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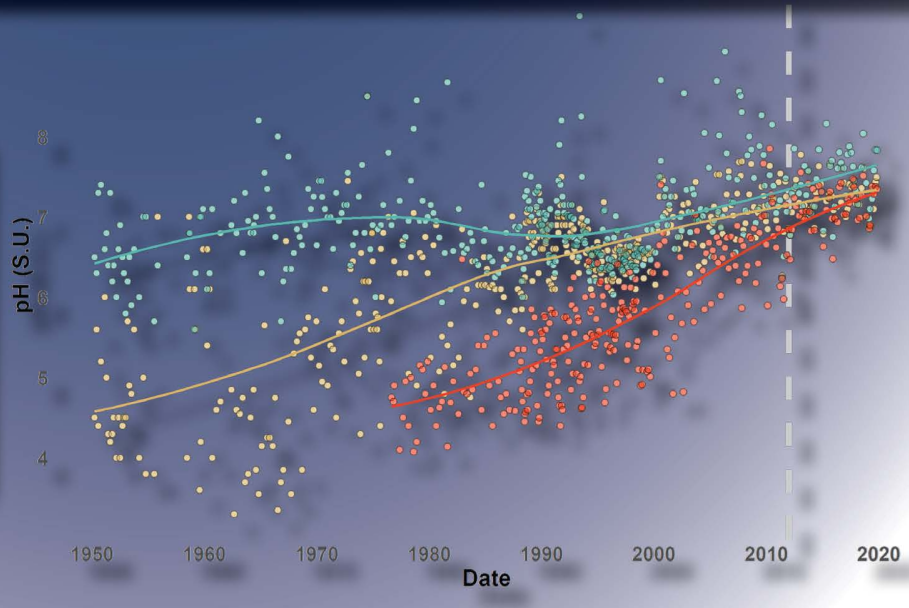
DEPARTMENT OF ENVIRONMENTAL PROTECTION

Office of Water Programs
Bureau of Clean Water

Assessment Methodology for Streams and Rivers
2021



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OFFICE OF WATER PROGRAMS

BUREAU OF CLEAN WATER

ASSESSMENT METHODOLOGY FOR STREAMS AND RIVERS

2021

Chapter 2 Biological Assessment Methods

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TABLE OF CONTENTS

ABBREVIATIONS

ALU	Aquatic Life Use
BCG	Biological Condition Gradient
CIM	Continuous Instream Monitoring
CV	Coefficient of Variation
CWA	Clean Water Act
CWA	Cold Water Assemblage
CWF	Cold Water Fishes
DAG	Drainage Area Group
DEP	Pennsylvania Department of Environmental Protection
DRBC	Delaware River Basin Commission
ECD	Eutrophication Cause Determination
EPT	Ephemeroptera, Plecoptera, and Trichoptera
EV	Exceptional Value
FCATW	Fish Consumption Advisory Technical Workgroup
FS	Freestone
GIS	Geographic Information System
HQ	High Quality
IBI	Index of Biotic Integrity
LS	Limestone
PCB	Polychlorinated Biphenyls
PFAS	Per- and Polyfluoroalkyl Substances
PFBC	Pennsylvania Fish and Boat Commission
PFOS	Perfluorooctane Sulfonic Acid
RU	Recreational Use
SSWAP	Statewide Surface Water Assessment Program
SWMMI	Semi-Wadeable Multimetric Index
TMDL	Total Maximum Daily Load
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
WC	Water Contact Sports
WQS	Water Quality Standards

CHAPTER 1 INTRODUCTION

Chapter 1 Introduction

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PURPOSE

Conceptual Framework

This document details the suite of methods currently used by the Pennsylvania Department of Environmental Protection (DEP) to assess surface waters for their attainment of protected designated and existing uses (further described below). This book is part of a larger conceptual framework (Figure 1) that DEP uses to collect quality data and make scientifically defensible decisions on various surface water matters across Pennsylvania. The data collections protocols established in *Water Quality Monitoring Protocols for Streams and Rivers* (Monitoring Book, Lookenbill and Whiteash 2021) and evaluation methods that stem from them are based on peer reviewed technical reports and published literature. This foundation ensures that not only are DEP data collection protocols and evaluation methods founded on defensible scientific information, but that they also remain dynamic as new technology and innovation emerges. At this time, lake assessment methods are not included in this document.

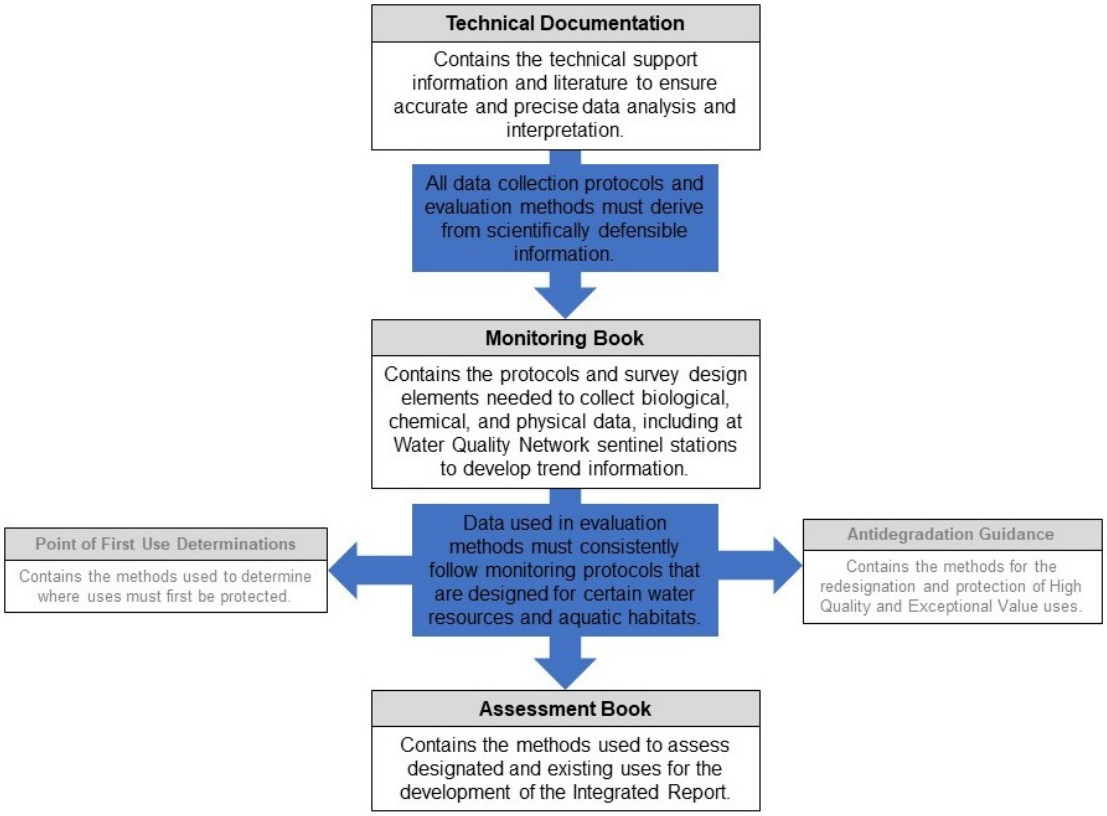

Figure 1. Conceptual framework that DEP uses to make scientifically defensible data collections and decisions. The conceptual progression for making use assessment determinations is highlighted for this book.

Federal Requirements

The primary purpose of this assessment methodology is to satisfy reporting requirements of the federal Clean Water Act (CWA) sections 303(d), 305(b), and 314. 33 U.S.C.A. §§ 1313(d), 1315(b) and 1324. The CWA requires Pennsylvania's Integrated Water Quality Monitoring and Assessment Report (Integrated Report) to be submitted by April 1st of all even numbered years. The timely completion of the Integrated Report satisfies at least one requirement for states to receive Section 106 Water Pollution Control grant funding, which is a critical resource for DEP water quality protection programs. Section 303(d) is composed of a list of waters (Category 5 and 5alt in Table 1) that will not meet all water quality standards (WQS) after implementation of discharge controls and require the development of Total Maximum Daily Loads (TMDL). Section 305(b) of the CWA requires a report on the status of all waters within the state. As a part of section 305(b), section 314 also requires states to assess and report the status of publicly owned lakes. Pennsylvania's Integrated Water Quality Monitoring and Assessment Report (Integrated Report) is the vehicle by which the above information is submitted to the United States Environmental Protection Agency (USEPA). The Integrated Report is made up of a categorical list of assessment determinations (Table 1). These categories help communicate the status of each assessment determination for the numerous waterbodies in Pennsylvania.

Table 1. Listing categories used to develop the Integrated Report.

Category	Description
1	Waters attaining all uses
2	Waters where some, but not all uses are attaining. Attainment status of the remaining uses may be unknown because data are insufficient to categorize the water or it may be impaired.
3	Waters for which there are insufficient or no data to determine if uses are met.
4a	Waters impaired for one or more uses, not needing a TMDL, because it has been completed.
4b	Waters impaired for one or more uses, not needing a TMDL, because uses are expected to be attained within a reasonable timeframe.
4c	Waters impaired for one or more uses, not needing a TMDL, because the impairment is not related to a pollutant.
5	Waters impaired for one or more uses by a pollutant that require the development of a TMDL.
5alt	Waters impaired for one or more uses by a pollutant that are selected for alternative restoration plan implementation.

Pennsylvania WQS and Assessments

DEP works under a state regulatory framework to make assessment determinations that ultimately create the Integrated Report. This framework includes 25 Pa. Code Chapter 93, (Pennsylvania's WQS) and Chapter 96 (WQS implementation). When making assessments, the most relevant parts of WQS are the protected uses and water quality criteria. Waters can have more than one protected use, and each use may have many applicable criteria. Each criterion may also have different implementation requirements through Chapter 96. Therefore, another purpose of this assessment methodology is to articulate how data are used within this regulatory framework to make assessment determinations.

Protected Uses in Pennsylvania

Protected uses fall into four main categories which include Aquatic Life, Water Supply, Recreation and Fish Consumption, and Special Protection. Aquatic Life Uses (ALU) include Cold Water Fishes (CWF), Warm Water Fishes (WWF), Trout Stocking (TSF), and Migratory Fishes (MF). Water Supply uses include Potable Water Supply (PWS), Industrial Water Supply (IWS), Livestock Water Supply (LWS), Wildlife Water Supply (AWS), and Irrigation (IRS). Recreation and Fish Consumption uses include Boating (B), Fishing (F), Water Contact Sports (WC), and Esthetics (E). Special Protection Uses include High Quality Waters (HQ) and Exceptional Value Waters (EV). In addition to the four main categories, an "Other" category lists the Navigation (N) use. See 25 Pa. Code § 93.3 for definitions of these uses.

For the purposes of this book, an "assessment determination" is the decision whether a waterbody is meeting its established water quality standards. DEP has an obligation to assess all surface waters of Pennsylvania for the uses listed in § 93.3. To date, the assessment methods in this book allow for assessment determinations across all four main use categories. DEP will continue to refine and develop new assessment methods to achieve the goal of accurate and complete water quality assessment determinations across Pennsylvania.

Criteria and Assessment Methods

Assessment methods may incorporate 25 Pa. Code Chapter 93 criteria including general and specific water quality criteria (§ 93.6 and § 93.7, respectively), as well as Chapter 96 implementation. This ensures that assessment methods are congruent with applicable WQS and implementation (e.g., permitting). There are many more methods in Chapters 2–4 of this book that focus on the biological community aspects of water quality rather than chemical data comparisons to numeric criteria. This is because DEP relies heavily on biological data to make ALU assessment determinations. USEPA guidance says that in the absence of numeric criteria, the thresholds established in

assessment methods can be used as translators of narrative criteria (USEPA 2002). Therefore, if numeric criteria are not specifically identified in assessment methods, then it can be assumed that the general water quality criteria (narrative criteria) in § 93.6 are being assessed.

Other Purposes

The primary purpose of assessment methods is to create accurate and precise assessment determinations that make up Pennsylvania's Integrated Report. They are also meant to follow uses, criteria, and implementation found in state regulations. Yet, the production of useful tools (e.g., multimetric indices) that measure aspects of water quality may have additional and very relevant purposes, which include – but are not limited to – evaluation of permit compliance, source tracking, and measuring incremental progress. DEP encourages the use of assessment methods for additional purposes as long as the applicable data collection protocols established in the Monitoring Book (Lookenbill and Whiteash 2021) are followed. Using assessment methods for other purposes must also consider how each data collection protocol and assessment method was developed, because the divergence of purposes may not produce scientifically valid results. For instance, applying multimetric index calculations from a wadeable stream macroinvertebrate assessment method using data collected along the banks of a large non-wadeable river is not appropriate. This is because the macroinvertebrate data collection in the non-wadeable river is not consistent with wadeable stream data collection protocols, and macroinvertebrate communities are naturally different between these waterbody types.

Previous Assessment Methods

DEP has gone through several iterations of assessment methods through the history of the program. Changes to assessment methods and additions of new assessment methods exemplify the increase of DEP's scientific understanding and methodological rigor. Consequently, it is important that new versions of assessment methods are used when available. To meet federal requirements, new draft assessment methods and significant revisions to existing methods are publicly participated and made publicly available through DEP's website. Superseded assessment methods are archived internally for record keeping purposes.

Finalization of the public participation process marks the period when the assessment method can be used for assessment determinations. Generally, there is a lag of about 2 to 4 years between when new assessment methods are developed and when assessment determinations using the new methods appear in the Integrated Report. Assessment determinations that used old methods remain valid until the waterbody is reassessed using a current assessment method.

NAVIGATING THE BOOK

Chapters 2–4 constitute the specific methods DEP has developed and uses when making assessment determinations. These assessment methods fall into 3 categories of data collection: biological, chemical, and physical. The structure of this book is designed to reflect the structure of the Monitoring Book (Lookenbill and Whiteash 2021) so that users can easily transition between the data collection (monitoring) aspects and assessment aspects. For accurate and consistent assessments to occur, data collection procedures must follow applicable protocols established in the Monitoring Book (Lookenbill and Whiteash 2021).

Each assessment method must follow general guidelines for making assessment determinations so that decisions are transparent and consistently made throughout Pennsylvania. General guidelines for assessments are provided in Chapter 5. This Chapter also provides guidelines for conducting a “delisting,” which is the determination that either the waterbody has been restored to meeting the use, or that a cause or causes of impairment no longer apply. A critical component in conducting good assessments is accurately identifying the source of the impairment (where the impairment originates from) and the cause of impairment (the pollutant or pollution that’s degrading the waterbody). Without a source and cause defined, it is difficult for meaningful and effective restoration efforts to occur. Chapter 6 identifies and describes how DEP makes source and cause determinations. In some cases, sources and/or causes of impairment may require developing unique determination methods. For example, DEP has created the atmospheric deposition source and cause determination method (Shank 2021) to facilitate differentiation between anthropogenic impacts and natural conditions. DEP will continue to develop new source and cause determination methods when specific needs arise.

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CHAPTER 2 BIOLOGICAL ASSESSMENT METHODS

2.1 WADEABLE FREESTONE RIFFLE-RUN STREAM MACROINVERTEBRATE ASSESSMENT METHOD

Chapter 2 Biological Assessment Methods

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INTRODUCTION

This assessment method is designed to make Aquatic Life Use (ALU) assessment determinations using benthic macroinvertebrate communities in Pennsylvania's wadeable, freestone, riffle-run streams. Through direct quantification of biological attributes along a gradient of conditions, the index of biotic integrity (IBI) provided in this assessment method measures the extent to which anthropogenic activities compromise a stream's ability to support healthy aquatic communities (Davis and Simon 1995). This IBI may also help guide and evaluate aquatic resource legislation, policy, goals, and management strategies (Davis and Simon 1995, Davies and Jackson 2006, Hawkins 2006). Full technical documentation of this assessment method can be found in the technical report entitled *A Benthic Index of Biotic Integrity for Wadeable Freestone Streams in Pennsylvania* (Chalfant 2012). To use this method for assessment determination purposes data collection must follow applicable protocols established in the Monitoring Book (Lookenbill and Whiteash 2021).

THE METRICS

Several different metric combinations were evaluated during index development. The following six metrics were selected for inclusion in the IBI based on various performance characteristics. These six metrics all exhibited a strong ability to distinguish between relatively pristine and heavily impacted conditions. In addition, these six metrics measure different aspects of the benthic macroinvertebrate communities. When used together in a multimetric index, these six metrics provide a solid foundation for assessing the biological condition of benthic macroinvertebrate assemblages in Pennsylvania's wadeable, freestone, riffle-run stream ecosystems.

Total Taxa Richness

This taxonomic richness metric is a count of the total number of taxa in a sub-sample. Generally, this metric is expected to decrease with increasing anthropogenic stress to a stream ecosystem, reflecting loss of taxa and increasing dominance of a few pollution-tolerant taxa. Other benefits of including this metric include its common use in many biological monitoring and assessment programs in other parts of the world as well as its ease of explanation and calculation.

Ephemeroptera + Plecoptera + Trichoptera Taxa Richness (Pollution Tolerance Values 0-4 only)

This taxonomic richness metric is a count of the number of taxa belonging to the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) in a sub-sample –

common names for these orders are mayflies, stoneflies, and caddisflies, respectively. The aquatic life stages of these three insect orders are generally considered sensitive to, or intolerant of, many types of pollution (Lenat and Penrose 1996), although sensitivity to different types of pollution varies among taxa in these insect orders. The version of this metric used here only counts EPT taxa with pollution tolerance values (PTVs) of 0 to 4, excluding a few of the most tolerant mayfly and caddisfly taxa. This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of taxa from these largely pollution-sensitive orders. This metric has a history of use across the world and is relatively easy to use, explain, and calculate (Lenat and Penrose 1996).

Beck's Index (version 3)

This taxonomic richness and tolerance metric is a weighted count of taxa with pollution tolerance values of 0, 1, or 2. The name and conceptual basis of this metric are derived from the water quality work of William H. Beck in Florida (Beck 1955). This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of pollution-sensitive taxa. It should be noted that the version of the Beck's Index metric used for this project, although similar in name and concept, differs slightly in its calculation from the Beck's Index used in DEP's multihabitat protocol for assessing biological condition of low gradient, pool-glide type streams.

Shannon Diversity

This community composition metric measures taxonomic richness and evenness of individuals across taxa of a sub-sample. This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting loss of pollution-sensitive taxa and increasing dominance of a few pollution-tolerant taxa. The name and conceptual basis for this metric are derived from the information theory work of Claude Elwood Shannon (Shannon 1948).

Hilsenhoff Biotic Index

This community composition and tolerance metric is calculated as an average of the number of individuals in a sub-sample, weighted by pollution tolerance values. Developed by William Hilsenhoff, the Hilsenhoff Biotic Index (Hilsenhoff 1977, 1987, 1988, Klemm et al. 1990) generally increases with increasing ecosystem stress, reflecting increasing dominance of pollution-tolerant organisms.

Percent Sensitive Individuals (Pollution Tolerance Values 0-3 only)
This community composition and tolerance metric is the percentage of individuals with pollution tolerance values of 0 to 3 in a sub-sample and is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting loss of pollution-sensitive organisms.

Example calculations for each metric are provided below for a sub-sample from Lycoming Creek in Lycoming County collected November 19, 2001 (Table 1).

Table 1. Benthic macroinvertebrate sub-sample from Lycoming Creek in Lycoming County collected November 19, 2001

Taxa Name	Number of Individuals	Pollution Tolerance Value
Acentrella	1	4
Isonychia	4	3
Epeorus	6	0
Leucrocuta	1	1
Rhithrogena	9	0
Stenonema	8	3
Ephemerella	32	1
Serratella	1	2
Paraleptophlebia	4	1
Pteronarcys	1	0
Taeniopteryx	1	2
Leuctra	2	0
Agnetina	1	2
Paragnetina	1	1
Chimarra	1	4
Dolophilodes	1	0
Cheumatopsyche	25	6
Hydropsyche	22	5
Rhyacophila	16	1
Glossosoma	2	0
Brachycentrus	3	1
Micrasema	1	2
Apatania	2	3
Psilotreta	1	0
Psephenus	3	4
Optioservus	7	4
Atherix	1	2
Antocha	2	3
Hexatoma	5	2
Prosimulium	1	2
Chironomidae	49	6
Ancylidae	2	7
Oligochaeta	1	10

Total Taxa Richness

There are ***33 taxa*** in this sub-sample, so
Total Taxa Richness **= 33**

EPT Taxa Richness (PTV 0-4 only)

There are:
9 Ephemeroptera taxa (Acentrella, Isonychia, Epeorus, Leucrocuta, Rhithrogena, Stenonema, Ephemerella, Serratella, Paraleptophlebia),
5 Plecoptera taxa (Pteronarcys, Taeniopteryx, Leuctra, Agnetina, Paragnetina) and
8 Trichoptera taxa (Chimarra, Dolophilodes, Rhyacophila, Glossosoma, Brachycentrus, Micrasema, Apatania, Psilotreta) in this sub-sample *with PTVs* $\leq$ *4*, so:
EPT Taxa Richness (PTV 0-4 only) = 9 + 5 + 8
EPT Taxa Richness (PTV 0-4 only) **= 22**

Beck's Index (version 3)

$$= 3 * (n_{taxaPTV0}) + 2 * (n_{taxaPTV1}) + 1 * (n_{taxaPTV2})$$

Where $n_{taxaPTV0}$ is the number of taxa with a PTV attribute of 0, $n_{taxaPTV1}$ is the number of taxa with a PTV attribute of 1, and $n_{taxaPTV2}$ is the number of taxa with a PTV attribute of 2.

There are 7 taxa in this sub-sample with PTV = 0. There are 6 taxa in this sub-sample with PTV = 1. There are 7 taxa in this sub-sample with PTV = 2, so

Beck's Index (version 3) = 3(7) + 2(6) + 1(7)
Beck's Index (version 3) = 21 + 12 + 7
Beck's Index (version 3) = **40**

Hilsenhoff Biotic Index

$$= \sum_{i=0}^{10} [(i * n_{indvPTVi})] \Big/ N$$

where $n_{indvPTVi}$ = the number of individuals in a sub-sample with PTV of i and N = the total number of individuals in a sub-sample

In this sub-sample,

there are 22 individuals with PTV = 0, there are 22 individuals with PTV = 5
there are 57 individuals with PTV = 1, there are 74 individuals with PTV = 6
there are 11 individuals with PTV = 2, there are 2 individuals with PTV = 7
there are 16 individuals with PTV = 3, there are 0 individuals with PTV = 8 or 9, and
there are 12 individuals with PTV = 4, there is 1 individual with PTV = 10.

There is a total of 217 individuals in this sub-sample, so

Hilsenhoff Biotic Index = [(0 * 22) + (1 * 57) + (2 * 11) + (3 * 16) + (4 * 12) + (5 * 22) + (6 * 74) + (7 * 2) + (8 * 0) + (9 * 0) + (10 * 1)] / 217

Hilsenhoff Biotic Index = **3.47**

Shannon Diversity Index

$$= -1 \left(\sum_{i=1}^{Rich} [(n_i/N)\ ln(n_i/N)] \right)$$

where n_i = the number of individuals in each taxon (relative abundance); N = the total number of individuals in a sub-sample; and Rich = the total number of taxa in a sub-sample (total taxa richness).

There are 33 taxa in this sub-sample. The numbers of individuals in each taxon are shown in the table above. There are a total of 217 individuals in the sub-sample, so

Shannon Diversity Index = – 1[(1 / 217) *ln* (1 / 217) + (4 / 217) *ln* (4 / 217) + (6 / 217) *ln* (6 / 217) + (1 / 217) *ln* (1 / 217) + (9 / 217) *ln* (9 / 217) +(8 / 217) *ln* (8 / 217) +

(32 / 217) *ln* (32 / 217) + (1 / 217) *ln* (1 / 217) +
… *(do this for all 33 taxa)*
… (1 / 217) *ln* (1 / 217)]

Shannon Diversity Index = **2.67**

Percent Sensitive Individuals (PTV 0-3 only)

$$= \left(\sum_{i=0}^{3} n_{indvPTVi} \Big/ N \right) * 100$$

where $n_{indvPTVi}$ = the number of individuals in a sub-sample with PTV of i and N = the total number of individuals in a sub-sample

In this sub-sample,

there are 22 individuals with PTV = 0,
there are 57 individuals with PTV = 1,
there are 11 individuals with PTV = 2, and
there are 16 individuals with PTV = 3.

There are a total of 217 individuals in this sub-sample, so
Percent Sensitive Individuals (PTV 0-3 only) = (22 + 57 + 11 + 16) / 217 *100
Percent Sensitive Individuals (PTV 0-3 only) = 106 / 217 * 100
Percent Sensitive Individuals (PTV 0-3 only) = **48.8%**

THE INDEX

An index is simply a means to integrate information from various metrics of biological integrity (Barbour et al. 1999). In order to compare and combine sundry measures (e.g., percentage of individuals, counts of taxa, unitless numbers) of biological condition in a meaningful manner, it is necessary to standardize metrics with some mathematical transformation that results in a logical progression of values (Barbour et al. 1995).

To account for natural changes in benthic biota with stream size, different metric standardization values for samples from larger streams and smaller streams were developed for this IBI. Data suggest that the small stream approach is usually appropriate for first, second, and third order streams (using the Strahler stream ordering system) draining less than 25 to 50 mi^2, while the large stream approach is usually

appropriate for fifth order and larger streams draining more than 50 mi^2. More detailed guidelines for deciding whether to apply the large-stream or small-stream metric standardization values to a sample are discussed below.

The one selected core metric that increases in value with increasing anthropogenic stress – Hilsenhoff Biotic Index – was standardized to approximately the 5th percentile of metric scores for all samples from smaller streams and for all samples from larger streams in the IBI development dataset to arrive at the respective small-stream and large-stream standardization values. Core metrics that decrease in value with increasing stress – Total Taxa Richness, EPT Taxa Richness, Beck's Index, Shannon Diversity, and Percent Sensitive Individuals – were standardized to approximately the 95th percentile of metrics scores for all samples from smaller streams and for all samples from larger streams in the IBI development dataset to set the respective small-stream and large-stream standardization values (Table 2).

Table 2. The small-stream and large-stream standardization values used for each core metric.

Metric	Metric Standardization Values	
	smaller streams most 1st to 3rd order < 25 square miles	**larger streams** most 5th order and larger > 50 square miles
Total Taxa Richness	33	31
EPT Taxa Richness	19	16
Beck's Index	38	22
Hilsenhoff Biotic Index	1.89	3.05
Shannon Diversity	2.86	2.86
Percent Sensitive Individuals	84.5	66.7

To calculate the index of biological integrity, observed metric values are first standardized using the standardization values shown in the table immediately above and the following standardization equations.

The Hilsenhoff Biotic Index metric values are expected to increase in value with increasing anthropogenic stress and are standardized using the following equation:

Hilsenhoff Biotic Index standardized score =
(10 – observed value) / (10 – standardization value) * 100

The other five core metrics values are expected to decrease in value with increasing anthropogenic stress and are standardized using the following equation:

Standardized metric score = observed value / standardization value * 100

Once the observed metric values are standardized, the standardized metric scores are adjusted to maximum value of 100 if necessary. By standardizing metrics and setting a maximum value of 100 for the standardized metrics, the resulting adjusted standardized metric scores can range from maximum values of 100 to minimum values of zero, with scores closer to zero corresponding to increasing deviation from the expected reference condition and progressively higher values corresponding more closely to the biological reference condition (Barbour et al. 1995). This approach establishes upper bounds on the expected condition and moderate effects of metrics that may respond in some manner other than a monotonic response to stress. The index of biological integrity is calculated by calculating the arithmetic mean of these adjusted standardized metric values for the six metrics, resulting in a multimetric index of biological integrity score that can range from 0 to 100. To get a score of zero, a sample would have to contain no organisms at all.

In order to incorporate the variability of metric scores with annual seasons in setting biological expectations, DEP chose to implement different use attainment benchmarks as discussed below rather than adjust metric standardization values.

The sample from Lycoming Creek presented above was collected from a fifth order site draining approximately 173 mi^2 of land, so we will apply the large-stream metric standardization values in the example metric standardization and index calculations presented below (Table 3). For a small-stream sample, we would simply substitute the small-stream metric standardization values in place of the large-stream metric standardization values – the rest of the index calculation process is the same regardless of stream size.

Table 3. Index calculation process for Lycoming Creek.

Metric	Standardization Equation (using large-stream standardization values)	Observed Metric Value	Standardized Metric Score	Adjusted Standardized Metric Score Maximum = 100
Total Taxa Richness	(observed value / 31) * 100	33	106.5	100
EPT Taxa Richness	(observed value / 16) * 100	22	137.5	100
Beck's Index	(observed value / 22) * 100	40	181.8	100
Hilsenhoff Biotic Index	[(10 – observed value) / (10 – 3.05)] * 100	3.47	94.0	94.0
Shannon Diversity	(observed value / 2.86) * 100	2.67	93.4	93.4
Percent Sensitive Individuals	(observed value / 66.7) * 100	48.8	73.2	73.2
Average of standardized core metric scores = **IBI Score =**				**93.4**

AQUATIC LIFE USE ATTAINMENT BENCHMARKS

Due to the influences of annual seasons and drainage area seen in the dataset, DEP recognizes different assessment tools and use attainment thresholds are appropriate for samples collected during different times of the year and from different size stream systems. It is noted that some site-specific exceptions to any thresholds may exist because of local scale natural limitations (e.g., habitat availability) on biological condition (Hughes 1995).

Based on the results of technical analyses, professional workshops, feedback from DEP biologists and other colleagues, as well as policy considerations, DEP implements a multi-tiered benchmark decision process for wadeable, freestone, riffle-run streams in Pennsylvania that incorporates stream size and sampling season as factors for determining ALU attainment and impairment based on benthic macroinvertebrate sampling. A simplified flowchart of this decision process is outlined in the diagram below (Figure 1). Although this simplified decision matrix should guide most assessment decisions for benthic macroinvertebrate samples from Pennsylvania's wadeable, freestone, riffle-run streams using the collection and processing methods discussed

above, situations exist where this simplified assessment schematic will not apply exactly as outlined – some such situations are discussed in the following text.

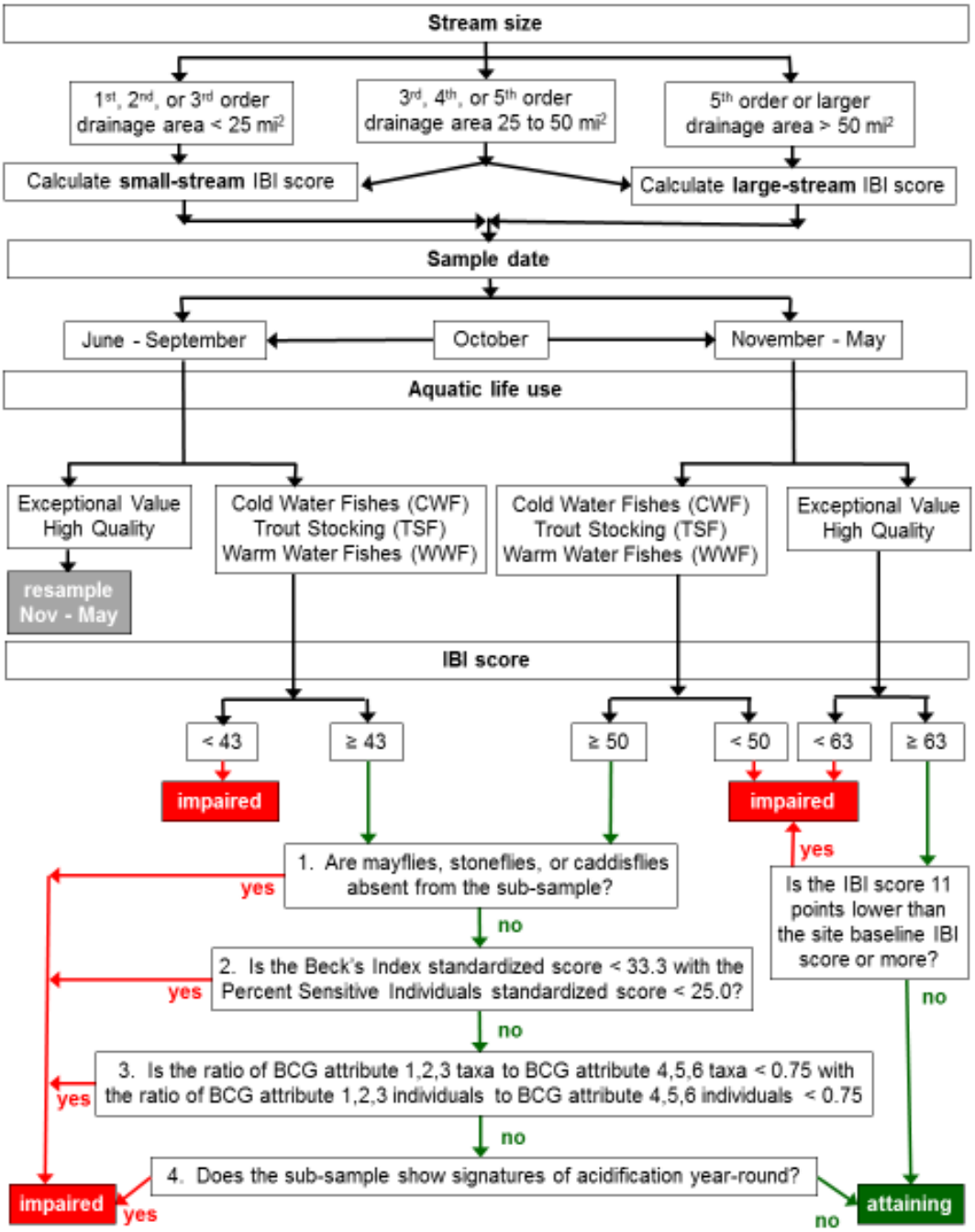

Figure 1. Assessment flowchart for wadeable, freestone, riffle-run streams.

The first step in the ALU assessment process for wadeable, freestone, riffle-run streams in Pennsylvania based on benthic macroinvertebrate sampling considers stream size. DEP does not feel that it is appropriate to set a single cutoff drainage area or stream order threshold to define which set of metric standardization values and which resulting IBI (i.e., large-stream or small-stream) should be applied. However – as stated above – data suggest that the small-stream approach is usually appropriate for samples from first, second, and third order streams draining less than 25 mi^2 of land, while the large-stream approach is usually appropriate for samples from fifth order and larger streams draining more than 50 mi^2.

There are many important considerations when deciding whether to apply the small-stream or large-stream metric standardization values to a sample. Many stream systems experience a variety of changes as they flow from headwaters on downstream. These changes include, but are certainly not limited to, changes in canopy shading, energy dynamics, algal growth, erosional and depositional patterns, habitat distributions, water temperature, and flow regimes. These shifts manifest themselves uniquely in each watershed. Streams in more northern, high elevation, high relief areas of the state may maintain cooler water, flashier flows, larger-particle substrates, and other characteristics typical of smaller streams at comparable drainage areas or stream orders when compared with streams in more southern, low elevation, low relief areas of the state. Local climatological and geological patterns also affect a stream's character.

When deciding which set of metric standardization values (i.e., small-stream or large-stream) to apply, care should be taken not to conflate human-induced changes to streams with natural landscape and climatological variations. For example, a stream draining 26 mi^2 of mostly corn and soybean fields with little forested riparian buffer may experience warmer water temperatures and more silted substrates than a stream of similar size draining a more forested watershed. The warmer water and more silted substrates of the agricultural stream may be characteristics typical of larger streams, but if those characteristics are primarily human-induced, then that argues against applying the large-stream metric standardization values based on the presence of those characteristics in the stream.

For streams of intermediate size (i.e., third, fourth, and some fifth order streams draining between 25 and 50 mi^2 of land), it will often be informative to consider both the small-stream and large-stream IBI scores and associated benchmarks. For example, if a sample from a fourth order site draining 30 mi^2 scores 77.0 on the small-stream IBI and 90.2 on the large-stream IBI and passes the additional screening questions, both approaches indicate ALU attainment, so the use assessment decision is the same regardless of which set of metric standardization values is applied. In another instance,

a sample collected in mid-March from a site draining 36 mi^2 may score 44.1 on the small-stream IBI – indicating impairment – while scoring 51.2 on the large-stream IBI – indicating possible attainment. Here, the small-stream and large-stream IBI score assessment decisions diverge. In such situations, it may be especially useful to consider the additional screening questions – detailed below – when making an assessment decision.

The second step in the ALU assessment process for wadeable, freestone, riffle-run streams in Pennsylvania based on benthic macroinvertebrate sampling considers sampling season. Samples collected during summer and early autumn months (i.e., June through September) are held to different IBI attainment thresholds than samples collected November through May since benthic macroinvertebrate communities in most wadeable, freestone, riffle-run streams in Pennsylvania exhibit consistent patterns of lower taxonomic diversity and organismal abundance during the summer and early autumn months compared with other times of the year. These seasonal index periods are intended as general guidelines and may vary slightly year-to-year depending on local climatological conditions. For example, a sample collected from a low elevation, low latitude stream during the last week of May in a particularly hot, dry year may be more properly evaluated using procedures set forth for the summer months – especially if many mayflies have already emerged from the stream – while a sample collected from a high elevation, high latitude location during the first week of June in an uncharacteristically cool, wet year may be more properly evaluated using the November to May procedures – especially if many mayfly nymphs are still present in the benthos.

October often is a transitional time for benthic macroinvertebrate communities in Pennsylvania with samples from earlier in the month resembling late summer communities (e.g., relatively low diversity and abundance) and samples from later in the month resembling early winter communities (e.g., increasing abundance of winter stoneflies). Therefore, depending on local climate, basin geology, and other factors discussed above (e.g., latitude, elevation, basin relief) samples from October may be evaluated using the June to September benchmarks or the November to May benchmarks. DEP advises against sampling in mid-October to avoid these issues.

For samples collected between November and May, IBI scores < 50 result in ALU impairment. Samples collected during these months scoring ≥ 50 on the appropriate IBI are subject to four screening questions before the ALU can be considered attaining.

These additional screening questions are:

1. **Are mayflies, stoneflies, or caddisflies absent from the sub-sample?** Organisms representing these three taxonomic orders are usually found in most healthy wadeable, freestone, riffle-run streams in Pennsylvania. If any or all of these orders are absent from a sample, this strongly suggests some sort of anthropogenic impact. Samples where one of these taxonomic orders is absent due to natural conditions (e.g., mayflies absent from a low-pH tannic stream) should be evaluated accordingly. *This question must be applied to small-stream samples collected between November and May, but does not have to be applied to samples from larger streams and samples collected between June and September.*

2. **Is the standardized metric score for the Beck's Index metric < 33.3 with the standardized metric score for the Percent Sensitive Individuals metric < 25.0?** Although these two metrics go into the IBI calculations, this screening question serves to double check that a sample has substantial richness and abundance of the most sensitive organisms. *This question must be applied to all samples.*

3. **Is the ratio of Biological Condition Gradient (BCG) attribute 1,2,3 taxa to BCG attribute 4,5,6 taxa < 0.75 with the ratio of BCG attribute 1,2,3 individuals to BCG attribute 4,5,6 individuals < 0.75?** This screening question evaluates the balance of pollution tolerant organisms with more sensitive organisms in terms of taxonomic richness and organismal abundance. By using the BCG attributes to measure pollution tolerance, this screening question serves as a check against the IBI metrics which account for pollution sensitivity based only on PTVs. *This question must be applied to small-stream samples collected between November and May, but does not have to be applied to samples from larger streams and samples collected between June and September.*

4. **Does the sub-sample show signatures of acidification year-round?** The primary acidification signatures in a sub-sample include low mayfly abundance and low mayfly diversity (i.e., scarce mayfly individuals and few mayfly taxa), especially when combined with high abundance of Amphinemura and/or Leuctra stoneflies, occasionally combined with high abundance of Simuliidae and/or Chironomidae individuals. A sub-sample with < 3 mayfly taxa, < 5% mayfly individuals, and > 25% Leuctra and/or Amphinemura stoneflies indicates likely acidification impacts. Acidification effects on benthic macroinvertebrate communities are often most

pronounced in small streams with low buffering capacity during the spring months when snowpacks melt and vernal rains are frequent. While it can be difficult to determine if low pH conditions in a stream are natural or more attributable to anthropogenic acidification, sampling of water chemistry and/or fish communities in addition to benthic macroinvertebrate communities can help inform assessment of acidic in-stream conditions. With this protocol, DEP will only impair sites that show persistent acidification signatures year-round. In other words, if a sample has no mayflies and is dominated by Leuctra and Amphinemura in the spring, but a November sample from the same site contains three or more mayfly taxa or over five percent mayfly individuals, the ALU will not be considered impaired because the stream exhibits the ability to recover biological integrity in the fall and winter months. If a spring sample shows acidification signatures, a late fall or early winter sample must be collected before making an ALU assessment decision. *This question must be applied to all samples.*

If the answer to these four screening questions (if applicable) is yes for a sample collected between November and May with an IBI score ≥ 50, then the sample is considered impaired without compelling reasons otherwise. If the answer to these questions (if applicable) is no for a sample collected between November and May with an IBI score ≥ 50, then the ALU represented by the sample can be considered attaining unless other information (e.g., water chemistry) indicates the ALU may not be fully supported at that location.

For samples collected between June and September, the same logic applies as for samples collected between November and May, but the attainment/impairment threshold is lowered to 43 instead of 50. For samples collected in the summer and early autumn time frame, the absence of mayflies – and in some instances, stoneflies – in samples collected immediately after seasonal hatches may be relaxed in some cases. Because benthic diversity may be underrepresented in summer and early autumn samples DEP encourages monitoring in the November to May timeframe if possible. Benthic macroinvertebrate sampling for determining ALU support should only be conducted from June to early October if sampling during other seasons is not possible due to hazardous conditions such as high, fast stream flow.

Limestone Influence

As discussed in the introduction, DEP deploys a different data collection protocol (Williams 2017b) and assessment methodology (Williams 2017a) for limestone spring streams whose flow is mostly or entirely derived from groundwater in areas with substantial primary calcareous geologies than for freestone streams. The sampling methodology and assessment protocol for these limestone spring streams incorporate the understanding that streams in areas receiving a substantial amount of flow from groundwater attributable to karst geologies often naturally have less diverse benthic macroinvertebrate communities than streams draining freestone geologies. This lower benthic macroinvertebrate community diversity in limestone spring streams is attributable in large part to less variable flow and thermal characteristics of such systems when compared with freestone streams that often exhibit flashier flows and a wider range of temperatures.

Some streams in Pennsylvania drain basins underlain partially by freestone geologies and partially by calcareous geologies. Such streams are often encountered in central regions of the state – especially in upper portions of the Juniata River basin – where they drain sandstone and/or quartzite upland ridges, steep shale slopes, and lower gradient calcareous valley floors. The calcareous valley geologies in these basins contributes to relatively high alkalinities and relatively high and consistent base flows in streams – characteristics of limestone spring streams – when compared with streams draining basins with no calcareous geologies. However, the upland sandstone, quartzite, and shale areas of these basins often contribute substantial surface runoff, which leads to surges in flow during rainfall and snowmelt events and dilution of alkalinity derived from the calcareous valleys. These streams – often referred to as “limestone-influenced” – exhibit some characteristics of limestone spring streams and some characteristics of freestone streams.

We often see substantial agriculture in the fertile valleys of these limestone-influenced streams, which makes it difficult to definitively establish reference conditions specific to these unique streams. However, there is evidence that the benthic macroinvertebrate communities in limestone-influenced streams are naturally less diverse than in freestone streams of similar size and with similar land uses. This lower diversity of benthic macroinvertebrate communities in limestone-influenced streams likely reflects the less variable flow and thermal patterns in these streams caused by the stabilizing influence of the substantial groundwater flowing into the streams through the calcareous valley geologies. Commonly, the benthic macroinvertebrate communities in limestone-influenced streams exhibit relatively low stonefly diversity and abundance when compared with streams of similar size and condition that drain freestone geologies.

