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Assessing the Relationship Between Laboratory Whole Effluent Toxicity Test Data and In-Stream Biological Communities

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Abstract The ability of whole effluent toxicity (WET) tests to predict in-stream effects to periphyton, benthic macroinvertebrates, and fish in a habitat-impaired stream was assessed. Habitat assessment data were useful in interpreting in-stream conditions for periphyton and benthic macroinvertebrates. Various periphyton and macroinvertebrate metrics identified siltation effects as opposed to water quality effects in-stream. Pathogen effects noted in fathead minnow WET tests were not reflected in the fish community. Overall, in-stream biological conditions confirmed the absence of water quality-related effects as predicted by WET tests.

Keywords WET · Fish · Macroinvertebrates · Periphyton

Chronic (7-day) whole effluent toxicity (WET) tests are widely applied in National Pollutant Discharge Elimination System (NPDES) programs. The ability of WET tests to predict in-stream effects can be highly dependant on site-specific factors (Ausley 2000). Diamond and Daley (2000) found the best relationships between WET and in-stream conditions when effluents comprised greater than 80% of stream flow. Taken together, *Ceriodaphnia dubia*, fathead minnow (*Pimephales promelas*), and *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) toxicity test results were effective in identifying toxicants related to land-use practices (DeVlaming et al. 2000). Eagleson et al. (1990) found agreement between *C. dubia* WET tests and toxicity as reflected by benthic macroinvertebrate surveys. Diamond and Stribling (2007) found a general lack of agreement between WET test results and in-stream effects when multiple sites were compared, but indicated some uncertainty in those findings due to data quality issues with some WET tests.

The receiving stream evaluated in this study (Fig. 1) is located in Tennessee, and consists entirely of effluent flow under dry weather conditions. No stream reaches exist upstream of the effluent discharge originating the study stream. The effluent consists of contact and non-contact cooling waters from a manufacturing facility. Streams in the region are generally impaired due to siltation as a result of agricultural land uses and the natural sediment accumulation as a result of the flat topography. A goal of this study was to compare two physically similar, habitat-impaired streams using standard biological metrics, and identify biological metrics useful in identifying stressors consistent with the findings of habitat evaluations. The primary goal of this study was determining whether water quality as measured by WET is further impacting in-stream biota. Study design included the use of laboratory WET test organisms to reflect in-stream communities: *P. subcapitata* to represent primary producers, *Ceriodaphnia dubia* to represent invertebrates, and *Pimephales promelas* to represent fish. A nearby same-watershed reference stream with similar land uses and equivalent habitat, stresses, and status was selected for comparison to the effluent-dominated stream.

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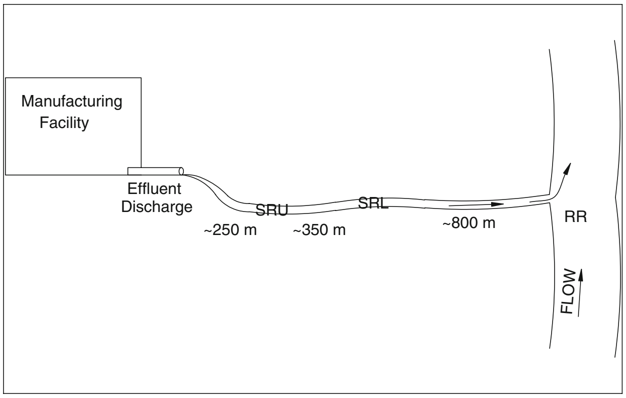

Fig. 1 Study reach upper (SRU), study reach lower (SRL), and reference reach (RR) sample locations

