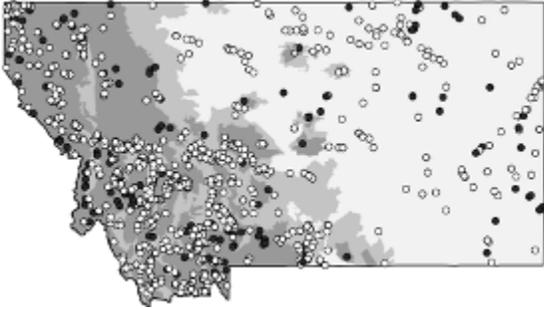


Addendum:

Eutrophication thresholds associated with benthic macroinvertebrate condition in Montana streams



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## 1.0 Introduction

The Montana Department of Environmental Quality (DEQ) sought further detail on work to support the narrative nutrient standards recently provided by Jonah Ventures (Schulte and Craine 2023) regarding thresholds in benthic macroinvertebrate community response to total nitrogen (TN). Questions were raised about the use of the midpoint in a four parameter logistic (FPL) regression (i.e., a nonlinear model resembling a sigmoid or S-shaped curve) as protective of aquatic life. We considered this concern in three parts: (1) a literature review of regime shifts and tipping points in ecological disturbance-response relationships; (2) a literature review of methods used to develop currently implemented or proposed thresholds of an environmental stressor related to aquatic life use; and (3) a comparison of FPL regression results to alternative models and U.S. Environmental Protection Agency (USEPA) frequency distribution-based guidance. Evidence is provided that the midpoint of a FPL model is a protective threshold because (1) it represents an inflection point in TN beyond which community structure and resilience to regime shifts dramatically change, (2) states define stressor thresholds at various positions along nonlinear curves - often after the initialization of community response and before saturation, (3) the midpoints of the FPL regression models in Schulte and Craine (2023) are consistent with those of piecewise linear regressions, generalized additive models, and regression tree models, and (4) the FPL midpoints correspond to 86th and 87th percentiles of reference site TN, which is consistent with the 86th percentile of reference sites associated with nutrient impacts in case studies of Montana streams (Suplee et al. 2007).

## 2.0 Regime shifts and tipping points: literature review

Ecological regime shifts (also called state changes or phase transitions) can be abrupt, persistent changes in the structure or function of a biological system, such as a population, assemblage, or ecosystem. A tipping point is the threshold at which a disturbance prompts a regime shift, potentially pushing the system to a state from which recovery to its original condition is difficult to reverse. Often, tipping points refer to discrete levels of disturbance that cause irreversible changes in the system state, leading to alternative stable states (different system states at the same level of disturbance, a phenomenon known as hysteresis) or collapse (Figure 1c,d). In the Montana macroinvertebrate metric-nutrient relationships analyzed in Schulte and Craine (2023), no models showed evidence of alternative stable states. Instead, all stressor-response relationships were nonlinear and unidirectional, with smooth transitions between states (i.e., low and high metric values) that were either gradual (Low Valleys and Transitional, Figure 1a) or abrupt (Mountains, Figure 1b). These relationships are characterized by a large response in the biological system (i.e., the metric) occurring across a small or large range of disturbance (i.e., nutrients), with the capacity for a linear recovery between states with reduction of disturbance. For example, Suplee et al. (2012) evaluated the success of a basin-wide voluntary nutrient reduction program to reduce algal biomass in the Clark Fork River in Montana. Downstream of the city of Missoula, average total phosphorus and total nitrogen concentrations decreased below

literature-derived saturation targets after 7 and 9 years, respectively, with corresponding decreases in algal biomass. At one site, total phosphorus concentrations were below saturation targets after the first year, and algal biomass decreased linearly until meeting the target after 8 years; algal biomass continued decreasing linearly through the rest of the 12-year record.