In light of these considerations, use attainment benchmarks may be justifiably relaxed for samples from limestone-influenced streams. The June to September IBI benchmark of 43 for freestone streams can be applied to limestone-influenced streams year-round, but the four screening question should still be applied as outlined above to samples from limestone-influenced streams to make ALU assessment decisions.

Antidegradation, Special Protection Considerations

The assessment decision process is somewhat different for streams with special protection uses of high-quality (HQ) or exceptional value (EV) waters. DEP will protect special protection streams based on a baseline IBI score determined by previous surveys. Subsequent samples from HQ and EV streams will be compared to the baseline IBI score for a given site using the IBI temporal precision estimates (Table 4). For example, if Mill Creek is designated HQ and a previous sample from a given site on Mill Creek using the protocol described above results in a mid-April IBI score of 78.0, this IBI score of 78.0 would be the baseline IBI score for that site. Future samples from that site collected November to May that score more than 10.0 IBI points below 78.0, would be considered impaired. Since DEP's sampling season for special protection surveys is November to May, we need not be concerned about how June to October samples compare to the baseline IBI – DEP will only make assessment decisions for HQ and EV streams based on samples collected November to May. The temporal precision estimate of 10.0 points is used because it approximates the October to May temporal precision estimate calculated in the table below. DEP will apply the more restrictive March to May and October to February temporal precision estimates – about 9.0 and 8.0 IBI points, respectively – to special protection use assessments if the situation is appropriate (Chalfant 2012). For example, if the baseline IBI was established in April, future March to May samples that score more than 9.0 points lower than the baseline will be considered impaired. Furthermore, any sample from an HQ or EV stream that scores less than 63.0 on the IBI will be considered impaired without compelling reasons otherwise (e.g., a stream was designated HQ or EV for a reason other than assessment of the benthic macroinvertebrate community).

Table 4. Temporal precision estimates for IBI scores and core metrics based on ANOVA results. The ANOVA mean square error (MSE) estimates intrasite standard deviation. Coefficients of variation (CV) were calculated for each sample pair (or triplet or quadruplet…) and then averaged across all sample pairs. "*s*" indicates standardized metric values. "*r*" indicates raw metric values.

Metric		small-stream						large-stream					
		November to May 384 samples from 137 sites			June to September 26 samples from 12 sites			November to May 78 samples from 26 sites			June to September 26 samples from 7 sites		
		ANOVA MSE	90% CI (1 sample)	%CV	ANOVA MSE	90% CI (1 sample)	%CV	ANOVA MSE	90% CI (1 sample)	%CV	ANOVA MSE	90% CI (1 sample)	%CV
IBI score		48.9	**9.0**	**8.8**	95.7	**12.5**	**19.6**	69.0	**10.6**	**10.3**	18.5	**5.5**	**4.8**
Total Taxa Richness	*s*	115.0	13.7	10.9	101.0	12.9	13.3	128.0	14.50	12.5	103.0	13.0	10.0
	r	16.6	5.2	13.2	16.1	5.14	14.8	15.5	5.05	13.2	12.1	4.5	11.3
EPT Taxa Richness (PTV 0-4 only)	*s*	138.0	15.0	18.5	89.5	12.13	23.8	185.0	17.44	17.3	78.8	11.4	10.7
	r	6.3	3.2	19.7	4.8	2.81	24.7	7.9	3.59	20.8	2.0	1.8	10.7
Beck's Index (version 3)	*s*	127.0	14.4	22.8	94.4	12.46	36.9	132.0	14.73	14.2	142.0	15.3	24.6
	r	21.9	6.0	23.7	17.9	5.42	37.5	16.0	5.13	19.7	10.4	4.1	26.4
Hilsenhoff Biotic Index	*s*	53.1	9.3	7.3	222.0	19.10	22.6	71.3	10.83	8.3	18.5	5.5	4.5
	r	0.4	0.8	15.6	1.5	1.57	21.2	0.4	0.81	15.4	0.1	0.4	6.1
Shannon Diversity	*s*	96.1	12.6	10.1	131.0	14.67	14.1	120.0	14.04	10.5	33.5	7.4	5.3
	r	0.1	0.4	10.7	0.1	0.45	14.4	0.1	0.42	10.8	0.0	0.2	5.7
% Sensitive Individuals (PTV 0-3 only)	*s*	215.0	18.8	23.6	361.0	24.36	65.7	337.0	23.53	27.7	133.0	14.8	16.5
	r	157.0	16.1	23.8	258.0	20.59	65.7	197.0	23.53	30.2	59.1	9.9	16.5

Applications and Exceptions

If a sample results in fewer than 160 total organisms in the entire sample, the IBI and assessment procedures may not apply exactly as outlined above. The IBI and associated benchmarks are calibrated for use with sub-samples containing 160 to 240 organisms, so applications of the IBI to samples containing less – or more – than the target number of organisms, cannot necessarily be assessed using the procedures and benchmarks outlined above. Low abundance of benthic organisms often indicates toxic pollution or severe habitat alterations, which must be considered in making holistic stream assessments.

The use assessment decision processes set forth above are intended as general guidelines, not as hard-and-fast rules. The procedures and guidelines discussed above will provide tenable assessments – as required by federal and state law – of benthic macroinvertebrate community conditions for the vast majority of samples collected from wadeable, freestone, riffle-run streams in Pennsylvania. However, as noted by Hughes (1995), there will be exceptional circumstances – such as those outlined in the Pennsylvania Code (2011: Title 25, Section 93.7(d). (b) relating to less restrictive uses) – when the above assessment procedures do not apply (e.g., there are no obvious sources of impairment and natural factors such as habitat availability or water chemistry limit biotic potential). In some situations, a biologist's local knowledge of conditions may warrant a decision not arrived at using these guidelines. Although the large-stream IBI appears to work well when applied to samples from large rivers (i.e., sites draining over 1,000 square miles), discretion must be used when applying this IBI to samples from such large rivers. These methods do not apply if a stream/river is not wadeable in over 90% or more of its channel area under base flow conditions for the river segment to be sampled or other situations not consistent with riffle and run dominated habitat. The relatively small dataset of samples from such large rivers used in the IBI development limits analysis of variability (i.e., estimates of spatial and temporal precision) in metric and IBI performance with samples from such large rivers.

In other situations, like when samples are heavily dominated by Prosimulium larvae – as discussed above – often this will unduly lower metric and IBI scores, confounding the assessment decision procedures outlined above. In such situations, the investigating biologist may have to re-sample the site after the seasonal Prosimulium larval boom, or the biologist may have to rely on a more qualitative analysis of metric scores, sample composition, and site conditions to arrive at an assessment decision. In any instance, evaluating stream samples requires mindfulness of conditions, and is not always a definite, exact exercise. A certain section of stream may represent a transition between pool-glide, low-relief, marshy, glaciated uplands where the substrate is mostly fine-grained sand and higher-gradient lower reaches filled with cobble-strewn riffles and

runs. Some years see cooler, wetter springs than other years. Nevertheless, for the vast majority of cases involving benthic macroinvertebrate samples from wadeable, freestone (and limestone-influenced), riffle-run streams in Pennsylvania using the protocols described above, the assessment procedures described here will lead to tenable ALU assessment decisions.

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2.2 WADEABLE LIMESTONE STREAM MACROINVERTEBRATE ASSESSMENT METHOD

Chapter 2 Biological Assessment Methods

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INTRODUCTION

This assessment method is designed to make Aquatic Life Use (ALU) assessment determinations using benthic macroinvertebrate communities in Pennsylvania's limestone streams. Limestone streams are streams formed or strongly influenced by limestone springs. All limestone streams are in limestone geology, but not all streams in limestone geology are limestone streams. To determine whether a stream is limestone, several parameters must be investigated. These parameters should be considered during the early stages of data collection and are consequently provided by Williams (2017). If these criteria are not met, the stream is likely not limestone, and another data collection protocol should be considered. To use this method for assessment determination purposes data collection must follow applicable protocols established in the Monitoring Book (Lookenbill and Whiteash 2021).

Limestone stream macroinvertebrate communities have low diversity, with only a few taxa showing high density. This is true to some degree in both reference and impaired streams. Table 1 lists the five most common taxa collected from limestone streams and shows how the composition of the macroinvertebrate communities changes from reference sites to impaired sites. This table clearly shows how different limestone macroinvertebrate communities are and how these differences could affect the metric selection process.

Table 1. Average percent of organisms per taxa collected per sample

Taxa	Common Name	TV	Reference Sites	Attaining Sites	Impaired Sites
Lirceus	Sowbugs	8	9.4 %	29.5 %	52.9 %
Gammarus	Scuds	6	25.0 %	10.7 %	12.7 %
Ephemerella	Mayflies	1	12.0 %	12.4 %	1.2 %
Optioservus	Riffle Beetles	4	11.6 %	5.8 %	1.8 %
Chironomidae	Midges	6	15.7 %	14.8 %	15.5 %
Total % Organisms			**73.6 %**	**73.2 %**	**84.0 %**

These five taxa account for 45,967 out of 58,010 organisms, or 79.2%, collected from 188 samples. Tolerance Value = TV

This document outlines the procedures for interpretation of samples collected from true limestone streams. The protocol was modified from the IBI for limestone streams technical report (Botts 2009). Technical details of the metric selection process and scoring are presented here.

THE METRICS

The following describes the metrics used to evaluate the macroinvertebrate communities in a limestone stream sample (Table 2).

Table 2. Metrics used to evaluate limestone stream samples

Category	Metric	Definition	Response to Pollution
Richness Measure	Total Taxa	Number of taxa in the subsample.	Decreases
	EPT Taxa	Number of taxa in the orders Ephemeroptera, Plecoptera, and Trichoptera.	Decreases
Tolerance/ Intolerance Measures	HBI	The biotic index and abundance of each taxa are used to find a biotic index for the sample.	Increases
	% Tolerant	Percent of organisms considered to be tolerant of pollution (HBI > 6).	Increases
	Beck's Index, 4	Taxa with a Hilsenhoff Biotic Index (HBI) of 0 or 1 are given 2 points and HBI of 2, 3, or 4 are given 1 point.	Decreases
Composition Measures	Shannon Diversity	Uses both taxa richness and abundance to measure general diversity and composition.	Decreases

The following provides a detailed explanation on how to calculate the six metric scores for limestone streams (Table 3). After the field and lab procedures have been completed, a macroinvertebrate list of 300 +/- 10% organisms will be produced.

Table 3. Taxa List for Letort Spring Run (20160330-0900-ablascovic)

Taxonomic Level	Taxa Name	# of Individuals	Hilsenhoff Score
Coleoptera	Optioservus	1	**4**
Diptera	Antocha	3	**3**
Diptera	Chironomidae	35	6
Ephemeroptera	Baetis	19	6
Ephemeroptera	Ephemerella	34	**1**
Gammarus	Gammarus	34	**4**
Lirceus	Lirceus	55	8
Oligochaeta	Oligochaeta	10	10
Trichoptera	Cheumatopsyche	17	6
Trichoptera	Chimarra	2	**4**
Trichoptera	Hydropsyche	80	5
Turbellaria	Turbellaria	1	8

Total Taxa

This metric sums the total number of taxa identified in the sub-sample (count the number of rows in the above table). In the Letort Spring Run sample, there are **12** taxa.

EPT Taxa

To calculate this metric, sum the total number of Mayfly (Ephemeroptera), Stonefly (Plecoptera), and Caddisfly (Trichoptera) taxa found in the sub-sample. In the above sample, Ephemeroptera are colored red and Trichoptera are colored blue; there are no Plecoptera in the sample:

Letort Spring Run:
Ephemeroptera = 2
Plecoptera = 0
Trichoptera = 3
5

Hilsenhoff Biotic Index (HBI)

This community composition and tolerance metric is calculated as an average of the number of individuals in a sub-sample, weighted by pollution tolerance values. Developed by William Hilsenhoff, the Hilsenhoff Biotic Index (Hilsenhoff 1977, 1987, 1988, Klemm et al. 1990) generally increases with increasing ecosystem stress, reflecting increasing dominance of pollution-tolerant organisms.

$$= \sum_{i=0}^{10} [(i * n_{indvPTVi})] \Big/ N$$

where $n_{indvPTVi}$ = the number of individuals in a sub-sample with PTV of i and

N = the total number of individuals in a sub-sample

Letort Spring Run:
In this sub-sample:

There are 0 individuals with PTV = 0,
there are 34 individuals with PTV = 1,
there are 0 individuals with PTV = 2,
there are 3 individuals with PTV = 3,
there are 37 individuals with PTV = 4,
there are 80 individuals with PTV = 5,
there are 71 individuals with PTV = 6,
there are 0 individuals with PTV = 7,
there are 56 individuals with PTV = 8,
there are 0 individuals with PTV = 9,
there are 10 individuals with PTV = 10.

There is a total of 291 individuals in this sub-sample, so

Hilsenhoff Biotic Index = [(0 * 0) + (1 * 34) + (2 * 0) + (3 * 3) + (4 * 37) + (5 * 80) + (6 * 71) + (7 * 0) + (8 * 56) + (9 * 0) + (10 *10)] / 291 =

Hilsenhoff Biotic Index = **5.38**

% Tolerant
This metric is the percent of organisms in the sub-sample considered to be tolerant of pollution (HBI > 6).

Letort Spring Run:
In this sub-sample, there are 66 individuals with PTV > 6 (values 7 through 10).

(66 / 291) * 100 = **22.7%**

Beck's Index, Version 4
Beck's Index, Version 4 is a pollution weighted taxa richness measure, based on Hilsenhoff Biotic Index Scores (HBI). Hilsenhoff's index measures the pollution tolerance of an organism on a scale of 0 to 10, where the organisms' tolerance level decreases with the score. Therefore, it differs from the Beck's Index used in the DEP Riffle/Run Freestone protocol. For Beck's Index, 4, taxa with a HBI score of 0 or 1 are given 2

points and HBI scores of 2, 3, or 4 are given 1 point. In the table, taxa with a score of 0 or 1 are highlighted in blue and scores of 2, 3, and 4 are highlighted in purple.

Letort Spring Run:

Total # of taxa with HBI score of 0 or 1 = **1**
2 pts. x 1 = **2**

Total # of taxa with HBI score of 2, 3, or 4 = **4**
1 pt. x 4 = **4**

2 + **4** = **6**

Shannon Diversity
This index measures taxa abundance and evenness in the sub-sample by dividing the # of individuals in a taxon by the total # of individuals in the sub-sample and then multiplying by the natural logarithm of this proportion. This is done for all taxa in the sub-sample; the products are then summed and the answer multiplied by -1.

$$= -1\left(\sum_{i=1}^{Rich}[(n_i/N)\ ln(n_i/N)]\right)$$

where n_i = the number of individuals in each taxon (relative abundance); N = the total number of individuals in a sub-sample; and Rich = the total number of taxa in a sub-sample (total taxa richness).

Letort Spring Run:

TaxaRich = 12
N = 291

(1/291) *ln* (1/291) + (3/291) *ln* (3/291) + (35/291) *ln* (35/291) + (19/291) *ln* (19/291) + (34/291) *ln* (34/291) + (34/291) *ln* (34/291) + (55/291) *ln* (55/291) + (10/291) *ln* (10/291) + (17/291) *ln* (17/291) + (2/291) *ln* (2/291) + (80/291) *ln* (80/291) + (1/291) *ln* (1/291)

See below for final answer:

-0.0194959562 + -0.0471619689 + -0.2547392859 + -0.1781745755 + -0.2508478806 + -0.2508478806 + -0.3148778505 + -0.1158329269 + -0.1659170745 + -0.0342280143 + -0.3549956378 + -0.0194959562 = -2.00615008 * -1 = **2.00615008 = 2.01**

INDEX OF BIOTIC INTEGRITY (IBI) SCORE

The individual metrics are scored using the standardization formulas as shown below (Tables 4 and 5). Table 4 scores the metrics that increase as conditions improve and Table 5 scores the metrics that increase as conditions degrade.

Table 4. Scoring metrics that increase with good stream conditions.

	Standard (Best Value)		
Metric	X_{95}	Xmin	Standardization Formula
Total Taxa	18.0	0	Score = (X/18.0) x 100
EPT Taxa	8.0	0	Score = (X/8.0) x 100
Beck's Index, 4	12.0	0	Score = (X/12.0) x 100
Shannon Diversity	2.13	0	Score = (X/2.13) x 100

Metrics such as % Tolerant and HBI increase with greater impairment. The lower the score for these metrics the better the ecological condition.

Table 5. Scoring metrics that increase with poor stream conditions.

	Standard (Best Value)		
Metric	X_5	Xmax	Standardization Formula
% Tolerant	1.5	100	Score = (100 - X/100 - 1.5) x 100
HBI	3.84	10	Score = (10 - X/10 - 3.84) x 100

Now that the six metric scores have been calculated, the scores are plugged into the normalized metric score equation: (Observed Value / 95th percentile) x 100. Some metrics may have a normalized score greater than 100 because normalization is based on the 95th percentile values of the statewide dataset. Normalized metric scores above 100 are adjusted to a score of 100. The adjusted metric scores for the six metrics are summed and then averaged to give the Total Biological Score. Table 6 below shows how to calculate the normalized metric scores and Total Biological Scores for the Letort Spring Run sample.

Table 6: Total Biological Score Calculation for Letort Spring Run

Metric	Equation	Observed Value	Normalized Metric Score	Adjusted Metric Score (Max = 100)
Total Taxa	(Observed / **18.0**) x 100	12	66.67	66.7
EPT Taxa	(Observed / **8.0**) x 100	5	62.5	62.5
Beck's Index, 4	(Observed / **12.0**) x 100	6	50	50
Shannon Diversity	(Observed / **2.13**) x 100	2.01	94.37	94.4
% Tolerant	[(100 - Observed) / (**100 - 1.5**)] x 100	22.7	78.4771574	78.5
HBI	[(10 - Observed) / (10 - 3.84)] x 100	5.38	75	75
Total Biological Score (IBI)				71.183333

AQUATIC LIFE USE BENCHMARK

The final score is compared to the values in Table 7, below, and assigned to one of four categories. Sites scoring less than 60 are considered impaired and should be placed on Integrated List Category 5 of impaired streams requiring TMDLs.

Table 7. Limestone Stream IBI Scoring Thresholds

	CWF	CWF	Impaired CWF	
Classification:	Reference	Attaining	Moderately	Severely
IBI Score	>73	73-60	<60-30	<30

Note: Less Than <60 is impaired

In the above example, Letort Spring Run has a final score of 71.2 and would be documented as attaining its ALU.

TEMPORAL PRECISION ESTIMATES

Temporal precision estimates were calculated to demonstrate the method's precision over time (Table 8). This used 193 temporally paired samples at 50 sites. Sites were sampled a minimum of two times and a maximum of nine times over a several-month to twelve-year period. Only samples collected from January through May were used. Sites were determined to be from "true" limestone streams. Samples from reference, attaining, and impaired sites were included in this analysis. The 90% confidence interval was 13.5. This indicates that samples collected from the same site over time may differ by approximately 13.5 points, but differences greater than or less than that number of points may indicate an anthropogenic or other change in the site.

Table 8. Temporal precision estimates for IBI scores and core metrics based on ANOVA results. The ANOVA mean square error (MSE) estimates intrasite standard deviation. Coefficients of variation (CV) were calculated for each sample pair (or triplet or quadruplet…) and then averaged across all sample pairs. "*s*" indicates standardized metric values. "*r*" indicates raw metric values.

Metric		**limestone** **January to May** 193 samples from 50 sites **ANOVA MSE**	**90% CI** (1 sample)	**%CV**
IBI score		**111.5**	**13.5**	**13.9**
Total Taxa Richness	*s*	197.7	18	16.5
	r	6.4	3.2	16.5
EPT Taxa Richness	*s*	354.9	24.2	34
	r	2.3	1.9	34
Beck's Index (version 4)	*s*	271.4	21.1	29.8
	r	3.9	2.5	29.8
Hilsenhoff Biotic Index	*s*	115.3	13.8	14.3
	r	0.4	0.8	9.4
Shannon Diversity	*s*	159.7	16.2	15.7
	r	0.1	0.3	15.7
% Tolerant Individuals (PTV 7-10 only)	*s*	360.5	24.3	25.7
	r	349.7	24	51.9

CONCLUSION

In 2009, DEP finalized a macroinvertebrate bioassessment protocol for assessing Pennsylvania's limestone streams. Using the field and laboratory methods outlined in DEP's protocol, a macroinvertebrate taxonomic list is produced. The taxonomic data is then used to calculate metrics and produce IBI score that accurately reflects the ecological conditions of the waterway. This IBI score is compared to the ALU attainment benchmarks to determine if the sample reach is attaining or impaired.

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2.3 WADEABLE MULTIHABITAT STREAM MACROINVERTEBRATE ASSESSMENT METHOD

Chapter 2 Biological Assessment Methods

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INTRODUCTION

This assessment method is designed to make Aquatic Life Use (ALU) assessment determinations using benthic macroinvertebrate communities in Pennsylvania's low-gradient streams. The USEPA's *Rapid Bioassessment Protocols for use in Wadeable Streams and Rivers* (Barbour et al.1999) describes two approaches to collecting macroinvertebrate community data. These approaches are the "riffle-run" approach and the "multihabitat" approach. Due to low-gradient streams typically lacking riffle-run habitat, the multihabitat approach is preferred. Multihabitat data collection involves sampling a variety of habitat types instead of sampling a single habitat. To use this method for assessment determination purposes data collection must follow applicable protocols established in the Monitoring Book (Lookenbill and Whiteash 2021). For detailed information on the development of this method, reference the full technical document available on the DEP website (McGarrell and Pulket 2007).

METRICS

The six core metrics listed in Table 1 were chosen because they were the most powerful in differentiating between reference and impaired low-gradient sites. These metrics are used to calculate a station's IBI score.

Table 1. Six Core Low-Gradient Metrics

Category	Metric	Definition	Response to Pollution
Richness Measure	Taxa Richness	Total number of taxa	Decreases
	EPT Taxa	Number of taxa in the orders Ephemeroptera, Plecoptera, and Trichoptera.	Decreases
Tolerance/ Intolerance Measures	Beck4	Taxa with a Hilsenhoff Biotic Index (HBI) of 0 or 1 are given 2 points and HBI of 2, 3, or 4 are given 1 point.	Decreases
Abundance Measures	# Mayfly Taxa	Total number of Mayflies (Ephemeroptera)	Decreases
	# Caddisfly Taxa	Total number of Caddisflies (Trichoptera)	Decreases
Composition Measures	Shannon Diversity	Uses both taxa richness and abundance to measure general diversity and composition.	Decreases

The following provides a detailed explanation on how to calculate the six metric scores for two low-gradient streams, Saw Creek and Wiconisco Creek. After the field and lab procedures have been completed, a macroinvertebrate list of 200 +/- 10% organisms will be produced. The following taxa lists are color coded to help distinguish the taxa and information that will be used to calculate the metrics.

Table 2. Taxa List for Saw Creek (20040406-1705-CAM)

Taxonomic Level	Taxa Name	Number of Individuals	Hilsenhoff Score	Functional Feeding Group
Diptera	Chironomidae	109	6	CG
Isopoda	Caecidotea	8	6	CG
Trichoptera	Pycnopsyche	16	**4**	SH
Ephemeroptera	Eurylophella	4	**4**	SC
Trichoptera	Platycentropus	2	**4**	SH
Diptera	Ceratopogonidae	3	6	PR
Bivalvia	Sphaeriidae	3	8	FC
Oligochaeta	Oligochaeta	3	10	CG
Trichoptera	Oecetis	1	8	PR
Hirudinea	Hirudinea	1	8	PR
Ephemeroptera	Stenonema	3	**3**	SC
Plecoptera	Amphinemura	3	**3**	SH
Trichoptera	Lype	7	**2**	CG
Plecoptera	Isoperla	3	**2**	PR
Plecoptera	Leuctra	5	**0**	SH
Trichoptera	Diplectrona	3	**0**	FC
Trichoptera	Wormaldia	1	**0**	FC
Trichoptera	Rhyacophila	3	**1**	PR
Trichoptera	Lepidostoma	1	**1**	SH
Plecoptera	Prostoia	3	**2**	SH
Trichoptera	Molanna	7	6	SC
Diptera	Simulium	13	6	FC
Diptera	Prosimulium	2	5	FC
Diptera	Pseudolimnophila	1	**2**	PR
Diptera	Dicranota	11	**3**	PR
Diptera	Tipula	1	**4**	SH

Table 3. Taxa List for Wiconisco Creek (20050525-1030-CAM)

Taxonomic Level	Taxa Name	# of Individuals	Hilsenhoff Score	Functional Feeding Group
Diptera	Chironomidae	151	6	CG
Isopoda	Caecidotea	1	6	CG
Trichoptera	Platycentropus	1	**4**	SH
Diptera	Ceratopogonidae	2	6	PR
Bivalvia	Sphaeriidae	3	8	FC
Oligochaeta	Oligochaeta	35	10	CG
Amphipoda	Crangonyx	3	**4**	CG
Odonata	Calopteryx	1	6	PR
Plecoptera	Leuctra	1	**0**	SH
Megaloptera	Sialis	1	6	PR
Odonata	Lestes	1	9	PR
Odonata	Ischnura	1	9	PR

EPT

To calculate this metric, sum the total number of Mayfly (Ephemeroptera), Stonefly (Plecoptera), and Caddisfly (Trichoptera) taxa found in the sub-sample:

Saw Creek		Wiconisco Creek	
Ephemeroptera	= 2	Ephemeroptera	= 0
Plecoptera	= 4	Plecoptera	= 1
Trichoptera	= 9	Trichoptera	= 1
	15		**2**

Taxa Richness

This metric sums the total number of taxa identified in the sub-sample (count the number of rows in the above tables):

Saw Creek = **26** Wiconisco Creek = **12**

Beck4

Beck4 is a pollution weighted taxa richness measure, based on Hilsenhoff Biotic Index Scores (HBI). Hilsenhoff's index measures the pollution tolerance of an organism on a scale of 0 to 10, where the organisms' tolerance level decreases with the score. This metric is a modification of Beck's Index; it was chosen because this version works better for low-gradient streams. Therefore, it differs from the Beck's Index used in the DEP

Riffle/Run Freestone protocol. For Beck4, taxa with a HBI score of 0 or 1 are given 2 points and HBI scores of 2, 3, or 4 are given 1 point. In the tables, scores of 0 and 1 are highlighted in blue and scores of 2, 3, and 4 are highlighted in purple.

Saw Creek	Wiconisco Creek
Total # of taxa with HBI score of 0 or 1 = **5**	Total # of taxa with HBI score of 0 or = **1**
2 pts. x 5 = **10**	2 pts x 1 = **2**
Total # of taxa with HBI score of 2, 3, or 4 = 11	Total # of taxa with HBI score of 2, 3, or 4 = 2
1 pt. x 11 = **11**	1 pt. x 2 = **2**
10 + **11** = **21**	**2** + **2** = **4**

Shannon Diversity

This index measures taxa abundance and evenness in the sub-sample by dividing the # of individuals in a taxon by the total # of individuals in the sub-sample and then multiplying by the natural logarithm of this proportion. This is done for all taxa in the sub-sample; the products are then summed and the answer multiplied by -1.

$$= -1\left(\sum_{i=1}^{Rich}[(n_i/N)\ ln(n_i/N)]\right)$$

where n_i = the number of individuals in each taxon (relative abundance); N = the total number of individuals in a sub-sample; and Rich = the total number of taxa in a sub-sample (total taxa richness).

Saw Creek	Wiconisco Creek
TaxaRich = 26	TaxaRich = 12
P = 217 (sum the 'Number of Individuals' column in Tables 2 and 3)	P = 201

p_i = this value is listed in the above tables in the Number of Individuals column.

Saw Creek

(109/217) *ln* (109/217) + (8/217) *ln* (8/217) + (16/217) *ln* (16/217)……(1/217) *ln* (1/217) = -2.12946 * -1 = **2.12946**

Wiconisco Creek

(151/201) *ln* (151/201) + (1/201) *ln* (1/201) + (1/201) *ln* (1/201)……(1/201) *ln* (1/201) = -0.875322793 * -1 = **0.87532**

Number of Caddisfly Taxa

To calculate this metric, sum the number of Caddisfly taxa present in the sub-sample.

Saw Creek	Wiconisco Creek
Trichoptera = 9	Trichoptera = 1

Number of Mayfly Taxa

Sum the total number of Mayfly taxa identified in the sub-sample.

Saw Creek	Wiconisco Creek
Ephemeroptera = 2	Ephemeroptera = 0

INDEX OF BIOTIC INTEGRITY (IBI) SCORE

Now that the six metric scores have been calculated, the scores are plugged into the normalized metric score equation: (Observed Value / 95th percentile) x 100. Some metrics may have a normalized score greater than 100 because normalization is based on the 95th percentile values of the statewide dataset. Normalized metric scores above 100 are adjusted to a score of 100. The adjusted metric scores for the six metrics are summed and then averaged to give the Total Biological Score. Tables 4 and 5 below show how to calculate the normalized metric scores and Total Biological Scores for Saw Creek and Wiconisco Creek.

Saw Creek's Raw Metric Scores	Wiconisco Creek's Raw Metric Score
EPT = 15	EPT = 2
Taxa Richness = 26	Taxa Richness = 12
Beck4 = 21	Beck4 = 4
Shannon Diversity = 2.12946	Shannon Diversity = 0.87532
# Of Caddisfly Taxa = 9	# Of Caddisfly Taxa = 1
# Of Mayfly Taxa = 2	# Of Mayfly Taxa = 0

Table 4. Total Biological Score Calculation for Saw Creek

Metric	Equation	Observed Value	Normalized Metric Score	Adjusted Metric Score (100 Max)
EPT	(Observed / **17**) x 100	15	88.2	88.2
Taxa Richness	(Observed / **31**) x 100	26	83.9	83.9
Beck4	(Observed / **22**) x 100	21	95.5	95.5
Shannon Diversity	(Observed / **2.43**) x 100	2.13	87.6	87.6
# Of Caddisfly Taxa	(Observed / **11**) x 100	9	81.8	81.8
# Of Mayfly Taxa	(Observed / **6**) x 100	2	33.3	33.3
		Total Biological Score (IBI)		**78.4**

Table 5. Total Biological Score Calculation for Wiconisco Creek

Metric	Equation	Observed Value	Normalized Metric Score	Adjusted Metric Score (100 Max)
EPT	(Observed / **17**) x 100	2	11.8	11.8
Taxa Richness	(Observed / **31**) x 100	12	38.7	38.7
Beck4	(Observed / **22**) x 100	4	18.2	18.2
Shannon Diversity	(Observed / **2.43**) x 100	0.88	36.2	36.2
# Of Caddisfly Taxa	(Observed / **11**) x 100	1	9.1	9.1
# Of Mayfly Taxa	(Observed / **6**) x 100	0	0	0
		Total Biological Score (IBI)		**19.0**

AQUATIC LIFE USE BENCHMARK

ALU attainment status of a given sample reach is determined by comparing its Total Biological Score to a use attainment benchmark. If the Total Biological Score of the

sample reach is less than the benchmark score, the sample reach is impaired. If the score is greater than or equal to the benchmark the sample reach is attaining.

Table 6. ALU Benchmark for Low-Gradient Streams

Multihabitat ALU Benchmark
55 (10th percentile)

Therefore, Saw Creek would be documented as attaining its ALU and Wiconisco Creek would be impaired for ALU.

TEMPORAL PRECISION ESTIMATE

The temporal precision is calculated using the 90% confidence interval and is typically used to show confidence around a change in the biological condition of a site. Available for this calculation were 25 temporally paired samples collected at 12 sites between 2003-2010. The 90% confidence interval was 13.2, indicating that measured changes in index score of 14 or greater are not likely due to natural variation.

CONCLUSION

In 2007, Pennsylvania Department of Environmental Protection finalized a macroinvertebrate bioassessment protocol for assessing Pennsylvania's low-gradient streams. Using the field and laboratory methods outlined in DEP's protocol, a macroinvertebrate taxonomic list is produced. The taxonomic data is then used to calculate metrics and produce a total biological (IBI) score that accurately reflects the ecological conditions of the waterway. This IBI score is compared to the ALU attainment benchmark of 55 to determine if the sample reach is attaining or impaired.

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2.4 SEMI-WADEABLE LARGE RIVER MACROINVERTEBRATE ASSESSMENT METHOD

Chapter 2 Biological Assessment Methods

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INTRODUCTION

This assessment method is designed to make Aquatic Life Use (ALU) assessment determinations using benthic macroinvertebrate communities in Pennsylvania's semi-wadeable rivers. Assessment of ALU in large semi-wadeable rivers can be a complex process. To appropriately assess biological communities in large rivers and to increase the efficiency of ALU assessments, DEP separates large rivers into two categories: semi-wadeable and non-wadeable. This assessment method is designed for semi-wadeable rivers within the Commonwealth. Semi-wadeable rivers are defined as predominantly free-flowing systems with drainage areas >1,000 mi^2, and have physical characteristics that allow for riffle and run sections to occur with relative frequency. These river systems tend to lack a well-defined and navigable U-shaped channel for any significant distance and frequently present difficulties for both wadeable and non-wadeable macroinvertebrate data collection methodologies. Well over half of the large rivers within the Commonwealth are considered semi-wadeable (Figure 1). Several studies have shown that semi-wadeable rivers can express substantial and reliable differences in water quality across their width for great distances. These chemical and physical differences drive variations observed in the macroinvertebrate communities that inhabit these regions (Guild et al. 2014, DEP 2014, Shull 2017). The water quality differences across the width of large semi-wadeable rivers are usually the result of major tributary inputs that do not mix. Additionally, each major tributary input is driven by both the natural and anthropogenic influences within the respective basin.

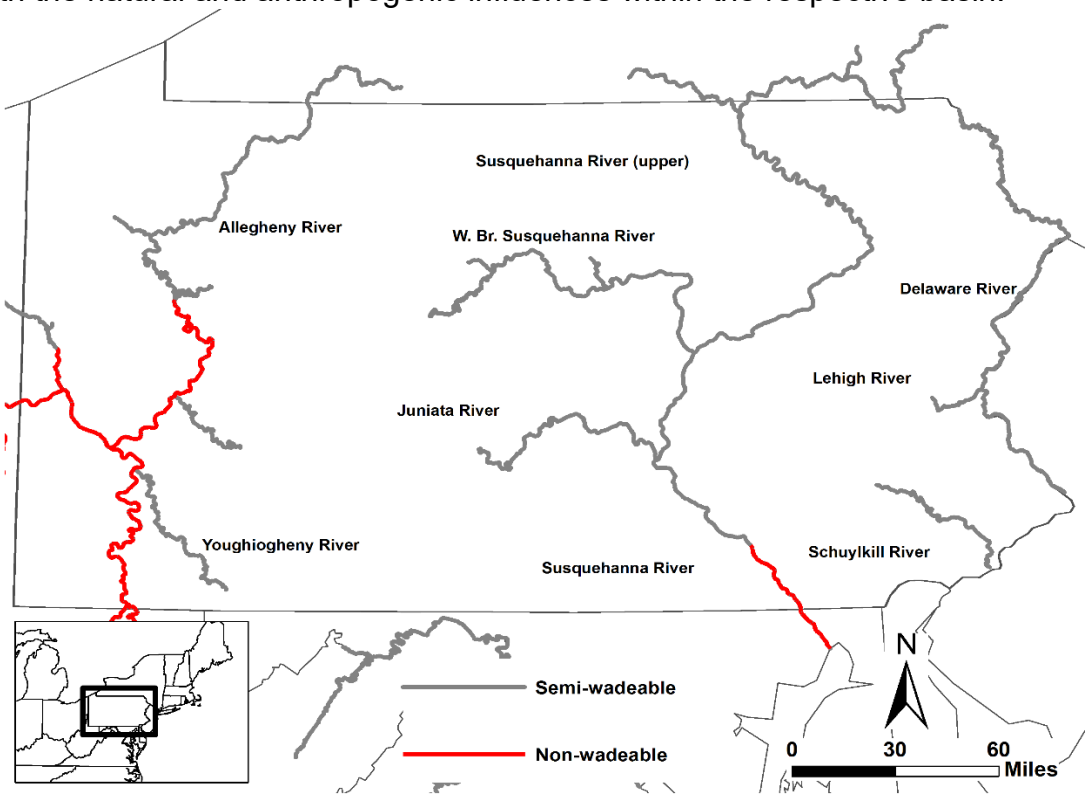

Figure 1. Large Rivers that are semi-wadeable and non-wadeable rivers throughout the Commonwealth. Assessment determinations will be made for semi-wadeable rivers

using this assessment method. DEP continues to develop assessment methods for non-wadeable large rivers.

No other large river biological assessment tool has sought to understand and deal with these chemical, physical, and biological differences at one location on a river separately. Yet, many large river collection methods have been created to capture and composite these variables into one measure; thereby, accounting for, but not giving heed to these important differential aspects (Applegate et al. 2007, Wessell et al. 2008, Blocksom and Johnson 2009, Weigel and Dimick 2011). Final assessments using these tools average or generalize biological condition to provide valuable assessment information, but they do not consider potentially important details in the environment. This effectively obscures the ability to account for biological community degradation within large and important zones on each river. It also reduces the ability to track major sources of impacts driving degradation. Even more problematic are the large river biological collection methods that only collect data along the shoreline of a large semi-wadeable river (Merritt et al. 2005, Angradi 2006, USEPA 2013). These methods are particularly questionable when making large scale inferences about water quality conditions, because shoreline habitats are likely affected by minor tributary influences and point source discharges that follow the shoreline in semi-wadeable rivers (DEP 2014, Shull and Pulket 2015). Consequently, this assessment method does not use shoreline collection methods in large semi-wadeable rivers when making large scale assessment determinations. DEP spent several years developing and refining the transect collection method, and because of this method, each semi-wadeable multimetric index (SWMMI) can not only be used to make assessment determinations that are reflective of overall water quality, but also produce results that retain the unique aspects of water quality variations. This should greatly improve the validity of each assessment on large semi-wadeable rivers, as well as provide important source tracking information for future restoration efforts, if needed.

The goal of this document is to lay the framework for how DEP intends on making ALU assessment determinations in large semi-wadeable rivers. The semi-wadeable large river technical report (Shull 2017) goes into much detail about evaluating the complexity of large semi-wadeable rivers and how these assessment tools were developed to compensate. Making accurate and defensible assessment decisions requires both a sufficient number of data types (e.g., physical, chemical, and biological) and a specificity of those data within a particular water influence (zone) – if needed – and season. Ultimately, ALU assessment determinations will be rather straightforward and similar to wadeable stream assessments if data can only be collected in one season and water influences are well mixed. However, ALU assessment determinations when water influences are not well mixed and when data are collected during both the summer and fall will require additional evaluation and discussion. To use this method for assessment

purposes data collection must follow the protocols established in the Monitoring Book (Lookenbill and Whiteash 2021).

Each reach of river is assessed by the macroinvertebrate collection site immediately downstream. The length of each assessed reach is then determined by where the next potential impact to water quality exists upstream (i.e., major tributary or developed area). Therefore, the location of each upstream macroinvertebrate collection site should reflect this pattern. More explicitly, each macroinvertebrate collection site along the longitudinal gradient is determined by several factors including, where sufficient riffle-run habitat exists, where changes in physiographic and demographic characteristics occur, and where additional major tributaries enter the system. Ideally, macroinvertebrate collection sites will occur at every viable riffle-run habitat, but at the very least, it is necessary to bracket major potential impacts to each system such as a major tributary or change in land use. In the example provided below (Figure 2), two semi-wadeable rivers converge to form another semi-wadeable river. Below this confluence, multiple water quality transects show that water influences do not mix so the non-mixing water influences were mapped. Hence, site 1 requires two unique 6D-200 samples composited completely within the delineated zone of each water influence. Additional macroinvertebrate collections sites (sites 2 – 5) are added upstream of the confluence to characterize each major water influence and to bracket the demographic characteristics across the drainage (e.g., communities, other land use transitions). It is important to note that water quality transect sites – used to delineate the area of specific water influences – can be collected at a higher frequency of locations than macroinvertebrate collections.

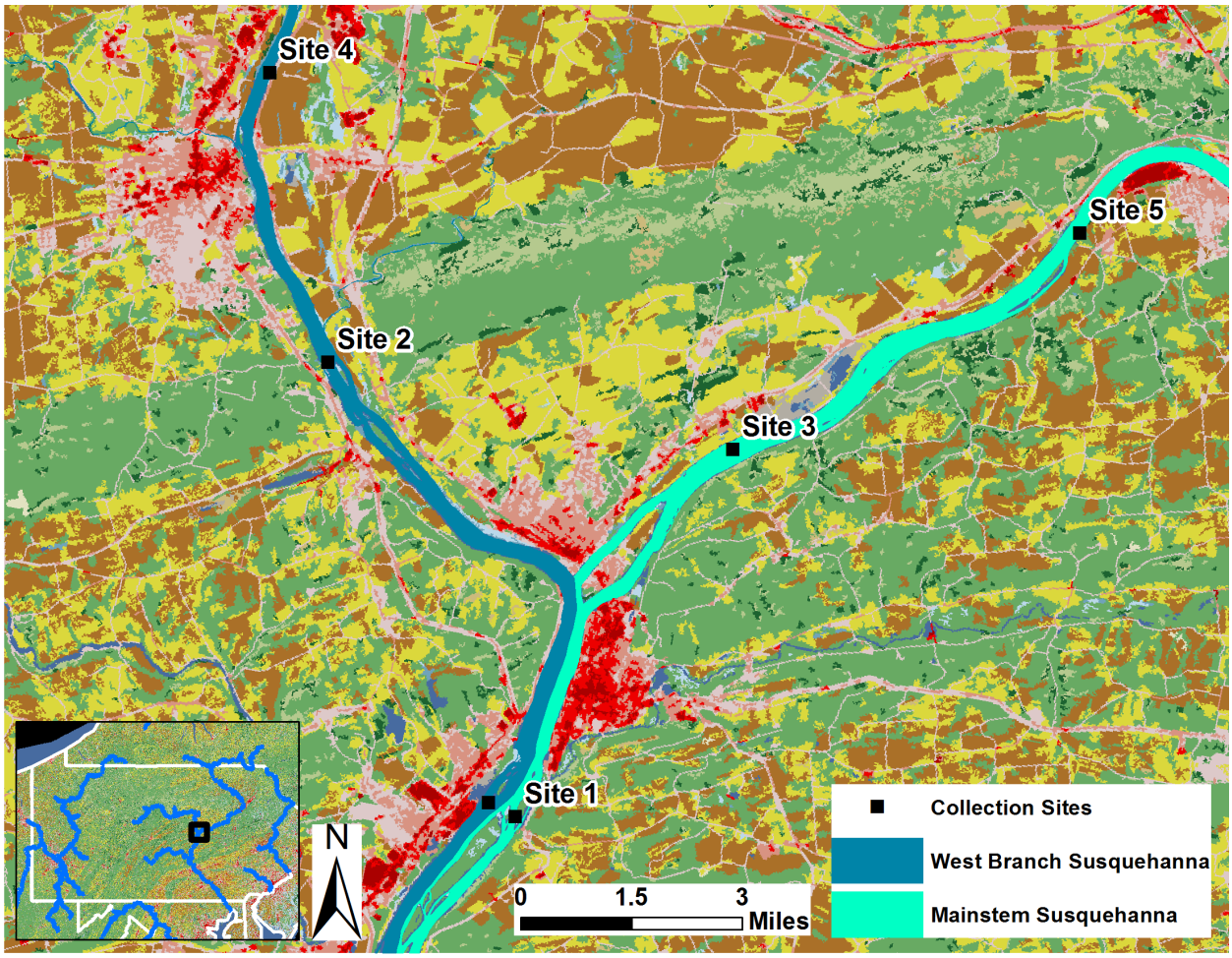

Figure 2. Macroinvertebrate collection sites on a large semi-wadeable river. Site locations were selected to bracket major land use changes and tributary inputs. Habitat assessments are required with each semi-wadable macroinvertebrate sample.