Materials and Methods

Chronic WET tests followed USEPA (2002) protocols and met all test acceptability criteria. Test water was USEPA moderately hard water in the fathead minnow and *C. dubia* tests, and culture water for the *P. subcapitata* tests. The *C. dubia* test assessed survival and reproduction, the fathead minnow test assessed survival and growth, and the *P. subcapitata* test assessed the number of viable cells. Fathead minnow tests were conducted with unaltered effluent and 0.45 *u*m-filtered effluent to control pathogen interference. The phenomenon of pathogen interference in fathead minnow chronic toxicity tests has been well documented (Grothe and Johnson 1996; Kszos et al. 1997; Downey et al. 2000; Geis et al. 2003), and typical symptoms of such interference include poor dose/response curves and poor intra-replicate mortality precision. USEPA (2002) acknowledges such interference, and allows test modifications to control fish pathogens. Symptoms typical of pathogen interference were observed in the present study's fathead minnow tests, and microscopic examinations (by a veterinary fish pathologist) of effluent-exposed fathead minnows confirmed the presence of filamentous iron bacteria and/or *Saprolegnia* as the agent interfering with the proper conduct of our WET tests. All stream reaches assessed were approximately 100 m in length. In the effluent-dominated study reaches, an upper and lower study reach (SRU and SRL) were established approximately 250 and 350 m downstream of the effluent discharge. SRL served as a quality assurance/quality control (QA/QC) site for duplicate biological sampling. The reference reach (RR) was established with similar in-stream habitat as the SR sites. Habitat quality was assessed using USEPA (1999) methods. Periphyton was collected in the proportion to which substrates occurred in each reach. A spatula was passed underneath an inverted Petri-plate to collect periphyton from a known area of silt substrates. Known solid surface areas were scraped directly into sample containers. Periphyton samples for all substrates were composited by reach, mixed by vigorous shaking, settled, and supernate containing algae was decanted. A sub-sample was preserved for taxonomic identification and enumeration, and the remaining sample utilized for chlorophyll-*a* analysis following Standard Methods number 10200H (APHA et al. 1992) using spectrophotometric techniques. Metrics recommended by Bahls (1993) were applied to periphyton data. Macroinvertebrate collections (USEPA 1999) consisted of 20 D-net jab-samples from all habitats (in proportion to occurrence) accounting for at least 5% of macroinvertebrate habitat in each reach. Samples were field-preserved prior to laboratory identification. Seven metrics were applied based on genus-level data as recommended for the ecoregion evaluated (TDEC 2003). Fish collections followed the general guidelines of USEPA (1999) using a backpack shocking unit and truck-mounted generator "drag-line" shocker. Any fish tumors, lesions, or pathogens were noted in field logbooks.

Results and Discussion

A 100% compliance rate with NPDES permit limits for WET was observed for *C. dubia* for the 4 years preceding this study (Table 1). The most sensitive test species was the fathead minnow. The facility's NPDES WET limit for the fathead minnow is based on 0.45 *u*m-filtered effluent samples (to control pathogen interference), and these tests indicated a 91% permit compliance rate. Tests with unfiltered effluent indicated a 76% permit compliance rate. No chronic WET was observed to *P. subcapitata*, the fathead minnow, or *C. dubia* in the year preceding this study. Likely pathogen-related effects to the fathead minnow were observed in 24% of WET tests. Duplicate WET tests immediately preceding field work indicted all 25% inhibition concentration (IC25) values were greater than 100% effluent.

SR and RR in situ analyses (Table 2) indicated pH, dissolved oxygen, temperature, and specific conductance

Table 1 Summary of chronic whole effluent toxicity test results

Species	Number of tests	Percent of tests with IC25 > 100% effluent[a]
C. dubia	23	100
Fathead minnow – filtered effluent	22	91
Fathead minnow – unfiltered effluent	17	76
P. subcapitata	5	100

[a] IC25 = 25% inhibition concentration. Data indicate compliance rate with NPDES permit limits for WET

Table 2 Results of in situ water quality analyses

Parameter	Units	SRU	SRL	RR
pH	s.u.	7.38	7.10	7.81
Dissolved oxygen	mg/L	8.2	7.3	9.0
Temperature	°C	27.3	23.6	25.3
Specific conductance	*u*mho/cm	183	183	582

(conductivity) values conducive to support of warmwater aquatic life. Cumulative habitat quality scores indicated "marginal" conditions in all reaches. "Poor" conditions were consistently observed for key in-stream parameters such as epifaunal substrate and sediment deposition, reflecting local watershed erosion. These findings corroborate those of state regulatory agencies who placed the reference stream on the 303(d) list for siltation. Negligible differences in habitat quality were observed between SR and RR sampling sites. Overall, the habitat quality data indicate that although in-stream habitat was in some ways impaired at the SR and RR sites, habitat quality was very similar at all sites, allowing evaluation of possible further impacts due to water quality as measured by WET test data.