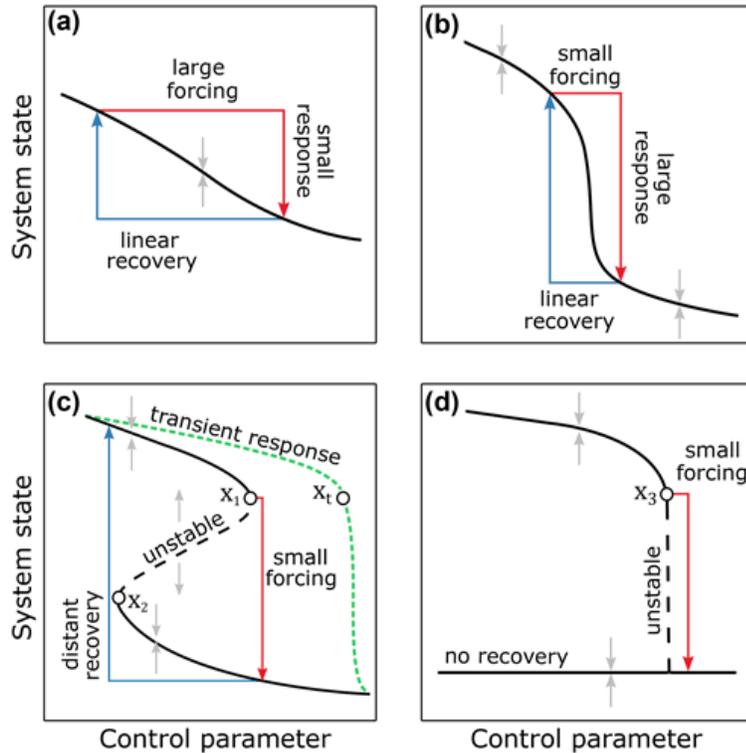


Figure 1. Conceptual behaviors of a system state undergoing a regime shift in response to changes in a disturbance (control parameter). A system can (a) have a small response across a wide range of disturbance and recover in a linear way, (b) have a large response across a small range of disturbance but still recover in a linear way, or (c-d) have a large response to a discrete disturbance with an irreversible regime shift. For (c) and (d), tipping points are denoted by open circles. For (a) and (b), tipping points may occur across the window of disturbance that causes a response, though the ability for these systems to recover often precludes the use of tipping point as a discrete value of disturbance. From Rosier et al. (2021).

Without a discrete shift in state, tipping points are defined across an interval of disturbance (Rosier et al. 2021). Often, thresholds (also called change points or breakpoints) are set at the levels of disturbance at which the slope of the model changes (e.g., piecewise linear regression, additive quantile regression, nonlinear regression), the cumulative sums of deviation from the overall mean significantly shifts (e.g., nonparametric change point analysis [nCPA]), or the curvature of the function changes (e.g., generalized additive models [GAMs]). Such thresholds often occur across broad ranges of disturbance and uncertainty. Setting a protective threshold therefore involves assessment of tolerance around uncertainty and risk (Mazor et al. 2022). The most protective thresholds can be set at the onset of any state change, the least protective thresholds after change has occurred and the system state has transitioned, and other thresholds

at levels of disturbance across which change is actively occurring but before a regime shift has completed (Dakos et al. 2015).

In Montana, the relationship between macroinvertebrate condition (as the Beck's Biotic Index version 3, or Becks3) and nutrients (as total nitrogen, TN) followed a decreasing, four parameter logistic regression (FPL, also called a sigmoidal or S-shaped nonlinear regression). Mathematically, decreasing FPL regressions are defined by three regions of change: the beginning of the downward slope from an initial asymptote (initialization), the point of maximum rate of change in the response (midpoint), and the leveling off of the downward slope into a second asymptote (saturation). The midpoint of a FPL regression is the inflection point of the curve where the rate of response begins to decline. In logistic population growth, for instance, the midpoint is the point of maximum growth rate. In dose-response curves, the midpoint is the half maximal effective concentration (EC50): the concentration of  $x$  required to obtain 50% of its maximal effect on  $y$ , or where 50% of the population exhibits a response. In general, this point reflects the maximum rate of change in the system and is typically seen as the tipping point for regime shifts in systems with alternative stable states or smooth transitions between states (Andersen et al. 2009). For example, in their study of eutrophication in a lake, Carpenter et al. (1999) defined a sigmoidal relationship between phosphorus concentrations and phosphorus loading. The midpoint of this relationship was determined to be the unstable equilibrium for a regime shift (Figure 2). Similarly, using a generalized additive model (GAM) to characterize the relationship between prey abundance ( $x$ ) and seabird breeding success ( $y$ ), Cury et al. (2011) described a sigmoidal pattern with a breakpoint approximately at the midpoint of the curve. Thus, multiple nonlinear modeling methods have identified significant thresholds at the point of  $x$  associated with the highest rate of change in  $y$ .