The DEP habitat data sheet for high gradient streams is used, which has undergone several iterations from Plafkin et al. (1989). This habitat evaluation uses a 12 parameter – 20-point scoring method. Currently, it is recommended that all 12 parameters are recorded when conducting habitat assessments in a semi-wadeable river. Although, instream parameters such as instream cover, epifaunal substrate, and embeddedness are the most reliable habitat indicators for large semi-wadeable rivers. Instream cover evaluates the percent makeup of the substrate (boulders, cobble, other rock material) and submerged objects (logs, undercut banks) that provide refuge for fish. Epifaunal substrate evaluates riffle quality, i.e. areal extent relative to stream width and dominant substrate materials that are present. Embeddedness estimates the percent (vertical depth) of the substrate interstitial spaces filled with fine sediments. These three instream habitat measurements can be summed to provide a possible range of 0 (indicating worst possible instream conditions) to 60 (indicating best possible instream conditions) points at each sampling site. Instream habitat totals that fall below 30 points

may be an indication of poor physical habitat conditions. The other parameters in the habitat assessment are also useful for informational purposes but tend to become difficult to measure as river size increases.

SWMMI CALCULATION AND PRECISION

The assessment method development process (Shull 2017) identified two different macroinvertebrate communities existing in large semi-wadeable rivers between the summer and fall seasons. The macroinvertebrate communities were shown to be different enough to justify creating two independent assessment tools for semi-wadeable rivers. For ALU assessment determinations, summer sampling is conducted between July 1st and September 30th and fall sampling is conducted between November 1st and December 31st. October sampling is not recommended if the intent is to make ALU assessment determinations as this is a critical transition period for the macroinvertebrate communities. Examples for each SWMMI (Summer and Fall) are provided to show the metric and index calculation process step-by-step. The summer and fall SWMMI calculations are separated into their respective sections for clarity. Many different metric combinations were evaluated during method development. Each SWMMI had six metrics selected for inclusion into the final index. All metrics, which are further defined and described in Shull (2017) exhibited a strong ability to distinguish between relatively unimpacted and heavily impacted conditions. In addition, these metrics measure different aspects of the benthic macroinvertebrate communities, but when used together in an index, they provide a solid foundation for assessing the biological condition of benthic macroinvertebrate communities in large semi-wadeable rivers. A complete list of taxa and their attributes is provided in Appendix B of Shull (2017).

Summer SWMMI

The following summer sample was collected in the Delaware River on September 9 (Table 1), 2016 and is used in the metric calculation and index standardization example below.

Table 1. Taxa list from a sample collected in the Delaware River on September 9, 2016.

Taxa Name	Number of Individuals
Acroneuria	1
Agnetina	2
Baetisca	1
Brachycentrus	1
Cheumatopsyche	14
Chimarra	7
Chironomidae	10
Corbiculidae	8
Helicopsyche	14
Hydrobiidae	13
Hydropsyche	7
Isonychia	11
Lepidostoma	2
Leucrocuta	8
Maccaffertium	16
Micrasema	1
Oecetis	2
Oligochaeta	7
Optioservus	25
Physidae	1
Plauditus	7
Stenelmis	14
Teloganopsis	22
Tricorythodes	3

Percent Tolerant Individuals using Biological Condition Gradient (BCG) attribute 5 (BCGpct5)

$$= \left(\sum n_{indvBCG5}/N\right)*100$$

Where $n_{indvBCG5}$ is the number of individuals in the subsample with a BCG value of 5, and N is the total number of individuals in the subsample.

Table 2. Taxa list from a sample collected in the Delaware River on September 9, 2016 with BCG attributes. The highlighted taxa have a BCG attribute of 5.

Taxa Name	Number of Individuals	BCG
Acroneuria	1	3
Agnetina	2	3
Baetisca	1	2
Brachycentrus	1	3
Cheumatopsyche	14	5
Chimarra	7	4
Chironomidae	10	5
Corbiculidae	8	5
Helicopsyche	14	3
Hydrobiidae	13	4
Hydropsyche	7	5
Isonychia	11	3
Lepidostoma	2	2
Leucrocuta	8	3
Maccaffertium	16	3
Micrasema	1	3
Oecetis	2	3
Oligochaeta	7	5
Optioservus	25	4
Physidae	1	5
Plauditus	7	
Stenelmis	14	5
Teloganopsis	22	3
Tricorythodes	3	5

There are 64 individuals with a BCG of 5, and a total of 197 individuals in the subsample.
(64/197)*100 = 32.5%

Percent Intolerant Individuals using Pollution Tolerance Value (PTV) attributes 0-3 (PTVpct03)

$$= \left(\sum_{i=0}^{3} n_{indvPTVi} / N \right) * 100$$

Where $n_{indvPTVi}$ is the number of individuals in a sub-sample with PTV of i, and N = the total number of individuals in the subsample.

Table 3. Taxa list from a sample collected in the Delaware River on September 9, 2016 with PTV attributes. The highlighted taxa have PTV attributes between 0 and 3.

Taxa Name	Number of Individuals	PTV
Acroneuria	1	0
Agnetina	2	2
Baetisca	1	4
Brachycentrus	1	1
Cheumatopsyche	14	6
Chimarra	7	4
Chironomidae	10	6
Corbiculidae	8	4
Helicopsyche	14	3
Hydrobiidae	13	8
Hydropsyche	7	5
Isonychia	11	3
Lepidostoma	2	1
Leucrocuta	8	1
Maccaffertium	16	3
Micrasema	1	2
Oecetis	2	8
Oligochaeta	7	10
Optioservus	25	4
Physidae	1	8
Plauditus	7	4
Stenelmis	14	5
Teloganopsis	22	2
Tricorythodes	3	4

There are 78 individuals with a PTV value of 0-3, and a total of 197 individuals in the subsample.
(78/197)*100 = 39.6%

Hilsenhoff Index using BCG attributes (BCGindex2)

$$= \sum_{i=1}^{6} [(i * n_{indvBCGi})] \Big/ N_{BCG}$$

Where $n_{indvBCGi}$ is the number of individuals in a sub-sample with a BCG of i, and N_{BCG} is the total number of individuals with BCG values in the subsample.

Table 4. Taxa list from a sample collected in the Delaware River on September 9, 2016 with BCG attributes. All taxa with a BCG attribute are highlighted.

Taxa Name	Number of Individuals	BCG
Acroneuria	1	3
Agnetina	2	3
Baetisca	1	2
Brachycentrus	1	3
Cheumatopsyche	14	5
Chimarra	7	4
Chironomidae	10	5
Corbiculidae	8	5
Helicopsyche	14	3
Hydrobiidae	13	4
Hydropsyche	7	5
Isonychia	11	3
Lepidostoma	2	2
Leucrocuta	8	3
Maccaffertium	16	3
Micrasema	1	3
Oecetis	2	3
Oligochaeta	7	5
Optioservus	25	4
Physidae	1	5
Plauditus	7	
Stenelmis	14	5
Teloganopsis	22	3
Tricorythodes	3	5

There are 0 individuals with a BCG of 1, 3 with a BCG of 2, 78 with a BCG of 3, 45 with a BCG of 4, 64 with a BCG of 5, 0 with a BCG of 6, and a total of 190 BCG individuals in the subsample.

[(1 * 0) + (2 * 3) + (3 * 78) + (4 * 45) + (5 * 64) + (6 * 0)]/190 = 3.89

Percent Dominant Taxon (pctDOM)

$$= \left(\sum n_{indvDOM}/N\right) * 100$$

Where $n_{indvDOM}$ is the number of individuals of the dominant taxon in the subsample, and N is the total number of individuals in the subsample.

Table 5. Taxa list from a sample collected in the Delaware River on September 9, 2016. The highlighted taxon is the dominant taxon.

Taxa Name	Number of Individuals
Acroneuria	1
Agnetina	2
Baetisca	1
Brachycentrus	1
Cheumatopsyche	14
Chimarra	7
Chironomidae	10
Corbiculidae	8
Helicopsyche	14
Hydrobiidae	13
Hydropsyche	7
Isonychia	11
Lepidostoma	2
Leucrocuta	8
Maccaffertium	16
Micrasema	1
Oecetis	2
Oligochaeta	7
Optioservus	25
Physidae	1
Plauditus	7
Stenelmis	14
Teloganopsis	22
Tricorythodes	3

There are 25 individuals of the dominant taxon, *Optioservus* spp., and a total of 197 individuals in the subsample.
(25/197)*100 = 12.7%

Percent Ephemeroptera using BCG attributes 1-3 (pctEbcg13)

$$= \left(\sum_{i=1}^{3} n_{EphemBCGi} / N \right) * 100$$

Where $n_{EphemBCGi}$ is the number of Ephemeroptera individuals in a sub-sample with BCG of i, and N = the total number of individuals in the subsample.

Table 6. Taxa list from a sample collected in the Delaware River on September 9, 2016 with BCG attributes. The highlighted taxa are Ephemeroptera taxa with a BCG attribute between 1 and 3.

Taxa Name	Number of Individuals	BCG
Acroneuria	1	3
Agnetina	2	3
Baetisca	1	2
Brachycentrus	1	3
Cheumatopsyche	14	5
Chimarra	7	4
Chironomidae	10	5
Corbiculidae	8	5
Helicopsyche	14	3
Hydrobiidae	13	4
Hydropsyche	7	5
Isonychia	11	3
Lepidostoma	2	2
Leucrocuta	8	3
Maccaffertium	16	3
Micrasema	1	3
Oecetis	2	3
Oligochaeta	7	5
Optioservus	25	4
Physidae	1	5
Plauditus	7	
Stenelmis	14	5
Teloganopsis	22	3
Tricorythodes	3	5

There are 58 Ephemeroptera individuals with BCG values of 1-3, and a total of 197 individuals in the subsample.
(58/197)*100 = 29.4%

Richness of Sensitive Ephemeroptera, Plecoptera, and Trichoptera taxa using BCG attributes 1-3 (richEPTbcg)

$$= n_{taxaEPTbcg}$$

Where $n_{taxaEPTbcg}$ is the number of taxa belonging to the orders Ephemeroptera, Plecoptera, and Trichoptera that have BCG attributes of 1-3.

Table 7. Taxa list from a sample collected in the Delaware River on September 9, 2016 with BCG attributes. The highlighted taxa are EPT taxa with a BCG attribute between 1 and 3.

Taxa Name	BCG
Acroneuria	3
Agnetina	3
Baetisca	2
Brachycentrus	3
Cheumatopsyche	5
Chimarra	4
Chironomidae	5
Corbiculidae	5
Helicopsyche	3
Hydrobiidae	4
Hydropsyche	5
Isonychia	3
Lepidostoma	2
Leucrocuta	3
Maccaffertium	3
Micrasema	3
Oecetis	3
Oligochaeta	5
Optioservus	4
Physidae	5
Plauditus	
Stenelmis	5
Teloganopsis	3
Tricorythodes	5

There are 5 Ephemeroptera taxa with BCG attributes of 1-3, 2 Plecoptera taxa with BCG attributes of 1-3, and 5 Trichoptera taxa with BCG attributes of 1-3.
5 + 2 + 5 = 12

Metric Standardization and Index Calculation

Final ceiling and floor standardization values are needed to standardize each metric (Table 8). All standardized metrics are then multiplied by 100 to get the metric standardized score, and the score must range between 0 and 100. Final adjusted metrics scores are then averaged to get a final Summer SWMMI score on a 0 to100 scale.

Table 8. Summer Metric Standardization Values

Metric	Floor Standardization (5^{th} percentile)	Ceiling Standardization (95^{th} percentile)
BCGpct5	28.5	80.6
PTVpct03	2.3	50.6
BCGindex2	3.76	4.76
pctDOM	14.4	46.8
pctEbcg13	0.4	49.7
richEPTbcg	1	10

For metrics like PTVpct03, pctEbcg13, and richEPTbcg (negative-response metrics), standardizations are calculated using the following equation:

(observed value - floor) / (ceiling - floor) * 100.

For metrics like BCGpct5, BCGindex2, and pctDOM (positive-response metrics) standardizations are calculated using the following equation:

(ceiling - observed value) / (ceiling - floor) * 100.

It is important to note that if a metric standardization score is < 0 then the score is set to 0, and if the metric standardization score is > 100 then the score is set to 100. This process creates the adjusted standardized metric score (Table 9).

Table 9. Raw metric values, standardized scores, and final summer SWMMI for a sample collected in the Delaware River on September 9, 2016.

Metric / SWMMI	Observed Value	Standardized Metric Score	Adjusted Standardized Metric Score
BCGpct5	32.5	92.3	92.3
PTVpct03	39.6	77.7	77.7
BCGindex2	3.89	86.7	86.7
pctDOM	12.7	105.2	100
pctEbcg13	29.4	59.1	59.1
richEPTbcg	12	122.2	100
Summer SWMMI	--	--	**86.0**

Summer Precision Estimates

Summer SWMMI methodological precision is calculated using the coefficient of variation intrasite replicate samples (samples collected at the same site on the same day). The summer SWMMI intrasite precision estimate was 8.8%, which was well below recommended limits (10 -15%, Stribling et al. 2008), indicated the summer SWMMI is a precise assessment tool. The summer SWMMI temporal precision is calculated using the 90% confidence interval and is typically used to show confidence around a change in biological condition at a site. The temporal precision estimate for the summer SWMMI using all available samples was 14.7, indicating that measured changes in index score of 15 or greater are not likely due to natural variation.

Fall SWMMI

The following fall sample was collected in the Delaware River on December 16, 2015 (Table 10) and is used in the metric calculation and index standardization example below.

Table 10. Taxa list from a sample collected on the Delaware River on December 16, 2015.

Taxa Name	Number of Individuals
Acroneuria	5
Cheumatopsyche	3
Chimarra	2
Chironomidae	65
Cultus	1
Epeorus	9
Ephemerella	53
Helopicus	2
Hydropsyche	15
Isonychia	3
Lepidostoma	5
Leucrocuta	5
Maccaffertium	28
Nematoda	1
Neophylax	1
Oligochaeta	4
Ophiogomphus	2
Optioservus	15
Oulimnius	1
Paraleptophlebia	2
Psephenus	1
Rhyacophila	2
Stenacron	1
Stenelmis	4
Taeniopteryx	1
Teloganopsis	6

Beck's Index using PTV attributes 0-2 (PTVBeck3)

$$= 3 * (n_{taxaPTV0}) + 2 * (n_{taxaPTV1}) + 1 * (n_{taxaPTV2})$$

Where $n_{taxaPTV0}$ is the number of taxa with a PTV attribute of 0, $n_{taxaPTV1}$ is the number of taxa with a PTV attribute of 1, and $n_{taxaPTV2}$ is the number of taxa with a PTV attribute of 2.

Table 11. Taxa list from a sample collected on the Delaware River on December 16, 2015 with PTV attributes. The highlighted taxa have a PTV between 0 and 3.

Taxa Name	PTV
Acroneuria	0
Cheumatopsyche	6
Chimarra	4
Chironomidae	6
Cultus	2
Epeorus	0
Ephemerella	1
Helopicus	2
Hydropsyche	5
Isonychia	3
Lepidostoma	1
Leucrocuta	1
Maccaffertium	3
Nematoda	9
Neophylax	3
Oligochaeta	10
Ophiogomphus	1
Optioservus	4
Oulimnius	5
Paraleptophlebia	1
Psephenus	4
Rhyacophila	1
Stenacron	4
Stenelmis	5
Taeniopteryx	2
Teloganopsis	2

There are 2 taxa with PTV attributes of 0, 6 taxa with PTV attributes of 1, and 4 taxa with PTV attributes of 2.

3 * (2) + 2 * (6) + 1 * (4) = 22

Richness of Sensitive Ephemeroptera, Plecoptera, and Trichoptera taxa using PTV attributes 0-4 (richEPTptv)

$$= n_{taxaEPTptv}$$

Where $n_{taxaEPTptv}$ is the number of taxa belonging to the orders Ephemeroptera, Plecoptera, and Trichoptera that have PTV attributes of 0-4.

Table 12. Taxa list from a sample collected on the Delaware River on December 16, 2015 with PTV attributes. The highlighted taxa are EPT taxa with a PTV between 0 and 4.

Taxa Name	PTV
Acroneuria	0
Cheumatopsyche	6
Chimarra	4
Chironomidae	6
Cultus	2
Epeorus	0
Ephemerella	1
Helopicus	2
Hydropsyche	5
Isonychia	3
Lepidostoma	1
Leucrocuta	1
Maccaffertium	3
Nematoda	9
Neophylax	3
Oligochaeta	10
Ophiogomphus	1
Optioservus	4
Oulimnius	5
Paraleptophlebia	1
Psephenus	4
Rhyacophila	1
Stenacron	4
Stenelmis	5
Taeniopteryx	2
Teloganopsis	2

There are 8 Ephemeroptera taxa with PTV attributes of 0-4, 4 Plecoptera taxa with PTV attributes of 0-4, and 4 Trichoptera taxa with PTV attributes of 0-4.
8 + 4 + 4 = 16

Percent Intolerant Individuals using PTV attributes 0-3 (PTVpct03)

$$= \left(\sum_{i=0}^{3} n_{indvPTVi} / N \right) * 100$$

Where $n_{indvPTVi}$ is the number of individuals in a sub-sample with PTV of i, and N = the total number of individuals in the subsample.

Table 13. Taxa list from a sample collected on the Delaware River on December 16, 2015 with PTV attributes. The highlighted taxa have a PTV between 0 and 3.

Taxa Name	Number of Individuals	PTV
Acroneuria	5	0
Cheumatopsyche	3	6
Chimarra	2	4
Chironomidae	65	6
Cultus	1	2
Epeorus	9	0
Ephemerella	53	1
Helopicus	2	2
Hydropsyche	15	5
Isonychia	3	3
Lepidostoma	5	1
Leucrocuta	5	1
Maccaffertium	28	3
Nematoda	1	9
Neophylax	1	3
Oligochaeta	4	10
Ophiogomphus	2	1
Optioservus	15	4
Oulimnius	1	5
Paraleptophlebia	2	1
Psephenus	1	4
Rhyacophila	2	1
Stenacron	1	4
Stenelmis	4	5
Taeniopteryx	1	2
Teloganopsis	6	2

There are 125 individuals with a PTV value of 0-3, and a total of 237 individuals in the subsample.
(125/237)*100 = 52.7%

Percent Ephemeroptera using BCG attributes 1-3 (pctEbcg13)

$$= \left(\sum_{i=1}^{3} n_{EphemBCGi} / N \right) * 100$$

Where $n_{EphemBCGi}$ is the number of Ephemeroptera individuals in a sub-sample with BCG of i, and N = the total number of individuals in the subsample.

Table 14. Taxa list from a sample collected on the Delaware River on December 16, 2015 with BCG attributes. The highlighted taxa are Ephemeroptera taxa with a BCG between 1 and 3.

Taxa Name	Number of Individuals	BCG
Acroneuria	5	3
Cheumatopsyche	3	5
Chimarra	2	4
Chironomidae	65	5
Cultus	1	1
Epeorus	9	2
Ephemerella	53	2
Helopicus	2	3
Hydropsyche	15	5
Isonychia	3	3
Lepidostoma	5	2
Leucrocuta	5	3
Maccaffertium	28	3
Nematoda	1	
Neophylax	1	3
Oligochaeta	4	5
Ophiogomphus	2	3
Optioservus	15	4
Oulimnius	1	2
Paraleptophlebia	2	2
Psephenus	1	4
Rhyacophila	2	2
Stenacron	1	4
Stenelmis	4	5
Taeniopteryx	1	3
Teloganopsis	6	3

There are 106 Ephemeroptera individuals with BCG values of 1-3, and a total of 237 individuals in the subsample.
(106/237)*100 = 44.7%

Total Taxa Richness (Richness)

$$= n_{taxa}$$

Where n_{taxa} is the total number of taxa in the subsample.

Table 15. Taxa list from a sample collected on the Delaware River on December 16, 2015.

Taxa Name
Acroneuria
Cheumatopsyche
Chimarra
Chironomidae
Cultus
Epeorus
Ephemerella
Helopicus
Hydropsyche
Isonychia
Lepidostoma
Leucrocuta
Maccaffertium
Nematoda
Neophylax
Oligochaeta
Ophiogomphus
Optioservus
Oulimnius
Paraleptophlebia
Psephenus
Rhyacophila
Stenacron
Stenelmis
Taeniopteryx
Teloganopsis

There are 26 taxa in the subsample.

Richness of taxa in the Functional Feeding Group (FFG) Scrapers (FFGrichSC)

$$= n_{sctaxa}$$

Where n_{sctaxa} is the number of scraper taxa.

Table 16. Taxa list from a sample collected on the Delaware River on December 16, 2015 with FFG attributes. The highlighted taxa are Scrapers within the FFG.

Taxa Name	Number of Individuals	FFG
Acroneuria	5	PR
Cheumatopsyche	3	FC
Chimarra	2	FC
Chironomidae	65	CG
Cultus	1	PR
Epeorus	9	SC
Ephemerella	53	CG
Helopicus	2	PR
Hydropsyche	15	FC
Isonychia	3	CG
Lepidostoma	5	SH
Leucrocuta	5	SC
Maccaffertium	28	SC
Nematoda	1	CG
Neophylax	1	SC
Oligochaeta	4	CG
Ophiogomphus	2	PR
Optioservus	15	SC
Oulimnius	1	SC
Paraleptophlebia	2	CG
Psephenus	1	SC
Rhyacophila	2	PR
Stenacron	1	SC
Stenelmis	4	SC
Taeniopteryx	1	SH
Teloganopsis	6	CG

There are 9 scraper taxa in the subsample.

Metric Standardization and Index Calculation

Final ceiling and floor standardization values are needed to standardize each metric. All standardized metrics are then multiplied by 100 to get the metric standardized score, and the score must range between 0 and 100. Final adjusted metrics scores are then averaged to get a final fall SWMMI score on a 0 to100 scale.

Table 17. Fall Metric Standardization Values

Metric	Floor Standardization (5^{th} percentile)	Ceiling Standardization (95^{th} percentile)
PTVBeck3	2	15
richEPTptv	2	15
PTVpct03	3.3	65.3
pctEbcg13	0	62.3
Richness	11	27
FFGrichSC	2	10

For all fall metrics (negative-response metrics), standardizations are calculated using the following equation:

(observed value - floor) / (ceiling - floor) * 100.

It is important to note that if a metric standardization score is < 0 then the score is set to 0, and if the metric standardization score is > 100 then the score is set to 100. This process creates the adjusted standardized metric score.

Table 18. Raw metric values, standardized scores, and final fall SWMMI for a sample collected in the Delaware River on December 16, 2015.

Metric / SWMMI	Observed Value	Standardized Metric Score	Adjusted Standardized Metric Score
PTVBeck3	22	153.8	100
richEPTptv	16	107.7	100
PTVpct03	52.7	79.7	79.7
pctEbcg13	44.7	71.7	71.7
Richness	26	93.7	93.7
FFGrichSC	9	87.5	87.5
Fall SWMMI	--	--	**88.8**

Fall Precision Estimates

Fall SWMMI methodological precision is calculated using the coefficient of variation intrasite replicate samples (samples collected at the same site on the same day). The

fall SWMMI intrasite precision estimate was 14.1%, which was within recommended limits (10 -15%, Stribling et al. 2008), indicating the fall SWMMI is a precise and repeatable assessment tool. The fall SWMMI temporal precision is calculated using the 90% confidence interval and is typically used to show confidence around a change in biological condition at a site. The temporal precision estimate for the fall SWMMI using all available samples was 12.8, indicating that measured changes in index score of 13 or greater are not likely due to natural variation.

AQUATIC LIFE USE ASSESSMENTS

Both SWMMIs (summer and fall) are accurate and precise tools for making ALU assessment determinations in semi-wadeable rivers. Ideally, assessment in large rivers will understand and compensate for the complexity of the biological communities that exist in these rivers. This assessment tool is a substantial step toward that ideal situation. It is important to note that the transect method can produce multiple SWMMI results at any given location based on the number of major water influences discovered during transect data collection. To address this issue, DEP will use transect data to create zones within each river to be assessed independently, if needed. For example, if transect data shows that 3 unique water quality zones exist, then DEP will use the SWMMI to assess each zone independently. This determination will result in more accurate assessments on large semi-wadeable rivers without ignoring major impacts, or averaging major impacts with better conditions. This method also creates the ability to source track major impacts. Linking large river impacts to sources will inform more appropriate Total Maximum Daily Load (TMDL) and TMDL alternative solutions. In addition, the transect method specifically targets observed variations in water quality and measures biological conditions within those regions; therefore, SWMMI scores between defined zones across the width of a river should not be averaged.

The summer SWMMI impairment threshold is 49 and the fall SWMMI impairment threshold is 57. More information on the development of these impairment thresholds is found in the development report (Shull 2017). SWMMI scores below these thresholds will indicate impaired ALU. Each SWMMI (summer and fall) is independently applicable when making ALU determinations. This is based on USEPA guidance, which mandates that all biological communities DEP has assessment methods for must be evaluated on a stand-alone basis (USEPA 2002). Consequently, each SWMMI is functionally equivalent to having two completely different biological assessment tools (e.g., fish MMI and a macroinvertebrate MMI). Therefore, it is not appropriate to average both SWMMI scores to obtain an overall result. It is also not appropriate to favor the results of one SWMMI over the other. DEP will always strive to collect as much information as possible to make the most accurate assessment decisions. However, based on

independent applicability, it is also understood that only one SWMMI (summer or fall) is required to make an ALU determination for a semi-wadeable river.

The following situation provides an example of this biological assessment rule. Multiple summer and fall samples were collected at the same site (Figure 3). Based on transect analysis the site had one homogeneous influence, so each macroinvertebrate sample was collected evenly across the entire width of the river during each visit. A total of five samples were collected: two samples during the summer and three samples during the fall. The summer samples consistently showed reduced, but attaining SWMMI scores, yet the fall samples resulted in impaired scores. The fall biological community was not supporting the ALU; therefore, DEP would determine that this section of the river is impaired. It may be concluded from this example that one SWMMI is more sensitive than the other; however, that is not the case. Examination of the entire development dataset showed no preference for one SWMMI consistently selecting for one assessment decision when biological communities were close to thresholds.

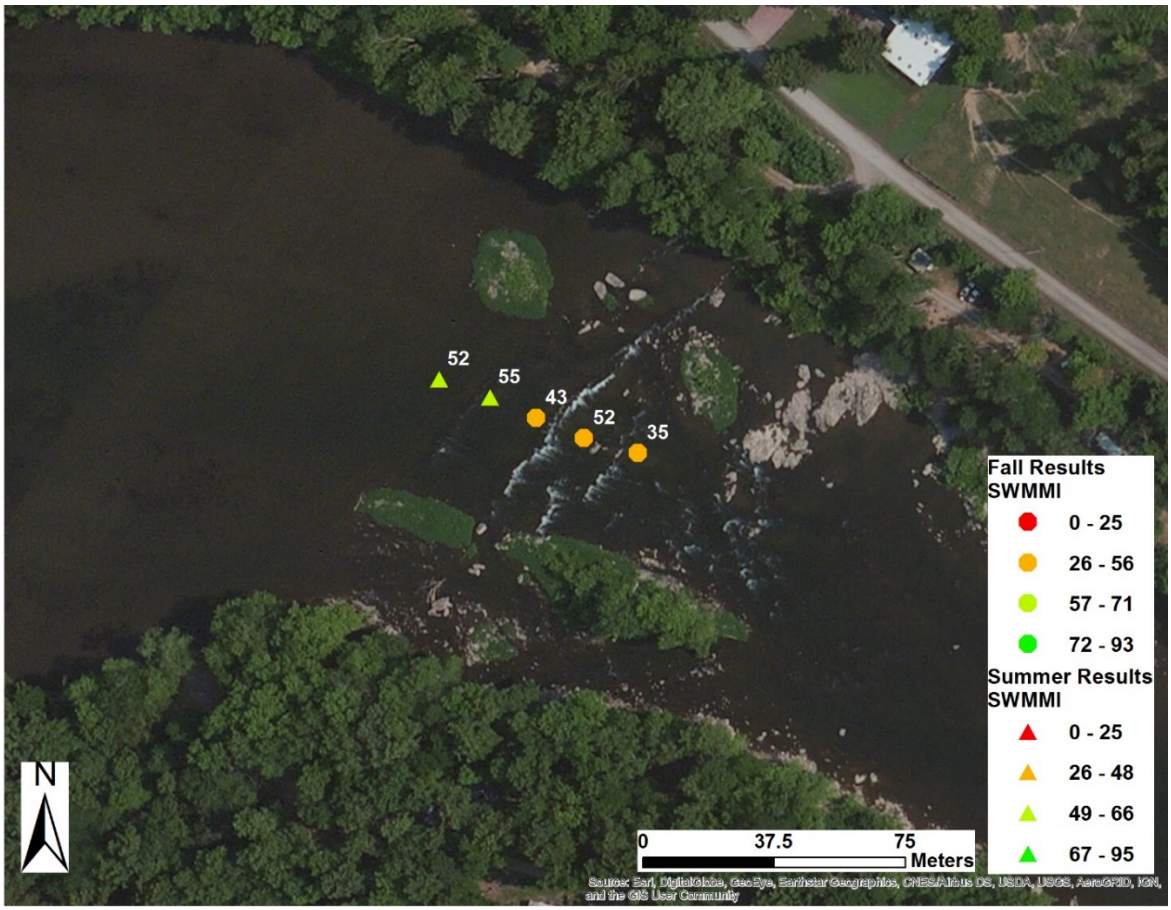

Figure 3. Multiple summer and fall SWMMI results over time at the same location on a semi-wadeable river. Location of points on the map do not indicate exact sample location; points were moved slightly to illustrate the results of sampling. Points are labeled with the respective SWMMI score.

ADDITIONAL APPLICATION CONSIDERATIONS

Data resulting from SWMMI scores may not be used in making ALU determinations in some situations. In fact, DEP uses the wadeable freestone riffle-run method developed by Chalfant (2012) for several other purposes, including, but not limited to cause and effect surveys and incremental improvement reports. These surveys can collect biological information in areas that are not appropriate for making ALU determinations. For example, two macroinvertebrate samples were collected on a semi-wadeable river near a city in Pennsylvania, just downstream of a sewage treatment plant. In this example, the SWMMI results showed that a major portion of this semi-wadeable river (laterally) was being impacted by a facility, perhaps, not operating within permitted limits. Sampling locations specifically targeted one city's sewage treatment facility, but were not necessarily representative of river conditions in this area. Therefore, it would not be appropriate to use these results in making assessment decisions on this river. However, this example does illustrate the usefulness of the semi-wadeable biological collection method for other purposes. This example also illustrates the necessity to differentiate between ALU assessments and reports on local scale impacts. All ALU assessments on semi-wadeable rivers should examine the longitudinal scale that each macroinvertebrate sample represents. If a macroinvertebrate sample is determined to be more representative of a local scale impact, then consideration of appropriate compliance actions may be appropriate.

The SWMMIs may also be used to evaluate whether conditions are degrading or improving at a given site (e.g., trend analysis). It is important to note that this is a different type of analysis than making assessment determinations using an impairment threshold. Methodological error is already incorporated during the development of the impairment threshold, so using variability measurements as "gray areas" while making assessment determinations is not appropriate (Stribling et al. 2008). However, for analyses such as trend analysis, the temporal precision estimate can be used to decide whether a macroinvertebrate community changes over time. When SWMMI scores at the same site change over time beyond the temporal precision estimate, there is a high level of confidence that the biological community change was driven by human influences. The summer SWMMI temporal precision estimate for all sites (where repeat data were available) was 14.7 points, which suggests that observed score changes at a site over time of 15 points or more can be considered a change in condition. The fall SWMMI temporal precision estimate for all sites (where repeat data were available) was 12.8 points, which suggests that observed score changes at a site over time of 13 points or more can be considered a change in condition.

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2.5 BACTERIOLOGICAL ASSESSMENT METHOD FOR WATER CONTACT SPORTS

Chapter 2 Biological Assessment Methods

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INTRODUCTION

This assessment method is designed to make assessment determinations on the Water Contact Sports (WC) Recreational Use (RU) using bacteriological data from Pennsylvania's surface waters. All waters of Pennsylvania are subject to the criterion for *Escherichia coli* (*E. coli*) and fecal coliform colony forming units per 100 milliliters (CFU/100 ml) in 25 Pa. Code § 93.7. The criterion specifies that during the swimming season (May 1st through September 30th), the maximum *E. coli* level shall be a geometric mean of 126 CFU/100 ml. The geometric mean for the samples collected in the waterbody should not be greater than 126 CFU/100 ml in any 30-day interval. There should not be greater than a 10% excursion frequency of 410 CFU/100ml for the samples collected in the same 30–day duration interval. For the remainder of the year (the non-swimming season), the maximum fecal coliform level shall be a geometric mean of 2,000 CFU/100 ml, based on a minimum of 5 consecutive samples collected on different days during a 30-day period.

The criterion specifically defines the sample duration during the bathing season and the sample magnitude, frequency, and duration during the non-bathing season for determining impairment for WC RU. This method defines the magnitude, frequency, and duration during the bathing season and remainder of the year as a sampling group consisting of at least 5 samples, each separated by at least 24 hours, spanning a maximum of 30 days and a minimum of 14 days, except as noted below in *Assessment Decisions*, *Exceptions to the 5-sample limit*. To use this method for assessment determination purposes, data collection must follow the Monitoring Book protocols in *Chapter 3.11, Bacteriological Data Collection Protocol* (Miller 2021).

DATA PROCESSING

A geometric mean is calculated for each sampling group consisting of multiple samples collected at each site on different days within a 14 to 30-day period. Geometric means are calculated by taking the natural logarithm (*ln*) of each sample result and then averaging the logarithm values. This average is then converted back to a normal value by computing the antilog. The following example illustrates this process (Table 1).

Table 1. Example of 5 *E. coli* samples collected at a single site during the swimming season for assessment determination purposes.

Sample	Result (cfu/100 ml)	*ln*(Result)
1	130	4.868
2	380	5.940
3	240	5.481
4	100	4.605
5	180	5.193
	Mean of *ln*(Results)	5.217
	Antilog of Mean	**184** cfu/100 ml

In the example above, the geometric mean of all 5 samples taken during the swimming season was 184, which is above the criterion for *E. coli* (126 cfu/100 ml), and no single sample was above 410 cfu/100 ml. For this reason, this stream segment would be considered not attaining the WC RU.

ASSESSMENT DECISIONS

The primary focus of WC RU assessments is to list waters that are impaired due to chronic long-term water quality impacts, and not acute or transitory situations. Hence, at least 5 samples should be collected, each separated by at least 24 hours, spanning a maximum of 30 days and minimum of 14 days.

DEP will assess waters as impaired for WC RU if there is one exceedance of the *E. coli* 30-day geometric mean criterion during the swimming season, or if there is one exceedance of the fecal coliform 30-day geometric mean criterion during the non-swimming season. The *E. coli* criterion also states that, no more than 10% of the samples collected from waters attaining recreational use exceed 410 CFU/100ml during the swimming season. This 10% excursion frequency will apply to assessment decisions on a case-by-case basis and only when there is enough data to support an impairment decision. Generally, the geometric mean shall be used to assess waterbodies because this value is more relevant to the long-term quality of a waterbody.

The primary waterbodies of concern are streams and rivers that are larger than Strahler Order 2. These streams are most frequently used by the public for swimming and full body immersion and thus the potential to contract illness due to waterborne pathogens is much more likely than small headwater streams. Although larger streams and rivers are a focus, it will not preclude smaller waters from assessment.

Exceptions to the 5-sample limit

The 2006 Integrated Report guidance (USEPA 2005), USEPA stressed the importance of not setting minimum sample size for data sets to assess attainment of WQS. Consequently, DEP will evaluate incomplete data sets (i.e., at least 3 samples in a 30-day period) as allowable in § 93.7 (i.e., no minimum number of samples established for *E. coli*). DEP will use its best professional judgment to evaluate the incomplete data and where samples document consistently low bacteria levels within mostly forested watersheds, DEP will consider attainment of the criterion if no likely sources of bacteria are present in the watershed. Conversely, for incomplete data sets that consistently document high bacteria counts (e.g., *E. coli* >410 CFU/100 ml), DEP will consider whether the waterbody is impaired for the WC RU.

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2.6 FISH TISSUE CONSUMPTION ASSESSMENT METHOD

Chapter 2 Biological Assessment Methods

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INTRODUCTION

This assessment method is designed to make Fishing (F) Use assessment determinations using fish tissue contaminant data from Pennsylvania's surface waters. Priority is given to surface waters that are targeted by anglers or subsistence populations. In surface waters that do not contain fishable populations of organisms, it may not be possible to assess fish consumption. To use this method for assessment determinations data collection must follow applicable protocols established in the Monitoring Book (Lookenbill and Whiteash 2021).

The importance of the fish tissue sampling and advisory issuance program was fully recognized in May 1986 with the signing of an interagency agreement between the Department of Environmental Resources (now DEP), Pennsylvania Department of Health, and Pennsylvania Fish Commission (now PFBC). This agreement was developed because "the agencies desire to pursue a systematic approach for the detection and evaluation of fish tissue contamination and to develop coordinated procedures for informing the public that may consume such fish of possible adverse health impacts." It listed the responsibilities of each agency and provided for the "timely joint issuance of a health advisory" when fish tissue contamination constituted a health risk. The first joint advisory was issued in June 1986 and included a number of waters throughout Pennsylvania. A new agreement, signed in 2002, added the Pennsylvania Department of Agriculture (PDA) to the fish consumption advisory program and established a two-tiered system for advisory decisions and issuance. A Fish Consumption Advisory Policy Workgroup was established to oversee the program and make management decisions. This workgroup includes deputy secretaries from the three cabinet agencies and the Executive Director of the PFBC. The existing staff-level workgroup was renamed the Fish Consumption Advisory Technical Workgroup (FCATW) and includes representatives of all four agencies. The technical workgroup coordinates routine program activities, such as sampling site identification and provides recommendations for advisory issuance or lifting to the policy workgroup.

DATA REVIEW

The annual data review process begins in late spring when the DEP Bureau of Labs (BOL) has finished analyzing the samples collected from the previous year. An initial review of the data is conducted to screen for anomalous results based on previous data and expected results for a species, sample size (average length and weight), lipid percentage or particular waterbody. If anomalous data are encountered, the BOL is requested to either verify the result or reanalyze the sample using a backup aliquot of the parent tissue. Once the results are final, the data is evaluated and compared to current advisory triggers. All recent tissue contaminant data is evaluated to determine

the possible need for an advisory for a particular waterbody and fish species. Sample results that exceed the 1 meal per week statewide advisory, but do not exceed the “Do Not Eat” threshold, are subject to a second verification sample before an advisory can be issued or lifted. A “Do Not Eat” advisory is issued if a single representative sample result exceeds the appropriate “Do Not Eat” trigger. The possibility of lifting or reducing a “Do Not Eat” consumption advisory also requires a verification sample. All issued advisories are considered impaired for Fishing Use.

ADVISORY TRIGGERS

PCBs and Chlordane

Currently, Pennsylvania’s program includes a mixture of risk assessment-based methods and United States Food and Drug Administration (USFDA) Action Levels that are used as the basis for issuing or lifting advisories. Risk assessment methods form the basis for meal-specific advisories due to PCBs, mercury, and chlordane. Advisories for other compounds use USFDA Action levels to issue “Do Not Eat” advice. Trigger levels for PCBs and chlordane are shown in Table 1.

PCB meal-specific advisories based on this method were issued for Lake Erie and Presque Isle Bay for 1997, and it was applied statewide in 1998. **Pennsylvania issued a general, statewide advisory recommending that anglers eat no more than one meal per week of recreationally caught sport fish in April 2001. As a result, only Groups 3-6 from Table 1 are now applicable.**

Mercury

Consumption advisories due to mercury in fish tissue are based on a health risk assessment developed by USEPA. The USEPA risk assessment was originally released in 1997. As a result of a request from Congress, USEPA contracted with the National Research Council (NRC) to review the risk assessment and prepare recommendations on the appropriate reference dose for mercury exposure. In July 2000, the NRC reported that the Reference Dose (RfD) for mercury, developed by USEPA, was a scientifically justifiable level for the protection of public health. As a result of this finding, USEPA recommended that sensitive individuals should eat no more than one meal per week of sport-caught fish. The USFDA and USEPA currently post these federal recommendations online. As noted above, Pennsylvania has issued a statewide one meal per week advisory that mirrors this federal advice. Pennsylvania also issues more protective mercury advisories on a site-specific basis, using the USEPA risk assessment and advisory triggers slightly modified from those in a September 1999 USEPA fact sheet. The trigger levels and meal recommendations are outlined in Table 1. Because a statewide one meal per week advisory has been issued, site-specific mercury advice begins at two meals per month. Meal-specific advisories for

mercury were first issued at the same time as the general, statewide advisory, April 11, 2001.

PFOS

Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent chemical substances that have been used extensively in the manufacturing of fire-fighting foams and non-stick materials based on resistance to heat, grease, oils, and water. PFAS chemical substances were originally developed for manufacturing during World War II, based on these unique properties. By the early 2000's technological advancements led to PFAS detection limits in the range of parts-per-billion (ppb). The ability to detect these substances led to a phase-out of PFAS chemicals based on human health concerns once they were detectable in some drinking water and fish tissue sources. Many of the PFAS compounds bio-accumulate, with long-chain substances – perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) – tending to bio-accumulate more than short-chain PFAS substances. Additionally, toxicity varies across PFAS substances with many being considered less toxic than PFOS (USEPA 2018).

Pennsylvania, through the FCATW, works collaboratively with the Great Lakes Consortium for Fish Consumption (Consortium). The Consortium was created in the 1980's to align protocols and communicate fish consumption advisories across shared waters of the United States and Canada. In 2019 the Consortium published "Best Practice for Perfluorooctane Sulfonate (PFOS) Guidelines" (PFOS-Best Practice, Consortium 2019) that summarizes the history, toxicity, and current advisories used by Consortium members for PFAS substances. The PFOS-Best Practice recommends meal frequencies for PFOS based on a drinking water reference dose (RfD) of $2x10^{-5}$ milligrams per kilogram per day (mg/kg/day, USEPA 2016). Recommended trigger values for meal frequencies were modified for Pennsylvania by converting ug/kg to ppm for standardized reporting and by removing the "two meal per week" frequency (10-20 ug/kg or 0.01-0.02 ppm). The decision to remove the original recommendation of "two meals per week" was based on the general statewide advisory being more restrictive at one meal per week. The FCATW voted to adopt the PFOS-Best Practice meal frequency advisory on March 23, 2021. Two meal frequencies, one meal per month and do not eat, were adopted for PFOS contamination (Table 1).

Table 1. Trigger levels for contaminant concentrations found in fish tissue and subsequent meal recommendations. Bold values represent meal frequencies that are more restrictive than general statewide advise. Concentration values are in ppm.

