mg/L un-ionized ammonia).

Results consistently differed between reference streams and control chambers. The purpose of the control chambers was to isolate treatment effects. It is clear that there was an effect on the caddis when transported and placed into an artificial stream. While no chemicals were introduced to the control there was higher net mesh variability observed compared to reference streams, presumably from the stress associated with transport to an artificial environment. This is supported by McElhorne (1987) who found changes in Trichoptera behavior and community structure when exposed to disturbances such as transport.

Results of this effort demonstrate the influence of elevated un-ionized ammonia concentrations on *Hydropsyche morosa* net construction and tending behavior. Combined results of field observations and laboratory experiments provide measures of natural variability and define specific whole organism responses to a common water quality problem. In contrast, most contemporary biological monitoring efforts focus on changes to algae, macrophyte, invertebrate, and/or fish community structure (Jones 1977, Statzner 1985, McElhone 1987, Basaguren 1990, Barbour 1999). While community endpoints are capable of measuring significant changes in environmental condition, biological responses to disturbance at the whole organism level must occur prior to changes in community structure (Anderson 1982, Evans 1991, Moller 1993, Manning and

Chamberlain 1994, Miller 1998). Early detection of watershed disturbance using whole organism indicators would allow management of disturbed areas prior to significant changes in community structure.

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