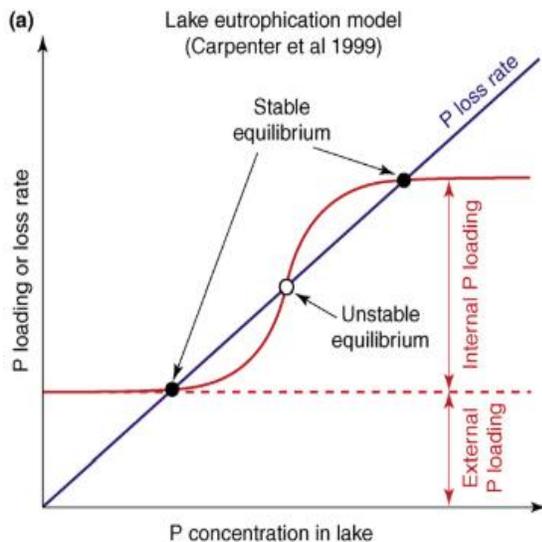


Figure 2. Model of an observed sigmoidal relationship between phosphorus concentration in a lake and phosphorus loading, with the tipping point (unstable equilibrium) set at the midpoint of the regression. From Carpenter et al. (1999).

Biological systems also maintain a measure of resilience: the ability to absorb disturbance without significant alteration of its state. A disturbance can trigger changes within the system, but these changes may be within the bounds of what the system can manage or recover from using its inherent adaptive processes. Therefore, the point where change in the response begins (the initialization point in a FPL regression) often falls within the system's natural variability or ability to recover and does not necessarily signify a tipping point of a regime shift (Folke et al. 2004, Rocha et al. 2015). Thus, setting a protective threshold at the initialization point may be overly protective and restrict attainability of general or managed use goals. Meanwhile, the point at which change in response levels off (the saturation point in a FPL regression) generally indicates the completion of a regime shift (Lees et al. 2006, Selkoe et al. 2015). While systems with smooth, nonlinear responses to disturbance can often recover from such a regime shift, the saturation point is not considered protective of the initial state, and substantial recovery efforts must be undertaken to return to the initial state (Rosier et al. 2021). For example, in their evaluation of a nutrient reduction program in the Clark Fork River, Suplee et al. (2012) found that algal biomass only declined significantly at nutrient concentrations near natural background – beyond the saturation breakpoint concentrations targeted by the reduction program.

### 3.0 Biological and nutrient criteria: literature review

Nutrient criteria thresholds are often defined using statistical methodologies like frequency distributions or aforementioned change point analyses that incorporate both empirical evidence and decision-makers' tolerance for risk and uncertainty (USEPA 2010, Evans-White et al. 2013). The USEPA, for example, suggests using the 75th percentile of nutrient concentrations from reference sites or the 25th percentile from impacted sites as thresholds (USEPA 2000). Yet, these percentiles may under- or overestimate the threshold of nutrient concentrations that empirically have an effect on biological responses in targeted studies (Suplee et al. 2007, Herlihy and Sifneos 2008; Morgan et al. 2012).

Similar to frequency distributions, change point analyses often require user decisions to define threshold confidence. For instance, with nonparametric change point analysis (nCPA), the probability of a change point in the response variable is determined at each value of the independent variable. This yields a cumulative probability distribution of a change point occurring at a given value of  $x$ . Studies have selected various probability thresholds as representative of significant change points, from 5% to 50% to 95% (King and Richardson 2003, Smith and Tran 2010). The 50% probability threshold is arguably the most commonly used (King and Richardson 2003), but this represents a decision by the user to accept an amount of uncertainty for the point at which the threshold occurs.

Techniques like piecewise regression and additive quantile regression smoothing (AQRS) are also used to identify breakpoints, and they commonly identify the onset or completion of change

when there is a smooth, nonlinear transition between states as opposed to the regions of high rates of change (Toms and Lesperance 2003). Thus, these methods may not account for resilience exhibited by ecological communities and therefore do not allow for some shifts in structure and function while maintaining overall integrity. For instance, the Minnesota Pollution Control Agency used AQRS to define nutrient thresholds for biological condition of fish and macroinvertebrates in streams (Heiskary and Bouchard Jr. 2015). Models yielded (1) upper breakpoint only, (2) lower breakpoint only, or (3) upper and lower breakpoints. To account for lower breakpoint models representing a saturation point, the midpoint between the lowest concentration and the breakpoint was considered protective based on Minnesota professionals' judgment. For models with upper and lower breakpoints, the midpoint was used as a protective threshold if the upper breakpoint was not significant.