Periphyton chlorophyll-*a* concentrations (Table 3) ranged from 0.05 to 1.76 mg/M^2. These values are very low and ecologically indistinguishable. Periphyton samples revealed a diatom-dominated assemblage at each site with little contribution to algal biomass from non-diatom flora. Non-diatom algae richness ranged from four to seven taxa in the SR samples as compared to six taxa at the RR, again indicating negligible differences in periphyton structure between the streams. Diatom richness values ranged from 34 to 41 in SR samples as compared to 38 taxa in RR samples. Forms of the genera *Nitzschia* and *Navicula* dominated the diatom assemblage at all sites, resulting in a relative percent abundance range of 66% to 87% among the three sites (data not shown).

These data imply no ecologically important differences in diatom community composition due to water quality between the SR and RR sites, as would be predicted by the lack of WET to *P. subcapitata*. The Shannon–Weiner Diversity Index (SWDI) value was similar (3.0–3.2) for all stream reaches. Pollution tolerance index (PTI) values closer to 1 indicate a diatom community tolerant of organic pollution, and values closer to three indicate a community favoring low-nutrient conditions. PTI values for the all reaches ranged from 2.0 to 2.2, suggesting negligible differences between streams. The siltation index (SI) is based on the relative abundance of taxa in assemblages considered motile. Periphyton identified from the study reaches that were incorporated in determination of the SI included 22 species of *Navicula,* 13 species of *Nitzschia,* four species of *Surirella*, and *Cymatopleura solea*. SI values from all samples ranged from 82.2% to 89.4%, depicting a dominant contribution of SI taxa at all sites. Based on the habitat conditions at each site, the diatom data suggest the diatom Siltation index is an appropriate and sensitive metric for these streams and can be used to further evaluate the results of habitat quality assessments. The diatom community at all sites exhibited similar richness values (34–41 taxa). The diatom data indicate no meaningful

Table 3 Chlorophyll-*a* and periphyton cell density

Parameter	Units	SRU	SRL	SRL – duplicate	RR
Chlorophyll-*a*	mg/m^2	0.08	0.88	0.05	1.76
Periphyton cell density	Cells/cm^2	41,387	32,165	56,851	20,346
Diatom					
Cell density	Cells/cm^2	26,767	22,043	27,992	9,582
Richness	Number of taxa	38	41	34	38
Diatom bioassessment					
Shannon–Wiener diversity index metric		3.1	3.2	3.0	3.1
Siltation index metric	Percent	89.4	89.4	82.2	87.5
Pollution tolerance index metric		2.1	2.2	2.2	2.0
Diversity metric score		3	3	3	3
Siltation metric Score		1	1	1	1
Pollution tolerance metric score		4	4	4	3
Lowest Score Biological Integrity		Poor	Poor	Poor	Poor
Impairment for biological integrity rating		Severe	Severe	Severe	Severe
Non-diatom algae					
Cell density	Cells/cm^2	14,620	10,122	28,859	10,764
Richness	Number of taxa	4	4	7	6

differences between SR and RR samples, agreeing with *P. subcapitata* WET tests that indicated a lack of toxicity.