Group	Meal Advice	PCB	Chlordane	Mercury	PFOS
1	UNRESTRICTED	0-0.05	0-0.15	0-0.12	
2	1 MEAL/WEEK, (*52 MEALS/YEAR*)	0.06-0.2	0.16-0.65	0.13-0.25	0.02-0.05
3	**2 MEALS/MONTH, (24 *MEALS/YEAR*)**			**0.26-0.50**	
4	**1 MEAL/MONTH, (*12 MEALS/YEAR*)**	**0.21-1.0**	**0.66-2.82**	**0.51-1.0**	**0.05-0.2**
5	**6 MEALS/YEAR**	**1.1-1.9**	**2.83-5.62**	**1.1-1.9**	
6	**DO NOT EAT**	**>1.9**	**>5.62**	**>1.9**	**>0.2**

USFDA Action Levels

USFDA Action Levels are regulatory standards applicable to commercial fish and other foodstuffs. These Action Levels are developed based on general consumption patterns and may include consideration of economic issues such as potential loss of food supply. The USFDA has acknowledged that Action Levels may not adequately protect sensitive individuals or those individuals who may consume larger quantities of recreationally caught sport fish. The work group has been unable to completely evaluate risk assessment-based methods for these contaminants due to resource constraints. In addition, evaluation of risk assessment-based methods for most of these contaminants has not been a priority because they are normally found in very low concentrations in Pennsylvania fish. The compounds for which USFDA Action Levels constitute advisory triggers are listed in Table 2.

Table 2. USFDA Action Level triggers for a recommendation of Do Not Eat.

Contaminant	FDA Action Level
Aldrin and Dieldren (sum)	0.3 ppm
Chlordecone (Kepone)	0.3 ppm
DDT, DDE, and TDE (sum)	5.0 ppm
Heptachlor and Heptachlor Epoxide (sum)	0.3 ppm
Mirex	0.1 ppm

ADVISORY DECISIONS

For the evaluation of advisories that are more restrictive than the statewide advisories (i.e., one meal per week), DEP evaluates all readily available tissue contaminant data to prepare for a meeting of the FCATW where final advisory decisions will be made. This meeting is held annually in early summer. These data are compared to the applicable advisory triggers to determine the possible need for an advisory for a particular waterbody and a specific species. The possibility of lifting or modifying an advisory is also considered during this evaluation. Once the advisories are agreed upon at the workgroup level, the FCATW considers the most appropriate spatial delineation of the advisory. The method for determining the advisory delineation area is based on the movement potential of fishes throughout a waterbody. The point or small reach where fish collection took place is located on a map, and major upstream and downstream landmarks (i.e., dams, roads, tributaries, other barriers) are located and evaluated as segment boundaries. Barriers, such as dams, are preferred because they block fish movement. Other boundaries are selected to be relatively easy for fishermen to recognize. Once the spatial delineation is determined, the official advisories are sent to the PFBC by August 1 for inclusion in the fishing regulations booklet for the next calendar year, and the advisory delineation is included on the 303(d) list of impaired waters. Additionally, DEP and the PFBC publish the advisories on their websites. Finally, a joint press release is usually issued in November to remind the public of the advisories.

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2.7 STREAM FISH ASSEMBLAGE ASSESSMENT METHOD

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INTRODUCTION

This assessment method is designed to make categorical Aquatic Life Use (ALU) assessment determinations using fish assemblages in Pennsylvania's lotic surface waters. Use of this method requires fish assemblage data collection follow the standardized DEP fish collection protocol (Wertz 2021a). Implementation of this method supports reporting requirements of the federal Clean Water Act (CWA) sections 303(d) and 305(b). Using biological indicators to assess attainment of water quality standards (WQS) (more specifically, aquatic life-based WQS) is considered a "core indicator" of attainment (USEPA 2002). Furthermore, bioassessment methods are considered "translators" of narrative criteria (USEPA 2002). Pennsylvania's narrative water quality standards are found at 25 Pa. Code § 93.6:

> ***25 Pa. Code § 93.6****. General water quality criteria*
> *(a) Water may not contain substances attributable to point or nonpoint source discharges in concentration or amounts sufficient to be inimical or harmful to the water uses to be protected or to human, animal, plant or aquatic life.*
> *(b) In addition to other substances listed within or addressed by this chapter, specific substances to be controlled include, but are not limited to, floating materials, oil, grease, scum and substances that produce color, tastes, odors, turbidity or settle to form deposits.*

This assessment method is based on the development of a thermal fish index (TFI, Wertz 2021), which followed methods commonly used to develop traditional bioassessment tools (e.g., Barbour et al. 1999). The development of the TFI differed from the development of other bioassessment tools in that TFI development included classifications not only for stream types such as freestone (FS) and limestone (LS), but also longitudinal drainage area groups (DAGs). Drainage area is considered a longitudinal variable as catchment size increases from headwater to mouth. For the TFI, six final DAGs classifications were determined by stream type and upper range of catchment area (km^2) as: LS<1000, FS<40, FS<150, FS<550, FS<6000, FS>6000 (Wertz 2021b). The resulting classification schema is similar to recent classification studies of fish distribution conducted by Olivero et al. (2015) and Troia and McManamay (2019). The DAG serves as the final classification group, wherein TFI-based assessments can be made. Conceptual frameworks similar to DAGs are commonly utilized for lotic waterbodies, as longitudinal (e.g., cold water vs. warm water, or headwater stream vs. large river) bioassessments have been investigated and classified for separate assessments using multi-metric approaches for both fish and macroinvertebrates (Lyons et al. 1996, Langdon 2001, Lyons et al. 2001, Hughes et al. 2004, Shull and Lookenbill 2017). Additional techniques to address longitudinal effects have been employed with traditional multi-metric indices (MMI) that scale metrics based on stream size. An example of this scaling would be maximum species richness (MSR) levels that can be used to standardize expected richness depending on stream size or

zoogeography (Fausch et al. 1984). The technique of standardizing MMI scores standardizes the measure of condition (good vs. poor) along a longitudinal gradient but in doing so, simultaneously reduces meaningful interpretation of the longitudinal effect, unless deconstructed to individual metrics. The intent here is not to discredit traditional MMIs but to provide insight into differences in strengths and weakness associated with each. As a standalone metric, the TFI: 1) provides a reliable, precise indicator of anthropogenic stress along a meaningful gradient of stream type and stream size and 2) is able to numerically characterize assemblages parallel to ALU definitions. However, the TFI intentionally does not discriminate or communicate other ecological factors that are typically conveyed in traditional MMIs. These factors include, but are not limited to: species richness, native status, reproductive strategies or feeding guilds. From a comparative perspective, a TFI-based assessment (TFI-BASS) may appear to be quite simple in design. In reality, a TFI-BASS should be viewed as a comprehensive assessment, in that: 1) all species and individuals within the assemblage are provided equal consideration based on relative abundance; 2) the TFI can be applied uniformly across the State, basins, or ecoregions; 3) the TFI has an ecologically meaningful output of assemblage thermal class (cold vs. warm) as opposed to a purely statistically-derived construct; and 4) the TFI exhibits fairly strong correlation with some other common bioassessment metrics and with indicators of water quality and habitat quality. For example, during preliminary data exploration the TFI exhibited fairly strong correlation with traditional metrics of biological condition gradient (BCG, Davies and Jackson 2006) and pollution tolerance; while responding as well as or better than traditional metrics to abiotic stress (Figure 1) based on Spearman’s correlation coefficients.

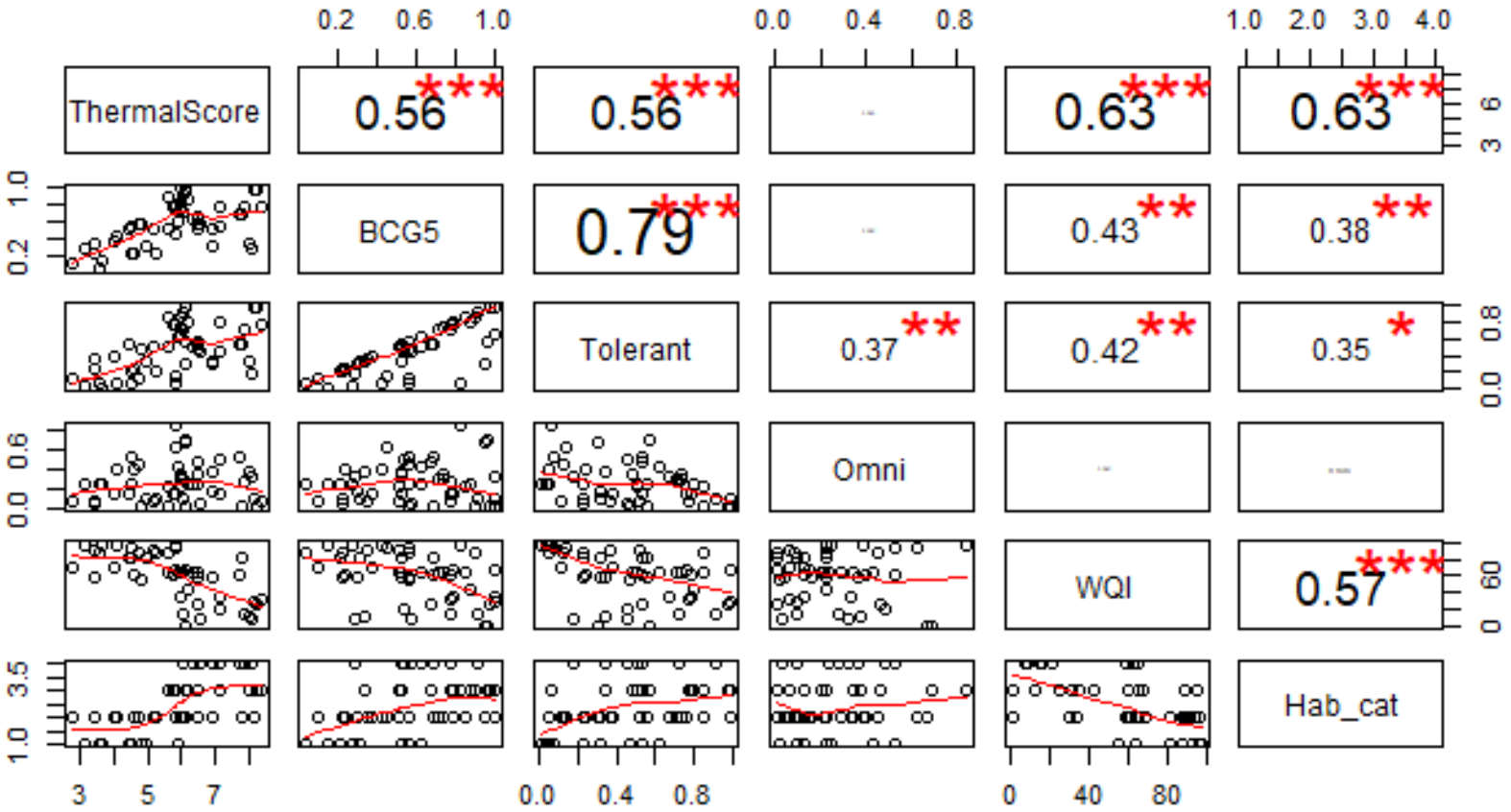

Figure 1. Pairwise comparison, using Spearman's correlation coefficient, of the thermal fish index score (ThermalScore) to traditional metrics; Biological Condition Gradient category 5 (BCG5), percent tolerant (Tolerant), percent omnivorous (Omni), water quality index (WQI) and habitat (Hab_cat) in FS<40 streams. Fitted red lines are LOESS smoothed. *** (P<0.001), ** (P<0.01), * (P<0.05)

The underlying concepts of the TFI provide valuable insight into the use of traditional metrics, notably, species richness. The TFI provides an ecologically relevant, numeric indicator of the shift from cold water assemblage (CWA) to transitional assemblage (TSA) to warm water assemblage (WWA), a transition which generally includes the initial displacement and eventual replacement of cold water species (see Appendix C for a taxonomic comparison). Displacement and replacement concepts are mechanisms that influence fish assemblage response to various stressors, including temperature (Dunham et al. 2002, Troia et al. 2015). The changes in relative abundance of species (or “turnover” based on displacement replacement), a key component of the TFI, has been recommended as an alternative to traditional biodiversity or species richness metrics for measuring environmental changes (Hillebrand et al. 2018). The natural transition from CWA (low species richness) to TSA (increasing species richness) is considered ecologically important. Conversely, the stress-induced transition from CWA to TSA may exhibit similar species richness to a natural TSA, however, in doing so spatially condenses the CWA (Figure 2, Wertz 2021). Lower species richness in a natural CWA represents a less disturbed system compared with increased species richness of a stress-induced TSA, challenging theories that imply increased species richness is always better. For example, aforementioned MSR levels incorporated into traditional MMIs score all increases in species richness as a positive response. While increased species richness in large warm water streams may be appropriately viewed this way, caution should be applied in naturally depauperate cold and cool water streams. This concept effectively requires that observed assemblages be compared to assemblages in least disturbed stream conditions with considerations for stream size to serve as a baseline comparison for ecological relevance. This baseline comparison, hereafter referred to as the relevant reference condition (RRC), requires an observed assemblage be compared to assemblages in least disturbed sites by DAG for TFI-BASS purposes. For some metrics or indices, additional filters (e.g., basin or ecoregion) may provide additional ecological relevance based on the pool of species regionally available, especially in the case of species richness metrics. Therefore, assessments of biological or ecological condition based on species richness or other important metrics without calibration of those metrics to a RRC should be avoided (Wertz 2021b).

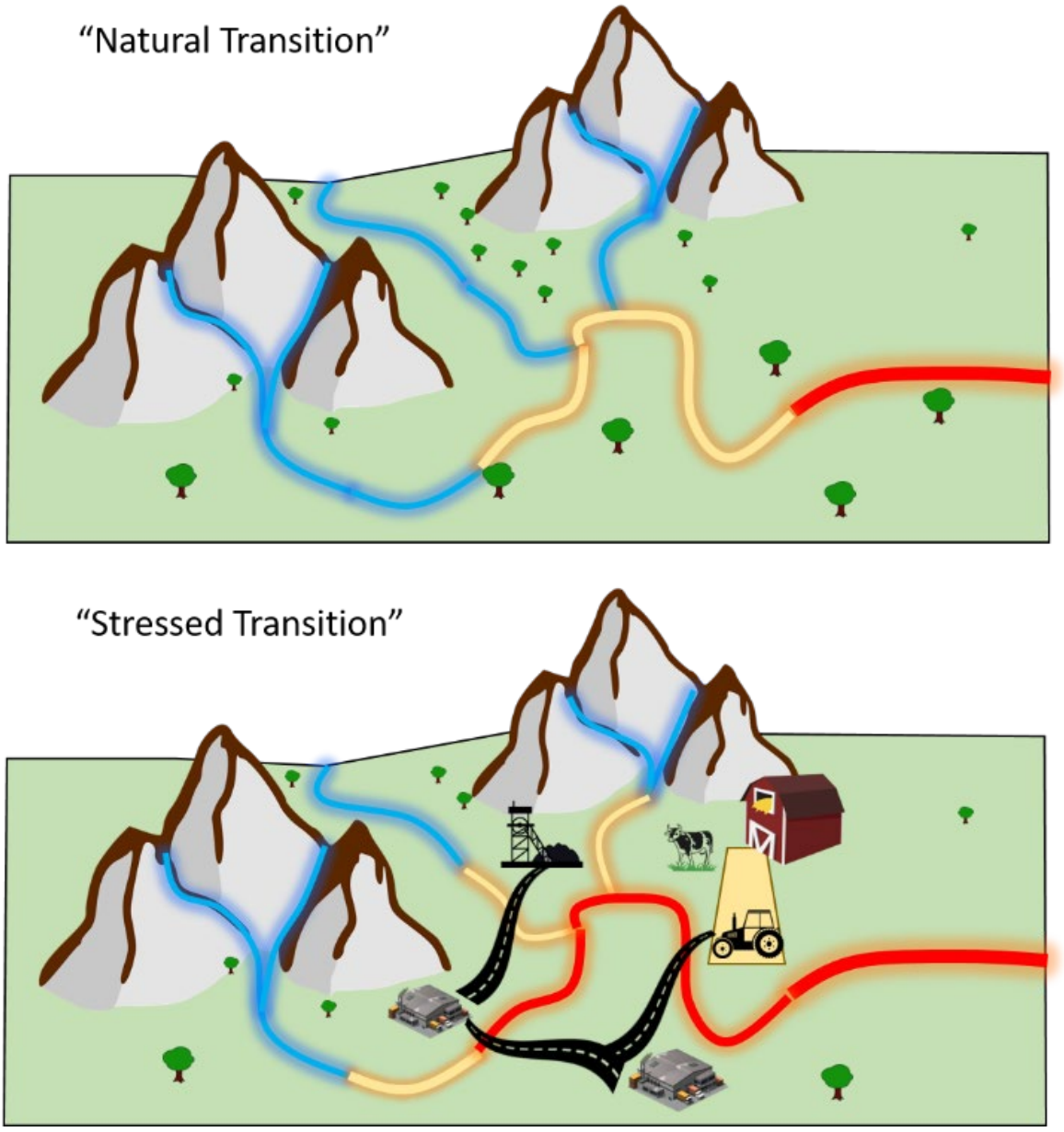

Figure 2. Theoretical example of natural longitudinal transition areas versus stress induced fish assemblage transitions. With applied stress to a cold water assemblage (CWA, blue), the CWA reduces, the transitional assemblage (TSA, yellow) is shifted upstream and the warm water assemblage (WWA, red) is expanded.

CALCULATION METHODS

After a sample has been processed in accordance with data collection protocol for fishes (Wertz 2021a), TFI calculation can be pursued. To calculate the TFI, the number of individuals within each thermal class, as a proportion (e.g., 0.20 cold water

individuals), is calculated. A weighted average is obtained by multiplying the numeric value for the thermal class by the proportion of individuals, summed across classes. The final value is then multiplied by two to expand and standardize the range from two to ten, coldest to warmest respectively. Calculation of the TFI follows:

$$TFI = \left(\sum_{1}^{5} NP_i\right) 2$$

where, *N* is the numeric value for the thermal class and *P* is the proportion of individuals at the i^{th} thermal class.

Once the TFI score has been calculated, the assemblage can be placed into one of the three aforementioned thermal assemblage classes: CWA (TFI scores 2.0 to 5.0), TSA (TFI scores 5.1 to 7.0) or WWA (TFI scores 7.1 to 10.0). The thermal assemblage classes are useful for describing and reporting ecologically meaningful assemblage groups based on a numerically-derived schema.

COLLECTION METHODS

When using the TFI for ALU assessments, the strict use of DEP collection protocols is necessary to making assessment determinations. Fish assemblage data not collected using DEP collection protocols are readily accepted by DEP but may be qualified based on the level of quality assurance and representativeness following methods for Outside Data outlined in the Assessment Book, Chapter 5 (see Section *Outside Data in Assessment Determinations*, Shull and Pulket 2021). Herein, the site selection process relating to representativeness, directly addressed in the data collection protocol for fishes (Wertz 2021a), is considered critical as site selection will influence the TFI score. Additionally, knowledge of the assessment method, stream types and appropriate DAGs should be considered in the site selection process; a desktop and field reconnaissance should be conducted prior to sampling to determine the appropriate DAG. To determine the appropriate DAG, two pieces of information are needed: stream type (LS or FS) and drainage area. Stream type is determined by the density of sinkholes in the upstream catchment area, where ≥ 0.03 sinkholes/km^2 is used as the inclusive criterion for limestone (karst) streams. This density is determined by first creating a polygon of the upstream catchment area of the sample site. The second piece of data needed is the sinkhole locations, specifically the “Digital data set of mapped karst features in south-central and southeastern Pennsylvania” (DCNR 2007). Finally, the number of sinkholes within the catchment (km^2) polygon can be summarized as a density, following:

$$Sinkhole\ Density = \frac{\#\ Sinkholes}{Catchment\ area}$$

Desktop reconnaissance should be followed by a field reconnaissance to confirm DAG. The field reconnaissance may reveal additional information that may be necessary to correctly classify DAG. For example, it is likely that small catchments within a larger karst system may not have any sinkholes measurable in the upstream catchment. This occurs by having an unmeasurable (without undertaking complex tracer studies) underground springshed that is larger than the measurable surface watershed. In this situation it would be most appropriate to classify the stream as LS. Additionally, proximal tributaries near the sample site should be evaluated for their influence on the representativeness of the site but also for their influence on the DAG. For example, DEP's fish collection method (Wertz 2021a) describes the potential for unrepresentative samples as a result of being too close to a proximal tributary or mouth. This effect becomes even more important when making TFI-BASS determinations as the DAG may change drastically just downstream of a nearby tributary by increasing the upstream catchment area. Simultaneously, the increase in catchment area may be aggravated if there are distinct temperature influences from the tributary, lowering the TFI score while increasing the DAG (and associated TFI impairment threshold). Similar situations may occur in close proximity to tailwaters (i.e., cold water or bottom-releases from impoundments) areas that may affect the TFI score. These examples reinforce the importance of proper site selection and representativeness for not only conducting fish assemblage surveys but also for making assessment determinations.

Prior knowledge of stream type and drainage size should also reduce complexities that may arise from a continuous metric (TFI) being nested within a hierarchical construct (DAG). For example, a freestone stream with a 39-km^2 drainage area (DAG=FS<40) has an impairment threshold of 4.8. However, a freestone stream with a drainage area only 2 km^2 larger (41km^2; DAG=FS<150) has an impairment threshold of 6.0 (Figure 3). It would be unrealistic to expect the fish assemblage to make such a radical transition in such a short distance. Therefore, a 10% buffer is placed on each DAG transition to minimize the effect of drastic transitions, hereafter referred to as the "grey zone" (Figure 4). The 10% buffer was determined based on best professional judgment as an initial starting point based on an evaluation of case studies in the TFI development dataset. DEP will reevaluate the 10% buffer during future recalibrations of the TFI.

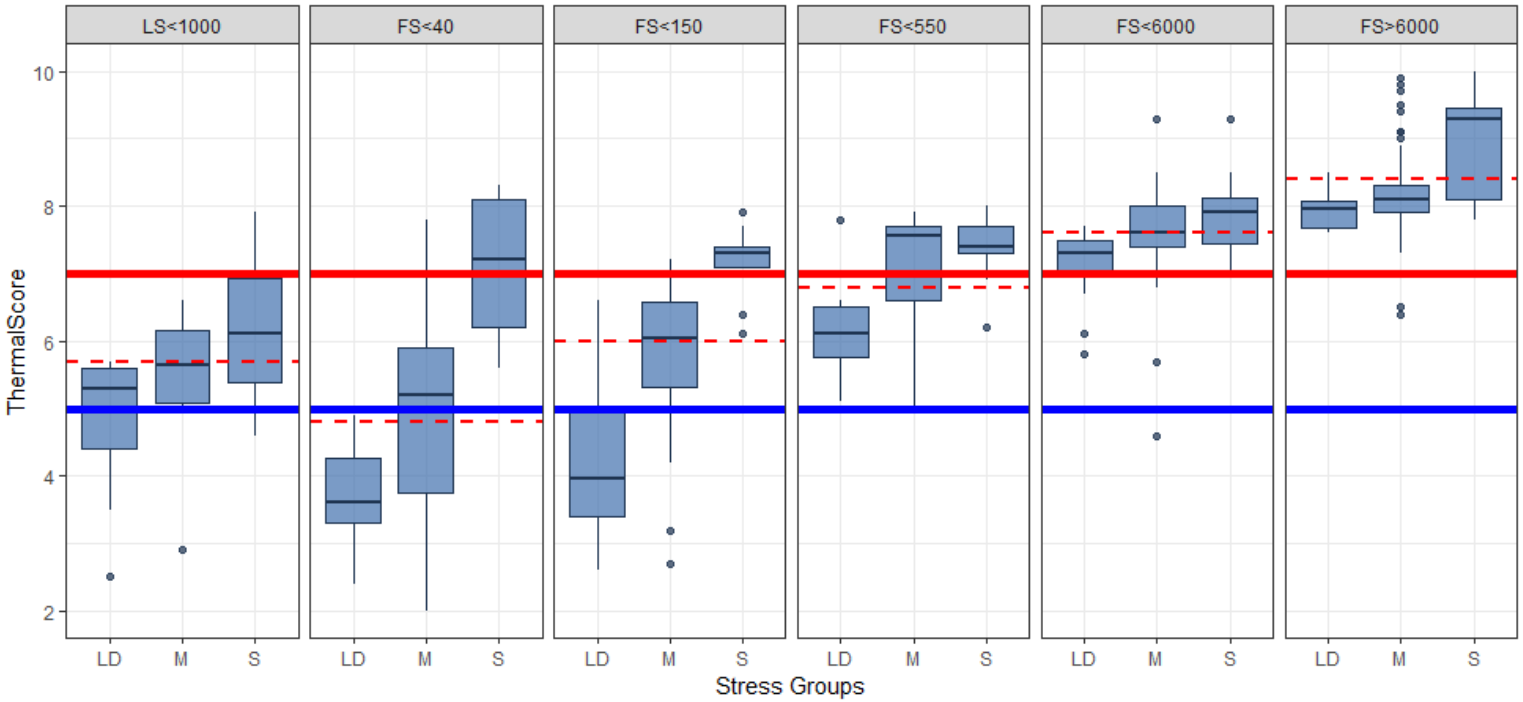

Figure 3. Boxplot of thermal fish index (TFI) scores (ThermalScore) for the final limestone (LS) and freestone (FS) drainage area groups (DAGs) (upper km^2 range). Stress groups are denoted as: Least Disturbed (LD), Moderate (M) and Stressed (S). Dotted red lines represents the 95th percentile of LD sites signifying the impairment threshold for each DAG. The solid blue line (TFI = 7.0) represents the upper limit for cold water assemblage and the solid red line (TFI = 5.0) represents the lower limit for warm water assemblage with the transitional assemblage range in between.

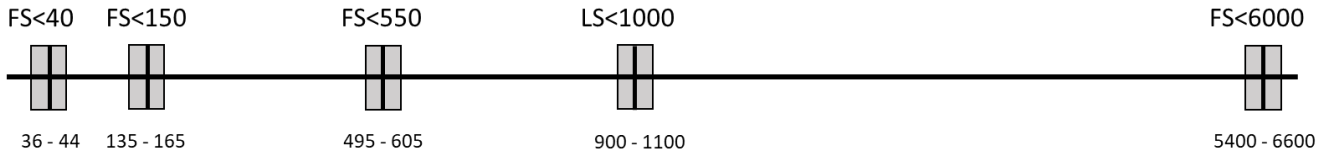

Figure 4. Drainage area groups and corresponding grey zone drainage area range (km^2); illustration not to scale.

If a site is in a grey zone, two options are available: 1) move the site either upstream or downstream (outside of grey zone) or 2) add a second site in either direction (outside of grey zone) to serve as supplementary evidence. Of the two choices, the latter is preferred if feasible. For example, Loyalhanna Creek (a FS stream) had two fish surveys: one upstream and one downstream of Latrobe. The upstream site had a drainage area of 503 km^2 (FS<550) and a TFI score of 6.7, which is considered attaining. The downstream site had a drainage area of 572 km^2 (FS<6000) and a TFI score of 7.2 (a TFI increase of 0.5 compared to the upstream site), which is also considered attaining. In this example when the 10% buffer rule is applied, both sites should be either moved or supplemented. In certain cases, there may be minimal flexibility on moving a site based on access or representativeness, and a supplemental site may be desired. In this specific example, the upstream site could be moved or supplemented to a site upstream with a drainage area ≤ 495 km^2. The downstream site could be moved or supplemented to a site further downstream with a drainage area between 605 km^2 and 900 km^2. To reduce bias and longitudinal data gaps, supplemental sites are preferred over moving sites in all grey zone cases. No assessments will be made on sites that are within a grey zone until future evaluations are conducted. It should be noted that while ALU assessments may be conducted on streams that inherently bracket influences (e.g., changes in land use or land cover), these assessments should not be confused with cause and effect surveys. Cause and effect surveys are used to evaluate local-scale impacts and are generally not considered representative of overall waterbody condition, whereas ALU assessments intend to represent overall waterbody conditions. Any TFI-BASS should follow DEP's typical minimum assessment lengths of ½ mile (Shull and Pulket 2021).

HABITAT CONSIDERATIONS

In the development of the TFI, habitat stress was an integral part of the stressor gradient and the TFI responded significantly to habitat alterations (Wertz 2021b, Figure 5). This response to habitat quality needs to be further discussed, as assessment determinations may also be affected. The TFI is likely not capable of discriminating between anthropogenically modified habitat and naturally unsuitable habitat conditions. For example, it is to be expected that samples collected in very low-gradient areas, or in and around natural marshes and wetlands, may have higher TFI scores than similarly sized high-gradient, cobble-dominated streams. This concept was evident in the results of slope being an important secondary factor identified in regression tree outputs during TFI development (Wertz 2021b). To this end, if a sample exceeds the appropriate TFI impairment threshold and unique habitat conditions are suspected, further investigations should be conducted before assessments are made. Furthermore, if habitat conditions are thought to be preventing or precluding attainment, an evaluation of these conditions should be made. As site-specific or general habitat conditions are identified, their inclusion into future calibration events will likely add precision and accuracy. For example, some streams will naturally flow subsurface for some distance during some or

all times of the year. By reducing fish migration, these general conditions may prevent ALU attainment upstream. Evaluations of habitat conditions with an emphasis on connectivity and influence (natural vs. anthropogenic) should be thoroughly documented in TFI-BASSs.

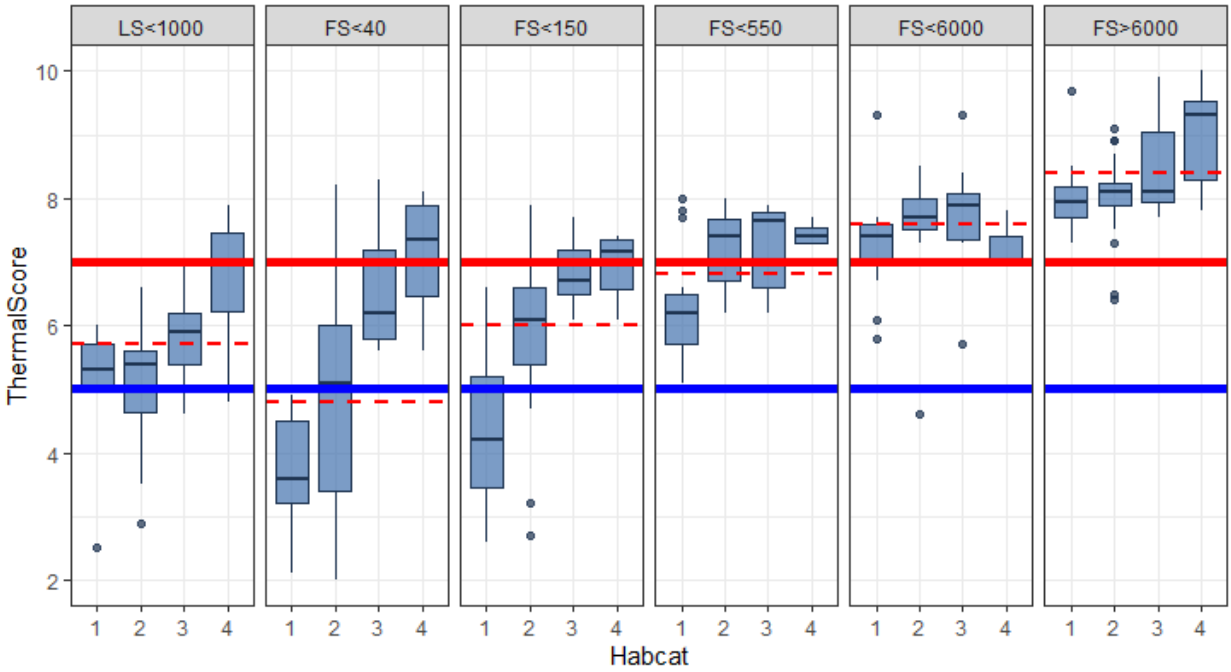

Figure 5. Boxplot of thermal fish index (TFI) scores (ThermalScore) for the final limestone (LS) and freestone (FS) drainage area groups (DAGs) (upper km^2 range). Habitat category (Habcat) groups 1-4 are on a gradient of good to poor, respectively. Dotted red lines represents the 95th percentile of least disturbed sites signifying the impairment threshold for each DAG. The solid blue line (TFI = 7.0) represents the upper limit for cold water assemblage and the solid red line (TFI = 5.0) represents the lower limit for warm water assemblage with the transitional assemblage range in between.

SAMPLE CONSIDERATIONS

Once the sample has been processed in accordance with collection methods (Wertz 2021a), the final assemblage data needs to be reviewed for representativeness before making assessment determinations. The sample is first evaluated for the number of individuals and species present. If fewer than 50 individuals are in the sample, the site should be evaluated for: 1) representative sampling as outlined in the collection methods (Wertz 2021a), 2) toxic conditions and 3) near-sterile conditions. If collection methods are suspected to be the cause of the low numbers, the site should be resampled as confirmation. If toxic conditions are suspected and supporting water quality evidence is present, categorical ALU impairment is justified. If near-sterile conditions are expected, the TFI should generally be considered representative of site conditions and ALU assessments can be pursued. The sample should then be

evaluated for the number of species present. A sample represented by only one species (or family for salmonids) is generally considered abnormal even in naturally depauperate headwater streams. In many headwater streams, trout will be the only species (or family) present in a sample, and this is often an indicator of acidified conditions because many of the species found in cold water environments have a lower tolerance to acidity (and associated effects) than trout (Johnson et al. 1987, Baker et al. 1996). This salmonid-dominated CWA has been an indicator of acidified conditions not only in the Northeastern U.S. (Baker et al. 1996) but across multiple continents (Schofield 1976). Consequently, when salmonids (one or more species) represent the entire assemblage, DEP's acid precipitation source and cause determination method (Shank 2021) along with habitat conditions (focusing on barriers to migration) should be investigated, and the sample could be considered to represent an ALU impairment. If the sample is represented by only one species (non-salmonid) and the number of individuals is ≥ 50, the TFI should generally be considered representative of site conditions and categorical ALU assessments can be pursued.

The salmonid-dominated assemblage indicator presented above uses species richness metrics along with TFI scores. Reporting traditional metrics (e.g., species richness, % omnivore species, % tolerant individuals) along with TFI scores may be helpful or even necessary in certain cases. For example, when assessing a stream with high specific conductance, it may be helpful to report TFI-BASS results along with the metric, % euryhaline individuals. In this example, the TFI-BASS will likely indicate a categorical ALU impairment and the metric % euryhaline individuals may help describe causal pathways. Reporting traditional metrics is encouraged but must be done using the RRC concept. Continuing the above example, if a sample has 40% euryhaline individuals, this metric provides little meaning without comparison to a RRC. If there is a TFI-BASS attaining stream is in the same basin and DAG as the one being investigated with 3% euryhaline individuals, meaningful inferences can be made.

When reporting the TFI scores, if identification of individuals is not at the species-level (e.g., hybrids, family or genus level), or the sample contains a species without an associated thermal preference score, the percentage of individuals not used in the TFI should be noted. Furthermore, when making an assessment with such data, this percentage should be taken into consideration. In the developmental analysis, 10% was used as a starting point based on best professional judgement and can be reevaluated during future recalibration events (Wertz 2021b) which can be applied as a general rule-of-thumb hereafter. For example, if 11% of the sample is not accounted for in the TFI, the decision to make an assessment may still be applicable but should be thoroughly evaluated and reported. An example of a thorough evaluation could include but is not limited to: 1) hybrid individuals represented by suspected parental species, 2) unidentified juveniles represented by suspected adults and 3) TFI scores similar across a higher taxonomic level. For example, some species of sculpins may be difficult to identify to species-level, increasing the percentage of individuals without a TFI score.

However, since all sculpins (family/genus level) found in Pennsylvania prefer cold (or cold-cool for Potomac Sculpin, *Cottus girardi*) habitats, the TFI score with > 10% of the individual fish excluded from the TFI score calculation could still be considered representative. Alternatively, minnows range from cold-cool to warm habitat preference, so the TFI would likely not be representative for a sample with > 10% of the individual fish identified as “unidentified minnows”.

Once the sample and corresponding TFI score are evaluated and considered representative of the waterbody (number of individuals/species in the sample, habitat representative of general stream conditions, TFI calculated from ≥ 90% of assemblage), the appropriate DAG determinations can be pursued. As noted previously, two pieces of information are necessary to determine the appropriate DAG: stream type and drainage area. If not directly obvious, the stream type is identified by having evaluated, through a desktop analysis, sinkhole densities in the upstream catchment. Once stream type is established, the appropriate DAGs and corresponding TFI impairment threshold is used to measure categorical ALU attainment. Impairment thresholds for each TFI DAG are considered numerical interpretations of the narrative criteria at 25 Pa. Code § 93.6(a) for making categorical ALU assessment determinations (USEPA 1990). The TFI impairment thresholds should not be confused with the TFI thermal assemblage classification thresholds (i.e., CWA at TFI scores ≤ 5.0, TSA at TFI scores ≤ 7.0 and WWA at TFI scores > 7.0). TFI-BASS assessment determinations can be aided using a flow chart (Figure 6). The TFI-BASS provides the first fish-based assessment tool for assessing categorical ALU across lotic waterbodies in Pennsylvania.

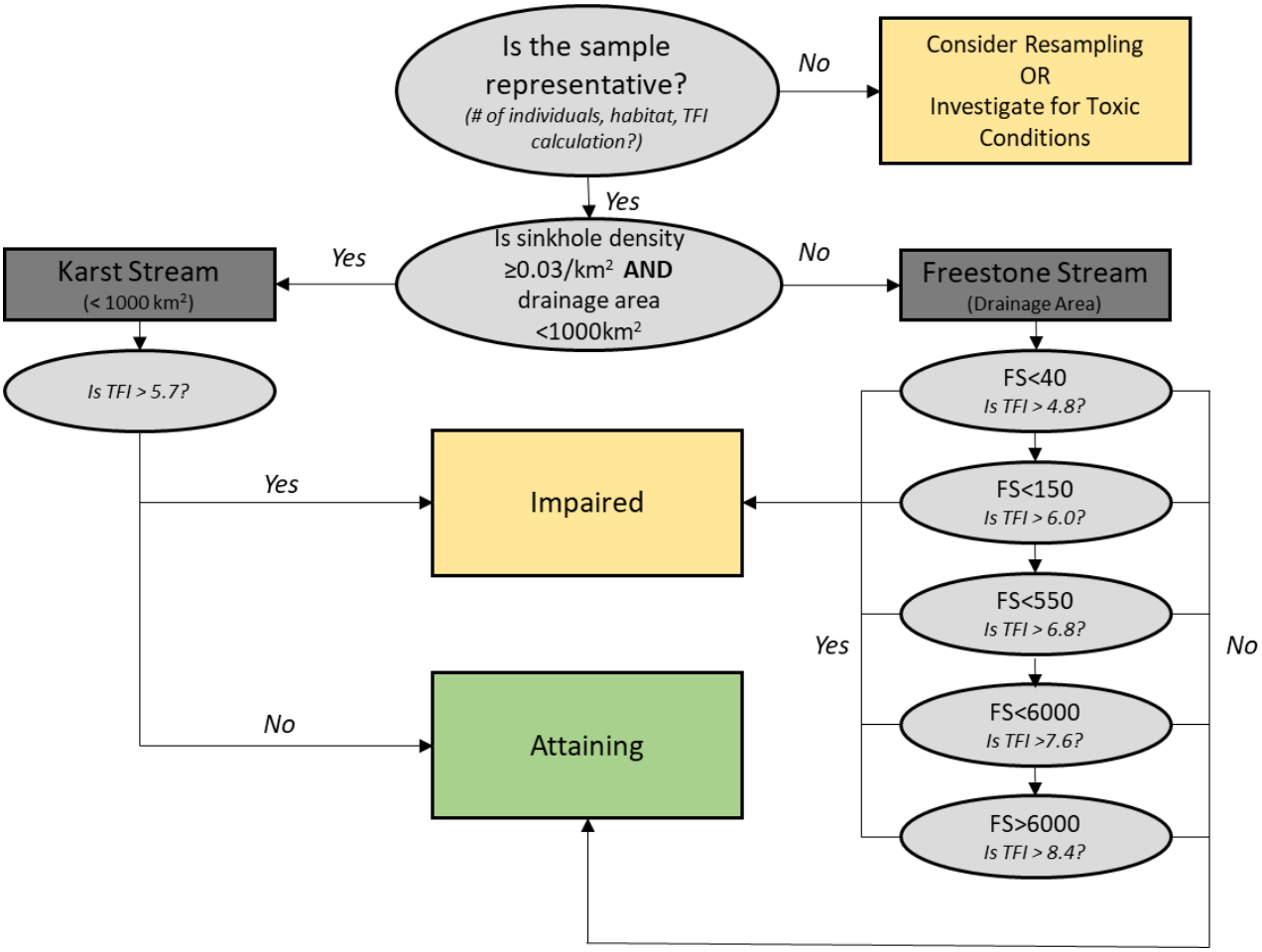

Figure 6. Flowchart to aid in Aquatic Life Use (ALU) assessments based on Thermal Fish Index (TFI) scores.

SOURCE/CAUSE DISCUSSION

The TFI-BASS is a biological assessment based on fish assemblages that was developed from, and considered calibrated to, water quality, habitat quality and temperature stress. Therefore, it is important to note that source/cause investigations resulting from TFI-BASS impairments should not be limited to temperature but should also extend to water quality and habitat quality. By identifying stressors that elicit significant metric (TFI) responses, causal inferences can be better focused. Herein, a general weight-of-evidence approach following Walters (2017a) should be conducted while focusing specifically on the three key variables (Figure 7). As described in Wertz (2021):

> *"…the effects of multiple stressors will be synergistic, antagonistic or additive to the TFI scores. For example, as water quality is reduced by agricultural activities and loss of riparian areas, changes to instream habitat and temperature will likely*

parallel, having a dramatic effect on the TFI. Alternatively, a stream with mining influences may have reduced water quality, without drastic changes in habitat and temperatures, which may have a smaller effect on the TFI. In other words, as the number of stressors and/or intensity of stressors increases, increases in the TFI are expected. This is a desired outcome from a management perspective, as measured improvements in individual stressors may result in measurable recovery. For example, best management practices applied to small reaches of a larger watershed may have localized, measurable biological effects."

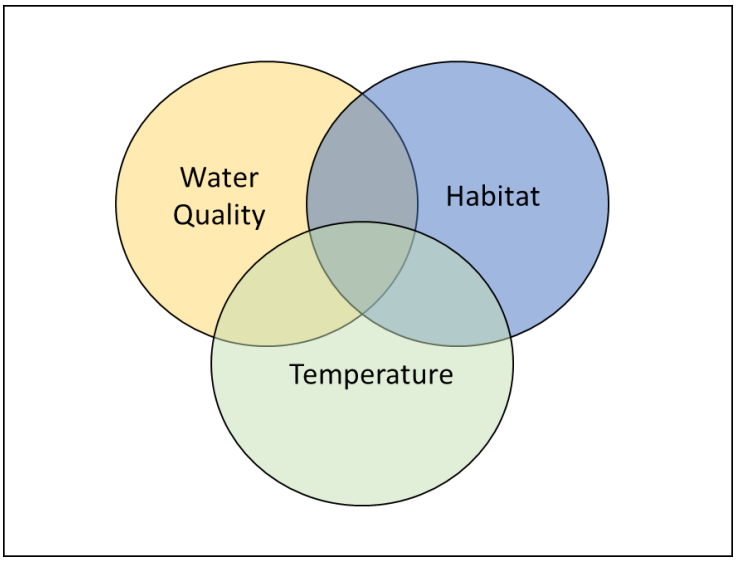

Figure 7. Venn diagram illustrating the three key variables used in developing and calibrating thermal fish index-based assessments.

The source/cause investigation should be completed according to DEP's general source and cause determination methods (Walters 2017a) and begin with the most obvious stressor(s) group of the three based on prior knowledge gained during site reconnaissance. Habitat stress will generally be the most obvious from a field investigation and subsequent stream habitat data collection (Lookenbill 2017) and physical habitat assessments (Walters 2017b) that may characterize a habitat modification cause of impairment. Generally, increases in sedimentation, siltation, embeddedness or impounding should be investigated and conveyed, as these were identified as important stress variables during TFI development (Wertz 2021b). Water quality and temperature data may not be obvious from a field investigation and may require additional data collection and evaluation.