Given variation among statistical methods and user decision points, many environmental management agencies use a variety of techniques for a weight-of-evidence approach to set thresholds in nutrient criteria. As a result, the delineation of thresholds that are protective of aquatic life is often based on the amount of weight professionals assign to each method and the degree of tolerance the users have for uncertainty and risk, as much as on statistically defined change points (Mazor et al. 2022). Final thresholds established by states therefore span a range of nutrient concentrations across the initialization, continuation, and saturation of change in biological communities. Thus, criteria vary in the amount of change in biological structure and function that is permissible.

Regardless of the variation in methods and decisions around defining thresholds, it is important to assess the rigor and protectivity of FPL regressions in environmental assessment given its less common use to set nutrient criteria or biological thresholds compared to methods like piecewise regression, AQRS, and nCPA. As reviewed in Section 2, sigmoidal disturbance-response curves are common in ecological systems that undergo regime shifts. While FPL regressions were not used explicitly as a method for defining thresholds, the Mississippi Department of Environmental Quality established changepoints in TN and total phosphorus (TP) relative to a site quality score (M-BISQ) based on regression tree models (Tetra Tech 2009). These changepoints were then validated using sigmoidal curves that characterized the shape of the nutrient-condition relationship. For both East and West Bioregions in Mississippi, the change points identified using regression tree models were near the midpoint or between the initialization and midpoint of the sigmoidal curve.

In Water Framework Directive guidance on best practices for establishing nutrient concentrations in the European Union (Phillips et al. 2018), binomial logistic models are recommended for binary response data of "good" or "bad" biological condition (such discrete categories were not available for Montana streams, hence the use of FPL). For datasets that do not have a wedge-shaped distribution of observations, Phillips et al. (2008) recommend using the

midpoint as the threshold criterion for supporting good ecological status, given a probability of 0.5 (Figure 3).

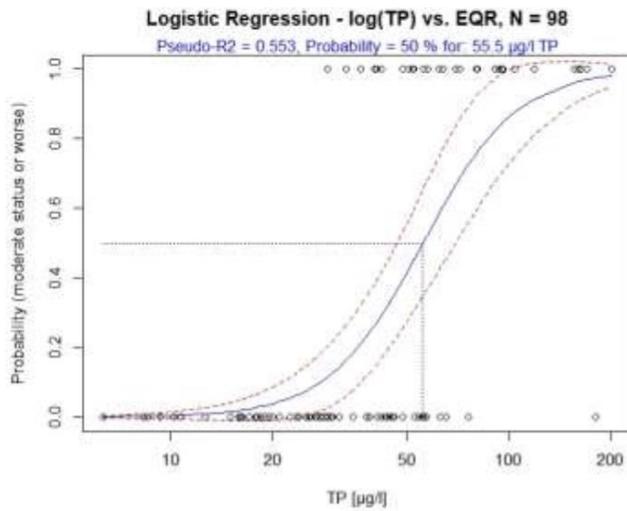


Figure 3. Binomial logistic regression between TP and ecological status (categorical: moderate or worse). The critical threshold is determined as the midpoint of the curve, which represents a 0.5 probability that a site is in moderate or worse condition. From Phillips et al. (2018).

In addition to indicators like reference site frequency distributions to identify effects of disturbance on aquatic life, the USEPA recommends the biological condition gradient (BCG) framework to describe biological change along gradients of stress. With BCG, sites are scored by the structural, functional, and taxonomic integrity of the community: BCG 1-2 represent natural condition or minimal loss of species and denote exceptional waters, BCG 3-4 represent some replacement of species and functions largely maintained, with BCG 4 often used as the threshold of general or modified use goals; and BCG 5-6 represent wide replacement by tolerant taxa and reduced functional complexity, marking degraded waters. The BCG is conceptualized as a decreasing logistic regression (Figure 4), in which BCG 2 represents the initialization of change (high quality waters), BCG 3 and 4 represent the midpoint of change (waters have been altered but are still protective of aquatic life), and BCG 5 represents the saturation of change (degraded waters). Beyond concept, the Minnesota Pollution Control Agency and Ohio Environmental Protection Agency both scored sites according to the BCG framework and found that the shape of the distribution also followed that of the logistic regression (Gerritsen et al. 2017, USEPA 2016).