Fish communities were compared only for the SRL and RR sites due to a culvert that precluded fish migration to the SRU site. Thirteen fish species were recorded from the RR, with centrarchids the most frequently encountered. The longeared sunfish (*Lepomis megalotis*) was the dominant RR taxon, comprising 40% of fish collected there. The SRL fish community was similar to the RR fish community in terms of the number of species observed (10), the contribution to the fish assemblage by centrarchids, and dominance of the longeared sunfish (42% SRL taxa). Seven species were common between SRL and RR sites, and included non-centrarchids such as the yellow bullhead catfish (*Ameiurus natalis*), blacktail shiner (*Cyprinella venusta*), bluntnose minnow (*Pimephales notatus*), and golden shiner. Fish observed at the RR and not the SRL site comprised 2% or less of the RR fish community, with the exception of the redfin shiner (15% of RR community). Similar trophic-levels and tolerance levels (intolerant to tolerant) were observed at the SRL and RR sites. There were no anomalies or lesions noted on any fish collected. The conclusion from the fish community data is that the effluent-dominated SRL site is comparable to reference conditions, as would be predicted by fathead minnow WET tests. No pathogen-related effects on receiving stream fish were observed despite pathogen interference in WET tests.

Benthic macroinvertebrate samples (Table 4) indicated similar numbers of taxa (35–39) at all sties. The abundance of aquatic worms (Oligochaetae) and midges (Chironomids), expressed as Percent O&C, reflects the substrate habitat quality (i.e., comprised primarily of sediment). Approximately 75%–95% of the organisms in all samples were O&C taxa. The contribution of "sensitive" EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa was low at all sites, consistent with substrate quality. The North Carolina Biotic Index (NCBI), ranging from 1 to 10 (ten indicating more tolerant organisms), provides a general assessment of water quality without regard to the type of pollution (Lenat 1993). The NCBI values were also similar at all sites. The Percent clinger metric is a physical attribute metric, reflecting the abundance of organisms preferring clean substrates. The percent clinger metric values are below 7.5% at the SRU and RR sites. This suggests sediment impairment more severe than at the SRL site. Differences in metric values for the parameters evaluated were negligible with respect to altering impairment category scores, with all sites indicating overall "slight" or "moderate" impairment. Metric values for a least-impacted ecoregion reference stream indicate much better conditions for a stream not in an agricultural watershed. This comparison indicates the importance of proper reference stream selection in study design. Overall, the macroinvertebrate data indicate a lack of water quality-related impairment.

The results of this study indicated good agreement between bioassessment results for a physically impaired effluent-dominated stream and laboratory WET test results for the source-water discharge. Overall, the bioassessment data indicated no water quality-related stream impairment. This study, incorporating assessments of three trophic levels, indicated that WET test data reflect in-stream conditions in an effluent-dominated stream. This is in keeping with the findings of Diamond and Daley (2000), and De-Vlaming et al. (2000). Study results also indicate that the *Ceriodaphnia dubia* chronic WET test may be an acceptable surrogate for assessing effects to benthic macroinvertebrates as demonstrated by Eagleson et al. (1990) and that the *P. subcapitata* chronic WET test may be a useful surrogate to assess effects to periphyton. While the general absence of WET to the fathead minnow as evaluated in chronic WET tests was reflected by the lack of differences between fish communities in the study and reference stream, the fathead minnow WET test indicated the presence of pathogens not reflected by in-stream fish communities. The importance of habitat assessments in supporting biological data interpretation was also illustrated, and the habitat assessment data confirmed that there were no important differences between the streams compared other than the presence of the effluent discharge. As indicated by the diatom siltation index, the pollution

Table 4 Summary of benthic macroinvertebrate metrics

Metrics	SRU	SRL	SRL – duplicate	RR	Ecoregion – reference
Taxa richness	35	37	29	39	38
EPT richness	1	5	3	2	10
Percent O&C	95.51	75.66	80.77	80.00	43.90
Percent EPT	0.21	18.48	14.34	12.81	37.00
Percent dominant taxon	40.17	34.90	37.41	21.80	34.00
Percent clingers	7.48	21.70	26.22	5.84	28.50
NCBI	8.25	6.41	6.32	7.65	6.00
Impairment category	Moderate	Slightly	Slightly	Moderate	Non-impaired

tolerance metric was not as sensitive to differences in water quality due to the dominance of *Nitzschia* and *Navicula*. Low values for the Percent EPT and Percent clinger metrics in combination with high values for the Percent O&C metric reflected the response of the benthic community to sedimentation. Proper interpretation of these data was also provided by the habitat assessment data.