To appropriately investigate and document a thermal modification cause, two major components are needed: a biological response and temperature data. The biological response is measured with a TFI-BASS impairment. The temperature data needed to support a thermal modification cause should be collected as a comparative study that includes both impacted and unimpacted areas. By demonstrating a significant difference

in temperature between ambient conditions and impacted areas, the thermal modification cause is supported. Ambient temperature conditions should be measured in areas that spatially and temporally represent the overall waterbody, meaning they should not be measured downstream of nearby tributaries that may influence the "reference condition" and should not be measured at times when differences are likely to occur. A thorough investigation of ambient conditions includes an average temperature of multiple samples that spatially represent the unimpacted area. Fish data should also be collected in the unaffected areas to bracket temperature stressors, but do not necessarily need to demonstrate attainment to apply a thermal modification cause to the impacted reach. For example, if a thermal discharge exists in a stream already stressed by siltation the "temperature reference" site may be impaired by siltation and the temperature-impacted site may be impaired by siltation and thermal modification. Due to the highly mobile nature of fishes, fish communities in close proximity to thermally modified areas will likely be affected. For example, thermal plumes exclude "cooler" fishes and provide areas suitable for reproduction of "warmer" fishes that will inevitably radiate to areas outside of the direct plume. This situation is dynamic and becomes challenging to directly quantify. To address this situation, if the "temperature reference" sites are impaired by the TFI-BASS with no evidence of cause, aside from proximity to temperature impacts, a "Thermal Modifications" cause (Appendix A) is justified.

To appropriately investigate and document a water quality or pollutant cause, two major components are needed: a biological response and pollutant data. The biological response is measured with a TFI-BASS impairment. The pollutant data needed to support a specific pollutant cause must be evaluated from a spatiotemporal perspective. In other words, the causal pollutant may not be directly obvious from a review of current data alone and should be augmented by historical data. Multiple spatial and temporal environmental variables must be considered, as recovery of fish assemblages is dependent upon multiscalar recolonization potential (Poff 1997). For example, where small-scale disturbance occurs and localized refugia (from a pollutant) are present, recovery may occur rapidly. Where large-scale disturbance occurs and no refugia are present, recovery may occur over years or decades (Detenbeck et al.1992). Where spatiotemporal pollutant data is available, and values are elevated, causal determinations will be fairly obvious. Where spatiotemporal pollutant data is not readily available or no links between pollutant and impairment are made, a cause of "Cause Unknown" (Appendix A) is justified.

NATURAL VARIATION

As sites are resampled through time, TFI scores will likely change. It is important to understand whether these changes in TFI scores are actually measuring degradation (increasing TFI scores), improvement (decreasing TFI scores) or are just natural variations. To provide insight into changes not associated with natural variation, TFI

precision estimates from repeated sites were evaluated. Precision measurements using coefficient of variation (CV) of the TFI score across replicate sites indicated that natural variation is low. The average TFI score CV across all DAGs was 4.3%, which translates to a TFI score ± 0.3 (Wertz 2021b), well under recommended threshold ranges of 10-15% (Stribling et al. 2008). The highest TFI CV (8.8%) was noted in the FS<150 DAG, likely caused by longitudinal shifts of assemblages that may be seasonally affected (i.e., cooler assemblages may retreat upstream as summer temperature increases). The TFI can be used as a tool to measure trends of improving/degrading conditions through time. As follow-up investigations are completed, TFI values greater than the average precision estimates for each DAG (Table 1) are considered outside of the range of natural variation and are likely caused by changing conditions.

Table 1. Precision estimates using coefficient of variation (CV) and corresponding thermal fish index (TFI) scores for repeated sites within each drainage area group (DAG).

DAG	CV %	TFI ±	*n*
LS<1000	4.0	0.3	16
FS<40	1.8	0.1	11
FS<150	8.8	0.7	59
FS<550	3.2	0.3	61
FS<6000	4.5	0.4	39
FS>6000	3.3	0.3	178

With appropriate implementation of this TFI-BASS method, DEP can use fish to identify and list impaired waters, to direct management strategies through source/cause evaluations, to document a change in conditions through the implementation of cause and effect survey design and to measure incremental progress.

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CHAPTER 3 CHEMICAL ASSESSESMENT METHODS

3.1 DISCRETE PHYSICOCHEMICAL ASSESSMENT METHOD

Chapter 3 Chemical Assessment Methods

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INTRODUCTION

This assessment method is designed to make assessment determinations based on discrete physical and chemical (physicochemical) data related to water quality criteria located in 25 Pa. Code Chapter 93. This document relies on contextual and conceptual discussions found in *Water Use Assessment Decision-making Based on Physicochemical and Bacteriological Sampling* (Whiteash 2021). It contains relatively little discussion of the planning and execution phases of physicochemical water quality sampling, such as outlining study objectives, choosing sampling plan designs, and setting data quality objectives, which are described in more detail within the Monitoring Book (Lookenbill and Whiteash 2021). This document aims to describe how the inherent variation and sampling error of physicochemical data are addressed by DEP in the use assessment process for discrete physicochemical water quality sampling data and to expand upon the general use assessment determination methods in Chapter 5 of this book (Shull and Pulket 2021), where appropriate.

Sampling Error

The inferential process of using discrete, spatiotemporally-limited observations (i.e., samples) to estimate a larger set of unobserved, continuously dynamic conditions can introduce uncertainty – called sampling error – into the use assessment determination process. Uncertainty attributable to analytical measurement techniques, known as measurement error, is discussed in the *Quality Assurance Manual for the Pennsylvania Department of Environmental Protection Bureau of Laboratories* (DEP 2016).

Ideally, physicochemical data will inform use assessment determinations by sampling frequently enough to minimize sampling error and to accurately characterize the conditions for each parameter of concern. These conditions should be characterized over a long enough time frame to account for variations in concentrations attributable to changes in all relevant factors. For many water quality parameters, including toxic substances listed in § 93.8a, this can only be achieved through very intense discrete sampling efforts, as they require physical site visits to collect data with handheld field meters and chemistry samples for laboratory analysis. When intense discrete sampling efforts are not possible, the resulting physicochemical data provides limited windows into the dynamic continuum of water quality conditions. However, more frequent sampling is possible for specific water quality criteria listed in § 93.7, through the deployment of automated, continuous instream monitoring (CIM) devices (see Chapter 3.2, *Continuous Physicochemical Assessment Method*, Hoger 2018).

Continuous instream monitoring devices can measure conditions frequently and can be deployed in remote locations. Monitoring water quality conditions at frequencies as high

as every 15 minutes minimizes the amount of time sample results must be extrapolated into unobserved time, thereby minimizing the potential for sampling error (Hoger 2018). Continuous instream monitoring devices can be set up to report observations via telemetry or through occasional retrievals and downloads. While CIM devices can provide extremely detailed, temporally-dense observational records, many such devices can only measure a few water quality parameters (e.g., dissolved oxygen, temperature, conductivity, pH) for which WQS exist.

Another approach to reduce sampling error in the absence of temporally-dense observations, is to collect enough information on other relevant variables (e.g., stream flow, precipitation, water temperature) to allow for confident extrapolation from observed conditions to unobserved conditions based on an empirical understanding of variability. A wide variety of interrelated factors can contribute to spatial and temporal variation in the concentrations of water quality parameters, such as precipitation, stream flow, geology, watershed drainage patterns, and anthropogenic influences. Different water quality parameters often vary in unique ways relating to these and other factors. For example, dissolved oxygen concentrations in streams often exhibit strong annual and diel patterns attributable to interrelated patterns of solar flux, stream temperature, and photorespiratory activity. Meanwhile, concentrations of total dissolved solids often vary much less with diel or annual patterns of solar flux, but instead vary primarily with stream flow and related patterns of surface runoff, geology, and groundwater flow patterns. Knowledge and understanding of such patterns can strengthen inferences about unobserved conditions (USEPA 2005).

Sampling and Criteria Frequency

Within the regulatory framework outlined above, DEP must determine if waterbodies meet WQS. In 25 Pa. Code Chapter 16 (relating to water quality toxics management strategy – statement of policy), § 16.21 states that aquatic life criteria for toxic substances are developed such that the frequency of occurrence is accounted for through the specification of factors appropriate to the criteria in Chapter 96 (relating to water quality standards implementation), but also, that the basis for the magnitude, duration, and frequency is described in criteria development rationale or other appropriate supporting documentation. Section 16.22 states that DEP looks to National guidelines (USEPA 1985) in establishing aquatic life criteria for toxic substances. In 25 Pa. Code Chapter 96 (relating to water quality standards implementation), § 96.3 (relating to water quality protection requirements) provides that – to protect existing and designated surface water uses – the water quality criteria described in Chapter 93 shall be achieved in all surface waters at least 99% of the time. For fish and aquatic life criteria for toxic substances, the National guidelines most often state that criteria excursions are to occur no more than once in three years on average (USEPA 1985).

For human health criteria for toxic substances, because EPA's derivation of the WQC recommendations "involves the calculation of the maximum water concentration for a pollutant that ensures drinking water and/or fish ingestion exposures will not result in human intake of that pollutant in amounts that exceed a specified level based upon the toxicological endpoint of concern" (USEPA 2000), it is understood that any human health criteria excursions are unacceptable. A number of interrelated considerations – discussed in more detail in Whiteash (2021) – must be addressed when assessing if waterbodies meet WQS "at least 99% of the time" or to determine if excursions occur more than "once in three years on average" based on physicochemical samples.

The frequency of "at least 99% of the time" addresses the temporal aspect of criteria for which this consideration is not otherwise explicitly specified in Chapter 93 or addressed in the criteria development documents for Nationally recommended criteria. Note that some water quality criteria in § 93.7 have frequency components explicitly specified as part of the criteria, including the ammonia nitrogen criterion for aquatic life and the bacteria criterion for water contact sports. The underlying concept in the phrase "at least 99% of the time" is straightforward: there is some acceptable frequency – albeit relatively low (i.e., ≤ 1% of the time) – at which water quality criteria excursions are allowed without constituting criteria exceedances.

The fish and aquatic life criteria for toxic substances are different with regard to temporal aspects because these criteria are based on USEPA's recommended frequencies, which most often state that excursions from the criteria are not to occur more than once in three years on average (USEPA 1985, USEPA 1991, USEPA 2002b). This concept is discussed further in the *Criteria Duration Considerations* section, below. As noted previously, the human health criteria for toxic substances consider any criteria excursions to be unacceptable (USEPA 2000). Determining if WQS are met at the appropriate frequency requires context-specific considerations that take into account the particular standard(s) being evaluated and the expected site-specific patterns of variability.

MAKING DISCRETE PHYSICOCHEMICAL ASSESSMENTS

DEP will follow the determination framework in Chapter 5 (Shull and Pulket 2021) of this book when making use assessments using physicochemical data. In order to have sufficient data for assessment determinations, physicochemical data should be collected according to the protocols established in the Monitoring Book (Lookenbill and Whiteash 2021), and data collections should consider the duration component of the water quality criterion, including the durations for toxic substance criteria as specified in Table 1 below. In the use assessment process, DEP must also consider the sampling

design employed – including critical sampling periods, when applicable – sample size, and quality assurance methods (discussed below).

Judgement-Based Sampling

Due to interrelated considerations of reasonable decision error rates, sample sizes, and extreme percentiles of frequency distributions, it will often be impractical to employ a probability-based sample design – discussed further in Whiteash (2021) – to assess against meeting WQS "at least 99% of the time" or allowing for one excursion in a three year period without collecting large numbers of samples. Especially when accounting for monitoring costs, the most resource-effective approach will often be to focus monitoring at times when excursions are most likely to occur, hereafter referred to as *critical sampling periods*. Collecting samples during these critical sampling periods will require an understanding of the variables at play. Some of the variables that should be considered are discussed further in Whiteash (2021). According to USEPA (2002a), critical sampling periods can be thought of as temporal "hot spots," and sampling that is targeted to observe these "hot spots" based on an understanding of context-specific variations is a targeted sampling approach referred to as "*judgment-based sampling*" (as contrasted with probability-based sampling). Since judgment-based sampling is not as suited to some forms of quantitative statistical analyses as probability-based sampling, assessment processes based on judgement-based sampling may involve a different analytical toolset than assessment processes based on probability-based sampling.

DEP does not discount any data or information from consideration, so no strict guidelines are set with regard to what sampling designs are acceptable for assessments; however, of the various sampling plan designs discussed by USEPA (2002a), DEP believes that the judgment-based sampling design is the most suited method to assess extreme, infrequent ends of water quality parameter distributions. A judgment-based sampling design offers the benefit of more resource-efficient sampling (i.e., needing fewer observations to achieve a given level of precision) than other sampling designs when a sound understanding of the sites and systems being sampled is incorporated (USEPA 2002a).

Sample Size and Representativeness

Depending on the data available, the criterion being assessed, or the ambient conditions of the waterbody, a single observation (sample) can represent different periods of time. These factors may suggest that more than one sample needs to be collected to confidently make an assessment determination. USEPA guidance discourages rigid minimum sample size requirements and requires States to evaluate all existing, readily available, and appropriate water quality-related data for determining

WQS attainment determinations (USEPA 2006, USEPA 2013). As a result of these factors and federal requirements, DEP recommends multiple sampling events for assessing any criterion but will evaluate all existing and readily available data when making assessments. More specifically, DEP encourages at least three sampling events within the criterion duration period. For example, the total iron criterion for aquatic life is written as a 30-day average, so DEP encourages at least three samples be collected within a 30-day period to compare conditions to the criterion.

DEP generally considers discrete samples to be representative of one day unless convincing evidence exists to suggest otherwise (e.g., a documented spill, influence of a known biological process, supporting high-frequency monitoring data). For most criteria, this strikes an acceptable balance between resource expenditure and sampling error. For some criteria, however, literal interpretation of criteria would result in sample collection that is very resource intensive and not feasible. For example, literal interpretation of the USEPA's national recommended toxic criteria would require samples to be collected at least once each day within a consecutive four-day period. Consequently, USEPA recommends that a single sample may represent periods longer than one day if conditions are stable (USEPA 1997). DEP conforms to this guidance and accepts that a sample collected for the assessment of time-averaged criteria can represent more than one day unless convincing evidence exists to suggest otherwise.

Criteria Duration, Frequency Considerations

Instantaneous Criteria

The allowable frequency of excursions depends on whether the criteria duration is instantaneous or time-averaged. For criteria expressed as instantaneous maxima or minima, there is no averaging period required to compare measured values to the criteria (i.e., the criteria magnitude duration is instantaneous). For these instantaneous criteria in § 93.7 (relating to specific water quality criteria), Table 3, the allowable frequency of excursions – unless otherwise specified as part of the criteria – follows Chapter 96, meaning that criteria must be met at least 99% of the time. For the human health criteria in § 93.8c (relating to human health and aquatic life criteria for toxic substances), Table 5, no excursions are allowable, as previously discussed above based on EPA's *Methodology for Deriving Ambient Water Quality Criteria for Human Health* (USEPA 2000). As such, excursions of instantaneous criteria in § 93.7 occurring on four or more separate days within twelve months typically constitute an exceedance, as excursions from the criteria magnitudes occur more than 1% of the time (i.e., 4 days / 365 days ≈ 1.1%) and therefore the criteria are met less than 99% of the time.

Time-Averaged Criteria

For the aquatic life criteria at § 93.8c (relating to human health and aquatic life criteria for toxic substances), Table 5, adopted based on the National recommended criteria

(see Table 1) for which the criteria durations are expressed as averages (e.g., seven-day, 30-day, or monthly averages), an excursion from the criteria is not to occur more than once in three years on average.

Other pollutants for which the criteria duration is expressed as averages – which includes several of the criteria in § 93.7 (relating to specific water quality criteria), Table 3, and the fish and aquatic life criteria at § 93.8c (relating to human health and aquatic life criteria for toxic substances) Table 5 that are not described in Table 1 below – any single averaging period showing an excursion from the mean concentration of the criterion will be considered an exceedance, and thus an impairment of the relevant protected use. For instance, a single, seven-day average dissolved oxygen observation below the criterion – which is considered a single excursion from the criterion – indicates an exceedance of the criterion, as there are 52 seven-day cycles per year, and one seven-day cycle is more than one percent of a year [100 * (1 seven-day cycle / 52 cycles / year) = 1.9% of the year]. Or, a single 30-day average ammonia concentration above the criterion magnitude indicates an of the criterion, as there are 12 30-day cycles per year, and one 30-day cycle is more than one percent of a year [100 * (1 30-day cycle / 12 cycles / year) ≈ 8.3% of the year]).

It is important to note that the criteria for toxic substances in § 93.8c do not specify durations. However, many of these fish and aquatic life criteria were adopted based on USEPA's *National Recommended Water Quality Criteria – Human Health Criteria* ("the national recommended criteria") (USEPA 2020), for which the aquatic life criterion maximum concentration (CMC, or acute criterion) and criterion continuous concentration (CCC, or chronic criterion) durations are provided in Table 1 below.

DEP will assess data based on the corresponding duration component each parameter's water quality criterion. The frequency components of the national recommended fish and aquatic life criteria for toxic substances indicate that more than one excursion in three years would result in a criterion exceedance and use impairment (USEPA 1985, USEPA 1991, USEPA 2002b).

Table 1. Criteria durations and other information for the fish and aquatic life criteria for toxic substances from § 93.8c (relating to human health and aquatic life criteria for toxic substances) including the Chemical Association System (CAS) number, Chemical Name, and durations for the Criterion Maximum Concentration (CMC, acute criterion) and Criterion Continuous Concentration (CCC, chronic criterion) as adopted from USEPA's national recommended criteria, identified by the Rationale Document.

CAS	Chemical Name	CMC Duration	CCC Duration	Rationale Document
333415	DIAZINON	1-hour average	4-day average	EPA-822-R-05-006
104405	NONYLPHENOL	1-hour average	4-day average	EPA-822-R-05-005
87865	PENTACHLORO-PHENOL	1-hour average	4-day average	EPA-820-B-96-001
7440382	ARSENIC (As3+)	1-hour average	4-day average	EPA-820-B-96-001
7440439	CADMIUM	1-hour average	4-day average	EPA-820-B-96-001
16065831	CHROMIUM III	1-hour average	4-day average	EPA-820-B-96-001
18540299	CHROMIUM VI	1-hour average	4-day average	EPA-820-B-96-001
7440508	COPPER	1-hour average	4-day average	EPA-820-B-96-001
7439921	LEAD	1-hour average	4-day average	EPA 440/5-84-027
7439976	MERCURY (Hg2+)	1-hour average	4-day average	EPA-820-B-96-001
7440020	NICKEL	1-hour average	4-day average	EPA-820-B-96-001
7782492	SELENIUM	1-hour average	4-day average	EPA-820-B-96-001
7440224	SILVER	Instantaneous	N/A	EPA 440/5-80-071
7440666	ZINC	1-hour average	1-hour average	EPA-820-B-96-001
57125	CYANIDE, FREE	1-hour average	4-day average	EPA-820-B-96-001
58899	gamma-BHC (LINDANE)	1-hour average	N/A	EPA-820-B-96-001
57749	CHLORDANE	Instantaneous	24-hour average	EPA 440/5-80-027
50293	4,4-DDT	Instantaneous	24-hour average	EPA 440/5-80-038
60571	DIELDRIN	1-hour average	4-day average	EPA-820-B-96-001
959988	alpha-ENDOSUL-FAN	Instantaneous	24-hour average	EPA 440/5-80-046
33213659	beta-ENDOSULFAN	Instantaneous	24-hour average	EPA 440/5-80-046
72208	ENDRIN	1-hour average	4-day average	EPA-820-B-96-001
76448	HEPTACHLOR	Instantaneous	24-hour average	EPA 440/5-80-052
1024573	HEPTACHLOR EPOXIDE	Instantaneous	24-hour average	EPA 440/5-80-052
--	PCB (Polychlorinated Biphenyls)	24-hour average	24-hour average	EPA 440/5-80-068
8001352	TOXAPHENE	1-hour average	4-day average	EPA 440/5-86-006
107028	ACROLEIN	1-hour average	1-hour average	Not Numbered
108883	TOLUENE	Not specified	Not specified	EPA 440/5-80-075

Fish and aquatic life criteria for toxic substances not described in Table 1 below, were determined by the Commonwealth rather than directly adopting USEPA's National recommendations (USEPA 1985). As such, the allowable frequency of occurrence follows Chapter 96 and criteria must be met at least 99% of the time.

As noted in the *Sample Size and Representativeness* section above, while DEP will evaluate all existing and readily available data, DEP encourages at least three samples be collected within the time-averaged criterion duration.

Quality Assurance

DEP makes every effort to verify the accuracy of all data used in the use assessment determination process. DEP strongly encourages anyone submitting data to familiarize themselves with DEP Bureau of Laboratories quality assurance and quality control procedures (DEP 2016) regarding record keeping, methods documentation, sampling techniques, selection of analytic laboratories, chain of custody concerns, and so forth. DEP will not exclude extreme values (outliers) from a dataset unless there is reason to believe the extreme value is invalid. For example, a dissolved oxygen concentration of 100 mg/L is physically impossible at tropospheric temperatures and pressures; it is likely that such a record is a typographical error actually meant to be 1 mg/L or 10 mg/L. Similarly, in a water temperature dataset submitted in degrees Celsius where one value is recorded at 72, it is highly unlikely this is a valid reading and may be recorded in degrees Fahrenheit.

Sample Precedence

In DEP's assessments of WQS, more recent data take precedence over older data, especially in situations where conditions have recently changed (e.g., installation of pollution remediation projects, alteration of permit limits in the watershed, changing land use patterns, discontinuation of combined sewer overflows). In some instances, older and newer data may be considered together to document temporal trends.

CONCLUSION

This document outlines much of what should be considered when conducting an assessment of water quality physicochemical data for making CWA 303(d) and 305(b) decisions. It should be read in its entirety and followed in combination with the protocols in the Monitoring Book (Lookenbill and Whiteash 2021) and Chapter 5 of this book (Shull and Pulket 2021). Additional technical information and rationale on physicochemical data collections and assessment decisions can be obtained in Whiteash (2021).

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3.2 CONTINUOUS PHYSICOCHEMICAL ASSESSMENT METHOD

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INTRODUCTION

This assessment method is designed to make assessment determinations based on continuous instream monitoring (CIM) data related to several water quality criteria located in 25 Pa. Code Chapter 93. Water quality data sondes record instream parameters such as temperature, specific conductance, pH, dissolved oxygen (DO), and turbidity at defined intervals. Because these intervals are sufficiently narrow (e.g., 30 minutes), these data are considered continuous, and are referred to as CIM data. The number of samples in a CIM dataset approximates a census of the water quality conditions, and therefore should be evaluated differently than a dataset of a limited number of discrete samples.

Some parameters directly recorded by CIM deployments (temperature, pH, and DO) have defined water quality standards (WQS) in 25 Pa. Code Chapter 93 that are implemented through Chapter 96 (WQS Implementation). These criteria are expressed as either a minimum or maximum concentration, or an arithmetic mean concentration over a defined period (§ 93.1). In addition to the parameters directly recorded by CIMs, discrete grab samples of many other water quality measures can be expanded into continuous datasets by modeling the relationship between the discrete grab samples and recorded CIM parameters. These model-derived continuous datasets may also have defined WQS.

This document largely focuses on evaluating CIM data (both directly measured and model-derived) for the purposes of assessing protected water uses; however, these analyses are also important tools to be used for other objectives, including stream use evaluations or cause and effect surveys.

CRITERIA IMPLEMENTATION

99% Rule

The frequency, or acceptance threshold, associated with Chapter 93 water quality criteria is given in § 96.3(c), which states,

“To protect existing and designated surface water uses, the water quality criteria described in Chapter 93 (relating to water quality standards), including the criteria in §§ 93.7 and 93.8a(b) (relating to specific water quality criteria; and toxic substances) shall be achieved in all surface waters at least 99% of the time…”

This WQS component introduces the allowance for temporary, rare digressions or excursions of water quality criteria (Whiteash 2021). Time, however, in “99% of the

time" is not defined in § 96.3(c), leaving the allowable occurrence of these digressions or excursions not fully described. Because CIMs record water quality parameters at such frequent intervals, the amount of interpolation between samples is very small, so the data are, in effect, a census of water quality, significantly reducing sampling error (see "Sampling Error" section of Whiteash 2021). This more thorough dataset and the resulting reduction in sampling error means that the application of the 99% rule can be applied over a large period for temporally comprehensive protection under the WQS.

Therefore, for the purpose of the assessment of CIM data with respect to § 93.7 Table 3, time, as a part of § 96.3(c), is defined as a rolling year. The length of one year provides the inclusion of all seasonal variation found in a temperate ecosystem, including various life cycles and reproductive signals that are often strongly tied to season. The use of a *rolling* year affords the opportunity to monitor for stressful conditions that may span other, arbitrary yearly divides such as calendar year or water year (October 1).

Because water quality is to be achieved "99% of the time", assessments will be made by determining whether digressions or excursions of criteria (see Whiteash 2021 for additional discussion on criteria durations, a.k.a., averaging periods for measuring concentrations to be compared to criteria) constitute greater than one percent of a year. To make this calculation for instantaneous criteria, the number of measurements outside of the criteria magnitude will be summed and a percent of a year (%Y) that those readings represent will be calculated, using the following equation:

$$\%Y = 100\left[\frac{n * i}{k}\right]$$

Where:

%Y = percent of a year
n = number of digressions
i = recording interval in minutes
k = A constant (525,600) equal to the number of minutes in a year (365 days * 24 hrs/day * 60 min/hr)

For criteria with time-averaged durations, see the *CIM Parameters with Established Criteria Durations* section below.

If %Y > 1, then the criterion is not achieved 99% of the time as required by § 96.3(c), and the waterbody is not attaining WQS. A summary of common recording intervals and

the number of readings – for criteria with instantaneous minima or maxima – that would constitute greater than one percent of a year is provided in Table 1.

Table 1. Common recording intervals and the number of readings necessary to represent greater than one percent of a year for criteria with instantaneous minima or maxima.

Recording Interval	Number of Readings
15 min	351
30 min	176
60 min	88

Because continuous monitoring equipment is left unattended for extended periods, CIM data are more susceptible to error from calibration drift or sensor fouling (see Quality Control Requirements below). This can result in the removal of portions of data from the dataset due to excess uncertainty in the accuracy of the data. The removal of data due to excess uncertainty makes it problematic to define time, with respect to the 99% rule, as the total number of readings in the dataset (rather than the rolling year as described above) because doing so would effectively lead to an increased likelihood of non-attainment for datasets with increased levels of uncertainty. For example, if a dataset of a criteria with instantaneous durations (e.g., temperature, pH) had 10,000 readings, and 90 of the readings were beyond the criterion magnitude, 0.9% of the dataset were digressions of the criterion. But if there was a period during the deployment that the sensor became excessively fouled, some data may need to be removed due to an overabundance of uncertainty in the accuracy of the measurements. If 1,200 readings were removed from the 10,000 readings, there would only be 8,800 readings remaining, and the 90 digressions would now represent 1.02% of the dataset. With increased uncertainty, data were necessarily removed; but, lowering the total number of readings increased the portion of the dataset that each digression represented, and in effect, increased uncertainty in the data made a non-attainment decision more likely. Therefore, the 99% rule is applied to a defined length of time (a rolling year) rather than the number of readings in a dataset.

The application of the 99% rule to a period of less than one year would also be problematic. Doing so would require a minimum period to be established because without a minimum, the period of record could be made so short as to necessitate only one reading to trigger non-attainment, and implementing a minimum period would, in effect, just establish that length of time as the *de facto* period to apply §96.3(c). In addition, application of §96.3(c) over a period shorter than a year (season, month, week, etc.) would drive the threshold for impairment exceptionally low, and cause a significant discrepancy between water quality criteria non-attainments, which were

established for the protection of uses, and observed impacts to uses. The same discrepancy would result if §96.3(c) were applied as a percent of days for which the criterion was exceeded. This application would mean that a single reading outside a listed criterion on four days throughout the entire year would represent non-attainment [100 * (4 / 365) = 1.1%].

Data Collection Requirements and Critical Time Periods

Even though the assessment decision is based on a 365-day period, it is not necessary that a full year of CIM data is collected. In some circumstances, the number of digressions or excursions, such that %Y > 1, may be observed in a rather short period. Focusing sampling effort during critical periods may give sufficient information to make an assessment decision while greatly reducing the amount of resources needed to conduct the survey.

If limited site-specific data are available, general knowledge of water quality processes can be used to determine critical periods and guide the period of record. For example, many water quality parameters are affected by seasonal change and their responses can, therefore, be predicted to a certain degree. DEP's CIM efforts have documented increases in pH values, increases in diel pH fluctuation, corresponding decreases in DO values, and increases in diel DO fluctuation from early spring through the fall. This correlates with increased photoperiod and increased air and surface water temperatures. The effect of increased temperature and photoperiod to increased instream production and respiration are well documented (Odum 1956, Strickland 1970, Neori and Holm-Hansen 1982, Raven and Geider 1988). An increased photoperiod with adequate nutrition will increase the standing biomass of photosynthetic organisms (Valenti et al. 2011). Photosynthesis and respiration throughout the day and community respiration at night results in diel fluctuation of pH and DO (Odum 1956, White et al. 1991, Wurts 2003). These processes indicate that during the growing season, pH is most likely to exceed the maximum criterion and DO to fall below the minimum criterion or 7-day average. If these criteria were the focus of the monitoring effort, the CIM deployment could be limited to this period to reduce resources while capturing the critical period.

DEP also recognizes that critical or limiting conditions may not be consistent year-to-year, and a single year of data may not accurately represent conditions that WQS were developed to protect. Typically, this is driven by the amount and timing of precipitation for a given period or year. Elevated precipitation will result in increased surface water discharge, which moderates limiting conditions characterized by temperature, pH and DO. DEP has documented in past surveys that elevated discharge can reduce daily DO, pH, and temperature fluctuations and increase daily minimum DO values and decrease

maximum pH and temperature values. When multiple years of data are collected, assessment decisions will be based on years where the most critical or limiting conditions exist. For instance, if two years of data are collected, and in the first year there are digressions of the maximum pH criterion greater than one percent of the time, and in the second-year, digressions are less than one percent of the time, it is likely that critical conditions existed in the first year that were not seen in the second, such as reduced amount or frequency of precipitation, or higher air temperatures. Therefore, the assessment decision will be based on the first year to be protective of critical periods. For this reason, it is also imperative to characterize conditions that drive critical or limiting conditions and reference those conditions as part of the protected use assessment and subsequent reassessments.

CIM Parameters with Established Criteria

Table 3 of § 93.7(a) provides criteria for three parameters that can be directly measured by CIM deployments. The first two parameters are pH and DO. These parameters often have significant changes throughout the day, driven by photosynthesis, making CIMs particularly useful in assessments. The applicable criterion for pH is 6.0 to 9.0, inclusive. The minimum DO criterion is 5.0 mg/L, but other, more stringent criteria are applied for certain waters or times of the year, including minimum 7-day durations (averaging periods).

A 7-day average can be for any period of that length; it is not tied to a calendar week or any other set period. To assess whether a digression has occurred, all possible 7-day periods should be calculated. For continuous data, this is accomplished by calculating a rolling mean over the period of record, where the averaging period is set to seven days or 168 hours (7 days x 24 hours). The completeness of the dataset over the 7-day durations should also be reviewed. Incomplete datasets (e.g., gaps due to sensor error, battery failure, or excessive fouling) should be evaluated carefully to ensure that any duration that contains a gap in the data is still representative of conditions for that duration. There is no minimum threshold of completeness necessary to calculate an average, however, no conditions should be over-represented due significant gaps in the dataset. Any calculated average that is determined to be non-representative due to gaps in the duration, should be excluded from assessment decisions. A single 7-day average DO below the criterion – which is considered a single excursion of the criterion – indicates non-attainment of the criterion, as there are 52 7-day cycles per year, and one 7-day cycle is more than one percent of a year [100 * (1 7-day cycle / 52 cycles / year) = 1.9% of the year].

Maximum temperature criteria are provided in § 93.7 for defined times of the year and water uses, which are applied to “*heated waste sources regulated under Chapters 92a*

and 96 and other sources where temperature limits are necessary to protect designated and existing uses". Continuous temperature data are not typically used to assess critical uses. DEP surface water quality data have consistently demonstrated a high degree of instream temperature variability that does not conform to the temperature criteria at § 93.7, where digressions of the criteria are not caused by thermal modification. An appropriate thermal evaluation includes a biological assessment based on instream flora and fauna to determine whether the biological community is affected by the thermal regime. Typically, fish community evaluations have the best resolution in characterizing a waterbody's thermal regime due to the effects to physiology and distribution patterns (Shuter et al. 1980, Ridgeway et al. 1991, Azevedo et al. 1998, Wehrly and Wiley 2003, Lyons et al. 2009). To qualify as a High Quality water under § 93.4b (a)(1)(i), a list of parameters, including temperature must be evaluated to meet the chemistry conditions. Continuous temperature could aid in this determination. The regulation states,

"The water has long-term water quality, based on at least one year of data which exceeds levels necessary to support the propagation of fish, shellfish and wildlife and recreation in and on the water by being better than the water quality criteria in § 93.7, Table 3… at least 99% of the time…"

In addition to the criteria listed in the Commonwealth's WQS, additional or more stringent criteria from the Delaware River Basin Commission (DRBC) water quality regulations, ORSANCO (Ohio River Valley Water Sanitation Commission) pollution control standards, and the Great Lakes Water Quality Agreement (GLWQA) are applicable as stated in §§ 93.2(b) and 93.9.

DETERMINATION OF SPATIAL EXTENT OF ASSESSMENT

While CIMs provide a thorough record of water quality conditions at a given point, additional data are necessary to understand the spatial extent to which the CIM data apply. To aid in this determination, discrete measurements should be collected throughout the area. The necessary spatial frequency of sampling will vary greatly depending on the stream, but these discrete measurements should target potential influences such as tributaries, discharges, or changes in land use that may significantly alter water quality (Shull and Pulket 2021). Transects at these additional points are helpful in determining any changes in mixing patterns (Hoger 2020). Most importantly, these measurements should be conducted during the critical periods of interest when water quality is suspected of exceeding criteria. Targeting these periods, at the periphery of expected values, provides the necessary information to characterize the spatial extent of an assessment.

Knowing when to sample is often informed by recent CIM readings and general knowledge of seasonal, daily, and weather-related trends in water quality. For example, if a CIM recently recorded digressions of the maximum pH criterion in a stream, additional measurements should be taken upstream and downstream of that CIM, and in and around any tributary or discharge. In this example, digressions are most likely to occur in the late afternoon as photosynthesis drives the pH higher. Reviewing the CIM record can give a more specific indication of the period of digressions, perhaps it was between 17:00 and 19:00. The additional samples should then be targeted for that period. Samples taken in the morning, even if they align with the CIM readings, are likely to be below the criterion and would not provide sufficient information to extend the assessment.

QUALITY CONTROL REQUIREMENTS

All CIM data to be used for assessment must follow quality control methods as described in the Continuous Physicochemical Data Collection Protocol (Hoger et al. 2017) including regular fouling and calibration checks of the equipment, discrete readings with a separate meter, appropriate corrections, and final independent approval of the data. Data that do not meet the usability threshold are removed from any assessment decisions. In addition, data are reviewed to determine if they are representative of the waterbody. Discrete water quality cross-section surveys (transects) are performed throughout the deployment, targeting various flows and water quality conditions to ensure that CIM data are representative of the targeted waterbody (Hoger 2020).

ASSESSMENT EXAMPLE

Continuous data were collected from late February to early November of 2016 on West Branch Octoraro Creek (WBOC), including temperature, specific conductance, pH, dissolved oxygen, and turbidity. After quality control checks were completed on the datasets, pH and dissolved oxygen data were compared to listed WQS criteria (Table 2). There were no digressions of DO criteria, but there were numerous digressions of the maximum pH criterion (Figure 1). Because the number of digressions represent greater than one percent of a year, there was an exceedance of the pH criterion and the WBOC was not attaining the Aquatic Life Use (ALU) at this location.

Table 2. Comparison of continuous data from WBOC to ALU WQS criteria.

Criterion	Number of Digressions	Percent of Year
pH 6.0 to 9.0, inclusive	299	1.71%

DO minimum, 5.0 mg/L	0	0.00%

While performing a routine maintenance visit on April 5, 2016, it was noted that the sonde had recorded numerous digressions of the maximum pH concentration. These digressions had taken place between roughly 12:00 and 19:00, with peak pH occurring around 16:00. To aid in delineating a potential assessment of WQS criteria, a field visit was scheduled near the peak time of day to take discrete readings throughout the watershed. Because of a rain event on April 7, and the likely suppression of the maximum pH that would result, the visit was delayed. After several days without any rain, the visit was completed on April 21 (discrete measurement at sonde location shown in Figure 1-B). Numerous readings were taken on WBOC and its tributaries above the continuous station (Figure 2) from 14:57 to 16:10, the peak of pH readings in the continuous dataset. No readings were taken downstream of the continuous station due to the effects of Octoraro Lake just a short distance downstream. The discrete readings indicate that the impairment should extend along the entire mainstem of WBOC above Octoraro Lake. A reading was taken in each of the four largest tributaries. Three of the four discrete measurements were well below the maximum pH criterion, while the fourth was slightly above. The slight digression in Bowery Run may not provide enough evidence to extend the impairment into this stream, and more information may be necessary.

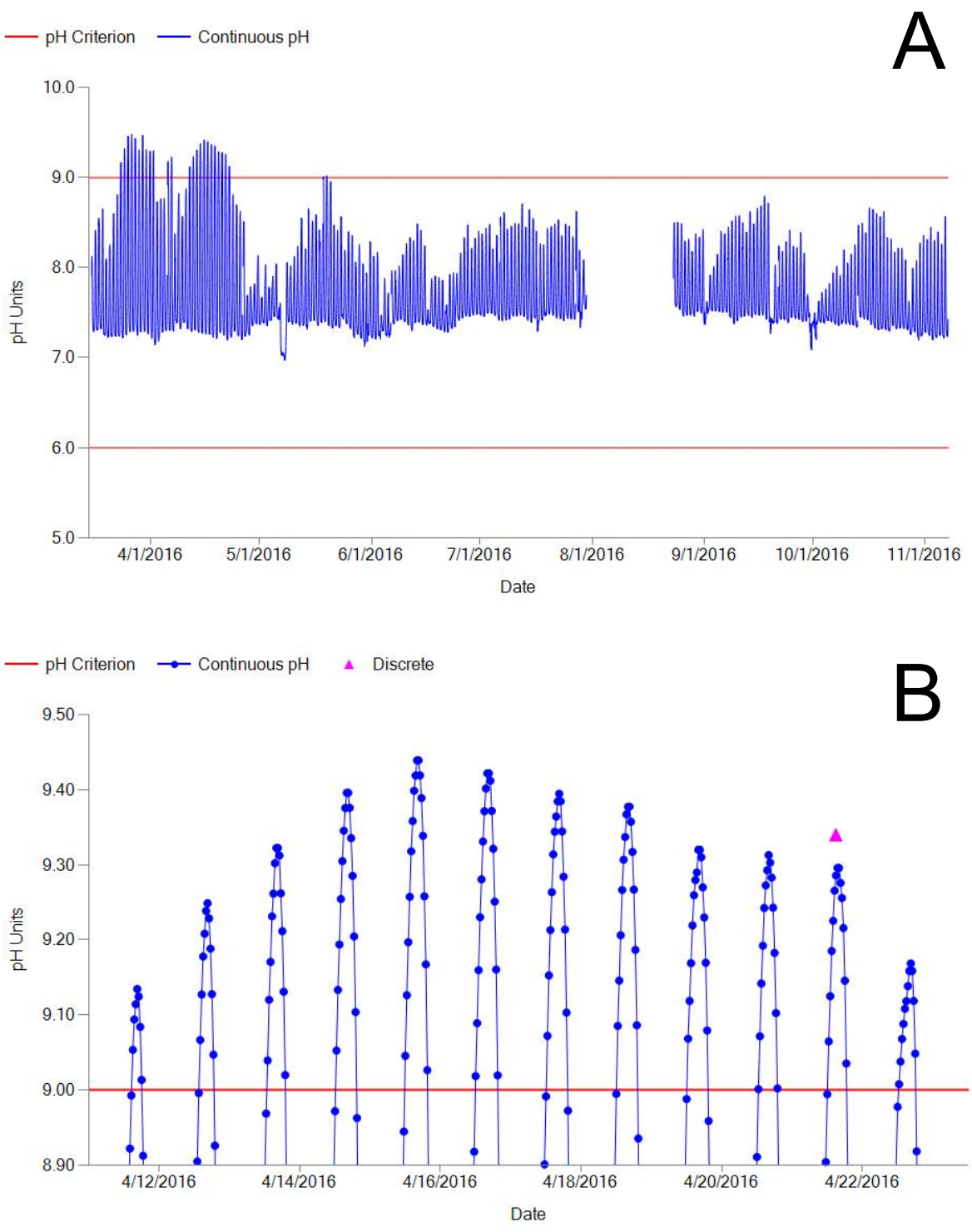

Figure 1. Continuous pH data (A) at WBOC shows digressions above the maximum WQS criterion magnitude. Individual points above the criterion maximum (B) are summed to calculate the percent of a year that these digressions represent.

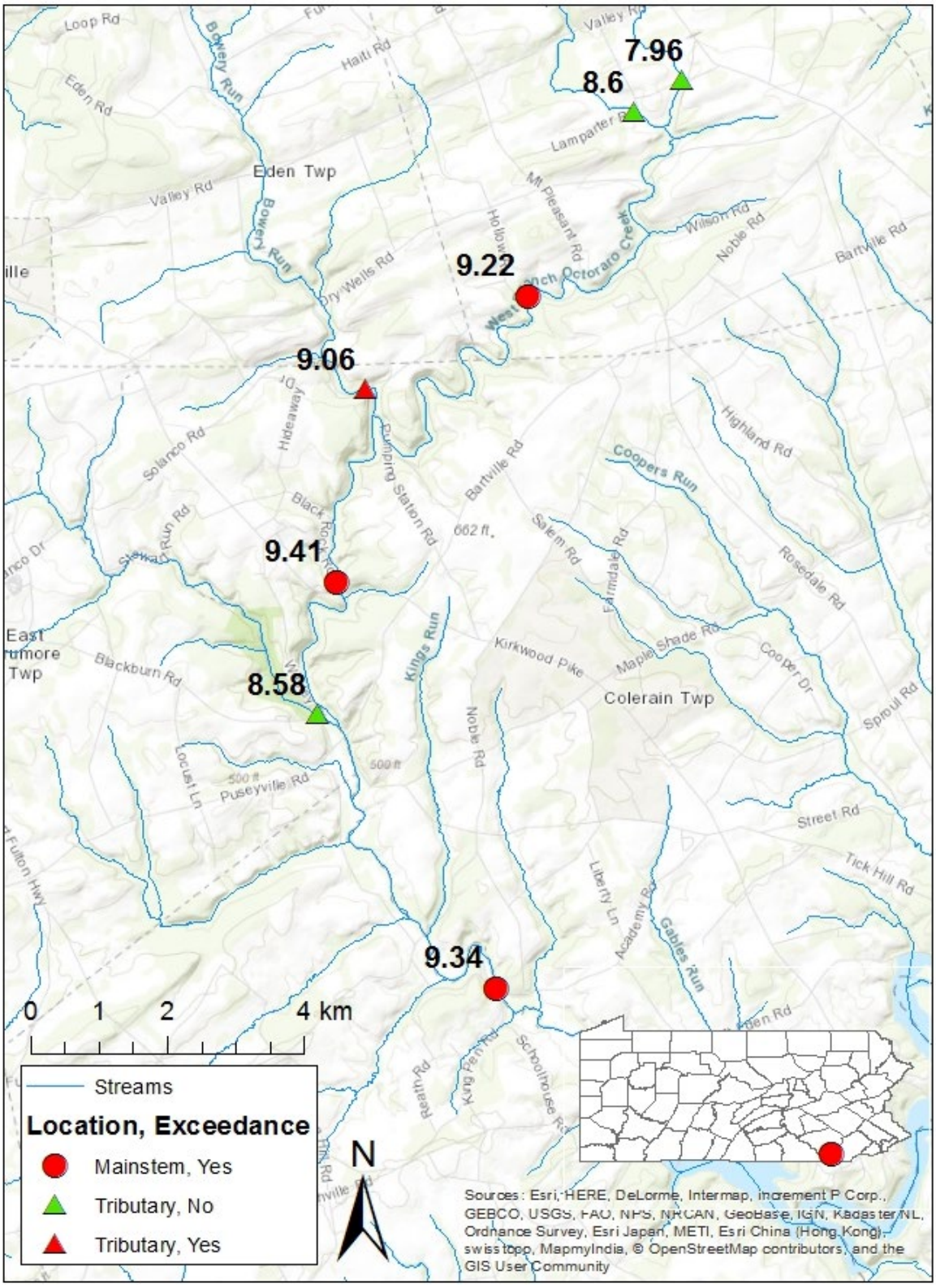

Figure 2. Discrete pH readings throughout the WBOC basin used to delineate an impairment. The furthest downstream location is the CIM station.

ASSESSMENTS USING DERIVED CIM DATA

Many water quality parameters of interest cannot be directly measured on a continuous basis. Auto-samplers could be used to collect discrete samples at regular intervals for analysis at the lab; however, this is a labor intensive and costly approach and is only realistic for relatively short periods of time. Though CIMs directly measure only a few parameters, some of the parameters have been shown to be highly correlated to other measures of water quality (e.g., Christensen et al. 2006, Foster and Graham 2016, Rasmussen et al. 2016). These relationships often have a strong physical basis, such as dissolved ions driving the specific conductance of water, or suspended sediment making water turbid. These relationships provide the opportunity to use easily-measured CIM parameters to accurately model numerous other parameters. The United States Environmental Protection Agency directs states to use all available water quality data in making assessment decisions (40 CFR 130.7(b)(5)) and, in guidance, specifies that models should be included in the data that are to be evaluated (US EPA 2005).

Models are developed by comparing discrete grab samples of the parameter(s) of interest to recorded CIM data. The discrete grab samples are collected directly over the CIM and should encompass the range of values observed in the CIM record. The number of samples necessary for the development of a strong model varies, but fewer samples are necessary if they are well distributed throughout the range of values (Rasmussen et al. 2009). Particular emphasis should be placed on collecting discrete samples when water quality is outside of criteria. Inclusion of these samples adds critical support to models resulting in exceedances of criteria. Both discrete grab samples and CIM data should be collected following established DEP protocols and undergo all quality control procedures prior to final model development. Review of CIM data during the period of record can aid in the timing and collection of discrete samples that are distributed throughout the range of values. Models should be considered site-specific, recognizing the potential for differences in the relationship between water quality constituents at each site.

Most models are based on continuous specific conductance or turbidity data, though continuous water temperature, continuous streamflow, and Julian day (day of the year) have been used to strengthen models. Examples of constituents and the explanatory variables included in their model are shown in Table 3. While the table provides many examples, the list is not comprehensive and strong models are probable for many other parameters.

Table 3. Example response and explanatory variables for models of derived CIM. Citation listed in the explanatory variable column(s) that the study used in the model. All models listed in the table had R^2 values of at least 0.8.

Response Constituent	Specific Conductance	Streamflow	Turbidity	Temperature	Julian day
Actinomycetes			5		5
Alkalinity	2, 4, 5, 11	4, 11			
Atrazine		10	10		
Bicarbonate	4, 11	4, 11			
Boron	13				
Calcium	4, 5, 11, 12, 13, 14		14		
Chloride	1, 2, 4, 5, 10, 11, 12, 13, 14, 15	1			
Dissolved nitrate	1			1	
Dissolved nitrate + nitrite	10		10	10	
Dissolved orthophosphorus		10			
Dissolved phosphorus		14			14
Dissolved solids	1, 2, 4, 5, 10, 11, 12, 13, 14				
E. coli			12, 14		14
Enterococci bacteria			12, 14		14
Fecal coliform bacteria		14	12, 14		
Fluoride	1	1			
Hardness	4, 11				
Magnesium	5, 12, 13				
Particulate phosphorus			14		
Sodium	1, 2, 4, 5, 11, 12, 13, 14	1			
Sulfate	1, 2, 4, 5, 11, 12, 13, 14		14		
Suspended sediment		4, 6, 11	1, 2, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14		
Total nitrogen		3	3, 5	5	
Total organic carbon			11		
Total organic N + NH3	1		1, 2, 4	1	
Total phosphorus	3	2	2, 3, 10, 11, 12, 13	3	
Total suspended solids	4		1, 2, 4, 11, 12, 13, 14		

1. Christensen 2001
2. Christensen et al. 2006
3. Christensen et al. 2002
4. Christensen et al. 2003
5. Foster and Graham 2016
6. Juracek 2011
7. Lee 2009
8. Lee et al. 2008
9. Maloney and Shull 2015
10. Mau et al. 2004
11. Rasmussen et al. 2016
12. Rasmussen et al. 2008
13. Rasmussen et al. 2005
14. Stone and Graham 2014
15. Trowbridge et al. 2010

The development of models should follow strict guidelines to ensure a consistent, empirical approach at building and evaluating the strength of each model. Comprehensive guidance is provided in Rasmussen et al. (2009) which includes multiple tests of the uncertainty of the model such as root-mean-squared error (RMSE), model standard percentage error (MSPE), and prediction error sum of squares (PRESS). Decreases in RMSE, MSPE, and PRESS indicate reduced uncertainty in the model. Coefficient of determination (R^2) and adjusted coefficient of determination (R^2a) are measures of the strength of the relationship between variables. A higher R^2 or R^2a indicates that a higher portion of the response variable is described by the model. These values range from -1 to 1, where -1 indicates perfect negative correlation, 1 indicates perfect positive correlation, and 0 indicates no correlation.

Though these statistics can describe the relative strength or weakness of a model, it is problematic to define an appropriate threshold of strength that must be achieved by a model before it should be used for assessment of a derived parameter. Alternatively, the use of prediction intervals or probability of digression calculations incorporate the uncertainty of the model into the calculation.

To illustrate the importance of the difference in these approaches consider the following examples. First imagine a set of criteria (e.g., R^2, MSPE, PRESS thresholds) were established to determine whether a model was sufficiently strong to be used for assessment of a derived parameter. Then consider that a model just barely achieved that minimum standard for use, and that model generated a derived dataset that contained values beyond an established criterion. If many of the values were calculated at just slightly beyond the established criterion (digressions of the criterion), it is possible that a significant portion of those values were below the criterion concentrations when the uncertainty of the model was incorporated. This would be analogous to a Type I error—a determination of non-attainment when the waterbody may be attaining.

The reverse could also happen. Imagine another model for a different set of data that just missed the standard for use, but, like the first example, some calculated values were beyond a criterion. If those values were well beyond the criterion, instead of slightly past it like in the first example, the degree to which they were beyond the criterion could mean that they would still likely be beyond the criterion, even if the higher uncertainty in the model was considered. This would be analogous to a Type II error—a determination of attainment when the water body is not attaining.

Because prediction intervals and probability of digression calculations incorporate the uncertainty of the model, these approaches reduce Type I and Type II error, and could

lead to a determination of attainment in the first example and non-attainment in the second example. The first method suggested in Appendix 3 of Rasmussen et al. (2009) to analyze derived data for criteria concentration digressions is to generate prediction intervals for cumulative frequency duration (CFD) curves. These curves show the proportion of values from the sample that fall below certain values. If 90 percent prediction intervals were then created around the CFD curve to assess based on a maximum criterion concentration, the lower prediction curve could be used to determine the percent of time that a digression of the criterion concentration occurred with 90% confidence. This could then be compared to the 99% rule to determine if an exceedance of the criterion occurred or if the waterbody was attaining; however, the percent of time in this calculation is based on the number of readings in the analysis and not one year. As discussed above, for the purposes of CIM assessment, the 99% rule should be applied to a 365-day period. Therefore, this method should not be used unless the calculation is adjusted.

An alternative method provided by Appendix 3 of Rasmussen et al. (2009) is to generate a probability of digression for each data point of the derived series using the following equation:

$$P=1-D\left[\frac{\text{x-Criterion}}{\text{RMSE}}\right]$$

Where

P = probability a digression of the criterion concentration occurred
D = cumulative distribution function for the standard normal curve (values found in tables provided in statistics textbooks)
x = model-computed value
RMSE = root-mean-square error, a measure of the variance between regression-computed and observed values

If the response variable was transformed in the model, both the model-computed value and the criterion must be transformed in the equation. For example, if the response variable was $\log_{10}$ transformed the equation would change to:

$$P=1-D\left[\frac{\log_{10}(x)-\log_{10}(\text{Criterion})}{\text{RMSE}}\right]$$

These probability of digression calculations can then be used to make assessment determinations on a derived dataset. All model-computed values with probability of digression greater than or equal to 0.9 (90%) are considered a digression of criteria. These digressions are then summed and a percent of a year that they represent

calculated. A number of digressions such that the percent of a year is greater than one percent indicates non-attainment of WQS criteria.

The selection of a 90% probability threshold was chosen because it is a common break point for describing probable occurrence in statistical measures (confidence intervals, tests of significance, etc.). Ninety-percent prediction intervals are used by United States Geological Survey (USGS) in their presentation and analysis of model-derived continuous data (e.g., USGS National Real-Time Water Quality website: http://nrtwq.usgs.gov/, Juracek 2011, Mau et al. 2004, Rasmussen et al. 2009, Rasmussen et al. 2016). In addition, DEP has used 90% as a threshold of significance for assessment methods in the past. For example, the limestone stream protocol (Williams 2017) and both the wadeable (Pulket 2017, Shull 2017) and semi-wadeable (Shull 2018) macroinvertebrate protocols all use 90% confidence intervals for the determination of precision estimates.

DELISTING CIM ASSESSMENTS

As previously discussed, critical conditions can vary greatly year to year. Therefore, to properly delist impaired waters, reassessment data should encompass conditions similar to those that existed during the original assessment period. For example, if one or more exceedances of criteria were determined for a stream with frequent digressions during a “dry” summer with infrequent rain, the reassessment data should also include a “dry” summer. It would be inappropriate to delist the stream using data from a “wet” summer with frequent rain and elevated flows as these are likely to moderate critical conditions.

A waterbody assessed with continuous data should not be delisted with discrete grab samples. Continuous data are temporally more comprehensive than discrete grab samples and should be used to delist a waterbody if a temporally-comprehensive, continuous dataset was the basis of the assessment. A waterbody assessed with discrete grab samples, however, can be delisted with continuous data, as continuous data are fundamentally discrete grab samples collected much more frequently.

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CHAPTER 4 PHYSICAL ASSESSMENT METHODS

4.1 PHYSICAL HABITAT ASSESSMENT METHOD

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2017

INTRODUCTION

This assessment method is designed to make Aquatic Life Use (ALU) assessment determinations using physical habitat data in Pennsylvania's wadeable streams. Physical habitat of the aquatic environment is a critical component of the overall ecological integrity of the aquatic community (Barbour et al. 1999). Therefore, assessment of the physical habitat is performed in conjunction with biological monitoring of all flowing waters. The instream habitat availability and condition is a major factor in determining the abundance and diversity of benthic macroinvertebrates and fish. Healthy, diverse aquatic communities in streams require a diversity of cover such as boulders, cobble and coarse woody debris (such as logs), interstitial space between cobble and boulder substrate largely free of fine sediment and sand, diverse flow regimes that provide slow and fast moving water as well as shallow and deep pools (Barbour et al. 1999). Accumulation of fine sediment and sand or deposition of other pollutants such as iron precipitate (yellow boy) can reduce or eliminate cover, interstitial space and deep pools degrading the habitat and impairing the ability of the stream or river to support healthy aquatic communities.

To assess ALU of flowing surface waters, DEP includes assessments of the physical habitat, whenever possible. To use this method for assessment determination purposes data collection must follow applicable protocols established in the Monitoring Book (Lookenbill and Whiteash 2021). There are two habitat assessment protocols based on flow regimes related to gradient or slope. The DEP habitat assessment for high gradient streams and semi-wadeable rivers (waters dominated by riffle-run habitat) is based on the habitat assessment published in Plafkin et al. (1989). The DEP method is a revision of the Plafkin et al. (1989) method, which had undergone several iterations during the 1990s. This habitat evaluation uses a twelve parameter – 20-point scoring method. The method for low-gradient streams and rivers (waters that lack riffles) is based on the Habitat Assessment and Physicochemical Parameters described in Barbour et al. (1999). The DEP assessment uses nine of the ten parameters of Barbour et al. (1999). More information on the data collection aspects of these parameters are found in Chapter 5.1 of DEP's Monitoring Book (Lookenbill 2017).

AQUATIC LIFE USE ASSESSMENT

Qualitative Method for High Gradient Streams and Rivers

Wadeable Streams

The threshold for ALU assessment impairment for high gradient riffle/run dominated wadeable (<1000 mi^2) streams is a total habitat score of 140 or less. Certain instream and riparian area habitat parameters are strong predictors of habitat degradation

leading to ALU impairment, and as a result, these parameters alone may warrant independent assessment decisions. These parameters are embeddedness, sediment deposition, condition of banks, and bank vegetative protection. The impairment threshold for the parameters of embeddedness + sediment deposition, or condition of banks + bank vegetative protection is a total score of 24 or less for either combination.

Semi-wadeable Rivers

Habitat assessments are required with each semi-wadable survey, but certain habitat parameters (e.g., riparian vegetation zone width) are difficult to measure as river size increases. All 12 parameters are recorded when conducting habitat assessments in semi-wadeable rivers, but instream parameters such as instream cover, epifaunal substrate, and embeddedness are the most reliable habitat indicators. These three instream habitat measurements can be summed to provide a possible range of 0 (indicating worst possible instream conditions) to 60 (indicating best possible instream conditions) points. Instream habitat totals that score 30 or less are an indication of instream habitat impairment.

Qualitative Method for Multihabitat/Low Gradient Streams and Rivers

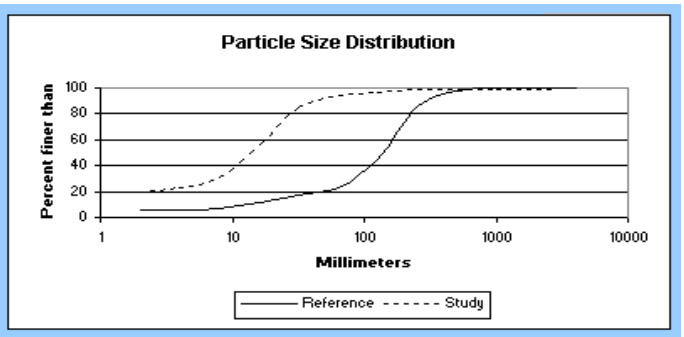
The threshold for ALU assessment impairment threshold for qualitative physical habitat of multihabitat/low gradient wadeable streams and rivers is 105 or less. Certain instream and riparian area habitat parameters are strong predictors of habitat degradation leading to ALU impairment and as a result these parameters alone warrant independent assessment decisions. These parameters are pool substrate characterization, sediment deposition, bank stability and bank vegetative protection. The impairment threshold for the parameters of pool substrate characterization + sediment deposition or bank stability + bank vegetative protection is a total score of 20 or less for either combination.

Quantitative Method for Stormwater Impacted Habitat

For stormwater-impacted sites where a pebble count analysis was conducted, collected data are plotted on graph paper or entered into Microsoft Excel spreadsheets and plotted electronically (Figure 1), as cumulative percentages for both reference and study streams. Particles 8 mm or smaller are of primary concern since they should have the most biological significance and are most likely to smother macroinvertebrate and fish spawning habitat. Reference streams should have no more than 15 percent of particles smaller than 8 mm. Impaired reaches, in general, are study streams with $\geq$35 percent of particles smaller than 8 mm. This threshold may be higher for certain types of streams, such as those with low gradient (Bevenger and King 1995).

Cumulative Distribution		
Class	Reference	Study
2	6	20
4	6	23
8	7	32
16	12	57
32	18	86
64	23	94
128	45	97
256	87	99
512	98	99
1024	100	99
2048	100	99
4096	100	100

Histogram		
Class	Reference	Study
2	6	20
4	0	3
8	1	9
16	5	25
32	6	29
64	5	9
128	23	3
256	42	2
512	11	0
1024	3	0
2048	0	0
4096	0	2



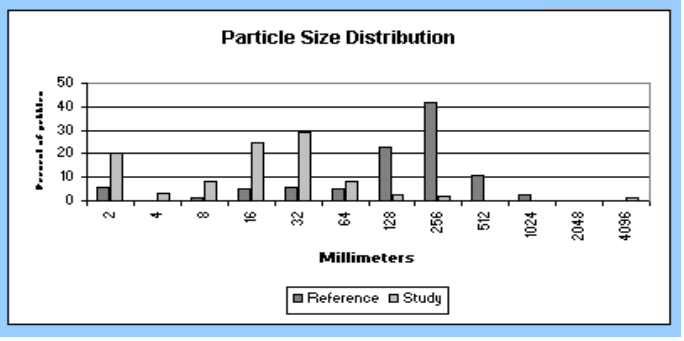


Figure 1. Example analysis of pebble count data.

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CHAPTER 5 ASSESSMENT DETERMINATION AND DELISTING METHODS

Chapter 5 Assessment Determination and Delisting Methods

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ASSESSMENT DETERMINATIONS

To meet the objective of creating accurate and precise determinations using the methods detailed above, DEP's assessments are conducted on a segment-by-segment basis of the National Hydrologic Dataset (NHD) flowline layer in a DEP Geographic Information System (GIS) application. Unlike most states that assess whole watersheds probabilistically, DEP conducts a statewide census primarily using targeted monitoring to identify individual stream reaches as attaining or impaired. This results in more detailed and accurate assessments of the waterbody, significantly reduces the need to revisit sites, and allows DEP to focus resources on only those segments of a waterbody that are not meeting applicable WQS.

Independent Applicability

The assessment methods detailed in Chapters 2–4 constitute the current "decision rules" DEP uses when making assessments. These methods are understood to be independently applicable when making assessment determinations. This is based on USEPA guidance, which says that all data must be evaluated on a stand-alone basis unless there is reason to place doubt on the quality of the data (USEPA 2002). An example of an exception to the independent applicability policy is with discrete and continuous physicochemical sampling methodologies for parameters that can be measured by both methods (e.g., pH, dissolved oxygen). Given that continuous datasets are more temporally comprehensive than discrete datasets, continuous datasets can be used to reassess or delist assessment determinations that were based on discrete datasets. Because of the ability to better capture important daily and seasonal variations with a continuous dataset, it is not recommended to use discrete data to reassess or delist a stream that was initially assessed with a continuous dataset. Continuous datasets are preferred for parameters where this technology is available.

Outside Data

In addition to the data DEP collects, DEP readily accepts and values all data from outside agencies and the public for use in making assessments. However, different data types and levels of quality assurance determine how those data can be used. DEP's tiered data acceptance strategies follow the same general tiered framework as described by the Chesapeake Bay Monitoring Cooperative's Prioritization Report (Chesapeake Bay Monitoring Cooperative 2017). Tier 1 data is generally defined as educational or environmental screening data that has known quality and a study plan, but does not follow DEP or USEPA quality assurance plans. These data will not be used for assessment determination purposes, but can be used by DEP to highlight areas of interest for future monitoring efforts. Tier 2 data have clearly defined quality assurance plans and procedures, but may not have followed DEP monitoring protocols established

in the Monitoring Book (Lookenbill and Whiteash 2021). These data may not be used for assessment determination purposes but can be used for other purposes such as trend or performance analysis. Tier 3 data are assessment level data that have approved quality assurance plans, follow appropriate study designs, and follow DEP monitoring protocols (Lookenbill and Whiteash 2021). Individuals seeking to provide DEP with Tier 3 data should also be trained and audited by DEP staff before submitting data.

Some interstate surface waters of Pennsylvania have water quality regulation through compact commissions. These waters are comprised of the Ohio River and Delaware River mainstems. The Ohio River Valley Water Sanitation Commission (ORSANCO) and the Delaware River Basin Commission (DRBC) have established methodology, in consultation with DEP and other compact states, to assess the attainment of WQS in compliance with CWA Section 305(b) and provide those results to the states and USEPA. DEP reviews these data and results to make appropriate assessments for both Section 303(d) and 305(b) in the Integrated Report. These assessments apply to the protected uses of the Ohio River from the confluence of the Allegheny and Monongahela Rivers to the PA/WV state line and for the West Branch Delaware River at the PA/NY state line and the mainstem Delaware River from the confluence of the East and West Branches through the Delaware estuary to the PA/DE state line.

Sample Design Considerations

Thoughtful study design and execution are critical to assuring water quality sampling efforts provide the information necessary to make assessment decisions. More information on acceptable sampling design procedures are found in DEP monitoring protocols (Lookenbill and Whiteash 2021). For assessment determination purposes, DEP utilizes both targeted and probabilistic sampling designs. However, DEP believes the targeted “judgment-based” sampling design is the most suited method to assess WQS and uses. Targeting sampling not only focuses in on sources and causes of potential impairment, it also delimits the spatial effect of the impact. This translates into more accurate assessments. In addition, properly implemented targeted sampling provides information that is necessary if a TMDL is developed. Probabilistic sampling designs can also be useful for assessment determination purposes, especially when waterbodies lack significant environmental stress or are rather homogeneous in land use. In these cases, probabilistic sampling can provide accurate information without overextending resources. When a probabilistic sampling design is employed, statistical analysis is conducted to determine miles of attaining and impaired stream miles. The results are then translated into assessment units for the Integrated Report. If probabilistic results return a significant mix of assessment decisions, then the watershed may be revisited using a targeted sampling design to obtain more detailed information for assessments.

Requirements for Making Assessments

Assessments will be completed with data that have been collected using appropriate sampling design, see the Monitoring Book (Lookenbill and Whiteash 2021). Sampling sites and locations are positioned to account for changes in water quality due to influences such as major tributaries, point and nonpoint source impacts, land use changes, soil characteristics, and geology. Additional samples are collected at the limits of these changes to effectively "bracket" potential sources of water quality differences. The minimum length of any assessment unit is typically ½ mile. Any assessment unit less than ½ mile may be considered a localized impact and likely will not be reported in the Integrated Report. There is no set maximum assessment unit length; however, the size is limited by the DEP GIS application to efficiently save and return results from the database. Approximately 55 segments of the NHD flowline is recommended as a maximum assessment unit length to avoid GIS application issues.

Decision Framework

DEP will implement the following framework when evaluating monitoring data in the use assessment decision process. The details of this appraisal process may vary from application to application based on the unique characteristics and contexts of each situation. However, DEP will follow this process as often as possible to maintain consistency in the use assessment decision process and so that interested stakeholders can clearly see how DEP evaluates data for assessments. The decision framework aims to document and communicate each step of the decision process in a clear, consistent manner addressing the study designs, data quality, data analysis, assumptions, uncertainties, and consequences associated with each use assessment decision. DEP attempts to be as concise as possible within this framework while not compromising adequate discussion of critical issues influencing the decisions.

(1) **Describe monitoring effort.** Describe the waterbody and the watershed, including basin size, land uses, geologies, and other characteristics. Discuss any germane history and context pertaining to the monitoring effort. To the extent possible, describe the motivations and intentions of the monitoring effort, including the individuals and organizations involved as well as the intended use of the information collected. Clearly state study goals. Describe and map monitoring locations. Include any relevant photographs.

(2) **Check data quality.** Evaluate any study plans and objectives, including sampling plan design details such as recordkeeping, data management,

training, sampling techniques, and analytical methods. Check data for typos and other anomalies. Document non-detects and censored data.

(3) Gather information on likely sources of variation. At a minimum, this information will typically include characterization – and quantification where possible – of tributary locations, upstream discharges, geologies, and land uses. Potential sources of this information include stream gages, climatological records, and discharge monitoring reports. Include maps, figures, and diagrams as needed. Discuss relevant physical, chemical, and biological processes and other potential sources of variation for the parameter(s) of concern. Address context-specific considerations (e.g., dams).

(4) Explore data. Perform various graphical analyses (e.g., histograms, probability distribution functions, boxplots, time-series plots, scatterplots with likely sources of variation, LOWESS) to visually explore and illustrate data characteristics. Document summary statistics (e.g., minimum, maximum, mean, median, standard deviation).

(5) Evaluate data representativeness. Evaluate how representative samples are of unmonitored conditions, mindful of the sampling plan design (e.g., sample collection frequency, locations, timing, targeting) and the likely sources of variation with special attention to any critical sampling times and locations. Consider if the system is likely to be spatially well-mixed at monitoring location(s) and how quickly conditions are likely to change in time.

(6) Describe the relevant standards. Identify which criteria are being evaluated and the uses to which they apply. Describe how the parameters of concern impact the protected use (i.e., exposure pathways, detrimental effects) being assessed. Review the associated regulatory language including any relevant criterion rationale documentation.

(7) Apply appropriate analytical procedures. Select and apply appropriate analytical techniques, mindful of the sampling plan design, monitoring objectives, and the relevant criteria, parameters, and context. State and verify any assumptions associated with each analytical technique. Evaluate decision error rates, if applicable. For hypothesis tests, evaluate null hypothesis choice. Discuss the magnitude, duration, and frequency of relevant criteria digressions, excursions, and/or exceedances.

(8) Consider other sources of relevant use assessment information. Additional sources of information may include: previous or concurrent monitoring efforts; data from water supply intakes; biological surveys; and discharge monitoring reports.

(9) Evaluate all relevant lines of evidence. Bring together the previous steps into a narrative that addresses contextual data interpretations, possible counter arguments, alternative decision choices, and decision consequences, including evaluation of decision error consequences. Explicitly address any policy ramifications if applicable.

(10) Decide. Decide what to do with the dataset and waterbody in question. At a minimum, each decision will include placing the waterbody in one of the Integrated Report categories.

Natural Conditions Exception

Natural quality is defined in 25 Pa. Code § 93.1 as "*The water quality conditions that exist or that would reasonably be expected to exist in the absence of human related activity.*" In accordance with the provisions of Pennsylvania's WQS, waters that have naturally occurring pollutant concentrations, or "natural quality," that prevent the attainment of an established use will not be assessed as impaired, if it can be demonstrated that anthropogenic sources do not cause or contribute to the non-attainment and the pollutant(s) of concern are generated by natural processes.

Reassessment of Previously Assessed Waters

DEP completed the first statewide ALU assessment of wadeable waters through the Statewide Surface Water Assessment Program (SSWAP) in 2006. The current assessment methodology is more rigorous than the SSWAP method and, as a result, the reassessment of waters is a high priority. The goal is to focus on reassessing areas where conditions have likely changed, or it is believed the water may have been listed in error. Additional reasons to reassess include confirmation of the original source and cause determination and collection of additional data necessary for TMDL development or alternative restoration plans. Following implementation of the TMDL targets and other restoration plans, reassessment should occur after sufficient time has passed to allow for recovery. In general, reassessment following implementation should occur five years after restoration activities have been completed, and if full restoration to WQS has not occurred, reassessment should occur at five-year intervals. It must also be noted that a high priority is placed on assessing the waters that have not yet been assessed any

use. Most of these waters are on large rivers where applicable biological assessment methods have not yet been developed.

INTEGRATED REPORT CATEGORY ASSIGNMENT

Chapter 1 introduced and described the Integrated Report Categories. This section describes the assignment of a waterbody segment to one of the Categories based upon the results of the assessment. Categories 1 and 2 are for waters attaining protected uses. Waterbody segments that have been assessed and are attaining all uses are assigned to Category 1. Waterbody segments that have been assessed and are attaining at least one use are assigned to Category 2. Category 3 is reserved for waters that are not assessed for any uses due to insufficient information to complete an assessment.

Impaired waters are assigned to Category 4 or 5. Waters assigned to Category 4 are impaired for one or more uses; however, these waters do not require a TMDL to be developed. Category 4 is comprised of 3 subcategories: 1) Category 4a applies when a TMDL has been completed and approved by USEPA; 2) Category 4b applies when a use impairment caused by a point source pollutant is being addressed by the state through other pollution control requirements and a schedule of compliance; and 3) Category 4c applies when a use is impaired, but the impairment is not caused by a pollutant (i.e., Flow Alterations, Habitat Modification, Water/Flow Variability, and Filling and Draining).

Waters assigned to Category 5 are impaired by pollutants for one or more uses and require the development of a TMDL. Category 5 has one subcategory, 5alt, that is comprised of waters that have been identified for water quality restoration through an alternative approach before a TMDL is completed.

DELISTING AND REMOVAL OF CAUSES FROM IMPAIRED CATEGORIES

Any removal of a pollutant on the 303(d) list (Category 5) is considered a “delisting” and is subject to USEPA review and approval. Delistings must come with reasoning and data to support the change. Removal of a cause of impairment from Category 4 is not strictly considered a “delisting” and is not required to be reviewed and approved by USEPA; however, DEP provides this information within the delisting documentation of each Integrated Report for transparency purposes.

Delisting Reasons

There are multiple reasons to remove a cause of impairment from a waterbody (Table 1). When conditions improve in impaired waters it is possible to remove a cause or causes of impairment from the impaired Categories (Category 4 or 5). In addition, if a cause of impairment is no longer appropriate, it can be removed despite the waterbody remaining impaired for other sources or causes. A delisting reason from Table 1 must be assigned to each cause that is being delisted. The refinement reason should only be used when clarifying the metals cause to a more specific metal, when specifying pH as high or low, and when removing cause unknown.

Table 1. USEPA Delisting Reasons

Delisted "Cause" Reason		Description
1	WQS_NEW_DATA	Applicable WQS attained; based on new data
2	WQS_RESTORATION_ACTIVITIES	Applicable WQS attained, due to restoration activities
3	WQS_LISTING_INCORRECT	Applicable WQS attained; original basis for listing was incorrect
4	WQS_STANDARDS_CHANGED	Applicable WQS attained, due to change in WQS
5	REFINEMENT	Clarification of listing cause
6	WQS_NEW_ASMT_METHOD	Applicable WQS attained, according to new assessment method
7	DELISTING_WQS_NOT_APPLICABLE	WQS no longer applicable
8	DELISTING_ORIG_INCORRECT	Data and/or information lacking to determine WQ status; original basis for listing was incorrect

Delisting Requirements

It takes the same or greater level of data rigor to delist a cause as it does to make the impairment determination. This documentation could include one year of Discharge Monitoring Reports (DMRs) with data showing the assessed use is meeting criteria, or data showing there is a different cause for the impairment.

To justify reasons 1-6 in Table 1, an assessment must be conducted to show the waterbody is now meeting criteria or that the cause has been appropriately refined. The data requirements to demonstrate these improvements are found in Table 2. The applicable data and a detailed map displaying the waterbody must be provided to DEP. Appendix B contains an example map of a stream delisting and details the information that should be depicted on the map. For ALU assessments, the station(s) and the new attaining index score(s) must be displayed. Recreational use assessments should show the station(s) on the map and display the attaining geometric mean(s). If an assessment is based on chemistry, the data showing attainment must be provided. Any other pertinent information or data to justify the delisting should also be provided.

Table 2. Data Requirements for Delisting 303(d) Waters

Assessed Use	Delisting Data Requirements
Aquatic Life - macroinvertebrate	Aquatic macroinvertebrate data, collected using DEP data collection protocols (Lookenbill and Whiteash 2021), that generates an IBI score above the attainment benchmark set by the sampling protocol. Multiple stations are required to bracket land use changes, nonpoint and point source influences, and any other influences that could affect water quality within the potential delisted waterbody.
Aquatic Life - chemistry	Chemistry results must demonstrate that the applicable criterion is being met as set forth in 25 Pa. Chemistry data used to delist must have been collected more recently than, and have been collected as frequent or more frequently than the data used to list the waterbody.
Recreation	The geometric mean of 5 consecutive samples collected on different days during a 30-day period must be below the criterion: during the swimming season, for *Escherichia coli*, a maximum 126 cfu/100 ml, and no greater than a 10% digression of 410 cfu/100 ml (for the same samples collected in the same 30-day duration interval); and during the remainder of the year, for fecal coliforms, 2,000 cfu/100 ml, as described in the Bacteriological Assessment Method for recreational use section of this book.
Potable Water Supply	Sampling should target the critical period when criteria digressions or excursions are expected. Samples must be collected at the point of withdrawal prior to the treatment process. Results must demonstrate that the applicable criterion is being met 99% of the time as set forth in 25 Pa. Code Chapters 93 and 96.
Fish Consumption	Fish tissue results showing the improved contamination level and the recommended fish advisory change.

Delisting Clarifications

Category 4b

Moving a pollutant from Category 5 to Category 4b (i.e., delisting) requires additional documentation that must be provided at the time of the assessment determination. According to USEPA's 2006 Integrated Report guidance document, DEP must document that the six following elements are addressed for a 4b delisting to be approved:

1. Identification of segment and statement of problem causing the impairment;
2. Description of pollution controls and how they will achieve water quality standards;
3. An estimate or projection of the time when WQS will be met;
4. Schedule for implementing pollution controls;
5. Monitoring plan to track effectiveness of pollution controls; and
6. Commitment to revise pollution controls, as necessary.

Details on each of the six elements are provided within USEPA's 2006 Integrated Report guidance document. It is important to note that certain Consent Order Agreements may also meet the six elements and can be used for approval.

To move a waterbody from Category 4b to either Category 1 or 2, documentation must be provided showing the facility is in compliance with their permit conditions and/or their discharge is no longer the cause of impairment.

Cause and Listing Date Refinement

The cause listing date is the year a cause of impairment is first reported on the Integrated Report. Each cause of impairment has its own listing date, or "Date First Listed". This information allows USEPA to track how long it takes TMDLs to be generated after a pollutant is placed on the 303(d) list. It is also useful information for other causes placed on Category 4c.

In most reassessment cases involving the same causes the listing date is carried over; however, if an existing TMDL does not address the new impairment (e.g., a new source of the pollutant enters the watershed that the TMDL does not cover), DEP may choose to create a new listing date (Figure 1). For example, if low pH from Acid Mine Discharges was a cause of impairment first listed in 2002, but a new assessment completed in 2021 determines that the existing TMDL (addressing only Acid Mine Discharges on a portion of the watershed) does not address the new source (e.g., Atmospheric Deposition), then the listing date for pH would be 2022. In this example, the new listing date acknowledges that the nature of the cause is now different and an existing TMDL does not cover the new impairment.

Given the previous example, it may be concluded that a cause of impairment can have multiple listing dates because of multiple sources; however, only one listing date can be assigned to a cause. This is due to the way the USEPA tracks listing date information through the Assessment, Total Maximum Daily Load (TMDL) Tracking and Implementation System (ATTAINS). In cases where a cause may have multiple sources, the oldest listing must be retained regardless of whether a new source (even if it is not covered by a TMDL) is discovered. For example, if Organic Enrichment was first listed in 2002 with a source of “Dam or Impoundment”, but a reassessment in 2021 determines a newly discovered source, “Combined Sewer Overflow”, is also contributing to the Organic Enrichment impairment, the listing date for Organic Enrichment would remain as 2002.

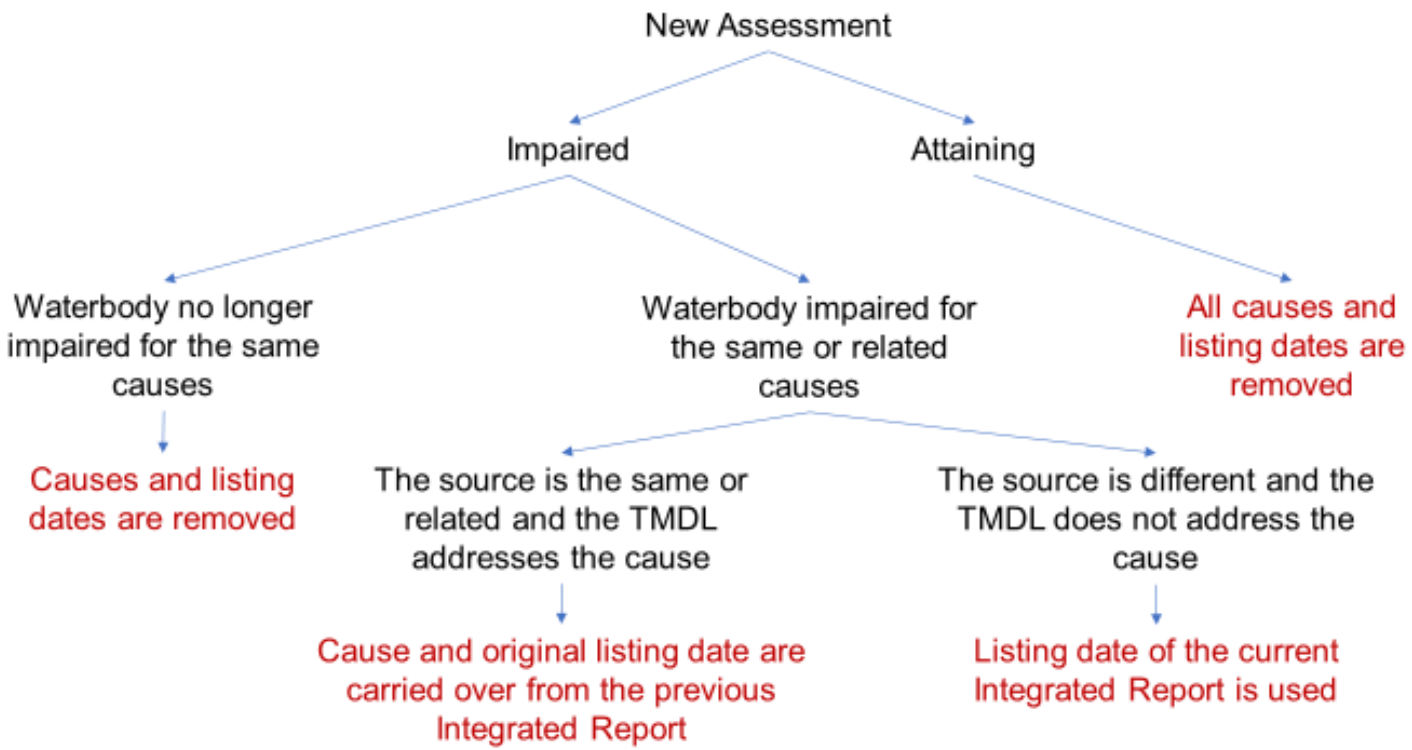

Figure 1. Cause and listing date decision process for reassessments.

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CHAPTER 6 SOURCE AND CAUSE DETERMINATION METHODS

6.1 GENERAL SOURCE AND CAUSE DETERMINATION METHODS

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INTRODUCTION

Once it is determined through the assessment determination process (Chapter 5) that one or more uses are impaired, the next steps are to identify the source and cause of the impairment. Section 303(d) of the CWA and 40 CFR § 130.7 (as it relates to total maximum daily loads and individual water quality-based effluent limitations) requires listing those waters impaired by pollutants that will not achieve WQS after the application of technology-based effluent limitations, of more stringent effluent limitations required by state requirements, and of other pollution control requirements (e.g., best management practices). This is an important component of the decision process as source and cause determinations distinguish waters that require the development of a TMDL versus waters that require some other method of restoration.

Sources of impairment can be divided into two general categories, point and non-point sources. In general, point sources are discharges from pipes or discrete conveyances. A “point source discharge” is defined in 25 Pa. Code § 93.1 as a pollutant source regulated under the National Pollution Discharge Elimination System (NPDES). Section 93.1 defines a “nonpoint source” as a pollution source which is not a point source discharge. An example of a nonpoint source is unconsolidated runoff coming over the land surface.

As with sources, causes of impairment can be assigned to two general categories: pollutant and pollution. “Pollutant” is defined in 25 Pa. Code Section 92a.2 as a contaminant or other alteration of the physical, chemical, biological or radiological integrity of surface water that causes or has the potential to cause pollution as defined in section 1 of The Clean Streams Law (35 P.S. §681.1). Examples of pollutants are substances such as iron, pesticides, pathogens, or sediment that prevent the attainment of uses. For the purpose of listing waters pursuant to section 303(d) of the CWA, “pollution” is described as habitat modifications and impacts related to water volume and/or flow. Any water impaired by a pollutant, and listed in Category 5, requires the development of a TMDL. Waters impaired by pollution do not require a TMDL and may be restored through other restoration methods.

GENERAL SOURCE AND CAUSE DETERMINATION METHOD

Source

The method to determine source of impairment relies heavily on a thorough reconnaissance and knowledge of the watershed that is being assessed. Prior to monitoring of the watershed, the investigator compiles all known point and nonpoint sources of pollution. Field reconnaissance should be conducted in addition to a desktop

reconnaissance of aerial photography to identify any potential sources of pollution. This information is then used in conjunction with sampling locations and data to assign the most probable source of the impairment. A full list of potential sources is provided in Appendix A.

Cause

Most causes of impairment can be determined in a similar manner as the source determination, thorough reconnaissance and knowledge of the watershed coupled with knowledge of ecological and biological responses to pollution and the applicable narrative and numeric water quality criteria. The causal identification process includes identifying all probable causes, evaluation of biological, physical, and chemical data and the observed response in the stream. Many causes of impairment will be obvious to the investigator such as excess sediment causing siltation impairments or metals precipitate covering the stream bed. Chemical impairments are determined through the analysis and evaluation of discrete and continuous water chemistry data and the applicable water quality criteria. A full list of potential causes and their context is provided in Appendix A.

There are instances when cause determination will require additional monitoring following specific protocols and a more structured casual identification process. This process may rely on a weight of evidence approach from multiple lines of evidence to arrive at the cause of an observed impairment. This will typically be the case when interpreting the narrative criteria at §93.6(a) and (b).

6.2 ATMOSPHERIC DEPOSITION SOURCE AND CAUSE DETERMINATION METHOD

Chapter 6 Source and Cause Determination Methods

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INTRODUCTION

This assessment method is designed to determine the source and cause of Aquatic Life Use (ALU) impairments in Pennsylvania's surface waters. Acidification was first documented in the rivers and streams of the northeastern US in the mid-20th century (Likens et al. 1972). This acidification was caused by fossil fuel combustion that resulted in elevated emissions of sulfur dioxide (SO_2), nitrogen oxides (NO_x), and ammonia (NH_3) that are deposited to the earth's surface in the form of wet and dry deposition (Driscoll et al. 2001), which is known as atmospheric deposition. Due to interaction with carbon dioxide in the atmosphere, the normal pH of rainwater is 5.7 (Likens et al. 1972). However, the pH of precipitation in PA during the mid-20th century could be as low as 4.0 and contain elevated concentrations of sulfate (SO_4) and nitrate (NO_3) (DeWalle et al. 1983).

One of the most significant ecological effects of atmospheric deposition is decreased buffering capacity and pH of streams in acid-sensitive watersheds that are typically small (i.e., headwaters), mountainous, forested, and infertile (Herlihy 1993). Decreased pH results in the mobilization of aluminum from soil and a shift in the form of aluminum in water from nontoxic organic forms to highly toxic inorganic forms (Mason and Seip 1985). Atmospheric deposition can result in chronic acidification, where streams exhibit depressed pH year-round. Alternatively, episodic acidification can result in streams with near-neutral pH during baseflow, experiencing substantial decreases in pH and increased concentrations of dissolved aluminum during snowmelt or high streamflow events. (Driscoll et al. 2001, Driscoll and Wang 2019).