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References

APHA, AWWA, WEF (1992) Standard methods for the examination of water and wastewater, 18th edn. American Public Health Assoc, Washington, DC

Ausley LW (2000) Reflection on whole effluent toxicity: the pellston workshops. Environ Toxicol Chem 19:1–2. doi:10.1897/1551-5028(2000)019<0001:ROWETT>2.3.CO;2

Bahls LL (1993) Periphyton bioassessment methods for montana streams. Water Quality Bureau, Montana Department of Health and Environmental Sciences, Helena Montana January 1993

DeVlaming V, Connor V, DiGiorgio C, Bailey HC, Deanovic LA, Hinton DE (2000) Application of whole effluent toxicity test procedures to ambient water quality assessment. Environ Toxicol Chem 19:42–62. doi:10.1897/1551-5028(2000)019<0042:AOWETT>2.3.CO;2

Diamond J, Daley C (2000) What is the relationship between whole effluent toxicity and in-stream biological condition? Environ Toxicol Chem 19:158–168. doi:10.1897/1551-5028(2000)019<0158:WITRBW>2.3.CO;2

Diamond J, Stribling J (2007) Evaluation of wet testing as an indicator of aquatic ecosystem health in effluent-dominated streams: a pilot study. Water Environment Research Federation Report 03-ECO-2T, Alexandria, VA

Downey PJ, Fleming K, Guinn R, Chapman N, Varner P, Cooney JD (2000) Sporadic mortality in chronic toxicity tests using *Pimephales promelas* (rafinesque): cases of characterization and control. Environ Toxicol Chem 19:248–255. doi:10.1897/1551-5028(2000)019<0248:SMICTT>2.3.CO;2

Eagleson KE, Lenat DL, Ausley LW, Winborne FG (1990) Comparison of measured in-stream biological responses with responses predicted using the ceriodaphnia chronic toxicity test. Environ Toxicol Chem 9:1019–1028. doi:10.1897/1552-8618(1990)9[1019:COMIBR]2.0.CO;2

Geis SW, Fleming K, Mager A, Reynolds L (2003) Modifications to the fathead minnow (*Pimephales promelas*) chronic test method to remove mortality due to pathogenic organisms. Environ Toxicol Chem 22:2400–2404. doi:10.1897/02-375

Grothe DR, Johnson DE (1996) Bacterial interference in whole effluent toxicity tests. Environ Toxicol Chem 15:761–764. doi:10.1897/1551-5028(1996)015<0761:SCBIIW>2.3.CO;2

Kszos LA, Stewart AJ, Sumner JR (1997) Evidence that variability in ambient fathead minnow short-term chronic tests is due to pathogenic interefernce. Environ Toxicol Chem 16:351–356. doi:10.1897/1551-5028(1997)016<0351:ETVIAF>2.3.CO;2

Lenat DR (1993) Development of a hilsenhoff-type biotic index for North Carolina, with tolerance values for southeastern stream macroinvertebrates. J North Am Benthol Soc 12:279–290. doi:10.2307/1467463

TDEC (2003) Quality system standard operating procedure for macroinvertebrate stream surveys. State of Tennessee Department of Environment and Conservation. Division of Water Pollution Control. March 2002 Revised November 2003

USEPA (1999) Rapid bioassessment protocols for use in wadeable streams and rivers – Periphyton, Benthic macroinvertebrates, and Fish, 2nd edn. United States Environmental Protection Agency, Office of Water, Washington, D.C EPA/841-B-99-002

USEPA (2002) Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, 4th edn. United States Environmental Protection Agency, Office of Water, Washington, D.C EPA-821-R-02-013