Atmospheric deposition impairment is difficult to detect using only biological assessment methods, particularly when the impairment is due to episodic acidification. Small, forested, headwater streams with low alkalinity are generally unproductive. Low numbers of benthic macroinvertebrates and fishes with relatively low diversity are frequently observed in these types of streams.

Macroinvertebrate metrics provide only an indirect indication of potential atmospheric deposition impairment. Macroinvertebrates in acidified streams are generally sensitive to organic pollution, so the benthic community will normally be dominated by taxa with low Hilsenhoff scores. Additionally, macroinvertebrates are able to recolonize stream reaches due to the short time between successive generations and adult life-stages capable of flight. Assuming that no major component of the benthic community is missing (e.g., mayflies), the biological assessment method may lead to the potentially erroneous conclusion of no biological impairment. However, when abundance and diversity are obviously low and community composition is abnormal (e.gl, < 3 mayfly

taxa, < 5% mayfly individuals, and > 25% Leuctra and/or Amphinemura stoneflies), macroinvertebrate assessment methods can support a decision of biological impairment due to atmospheric deposition (Shull 2017) when water chemistry measurements are supportive.

Fish communities provide more reliable evidence of atmospheric deposition effects due to their longer life spans. A fish community may slowly decline as year classes are lost to episodic acidification and sensitive species are eliminated from a given stream reach. Fish communities represented by fewer than 50 individuals and only one species (or family for salmonids) often indicates acidified conditions (Wertz 2021). Many non-salmonid species found in cold water environments have a lower tolerance to acidity and associated effects (Johnson et al. 1987, Baker et al. 1996). However, atmospheric deposition impacted streams that are located near healthy stream reaches may be re-colonized and conditions during the time of sampling may be misleading.

To definitively associate ALU impairment with an atmospheric deposition source determination, a water chemistry sample during a critical period (snowmelt or high streamflow event) must be associated with a biological sample. Water chemistry measurements during summer baseflow conditions will document the low alkalinity but may fail to detect a low pH or high dissolved aluminum event. Conversely, chemistry sampling during critical periods can reveal acutely toxic conditions that limit biological communities.

When these conditions are not observed and an atmospheric deposition impairment is suspected, a more detailed investigation may be warranted to conclusively identify an atmospheric deposition problem. Other evidence that may also trigger a detailed follow-up survey would include anecdotal information indicating a decline in a fishery; cessation of trout stocking by PFBC due to poor survival; and fisheries data documenting population changes and species loss over time.

ATMOSPHERIC DEPOSITION SOURCE AND CAUSE DETERMINATION

After biological sampling represents fish (fewer than 50 individuals and only one species/family) or macroinvertebrate (< 3 mayfly taxa, < 5% mayfly individuals, and > 25% Leuctra and/or Amphinemura stoneflies) communities typical of atmospheric deposition impacts, the best way to document atmospheric deposition impairment is to collect water samples during spring snowmelt or storm events that document conditions known to be acutely toxic. The most critical measurements are pH and dissolved aluminum. Low pH and high concentrations of dissolved aluminum have been linked to high fish mortality in studies of episodic acidification (Fiss and Carline 1993, Sharpe and

Drohan 1999). Dissolved inorganic monomeric aluminum is the aluminum species most strongly correlated to fish mortality, but analysis for this form of aluminum is more complicated than for the more traditional “total dissolved aluminum” concentration. Total dissolved aluminum concentrations obtained via the standard method of field filtration through a 0.45 µm filter are only weakly correlated with lethal response in fish, and are of limited value for identifying impairment due to acidification. An alternate dissolved aluminum analysis that correlates well with inorganic monomeric aluminum concentrations and is useful for identifying atmospheric deposition impairment is one conducted on water samples filtered through a 0.1 µm filter (Van Sickle et al. 1996).

Follow-up sampling to detect atmospheric deposition impairment should be concentrated during storm events and periods of heavy snowmelt. Ideally, water samples should be collected during peak flows to characterize worst-case conditions. Grab samples collected during high flow events should be adequate for most follow-up surveys. A low flow sample may be collected for comparison, but is not necessary. Standard Analysis Code 122 (SAC 122) has been established for use when investigating potential atmospheric deposition problems. The analyses conducted as part of SAC 122 are listed in Table 1. The most important parameters for identifying atmospheric deposition impairment are pH and dissolved monomeric aluminum concentrations (with 0.1 µm filtration). Prior to shipping the sample to the lab, a 125 ml aliquot must be filtered through a 0.1 µm filter.

If the high flow sample documents stressful conditions (i.e., low pH and high dissolved aluminum levels), then some degree of biological impairment is likely. Elevated dissolved aluminum concentrations (>150 µg/L) and low pH (<5.8) can be lethal to brook trout, depending on duration of exposure. When a stream survey documents pH depression and dissolved aluminum levels above 150µg/L (after 0.1 µm filtration), it is appropriate to consider the stream to be biologically impaired due to atmospheric deposition. For 303d list reporting purposes, pH will be the cause of impairment. Atmospheric deposition is the source of impairment.

Table 1. Analyses included under the Standard Analysis Code for atmospheric deposition samples (SAC 122).

Test Description	Units	Test Description	Units
Acidity Total	mg/L	Lithium Dissolved	µg/L
Alkalinity Total as CaCO3	mg/L	Lithium Total	µg/L
Alkalinity Bicarbonate	mg/L	Magnesium Dissolved	mg/L
Alkalinity Carbonate	mg/L	Magnesium Total	mg/L
Aluminum Dissolved	µg/L	Manganese Dissolved	µg/L
Aluminum Dissolved (Monomeric)	µg/L	Manganese Total	µg/L
Aluminum Total	µg/L	Nickel Dissolved	µg/L
Barium Total	µg/L	Nickel Total	µg/L
Boron Total	µg/L	Nitrate + Nitrite as N Dissolved	mg/L
Cadmium Dissolved	µg/L	Nitrate + Nitrite as N Total	mg/L
Calcium Dissolved	mg/L	Nitrogen Total	mg/L
Calcium Total	mg/L	Orthophosphate Dissolved	mg/L
Carbon Dioxide Total	mg/L	Orthophosphate Total	mg/L
Carbon Dissolved Organic	mg/L	Osmotic Pressure	mosm/kg
Carbon Total Organic	mg/L	pH (Field)	pH units
Chloride Total	mg/L	pH (Lab)	pH units
Copper Dissolved	µg/L	Potassium Total	mg/L
Copper Total	µg/L	Selenium Total	µg/L
Dissolved Oxygen % (Field)	%	Sodium Total	mg/L
Dissolved Oxygen (Field)	mg/L	Specific Conductance (Field)	umhos/cm
Dissolved Solids Total	mg/L	Specific Conductance (Lab)	umhos/cm
Hardness Total	mg/L	Strontium Total	µg/L
Iron Dissolved	µg/L	Sulfate Total	mg/L
Iron Total	µg/L	Temperature Water (Field)	C
Lead Dissolved	µg/L	Zinc Dissolved	µg/L
Lead Total	µg/L	Zinc Total	µg/L

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6.3 EUTROPHICATION CAUSE DETERMINATION METHOD FOR SMALL STREAMS (≤50 Mi^2 DRAINAGE AREA)

Chapter 6 Source and Cause Determination Methods

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INTRODUCTION

The USEPA describes nutrient pollution as one of America's most widespread, costly, and challenging environmental problems. Within the context of nutrient pollution of streams, the term eutrophication refers to the process by which elevated nutrient levels (especially phosphorus and nitrogen) stimulate the growth of algae and/or aquatic plants, and alters the quantity and quality of organic matter available as food for aquatic organisms. In addition to modifying the trophic structure of stream ecosystems, eutrophication can alter physical habitat conditions, stimulate the growth of toxin-producing algae, and can produce large daily (diel) fluctuations in dissolved oxygen (DO) and pH that, in some cases, fall below or rise above levels protective of aquatic life.

Over the past several years, DEP staff have collected nutrient; benthic chlorophyll-a; continuously monitored DO, pH, and water temperature; and benthic macroinvertebrate community data from small streams statewide. The technical background behind the development of the Eutrophication Cause Determination (ECD) protocol can be found in McGarrell (2018). The conceptual model shown in Figure 1 illustrates the cause/response relationships linking nutrient enrichment to stream biological integrity that was used as a framework for developing this ECD protocol. The ECD protocol provides a method for quantitatively assessing the impact of nutrient enrichment on Pennsylvania's small streams (drainage area ≤ 50 mi^2) The intended use of the ECD protocol is for determining if eutrophication is a cause of ALU impairment, under the context of nutrient enrichment, after DEP's Wadeable Freestone Riffle-Run Stream Macroinvertebrate Assessment Method indicates impairment.

The ECD protocol uses a multiple lines of evidence approach for determining if eutrophication is a cause of ALU impairment. Stream ecosystem parameters used in the protocol include: diel DO swing characteristics, water quality criteria for DO and pH, benthic chlorophyll-a concentration, diel DO swing-diel pH swing relationships, and diel DO swing- diel water temperature swing relationships. A graphical summary of the ECD Protocol is shown in (Figure 2).

THE EUTROPHICATION CAUSE DETERMINATION (ECD) PROTOCOL

Data Collection and Analysis

Baseflow (non-storm event) water column total phosphorus (TP) and total nitrogen (TN) samples are to be collected for laboratory analysis when continuous data sondes are first deployed, during each subsequent data sonde maintenance event (approximately

monthly), and when sondes are retrieved. Water column nutrient samples are to be collected and processed in accordance with Shull (2017).

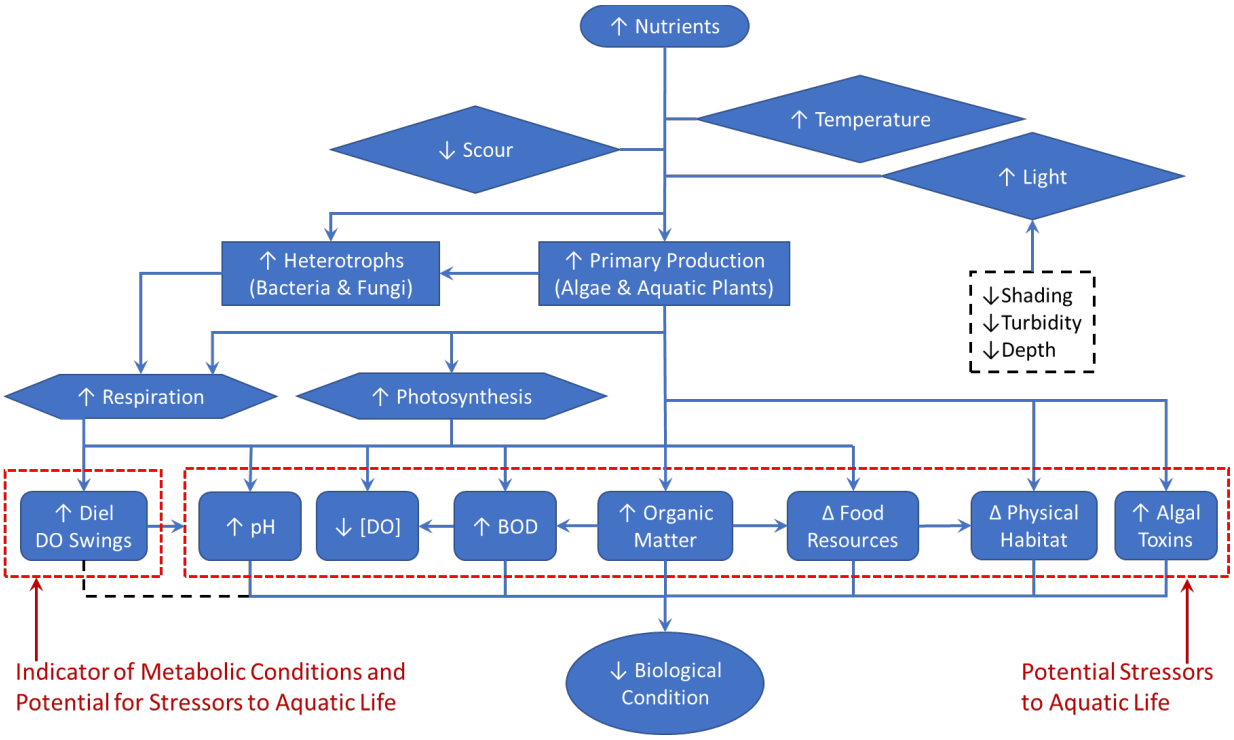


Figure 1. Conceptual model of how nutrient enrichment and eutrophication impact stream biological condition (modified from Heiskary and Bouchard 2015).

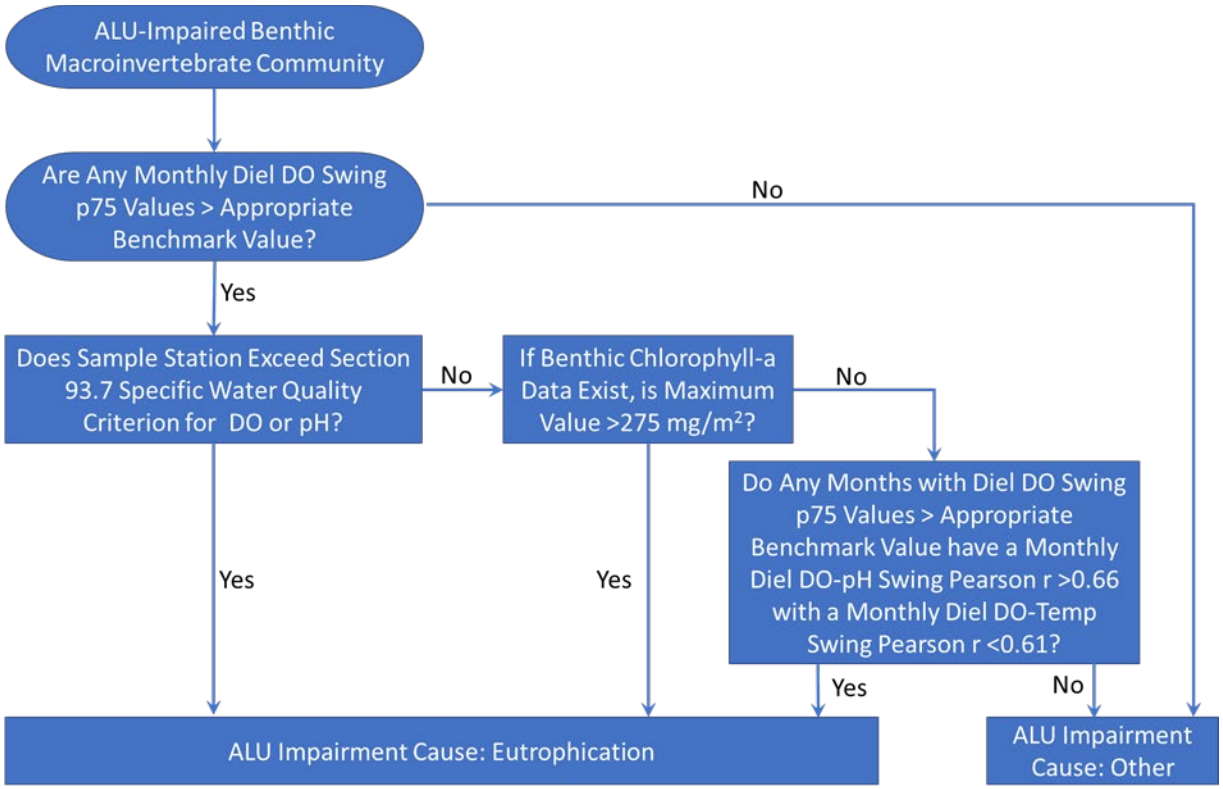


Figure 2. Graphical summary of the Eutrophication Cause Determination Protocol.

Photo-documenting or otherwise noting field observations of primary production levels (algal and/or aquatic macrophyte growth) at continuously monitored sample stations is an important part of the field data collection component of the ECD Protocol. Photographs that clearly show in-stream primary production levels should be taken on each sample station visit. At least one benthic periphyton sample should be collected at each sample station while the data sonde is deployed. Benthic periphyton samples are to be collected using DEP's Quantitative Benthic Epilithic (QBE) Periphyton Sampling Method (Butt 2017), and efforts should be made to collect samples when primary production rates appear to be relatively high, based on professional judgement and visual observations made during routine data sonde maintenance events.

Water column nutrient data and information pertaining to primary production levels can be very helpful when trying to ascertain the extent of nutrient enrichment at a specific reach of stream. In some cases, water column nutrient levels are excessively high and indicative of a nutrient-enriched system. However, some nutrient-enriched, highly productive stream reaches have very high diel DO swings that are strongly correlated with daily pH swings, but have very low water column phosphorus and nitrogen concentrations due to algal uptake of nutrients. In these cases, where elevated levels of primary production occur under seemingly low levels of nutrient enrichment, benthic chlorophyll-a concentration values and photo-documentation of excessive algal or aquatic macrophyte growth become even more important.

Continuously monitored DO, pH, and water temperature data are collected between March and October and are collected, graded, and approved for use in accordance with DEP's Continuous Physicochemical Data Collection Protocol (Hoger et al. 2017). Diel DO, pH, and water temperature swing values are calculated for days in which continuous data are collected over at least 75% of the day (e.g., a minimum of 36 readings at ½ hour intervals). Diel swing values are calculated as the difference between the maximum and minimum values recorded on a given day (Figure 3).

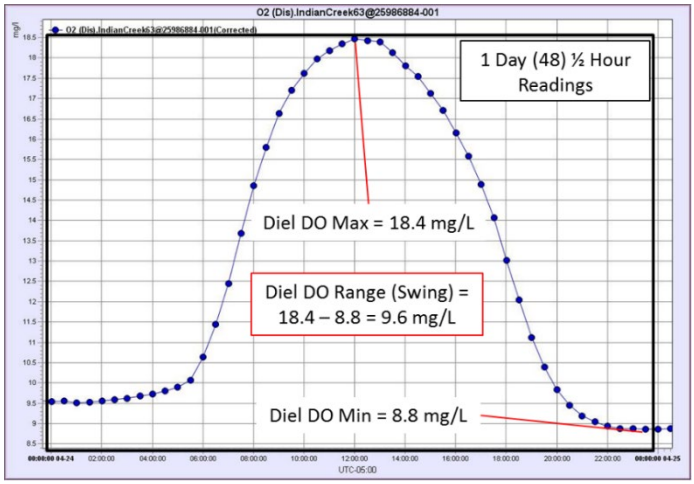


Figure 3. Graphical representation of the calculation of diel DO swing values from DO data monitored continuously over a period of 24 hours.

All useable diel DO swing values recorded within a given month are summarized using the 75th percentile (p75) value of the diel swing values recorded in that month. Diel DO swing p75 values are only generated for months that have usable diel DO swing values recorded for a minimum of 50% of days in that month. For example, if a sonde was deployed at Station X from March 1 to March 31, 2017, and yielded only 12 diel DO swing values, no p75 would be calculated for that month, because 12 days are less than 50% of the 31 days in March.

In addition to the requirement of having usable diel DO swing values recorded for a minimum of 50% of the days in a given month, a minimum of 15 pairs of diel DO-pH swing and diel DO-water temperature swing values are required for calculating monthly correlation values. Examples of how monthly diel DO swing p75 and correlation values are calculated are provided in Table 1 with results shown graphically in Figures 4 and 5.

Table 1. Example spreadsheet calculation of a monthly diel DO swing p75 value of 8.0 mg/L and monthly diel DO swing-diel pH swing and monthly diel DO swing-diel water temperature swing correlation coefficients of 0.95 and 0.14, respectively, from 31 days of data recorded at a small (drainage area ≤50 mi^2) ALU impaired stream in Physiographic Region A.

	A	B	C	D	E	F	G	H
1	Example Continuous Monitoring Data							
2	Date	Diel DO Swing (mg/L)	Diel pH Swing	Diel Water Temp Swing (C°)	Diel DO Swing p75 (mg/L)	Correlation Pairs (N)	Diel DO-pH Swing Correlation Coefficient r	Diel DO-Temp Swing Correlation Coefficient r
3	5/1/2013	7.1	1.5	4.8	8.0	31	0.95	0.14
4	5/2/2013	8.4	1.6	3.5				
5	5/3/2013	9.2	1.7	5.1	Formula in Cell E3		=PERCENTILE.INC(B3:B33,0.75)	
6	5/4/2013	9.2	1.6	1.7	Formula in Cell F3		=COUNT(B3:B33)	
7	5/5/2013	9.8	1.8	5.3	Formula in Cell G3		=CORREL(B3:B33,C3:C33)	
8	5/6/2013	9.1	1.6	4.3	Formula in Cell H3		=CORREL(B3:B33,D3:D33)	
9	5/7/2013	7.7	1.6	4.3				
10	5/8/2013	8.0	1.6	2.8				
11	5/9/2013	8.3	1.6	3.7				
12	5/10/2013	6.5	1.4	4.6				
13	5/11/2013	7.4	1.5	5.4				
14	5/12/2013	8.1	1.6	5.0				
15	5/13/2013	7.6	1.5	4.4				
16	5/14/2013	7.2	1.6	3.6				
17	5/15/2013	2.2	0.3	1.7				
18	5/16/2013	3.1	0.7	5.0				
19	5/17/2013	4.4	0.8	4.8				
20	5/18/2013	4.4	0.8	3.9				
21	5/19/2013	6.0	1.1	5.9				
22	5/20/2013	6.3	1.2	4.6				
23	5/21/2013	7.1	1.3	3.0				
24	5/22/2013	6.5	1.2	4.3				
25	5/23/2013	7.2	1.4	5.8				
26	5/24/2013	7.7	1.4	6.3				
27	5/25/2013	7.6	1.4	6.9				
28	5/26/2013	8.0	1.5	6.5				
29	5/27/2013	8.0	1.4	4.0				
30	5/28/2013	7.7	1.5	6.3				
31	5/29/2013	7.1	1.3	4.4				
32	5/30/2013	6.9	1.0	2.8				
33	5/31/2013	6.7	1.3	6.0				

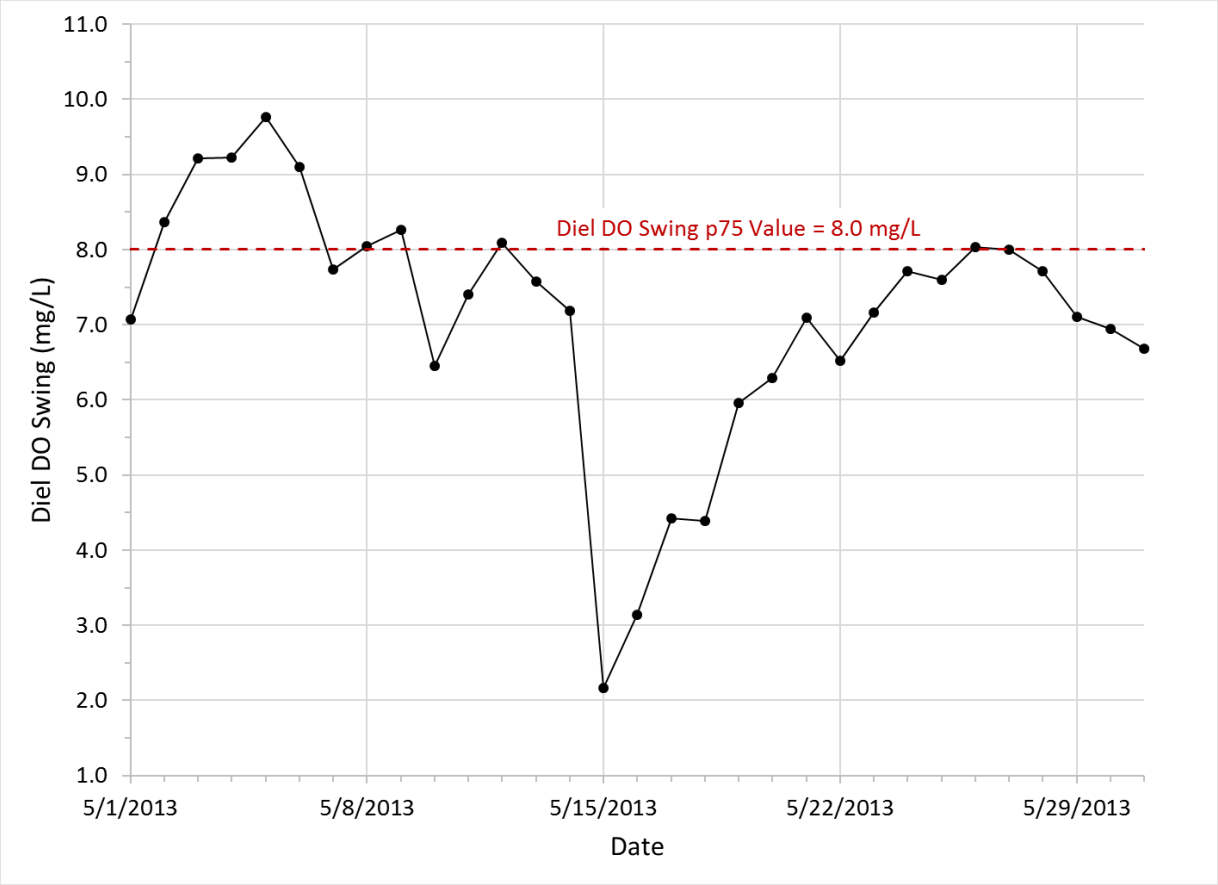
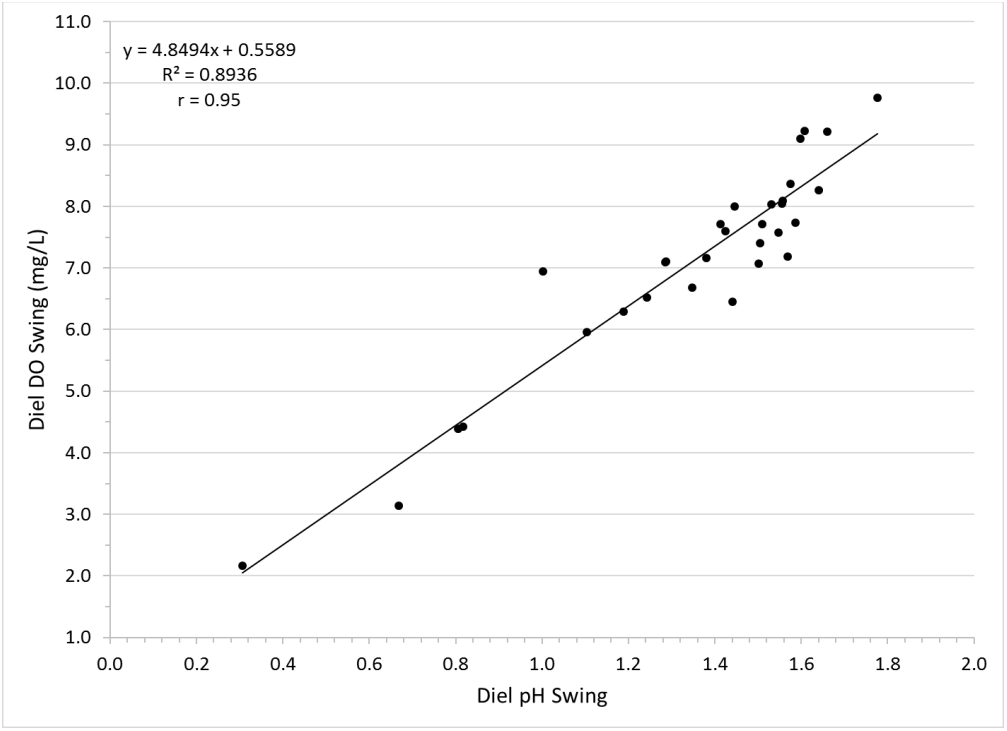
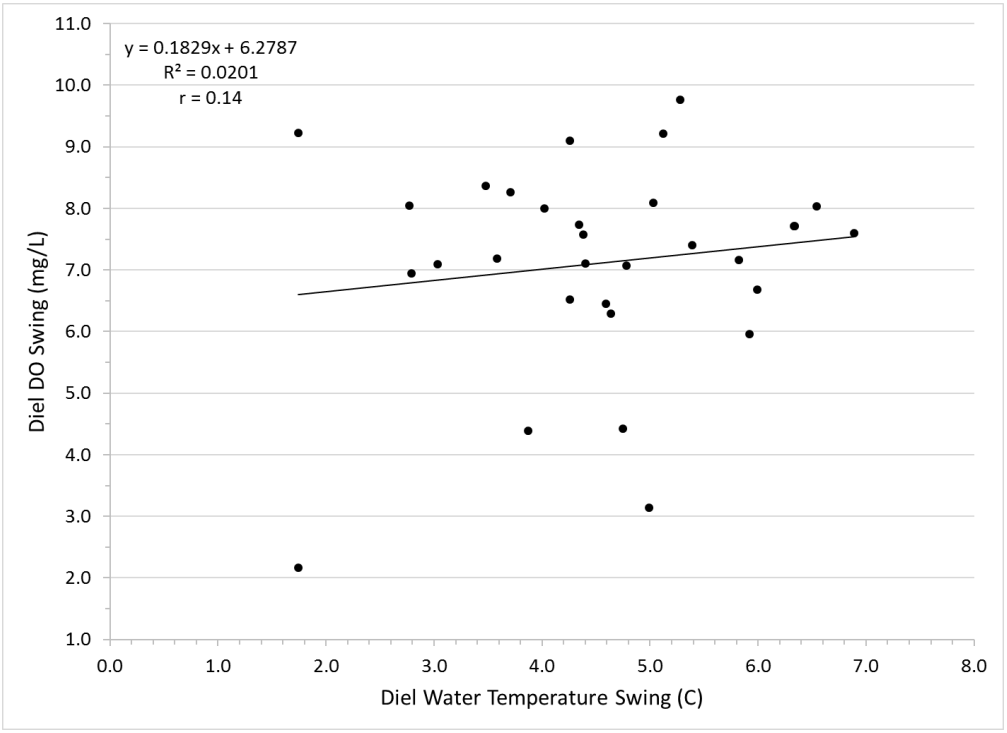


Figure 4. Graphical representation of data from Table 1 showing individual diel DO swing values and the monthly diel DO swing 75th percentile (p75) value of 8.0 mg/L.



(A)



(B)

Figure 5. Graphical representation of data from Table 1 showing (A) diel DO swing vs. diel pH swing values and corresponding monthly Pearson Correlation r-value of 0.95 and (B) diel DO swing vs. diel water temperature swing values and corresponding monthly Pearson Correlation r-value of 0.14.

Eutrophication Cause Determinations

The first step in the ECD Protocol is to determine if the ALU impaired stream is subject to excessive diel swings in DO. This is accomplished by comparing the monthly diel DO p75 values recorded at the ALU impaired stream to the benchmark values shown in Table 2. Separate diel DO swing benchmark values were developed within the context of 2-month sample periods and the Physiographic Regions shown in Figure 6.

If no monthly diel DO swing p75 values recorded at the ALU impaired stream exceed the appropriate Table 2 diel DO swing benchmark value, the cause of ALU impairment, is determined to be something other than eutrophication (Figure 2). If any monthly diel DO swing p75 value recorded at an ALU impaired stream segment exceeds the appropriate diel DO swing p75 benchmark value, eutrophication is identified as a cause of ALU impairment if:

1. The stream segment exceeds water quality criteria for DO or pH greater than 1% of the time, based on Hoger et al. (2017, Figure 2), or

2. Any benthic periphyton sample collected in the stream segment has a chlorophyll-a concentration >275 mg/m^2 (Figure 2), or

3. Any monthly diel DO swing p75 that exceeds the appropriate diel DO swing p75 benchmark value has a monthly diel DO swing-diel pH swing Pearson correlation r-value >0.66 with a monthly diel DO swing-diel water temperature swing Pearson correlation r-value <0.61 (Figure 2).

Table 2. Eutrophication Cause Determination Protocol benchmark values.

Monthly Diel DO Swing p75 Benchmark Values (mg/L)	**Physiographic Region**	
Sample Period	**A**	**B**
March-April	2.8	1.5
May-June	1.7	1.4
July-August	1.8	1.3
September-October	2.0	1.5
Maximum Benthic Chlorophyll-a Value (mg/m^2)	275	
Monthly Correlation Benchmark Values	**Pearson Correlation Coefficient (r)**	
Monthly Diel DO Swing-Diel pH Swing	>0.66	
Monthly Diel DO Swing-Diel Water Temperature Swing	<0.61	

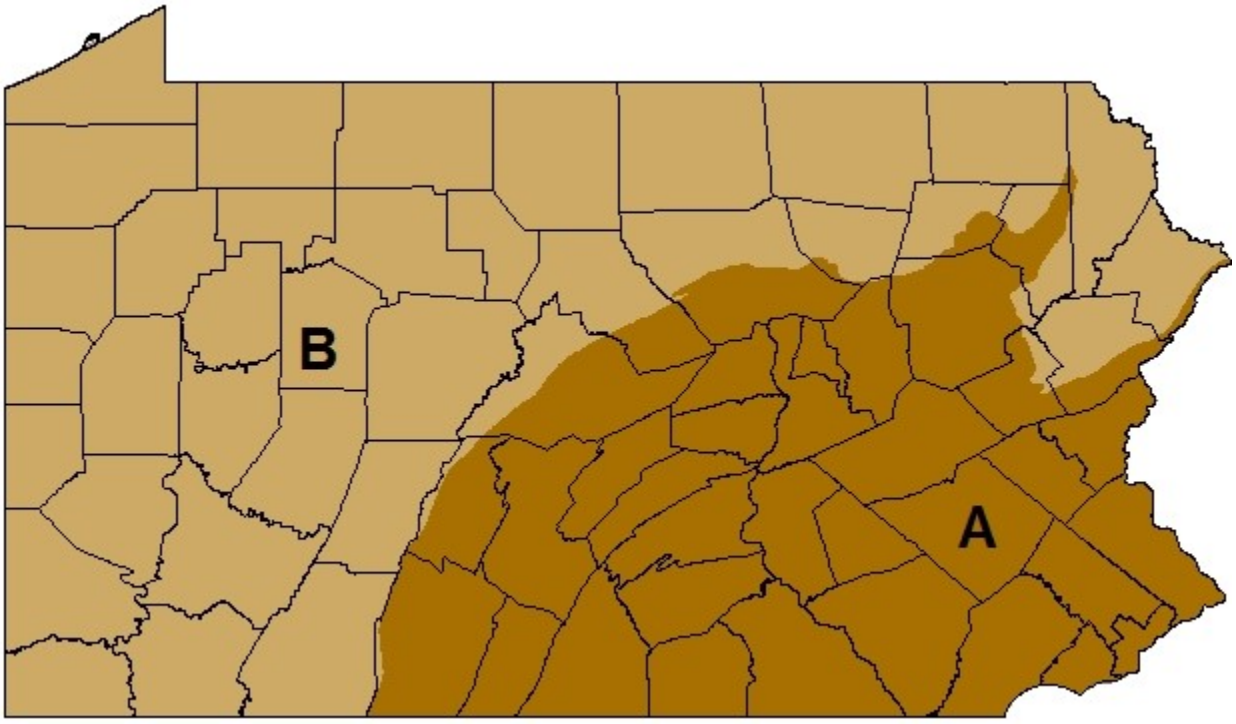


Figure 6. Eutrophication Cause Determination Protocol Physiographic Regions.

The following is an example application of the ECD Protocol to the data shown in Table 1. In this example, it is assumed that the stream segment meets water quality criteria for DO or pH and no benthic chlorophyll-a samples exceeded a concentration of 275 mg/m^2. Based on the ECD Protocol, eutrophication is identified as a cause of ALU impairment because the following conditions are met:

1. The monthly diel DO swing p75 value of 8.0 mg/L exceeds the benchmark value of 1.7 mg/L for Physiographic Region A streams during the May-June sample period, **AND**

2. The monthly diel DO-pH swing correlation r-value of 0.95 is >0.66, **AND**

3. The monthly diel DO-water temperature swing correlation r-value of 0.14 is <0.61.

In the example above, ECD Protocol results indicate the sample station has excessively high diel DO swings. Furthermore, the strong correlation between diel DO swings and diel pH swings, in conjunction with a weak correlation between diel DO swings and diel water temperature swings, indicates the excessive diel DO swings are related to stream metabolic processes (photosynthesis and respiration rates), not the water temperature conditions of the stream.

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APPENDIX A: SOURCES AND CAUSES

SOURCES

Below details the current list of available impairment sources allowable by the USEPA.

SOURCE CODE	SOURCE DESCRIPTION
110	ABOVE GROUND STORAGE TANK LEAKS (TANK FARMS)
700	ACCIDENTAL RELEASE/SPILL
5800	ACID MINE DRAINAGE
1100	AGRICULTURAL RETURN FLOWS
1150	AGRICULTURAL WATER DIVERSION
1000	AGRICULTURE
8150	AIRPORTS
1600	ANIMAL FEEDING OPERATIONS (NPS)
1200	ANIMAL HOLDING/MANAGEMENT AREAS
1250	ANIMAL SHOWS AND RACETRACKS
1300	AQUACULTURE (NOT PERMITTED)
1400	AQUACULTURE (PERMITTED)
8100	ATMOSPHERIC DEPOSITION
1450	AUCTION BARNS
500	BALLAST WATER RELEASES
505	BARGE CANAL IMPACTS
7150	BASEFLOW DEPLETION FROM GROUNDWATER WITHDRAWALS
6400	BROWNFIELD (NON-NPL) SITES
510	CARGO LOADING/UNLOADING
6450	CERCLA NPL (SUPERFUND) SITES
515	CHANGES IN TIDAL CIRCULATION/FLUSHING
7750	CHANNEL EROSION/INCISION FROM UPSTREAM HYDROMODIFICATIONS
7100	CHANNELIZATION
710	CHEMICAL LEAK/SPILL
5300	COAL MINING
5400	COAL MINING (SUBSURFACE)
5600	COAL MINING DISCHARGES (PERMITTED)
400	COMBINED SEWER OVERFLOWS
8200	COMMERCIAL DISTRICTS (INDUSTRIAL PARKS)
8250	COMMERCIAL DISTRICTS (SHOPPING/OFFICE COMPLEXES)
520	COMMERCIAL HARBOR AND PORT ACTIVITIES
1500	CONFINED ANIMAL FEEDING OPERATIONS - CAFOS (POINT SOURCE)
1550	CONFINED ANIMAL FEEDING OPERATIONS (NPS)
3000	CONSTRUCTION
3050	CONSTRUCTION STORMWATER DISCHARGE (PERMITTED)
120	COOLING WATER INTAKE STRUCTURES (IMPINGEMENT OR ENTRAINMENT)
1050	CROP PRODUCTION (CROP LAND OR DRY LAND)
7350	DAM OR IMPOUNDMENT
600	DEICING (STORAGE/APPLICATION)
6100	DISCHARGES FROM BIOSOLIDS (SLUDGE) STORAGE, APPLICATION OR DISPOSAL

7200	DREDGE MINING
7050	DREDGING (E.G., FOR NAVIGATION CHANNELS)
8050	EROSION FROM DERELICT LAND (BARREN LAND)
2300	FOREST ROADS (ROAD CONSTRUCTION AND USE)
8710	GOLF COURSES
1350	GRAZING IN RIPARIAN OR SHORELINE ZONES
7550	HABITAT MODIFICATION - OTHER THAN HYDROMODIFICATION
5700	HARDROCK MINING DISCHARGES (PERMITTED)
1750	HARVESTING/RESTORATION/RESIDUE MANAGEMENT
5150	HEAP-LEACH EXTRACTION MINING
8300	HIGHWAY/ROAD/BRIDGE RUNOFF (NON-CONSTRUCTION RELATED)
3100	HIGHWAYS, ROADS, BRIDGES, INFRASTRUCTURE (NEW CONSTRUCTION)
6150	HISTORIC BOTTOM DEPOSITS (NOT SEDIMENT)
7850	HYDROSTRUCTURE IMPACTS ON FISH PASSAGE
6200	ILLEGAL DUMPS OR OTHER INAPPROPRIATE WASTE DISPOSAL
4010	ILLICIT CONNECTIONS/HOOK-UPS TO STORM SEWERS
3150	IMPACTS FROM GEOTHERMAL DEVELOPMENT
7400	IMPACTS FROM HYDROSTRUCTURE FLOW REGULATION/MODIFICATION
6250	IMPACTS FROM LAND APPLICATION OF WASTES
8750	IMPACTS FROM RESORT AREAS
8350	IMPERVIOUS SURFACE/PARKING LOT RUNOFF
130	INDUSTRIAL LAND TREATMENT
100	INDUSTRIAL POINT SOURCE DISCHARGE
140	INDUSTRIAL THERMAL DISCHARGES
150	INDUSTRIAL/COMMERCIAL SITE STORMWATER DISCHARGE (PERMITTED)
800	INTRODUCTION OF NON-NATIVE ORGANISMS (ACCIDENTAL OR INTENTIONAL)
6000	LANDFILLS
6300	LEAKING UNDERGROUND STORAGE TANKS
7900	LITTORAL/SHORE AREA MODIFICATIONS (NON-RIVERINE)
1800	LIVESTOCK (GRAZING OR FEEDING OPERATIONS)
1850	MANAGED PASTURE GRAZING
1900	MANURE LAGOONS
1950	MANURE RUNOFF
525	MARINA BOAT CONSTRUCTION
530	MARINA BOAT MAINTENANCE
535	MARINA FUELING OPERATIONS
540	MARINA RELATED SHORELINE HABITAT DEGRADATION
545	MARINA/BOATING PUMPOUT RELEASES
550	MARINA/BOATING SANITARY ON-VESSEL DISCHARGES
555	MARINAS AND RECREATIONAL BOATING
5250	MINE TAILINGS
5000	MINING
8760	MOTORIZED WATERCRAFT
5050	MOUNTAINTOP MINING

8000	MUNICIPAL (URBANIZED HIGH DENSITY AREA)
200	MUNICIPAL POINT SOURCE DISCHARGES
8600	NATURAL SOURCES
160	NPS POLLUTION FROM MILITARY BASE FACILITIES (OTHER THAN PORT FACILITIES)
560	NPS POLLUTION FROM MILITARY PORT FACILITIES
8770	OFF-ROAD VEHICLES
6500	ON-SITE TREATMENT SYSTEMS (SEPTIC SYSTEMS AND SIMILAR DECENTRALIZED SYSTEMS)
5350	OPEN PIT MINING
720	OTHER SPILL RELATED IMPACTS
8780	OTHER TURF MANAGEMENT
230	PACKAGE PLANT OR OTHER PERMITTED SMALL FLOWS DISCHARGES
5500	PETROLEUM/NATURAL GAS ACTIVITIES
5850	PETROLEUM/NATURAL GAS PRODUCTION ACTIVITIES (PERMITTED)
5950	PIPELINE BREAKS
5450	PLACER MINING
8740	POLLUTANTS FROM PUBLIC BATHING AREAS
3250	POST-DEVELOPMENT EROSION AND SEDIMENTATION
5550	POTASH MINING
6550	RCRA HAZARDOUS WASTE SITES
8700	RECREATION AND TOURISM (NON-BOATING)
6600	RELEASES FROM WASTE SITES OR DUMPS
7600	REMOVAL OF RIPARIAN VEGETATION
4300	RESIDENTIAL DISTRICTS
4350	RURAL (RESIDENTIAL AREAS)
610	SALT STORAGE SITES
565	SALTWATER INTRUSION
5650	SAND/GRAVEL/ROCK MINING OR QUARRIES
410	SANITARY SEWER OVERFLOWS (COLLECTION SYSTEM FAILURES)
570	SEAFOOD PROCESSING OPERATIONS
6350	SEPTAGE DISPOSAL
4020	SEWAGE DISCHARGES IN UNSEWERED AREAS
8720	SHALLOW LAKE/RESERVOIR
575	SHIPBUILDING, REPAIRS, DRYDOCKING
2000	SILVICULTURE ACTIVITIES
2100	SILVICULTURE HARVESTING
2200	SILVICULTURE, FIRE SUPPRESSION
3200	SITE CLEARANCE (LAND DEVELOPMENT OR REDEVELOPMENT)
9000	SOURCE UNKNOWN
9100	SOURCES OUTSIDE STATE JURISDICTION OR BORDERS
1650	SPECIALTY CROP PRODUCTION
730	SPILLS FROM TRUCKS OR TRAINS
7700	STREAMBANK MODIFICATIONS/DESTABILIZATION
5200	SUBSURFACE (HARDROCK) MINING
5100	SURFACE MINING

7500	SURFACE WATER DIVERSIONS
7250	SURFACE WATER WITHDRAWALS
7300	TRANSFER OF WATER FROM AN OUTSIDE WATERSHED
5750	UIC WELLS (UNDERGROUND INJECTION CONTROL WELLS)
6050	UNPERMITTED DISCHARGE (DOMESTIC WASTES)
170	UNPERMITTED DISCHARGE (INDUSTRIAL/COMMERCIAL WASTES)
1700	UNRESTRICTED CATTLE ACCESS
4000	URBAN RUNOFF/STORM SEWERS
8730	WASTES FROM PETS
7650	WATER DIVERSIONS
810	WATERFOWL
740	WATERSHED RUNOFF FOLLOWING FOREST FIRE
7950	WETLAND DRAINAGE
820	WILDLIFE OTHER THAN WATERFOWL
4310	YARD MAINTENANCE

CAUSES

Below details the current list of available impairment causes allowable by the USEPA. The context that USEPA places each cause into is also provided for reference purposes; however, categories are not used as causes of impairment.

CAUSE CODE	CAUSE DESCRIPTION	CAUSE CONTEXT	POLLUTANT?
2210	ALGAE	ALGAL GROWTH	Y
2211	ALGAL TOXINS	ALGAL GROWTH	Y
2212	BROWN TIDE	ALGAL GROWTH	Y
2213	CHLOROPHYLL-A	ALGAL GROWTH	Y
2216	CURLY-LEAF PONDWEED	ALGAL GROWTH	Y
2217	FANWORT	ALGAL GROWTH	Y
2218	HARMFUL ALGAL BLOOMS	ALGAL GROWTH	Y
2219	HYDRILLA	ALGAL GROWTH	Y
2220	SEA LETTUCE	ALGAL GROWTH	Y
2221	SUSPENDED ALGAE	ALGAL GROWTH	Y
600	AMMONIA, UN-IONIZED	AMMONIA	Y
6000	ABNORMAL FISH DEFORMITIES, EROSIONS, LESIONS, TUMORS (DELTS)	BIOTOXINS	Y
6001	BIOTOXINS	BIOTOXINS	Y
6002	CYANOBACTERIA HEPATOTOXIC MICROCYSTINS	BIOTOXINS	Y
6003	CYANOBACTERIA HEPATOTOXIC NODULARINS	BIOTOXINS	Y
6004	CYANOBACTERIA NEUROTOXIC ANATOXINS	BIOTOXINS	Y
6005	CYANOBACTERIA NEUROTOXIC SAXITOXINS	BIOTOXINS	Y
0	CAUSE UNKNOWN	CAUSE UNKNOWN	Y
700	CHLORINE	CHLORINE	Y
701	CHLORINE DIOXIDE	CHLORINE	Y
702	CHLORINE, RESIDUAL (CHLORINE DEMAND)	CHLORINE	Y
703	FREE CHLORINE	CHLORINE	Y
425	2,3,7,8-TETRACHLORODIBENZOFURAN	DIOXINS	Y
422	2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN	DIOXINS	Y
423	DIBENZOFURAN	DIOXINS	Y
420	DIOXIN	DIOXINS	Y
424	FURAN COMPOUNDS	DIOXINS	Y
1601	ALTERATION IN STREAM-SIDE OR LITTORAL VEGETATIVE COVERS	HABITAT ALTERATIONS	N
1602	FISH PASSAGE BARRIER	HABITAT ALTERATIONS	N
1600	HABITAT ALTERATIONS	HABITAT ALTERATIONS	N
1603	LOSS OF INSTREAM COVER	HABITAT ALTERATIONS	N
1604	OTHER ANTHROPOGENIC SUBSTRATE ALTERATIONS	HABITAT ALTERATIONS	N
1605	PHYSICAL SUBSTRATE HABITAT ALTERATIONS	HABITAT ALTERATIONS	N
991	DEWATERING	HYDROLOGIC ALTERATION	N
990	FLOW REGIME MODIFICATION	HYDROLOGIC ALTERATION	N
992	SALINITY CHANGE DUE TO CHANGE IN FLOW	HYDROLOGIC ALTERATION	N
993	STREAM MODIFICATION	HYDROLOGIC ALTERATION	N
994	WETLANDS DRAINAGE	HYDROLOGIC ALTERATION	N
995	WETLANDS DREDGED/FILLED	HYDROLOGIC ALTERATION	N
510	MERCURY	MERCURY	Y
502	METHYLMERCURY	MERCURY	Y
503	ALUM IN SEDIMENT	METALS (OTHER THAN MERCURY)	Y
504	ALUMINUM	METALS (OTHER THAN MERCURY)	Y
505	ARSENIC	METALS (OTHER THAN MERCURY)	Y
506	BERYLLIUM	METALS (OTHER THAN MERCURY)	Y
507	CADMIUM	METALS (OTHER THAN MERCURY)	Y

508	CHROMIUM	METALS (OTHER THAN MERCURY)	Y
509	COBALT	METALS (OTHER THAN MERCURY)	Y
511	COPPER	METALS (OTHER THAN MERCURY)	Y
512	IRON	METALS (OTHER THAN MERCURY)	Y
513	LEAD	METALS (OTHER THAN MERCURY)	Y
514	MANGANESE	METALS (OTHER THAN MERCURY)	Y
500	METALS	METALS (OTHER THAN MERCURY)	Y
515	MOLYBDENUM	METALS (OTHER THAN MERCURY)	Y
516	NICKEL	METALS (OTHER THAN MERCURY)	Y
517	SELENIUM	METALS (OTHER THAN MERCURY)	Y
518	SILVER	METALS (OTHER THAN MERCURY)	Y
519	STRONTIUM	METALS (OTHER THAN MERCURY)	Y
520	THALLIUM	METALS (OTHER THAN MERCURY)	Y
521	TITANIUM	METALS (OTHER THAN MERCURY)	Y
522	VANADIUM	METALS (OTHER THAN MERCURY)	Y
523	ZINC	METALS (OTHER THAN MERCURY)	Y
2201	AQUATIC PLANTS (MACROPHYTES)	NOXIOUS AQUATIC PLANTS	Y
2200	NOXIOUS AQUATIC PLANTS	NOXIOUS AQUATIC PLANTS	Y
2202	NON-NATIVE AQUATIC PLANTS	NUISANCE EXOTIC SPECIES	Y
2203	NON-NATIVE FISH/SHELLFISH/ZOOPLANKTON	NUISANCE EXOTIC SPECIES	Y
2205	NOXIOUS AQUATIC PLANTS NATIVE	NUISANCE EXOTIC SPECIES	Y
2204	ZEBRA MUSSEL, DREISSENA POLYMORPH	NUISANCE EXOTIC SPECIES	Y
901	EUTROPHICATION	NUTRIENTS	Y
902	NITRATE	NUTRIENTS	Y
903	NITRATE/NITRITE (NITRITE + NITRATE AS N)	NUTRIENTS	Y
904	NITRITE	NUTRIENTS	Y
905	NITROGEN	NUTRIENTS	Y
906	NITROGEN, AMMONIA	NUTRIENTS	Y
907	NITROGEN, NITRATE	NUTRIENTS	Y
908	NITROGEN, NITRITE	NUTRIENTS	Y
900	NUTRIENTS	NUTRIENTS	Y
909	PHOSPHATE	NUTRIENTS	Y
910	PHOSPHORUS	NUTRIENTS	Y
911	TOTAL KJEHLDAHL NITROGEN (TKN)	NUTRIENTS	Y
1901	DIESEL FUEL	OIL AND GREASE	Y
1900	OIL AND GREASE	OIL AND GREASE	Y
1902	PETROLEUM HYDROCARBONS	OIL AND GREASE	Y
1903	RESIDUAL SURFACE AND SUB-SURFACE OIL/TAR BALLS/TAR MATS	OIL AND GREASE	Y
920	BIOCHEMICAL OXYGEN DEMAND (BOD)	ORGANIC ENRICHMENT/OXYGEN DEPLETION	Y
921	CHEMICAL OXYGEN DEMAND (COD)	ORGANIC ENRICHMENT/OXYGEN DEPLETION	Y
922	DISSOLVED OXYGEN	ORGANIC ENRICHMENT/OXYGEN DEPLETION	Y
923	ORGANIC ENRICHMENT	ORGANIC ENRICHMENT/OXYGEN DEPLETION	Y
924	SEDIMENT OXYGEN DEMAND	ORGANIC ENRICHMENT/OXYGEN DEPLETION	Y
925	TOTAL ORGANIC CARBON (TOC)	ORGANIC ENRICHMENT/OXYGEN DEPLETION	Y
2701	DEBRIS	OTHER CAUSE	Y
2700	OSMOTIC PRESSURE	OTHER CAUSE	Y
2702	PAPER SLUDGE	OTHER CAUSE	Y
2703	RESIDUES	OTHER CAUSE	Y
2704	SCUM/FOAM	OTHER CAUSE	Y
2705	SLUDGE	OTHER CAUSE	Y
2706	SURFACTANTS	OTHER CAUSE	Y
1701	BACTERIA (OYSTER WATERS)	PATHOGENS	Y
1702	ENTEROCOCCUS	PATHOGENS	Y

1703	ESCHERICHIA COLI (E. COLI)	PATHOGENS	Y
1704	FECAL COLIFORM	PATHOGENS	Y
1700	PATHOGENS	PATHOGENS	Y
1705	TOTAL COLIFORM	PATHOGENS	Y
1706	VIRUSES (ENTERIC)	PATHOGENS	Y
201	1,2-DIBROMO-3-CHLOROPROPANE	PESTICIDES	Y
202	1,2-DICHLOROETHANE	PESTICIDES	Y
203	1,2-DICHLOROPROPANE	PESTICIDES	Y
204	1,3-DICHLOROPROPENE	PESTICIDES	Y
205	2,3-DICHLOROPROPENE	PESTICIDES	Y
206	2,4,5-TP (SILVEX)	PESTICIDES	Y
207	2,4,5-TRICHLOROPHENOL	PESTICIDES	Y
208	2,4-DINITROPHENOL	PESTICIDES	Y
209	2-METHYLNAPHTHALENE	PESTICIDES	Y
210	4,4'-DDD	PESTICIDES	Y
211	4,4'-DDE	PESTICIDES	Y
212	4,4'-DDT	PESTICIDES	Y
213	ACROLEIN	PESTICIDES	Y
214	ACRYLONITRILE	PESTICIDES	Y
215	ALACHLOR	PESTICIDES	Y
216	ALDRIN	PESTICIDES	Y
217	ATRAZINE	PESTICIDES	Y
218	BENTAZON	PESTICIDES	Y
219	BETA-BHC	PESTICIDES	Y
220	BETA-ENDOSULFAN (ENDOSULFAN 2)	PESTICIDES	Y
221	BHC	PESTICIDES	Y
430	CHLORDANE	PESTICIDES	Y
222	DDD (DICHLORODIPHENYLDICHLOROETHANE)	PESTICIDES	Y
223	DDE (DICHLORODIPHENYLDICHLOROETHYLENE)	PESTICIDES	Y
224	DDT (DICHLORODIPHENYLTRICHLOROETHANE)	PESTICIDES	Y
225	DDT METABOLITES	PESTICIDES	Y
226	DIAZINON	PESTICIDES	Y
227	DIELDRIN	PESTICIDES	Y
228	DIMETHYL PHTHALATE	PESTICIDES	Y
229	DIQUAT	PESTICIDES	Y
230	DIURON	PESTICIDES	Y
231	ELDRIN	PESTICIDES	Y
232	ENDOSULFAN	PESTICIDES	Y
233	ENDOSULFAN SULFATE	PESTICIDES	Y
234	ENDOTHALL	PESTICIDES	Y
235	ENDRIN	PESTICIDES	Y
236	ENDRIN ALDEHYDE	PESTICIDES	Y
237	EPTC	PESTICIDES	Y
238	ETHELYNE DIBROMIDE	PESTICIDES	Y
239	ETHOPROP	PESTICIDES	Y
240	FIPRONIL	PESTICIDES	Y
241	FLUOMETURON	PESTICIDES	Y
242	FONOFOS	PESTICIDES	Y
243	FORMALDEHYDE	PESTICIDES	Y
244	GLYPHOSATE	PESTICIDES	Y
245	GUTHION	PESTICIDES	Y
246	HEPTACHLOR	PESTICIDES	Y
247	HEPTACHLOR EPOXIDE	PESTICIDES	Y
248	HEXACHLOROBENZENE	PESTICIDES	Y
249	HEXACHLOROCYCLOHEXANE (HCH)	PESTICIDES	Y
250	HEXACHLOROPHENE	PESTICIDES	Y

251	HEXAZINONE	PESTICIDES	Y
252	INDENO[1,2,3-CD]PYRENE	PESTICIDES	Y
253	KEPONE	PESTICIDES	Y
254	MALATHION	PESTICIDES	Y
255	METHANOL	PESTICIDES	Y
256	METHYL BROMIDE	PESTICIDES	Y
257	METOLACHLOR	PESTICIDES	Y
421	MIREX	PESTICIDES	Y
258	NAPHTHALENE	PESTICIDES	Y
259	NAPROPAMIDE	PESTICIDES	Y
260	NITROFEN	PESTICIDES	Y
261	ORYZALIN	PESTICIDES	Y
262	OXADIAZON	PESTICIDES	Y
263	OXAMYL (VYDATE)	PESTICIDES	Y
264	OXYFLUORFEN	PESTICIDES	Y
265	P,P' DDD	PESTICIDES	Y
266	PERMETHRIN	PESTICIDES	Y
200	PESTICIDES	PESTICIDES	Y
267	PYRETHROIDS	PESTICIDES	Y
268	QUINTOZENE	PESTICIDES	Y
269	SIMAZINE	PESTICIDES	Y
270	SIMETRYN	PESTICIDES	Y
271	XYLENE	PESTICIDES	Y
1001	ALKALINITY	PH/ACIDITY/CAUSTIC CONDITIONS	Y
1000	PH	PH/ACIDITY/CAUSTIC CONDITIONS	Y
1002	PH, HIGH	PH/ACIDITY/CAUSTIC CONDITIONS	Y
1003	PH, LOW	PH/ACIDITY/CAUSTIC CONDITIONS	Y
410	POLYCHLORINATED BIPHENYLS (PCBS)	POLYCHLORINATED BIPHENYLS (PCBS)	Y
3000	ALPHA PARTICLES	RADIATION	Y
3001	BARIUM	RADIATION	Y
3002	BETA PARTICLES AND PHOTON EMITTERS	RADIATION	Y
3003	CESIUM	RADIATION	Y
3004	RADIATION	RADIATION	Y
3005	RADIUM	RADIATION	Y
3006	TRITIUM	RADIATION	Y
3007	URANIUM	RADIATION	Y
1310	CHLORIDE	SALINITY/TOTAL DISSOLVED SOLIDS/CHLORIDES/SULFATES	Y
1301	SALINITY	SALINITY/TOTAL DISSOLVED SOLIDS/CHLORIDES/SULFATES	Y
1302	SODIUM	SALINITY/TOTAL DISSOLVED SOLIDS/CHLORIDES/SULFATES	Y
1303	SPECIFIC CONDUCTIVITY	SALINITY/TOTAL DISSOLVED SOLIDS/CHLORIDES/SULFATES	Y
1304	SULFATE	SALINITY/TOTAL DISSOLVED SOLIDS/CHLORIDES/SULFATES	Y
1300	TOTAL DISSOLVED SOLIDS (TDS)	SALINITY/TOTAL DISSOLVED SOLIDS/CHLORIDES/SULFATES	Y
1100	SILTATION	SEDIMENT	Y
2050	COLOR	TASTE, COLOR, AND ODOR	Y
2000	ODOR	TASTE, COLOR, AND ODOR	Y
2001	TASTE	TASTE, COLOR, AND ODOR	Y
1400	THERMAL MODIFICATIONS	TEMPERATURE	Y
100	TOXICITY	TOTAL TOXICS	Y
4000	ANTIMONY	TOXIC INORGANICS	Y
4001	ASBESTOS	TOXIC INORGANICS	Y
4002	BORON	TOXIC INORGANICS	Y
4003	CYANIDE	TOXIC INORGANICS	Y
4004	FLUORIDE	TOXIC INORGANICS	Y

4005	HYDROGEN SULFIDE	TOXIC INORGANICS	Y
4006	PERCHLORATE	TOXIC INORGANICS	Y
5000	1,1,1,2-TETRACHLOROETHANE	TOXIC ORGANICS	Y
5001	1,1,1-TRICHLOROETHANE	TOXIC ORGANICS	Y
5002	1,1,2,2-TETRACHLOROETHANE	TOXIC ORGANICS	Y
5003	1,1,2-TRICHLOROETHANE	TOXIC ORGANICS	Y
5004	1,1-DICHLORO-1,2,2-TRIFLUOROETHANE	TOXIC ORGANICS	Y
5005	1,1-DICHLOROETHANE	TOXIC ORGANICS	Y
5006	1,1-DICHLOROETHYLENE	TOXIC ORGANICS	Y
5007	1,2,3,4-TETRACHLOROBENZENE	TOXIC ORGANICS	Y
5008	1,2,4,5-TETRACHLOROBENZENE	TOXIC ORGANICS	Y
5009	1,2,4-TRICHLOROBENZENE	TOXIC ORGANICS	Y
5010	1,2,4-TRIMETHYLBENZENE	TOXIC ORGANICS	Y
5011	1,2-BUTYLENE OXIDE	TOXIC ORGANICS	Y
5012	1,2-DIBROMOETHANE	TOXIC ORGANICS	Y
5013	1,2-DICHLOROBENZENE	TOXIC ORGANICS	Y
5014	1,2-DICHLOROETHYLENE	TOXIC ORGANICS	Y
5015	1,2-DIPHENYLHDRAZINE	TOXIC ORGANICS	Y
5016	1,2-DIPHENYLHYDRAZINE	TOXIC ORGANICS	Y
5017	1,2-PROPANEDIOL	TOXIC ORGANICS	Y
5018	1,3-BUTADIENE	TOXIC ORGANICS	Y
5019	1,3-DICHLOROBENZENE	TOXIC ORGANICS	Y
5020	1,4-DICHLOROBENZENE	TOXIC ORGANICS	Y
5021	1,4-DIOXANE	TOXIC ORGANICS	Y
5022	2,2'-DICHLORODIETHYL ETHER	TOXIC ORGANICS	Y
5023	2,2'-DICHLORODIISOPROPYL ETHER	TOXIC ORGANICS	Y
5024	2,4,6-TRICHLOROPHENOL	TOXIC ORGANICS	Y
5025	2,4-DIAMINOTOLUENE	TOXIC ORGANICS	Y
5026	2,4-DICHLOROPHENOL	TOXIC ORGANICS	Y
5027	2,4-DIMETHYLPHENOL	TOXIC ORGANICS	Y
5028	2,4-DINITROTOLUENE	TOXIC ORGANICS	Y
5029	2,5-DICHLOROPHENOL	TOXIC ORGANICS	Y
5030	2,6-DINITROTOLUENE	TOXIC ORGANICS	Y
5031	2-ACETYLAMINOFLUORENE	TOXIC ORGANICS	Y
5032	2-BUTANONE	TOXIC ORGANICS	Y
5033	2-CHLOROETHYL VINYL ETHER	TOXIC ORGANICS	Y
5034	2-CHLORONAPHTHALENE	TOXIC ORGANICS	Y
5035	2-CHLOROPHENOL	TOXIC ORGANICS	Y
5036	2-ETHOXYETHANOL	TOXIC ORGANICS	Y
5037	2-HEXANONE	TOXIC ORGANICS	Y
5038	2-METHOXYETHANOL	TOXIC ORGANICS	Y
5039	2-METHYLPHENOL	TOXIC ORGANICS	Y
5040	2-METHYLPYRIDINE	TOXIC ORGANICS	Y
5041	2-NITROPHENOL	TOXIC ORGANICS	Y
5042	3,3'-DICHLOROBENZIDINE	TOXIC ORGANICS	Y
5043	3,3'-DIMETHOXYBENZIDINE	TOXIC ORGANICS	Y
5044	3,3'-DIMETHYLBENZIDINE	TOXIC ORGANICS	Y
5045	3,4-DICHLOROPHENOL	TOXIC ORGANICS	Y
5046	3-CHLOROPHENOL	TOXIC ORGANICS	Y
5047	4,4-DICHLORO-2-BUTENE	TOXIC ORGANICS	Y
5048	4,4'-ISOPROPYLIDENEDIPHENOL	TOXIC ORGANICS	Y
5049	4,4'-METHYLENEBIS	TOXIC ORGANICS	Y
5050	4-AMINOBIPHENYL	TOXIC ORGANICS	Y
5051	4-BROMOPHENYLPHENYL ETHER	TOXIC ORGANICS	Y
5052	4-CHLORO-3-METHYLPHENOL (3-METHYL-4-CHLOROPHENOL)	TOXIC ORGANICS	Y
5053	4-CHLOROPHENOL	TOXIC ORGANICS	Y

5054	4-DIMETHYLAMINOAZOBENZENE	TOXIC ORGANICS	Y
5055	4-METHYL-2-PENTANONE (MIBK)	TOXIC ORGANICS	Y
5056	4-METHYLPHENOL	TOXIC ORGANICS	Y
5057	4-NITROPHENOL	TOXIC ORGANICS	Y
5058	5-NITRO-O-TOLUIDINE	TOXIC ORGANICS	Y
5059	ACENAPHTHENE	TOXIC ORGANICS	Y
5060	ACENAPHTHYLENE	TOXIC ORGANICS	Y
5061	ACETALDEHYDE	TOXIC ORGANICS	Y
5062	ACETOCHLOR	TOXIC ORGANICS	Y
5063	ACETONE	TOXIC ORGANICS	Y
5064	ACETONITRILE	TOXIC ORGANICS	Y
5065	ACRYLAMIDE	TOXIC ORGANICS	Y
5066	ALKYLBENZENE	TOXIC ORGANICS	Y
5067	ALLYL ALCOHOL	TOXIC ORGANICS	Y
5068	ALLYL CHLORIDE	TOXIC ORGANICS	Y
5069	ALPHA-BNC	TOXIC ORGANICS	Y
5070	ALPHA-NAPHTHYLAMINE	TOXIC ORGANICS	Y
5071	ANILINE	TOXIC ORGANICS	Y
5072	ANTHRACENE	TOXIC ORGANICS	Y
5073	BENTAZONE	TOXIC ORGANICS	Y
5074	BENZ[A]ANTHRACENE	TOXIC ORGANICS	Y
5075	BENZAL CHLORIDE	TOXIC ORGANICS	Y
5076	BENZENE	TOXIC ORGANICS	Y
5077	BENZIDINE	TOXIC ORGANICS	Y
5078	BENZO[A]ANTHRACENE	TOXIC ORGANICS	Y
5079	BENZO[A]PYRENE	TOXIC ORGANICS	Y
5080	BENZO[A]PYRENE (PAHS)	TOXIC ORGANICS	Y
5081	BENZO[B,K]FLUORANTHENES	TOXIC ORGANICS	Y
5082	BENZO[B]FLUORANTHENE	TOXIC ORGANICS	Y
5083	BENZO[B]FLUORENE	TOXIC ORGANICS	Y
5084	BENZO[G,H,I]PERYLENE	TOXIC ORGANICS	Y
5085	BENZO[K]FLUORANTHENE	TOXIC ORGANICS	Y
5086	BENZO[K]FLUORENE	TOXIC ORGANICS	Y
5087	BENZOFLUORANTHENES TOTAL (B+K+J)	TOXIC ORGANICS	Y
5088	BENZOIC ACID	TOXIC ORGANICS	Y
5089	BENZOPYRENE	TOXIC ORGANICS	Y
5090	BENZOYL CHLORIDE	TOXIC ORGANICS	Y
5091	BENZYL ALCOHOL	TOXIC ORGANICS	Y
5092	BENZYL CHLORIDE	TOXIC ORGANICS	Y
5093	BETA-NAPHTHYLAMINE	TOXIC ORGANICS	Y
5094	BIPHENYL	TOXIC ORGANICS	y
5095	BIS(2 ETHYLHEXYL)PHTHALATE	TOXIC ORGANICS	Y
5096	BIS(2 ETHYLHEXYL)PHTHALATE AND PHENOL	TOXIC ORGANICS	Y
5097	BIS(2-CHLORO-1-METHYLETHYL)	TOXIC ORGANICS	Y
5098	BIS(2-CHLOROETHOXY)METHANE	TOXIC ORGANICS	Y
5099	BIS(2-CHLOROISOPROPYL) ETHER	TOXIC ORGANICS	Y
5100	BIS(2-ETHYLHEXYL) PHTHALATE	TOXIC ORGANICS	Y
5101	BIS(N-OCTYL) PHTHALATE	TOXIC ORGANICS	Y
5102	BIS-2-CHLOROETHYL ETHER	TOXIC ORGANICS	Y
5103	BISPHTHALATE	TOXIC ORGANICS	Y
5104	BROMACIL	TOXIC ORGANICS	Y
5105	BROMODICHLOROMETHANE	TOXIC ORGANICS	Y
5106	BROMOFORM	TOXIC ORGANICS	Y
5107	BUTYL BENZYL PHTHALATE	TOXIC ORGANICS	Y
5108	BUTYRALDEHYDE	TOXIC ORGANICS	Y
5109	CARBON TETRACHLORIDE	TOXIC ORGANICS	Y
5110	CESETHYLATRAZINE	TOXIC ORGANICS	Y

5111	CHLORAMINES	TOXIC ORGANICS	Y
5112	CHLORINATED BENZENES	TOXIC ORGANICS	Y
5113	CHLORINATED PHENOLS	TOXIC ORGANICS	Y
5114	CHLOROACETIC ACID	TOXIC ORGANICS	Y
5115	CHLOROBENZENE (MONO)	TOXIC ORGANICS	Y
5116	CHLORODIBROMOMETHANE	TOXIC ORGANICS	Y
5117	CHLORODIFLUOROMETHANE	TOXIC ORGANICS	Y
5118	CHLOROETHANE	TOXIC ORGANICS	Y
5119	CHLOROFORM	TOXIC ORGANICS	Y
5120	CHLOROMETHANE	TOXIC ORGANICS	Y
5121	CHLOROMETHYL METHYL ETHER	TOXIC ORGANICS	Y
5122	CHLOROPHENYL-4 PHENYL ETHER	TOXIC ORGANICS	Y
5123	CHLOROPRENE	TOXIC ORGANICS	Y
5124	CHRYSENE	TOXIC ORGANICS	Y
5125	CIS-1,2-DICHLOROETHYLENE	TOXIC ORGANICS	Y
5126	COAL ASH	TOXIC ORGANICS	Y
5127	COAL TAR	TOXIC ORGANICS	Y
5128	CREOSOTE	TOXIC ORGANICS	Y
5129	CRESOL (MIXED ISOMERS)	TOXIC ORGANICS	Y
5130	CUMENE	TOXIC ORGANICS	Y
5131	CYCLOHEXANAMINE, N-ETHYL-1-PHENYL-	TOXIC ORGANICS	Y
5132	CYCLOHEXANE	TOXIC ORGANICS	Y
5133	CYMENE	TOXIC ORGANICS	Y
5134	DEETHYLATRAZINE	TOXIC ORGANICS	Y
5135	DESETHYLATRAZINE	TOXIC ORGANICS	Y
5136	DESISOPROYLATRAZINE	TOXIC ORGANICS	Y
5137	DI(2-ETHYLHEXYL) ADIPATE	TOXIC ORGANICS	Y
5138	DI-2-ETHYLHEXYL PHTHALATE	TOXIC ORGANICS	Y
5139	DIAMINOTOLUENE (MIXED ISOMERS)	TOXIC ORGANICS	Y
5140	DIBENZ[A,H]ANTHRACENE	TOXIC ORGANICS	Y
5141	DIBROMOCHLOROMETHANE	TOXIC ORGANICS	Y
5142	DICHLOROBENZENE (MIXED ISOMERS)	TOXIC ORGANICS	Y
5143	DICHLOROBROMOMETHANE	TOXIC ORGANICS	Y
5144	DICHLORODIFLUOROMETHANE	TOXIC ORGANICS	Y
5145	DICHLOROETHANE	TOXIC ORGANICS	Y
5146	DICHLOROETHANE/POLYCYCLIC AROMATIC HYDROCARBONS	TOXIC ORGANICS	Y
5147	DICHLOROETHYLENE/1,1-DCE	TOXIC ORGANICS	Y
5148	DICHLOROETHYLENES	TOXIC ORGANICS	Y
5149	DICHLOROMETHANE	TOXIC ORGANICS	Y
5150	DIETHYL PHTHALATE	TOXIC ORGANICS	Y
5151	DI-N-BUTYL PHTHALATE	TOXIC ORGANICS	Y
5152	DINITROTOLUENE	TOXIC ORGANICS	Y
5153	DI-N-OCTYL PHTHALATE	TOXIC ORGANICS	Y
5154	DISINFECTION BY-PRODUCTS	TOXIC ORGANICS	Y
5155	DODECYLBENZENE	TOXIC ORGANICS	Y
5156	EPICHLOROHYDRIN	TOXIC ORGANICS	Y
5157	ETHER, BIS CHLOROMETHYL	TOXIC ORGANICS	Y
5158	ETHYLBENZENE	TOXIC ORGANICS	Y
5159	ETHYLENE	TOXIC ORGANICS	Y
5160	ETHYLENE GLYCOL	TOXIC ORGANICS	Y
5161	ETHYLENE OXIDE	TOXIC ORGANICS	Y
5162	ETHYLENE THIOUREA	TOXIC ORGANICS	Y
5163	FLUORANTHENE	TOXIC ORGANICS	Y
5164	FLUORENE	TOXIC ORGANICS	Y
5165	FORMIC ACID	TOXIC ORGANICS	Y
5166	HALOMETHANES	TOXIC ORGANICS	Y

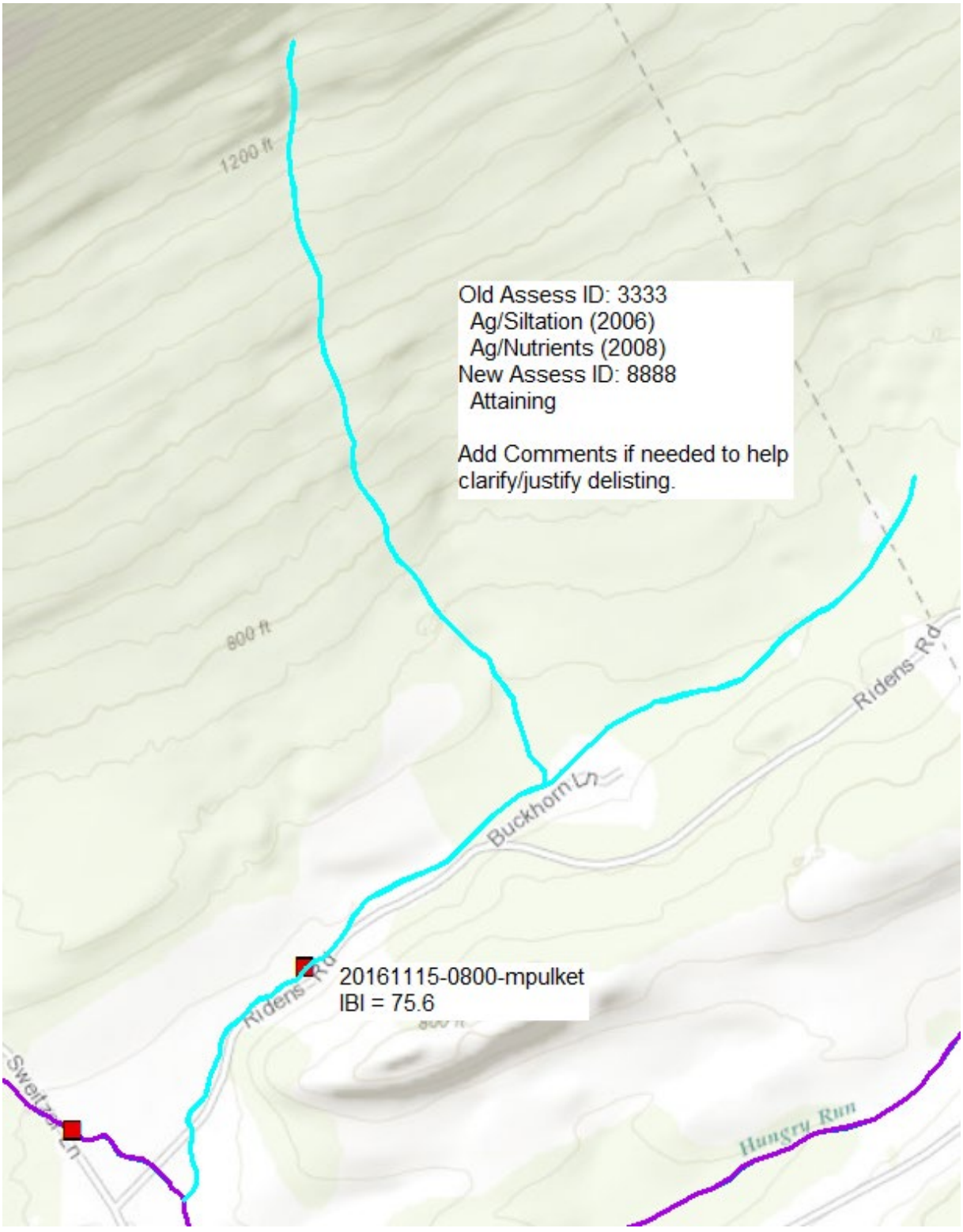
5167	HEXACHLOROBUTADIENE	TOXIC ORGANICS	Y
5168	HEXACHLOROCYCLOPENTADIENE	TOXIC ORGANICS	Y
5169	HEXACHLOROETHANE	TOXIC ORGANICS	Y
5170	HEXAMETHYLPHOSPHORAMIDE	TOXIC ORGANICS	Y
5171	HYDRAZINE	TOXIC ORGANICS	Y
5172	HYDROCARBONS	TOXIC ORGANICS	Y
5173	HYDROQUINONE	TOXIC ORGANICS	Y
5174	ISOBUTYRALDEHYDE	TOXIC ORGANICS	Y
5175	ISOPHORONE	TOXIC ORGANICS	Y
5176	ISOPROPANOL	TOXIC ORGANICS	Y
5177	ISOSAFROLE	TOXIC ORGANICS	Y
5178	MALEIC ANHYDRIDE	TOXIC ORGANICS	Y
5179	M-CRESOL	TOXIC ORGANICS	Y
5180	M-DICHLOROBENZENE	TOXIC ORGANICS	Y
5181	M-DINITROBENZENE	TOXIC ORGANICS	Y
5182	METHACRYLONITRILE	TOXIC ORGANICS	Y
5183	METHYL BLUE	TOXIC ORGANICS	Y
5184	METHYL CHLORIDE	TOXIC ORGANICS	Y
5185	METHYL ETHYL KETONE	TOXIC ORGANICS	Y
5186	METHYL HYDRAZINE	TOXIC ORGANICS	Y
5187	METHYL IODIDE	TOXIC ORGANICS	Y
5188	METHYL ISOBUTYL KETONE	TOXIC ORGANICS	Y
5189	METHYL METHACRYLATE	TOXIC ORGANICS	Y
5190	METHYL TERTIARY-BUTYL ETHER (MTBE)	TOXIC ORGANICS	Y
5191	METHYLENE BROMIDE	TOXIC ORGANICS	Y
5192	METHYLENE CHLORIDE	TOXIC ORGANICS	Y
5193	MTBE	TOXIC ORGANICS	Y
5194	N-BUTYL ALCOHOL	TOXIC ORGANICS	Y
5195	N-BUTYLBENZYLPHTHALATE	TOXIC ORGANICS	Y
5196	NITRILOTRIACETIC ACID	TOXIC ORGANICS	Y
5197	NITROBENZENE	TOXIC ORGANICS	Y
5198	NITRODIBUTYLAMINE,N	TOXIC ORGANICS	Y
5199	NITROGLYCERIN	TOXIC ORGANICS	Y
5200	NITROSODIETHYLAMINE,N	TOXIC ORGANICS	Y
5201	N-NITROSODIETHYLAMINE	TOXIC ORGANICS	Y
5202	N-NITROSODIMETHYLAMINE	TOXIC ORGANICS	Y
5203	N-NITROSO-DI-N-BUTYLAMINE	TOXIC ORGANICS	Y
5204	N-NITROSODIPHENYLAMINE	TOXIC ORGANICS	Y
5205	N-NITROSODIPROPYLAMINE	TOXIC ORGANICS	Y
5206	N-NITROSOMORPHOLINE	TOXIC ORGANICS	Y
5207	N-NITROSO-N-ETHYLUREA	TOXIC ORGANICS	Y
5208	N-NITROSO-N-METHYLUREA	TOXIC ORGANICS	Y
5209	N-NITROSOPIPERIDINE	TOXIC ORGANICS	Y
5210	N-NONYLBENZENE	TOXIC ORGANICS	Y
5211	NONYLPHENOL	TOXIC ORGANICS	Y
5212	O-CRESOL (2-METHYLPHENOL)	TOXIC ORGANICS	Y
5213	OCTACHLOROSTYRENE	TOXIC ORGANICS	Y
5214	OCTOCHLORONAPHTHALENE	TOXIC ORGANICS	Y
5215	O-DICHLOROBENZENE	TOXIC ORGANICS	Y
300	ORGANICS	TOXIC ORGANICS	Y
5216	O-TOLUIDINE	TOXIC ORGANICS	Y
5217	O-TOLUIDINE HYDROCHLORIDE	TOXIC ORGANICS	Y
5218	O-XYLENE	TOXIC ORGANICS	Y
5219	PAH1 - 2 & 3 RING POLYCYCLIC AROMATIC HYDROCARBONS	TOXIC ORGANICS	Y
5220	PAH2 - 4 RING POLYCYCLIC AROMATIC HYDROCARBONS	TOXIC ORGANICS	Y

5221	PAH3 - 5 & 6 RING POLYCYCLIC AROMATIC HYDROCARBONS	TOXIC ORGANICS	Y
5222	PARALDEHYDE	TOXIC ORGANICS	Y
5223	PCE	TOXIC ORGANICS	Y
5224	P-DICHLOROBENZENE	TOXIC ORGANICS	Y
5225	PENTACHLOROBENZENE	TOXIC ORGANICS	Y
5226	PENTACHLOROPHENOL (PCP)	TOXIC ORGANICS	y
5227	PERFLUOROOCTANE SULFONATE (PFOS)	TOXIC ORGANICS	Y
5228	PHENANTHRENE	TOXIC ORGANICS	Y
5229	PHENOL	TOXIC ORGANICS	Y
5230	PHTHALATE ESTERS	TOXIC ORGANICS	Y
5231	PHTHALIC ANHYDRIDE	TOXIC ORGANICS	Y
5232	PHTHLATE	TOXIC ORGANICS	Y
5233	PICRIC ACID	TOXIC ORGANICS	Y
5234	POLYBROMINATED BIPHENYLS	TOXIC ORGANICS	Y
5235	POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) (AQUATIC ECOSYSTEMS)	TOXIC ORGANICS	Y
5236	PROPIONALDEHYDE	TOXIC ORGANICS	Y
5237	PROPYLENE GLYCOL	TOXIC ORGANICS	Y
5238	PROPYLENE OXIDE	TOXIC ORGANICS	Y
5239	PYRENE	TOXIC ORGANICS	Y
5240	PYRIDINE	TOXIC ORGANICS	Y
5241	QUINOLINE	TOXIC ORGANICS	Y
5242	QUINONE	TOXIC ORGANICS	Y
5243	RDX (HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE)	TOXIC ORGANICS	Y
5244	SAFROLE	TOXIC ORGANICS	Y
5245	SEC-BUTYL ALCOHOL	TOXIC ORGANICS	Y
5246	STYRENE	TOXIC ORGANICS	Y
5247	TETRACHLOROETHYLENE	TOXIC ORGANICS	Y
5248	TETRACHLOROETHYLENE/PCE	TOXIC ORGANICS	Y
5249	THIOUREA	TOXIC ORGANICS	Y
5250	TOLUENE	TOXIC ORGANICS	Y
5251	TOTAL AROMATIC HYDROCARBONS	TOXIC ORGANICS	Y
5252	TOTAL BENZOFLUORANTHENES	TOXIC ORGANICS	Y
5253	TOTAL TRIHALOMETHANE (TTHM)	TOXIC ORGANICS	Y
5254	TRANS-1,2-DICHLOROETHENE	TOXIC ORGANICS	Y
5255	TRANS-1,2-DICHLOROETHYLENE	TOXIC ORGANICS	Y
5256	TRIBUTYLIN TBT (TRIBUTYLSTANNE)	TOXIC ORGANICS	Y
5257	TRICHLORINATED ETHANES	TOXIC ORGANICS	Y
5258	TRICHLOROETHYLENE (TCE)	TOXIC ORGANICS	Y
5259	TRICHLOROFLUOROMETHANE (CFC-11)	TOXIC ORGANICS	Y
5260	TRICLOPYR	TOXIC ORGANICS	Y
5261	TRIETHYLENE GLYCOL DICHLORIDE	TOXIC ORGANICS	Y
5262	VINYL ACETATE	TOXIC ORGANICS	Y
5263	VINYL BROMIDE	TOXIC ORGANICS	Y
5264	VINYL CHLORIDE	TOXIC ORGANICS	Y
5265	VINYLIDENE CHLORIDE	TOXIC ORGANICS	Y
5266	VOLATILE ORGANICS (VOCS)	TOXIC ORGANICS	Y
9998	TRASH	TRASH	Y
2100	TOTAL SUSPENDED SOLIDS (TSS)	TURBIDITY	Y
2500	TURBIDITY	TURBIDITY	Y

APPENDIX B: DELISTING EXAMPLE

HUC: 02050305
Assessed Use (e.g., Aquatic Life Use)
Delisting Reason: (e.g., WQS_NEW_DATA)

Title (e.g., Mill Creek Delisting)



Required Information:

1. Above the map, include the hydrologic unit code (HUC), the use being assessed, and the delisting reason (choose from Table 1 in Chapter 5).
2. Title the map with the waterbody name.
3. On or below the map, include the old Assessment ID with source(s) and cause(s) of impairment.
4. On or below the map, include all the source(s) and cause(s) of the new assessment. Even those causes that were retained from the old assessment. Include the new Assessment ID if available.
5. On the map, highlight or clearly depict the stream segment(s) or lake being delisted.
6. Label all stations with GISkey (yyyymmdd-HHMM-collector; e.g., 20060306-0800-mpulket) or unique station identifier.
7. Include the IBI score, geometric mean, and/or chemistry data when applicable.
8. Use an appropriate basemap layer; this example uses World Topographic Map.
9. The following information is not required but is very useful for tracking delistings.
    a. The listing date of the previous Sources/Causes (in parentheses on the map above)
    b. COMIDs of all the delisted segments
    c. Any comments or additional information to help clarify and justify the delisting.