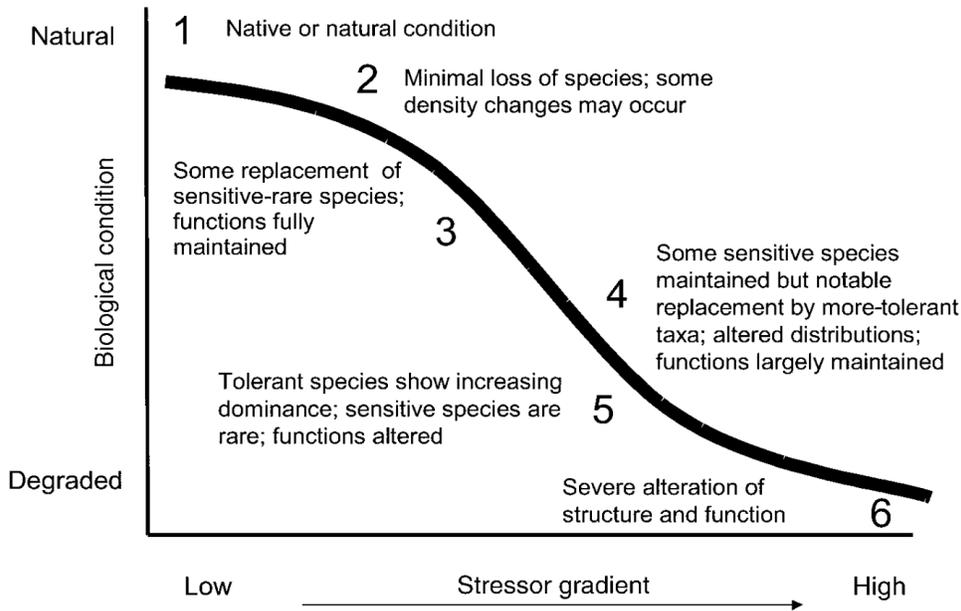


Figure 4. Conceptual diagram of the biological condition gradient (BCG) as a decreasing sigmoidal curve. According to USEPA guidance, BCG levels 3 and 4 represent somewhat altered biological communities but are still considered protective of aquatic life. From Davies and Jackson (2006).

All four of the aforementioned case studies (Minnesota, Mississippi, European Water Framework Directive, and USEPA’s BCG) identify and implement the midpoint of a sigmoidal curve as the threshold criterion for a stressor that is protective of aquatic life.

#### 4.0 Other EPA methods and Montana modeling results

In the absence of a modeled relationship between nutrient concentrations and biological condition, USEPA guidance is to use the 75th percentile of reference site nutrients, the 25th percentile of test site nutrients, or the midpoint of overlap between the reference 75th percentile and the test site 25th percentile (Figure 5, USEPA 2000). In the current analysis, the percentiles of reference site TN at the logistic regression midpoint were 86 and 85 in the Mountains and Low Valleys and Transitional, respectively (Table 1). These values are consistent with those reported in Suplee et al. (2007), who found that the 86th percentile of reference site TN and TP - not the 75th percentile recommended in USEPA guidance - was the mean and median percentile at which nutrient concentrations began to cause impacts to aquatic life in case studies in and around Montana.

The 75th percentile of reference site TN would yield approximately 21% (Mountains) and 15% (Low Valleys and Transitional) lower (i.e., more protective) TN concentrations than the values at the midpoint of the logistic regression. Yet, the recommendation for the 75th percentile of reference site TN is often paired with the 25th percentile of test site TN. In this case, USEPA guidance describes a presumptive relationship in which the 75th percentile of reference sites is less than the 25th percentile of test sites. However, the 25th percentile of test site TN was lower

than the 75th percentile of reference site TN in both regions (0.110 vs. 0.060 mg/L in the Mountains, 0.170 vs. 0.150 mg/L in the Low Valleys and Transitional; Table 1). Thus, in Montana, the distribution of reference and test sites does not follow the distribution presumed by USEPA percentile guidance, so using results from the modeled relationship is more appropriate.

Table 1. Summary statistics of four parameter logistic models developed by Schulte and Craine (2023).

Region	FPL midpoint: TN (mg/L)	FPL midpoint: Becks3	FPL midpoint: Reference percentile (TN)	FPL midpoint: Reference percentile (Becks3)	75th reference percentile: TN (mg/L)	75th reference percentile: Becks3	25th test percentile: TN (mg/L)	25th test percentile: Becks3
Mountains	0.139	35.09	86	89	0.110	44.00	0.060	44.00
Low Valleys and Transitional	0.199	18.68	85	77	0.170	21.50	0.150	24.13

It should be noted that USEPA’s approach (per Figure 5) has been critiqued in the scientific literature, where it is shown that the 75<sup>th</sup> percentile of reference stream site nutrient concentrations do not consistently overlap with the 25<sup>th</sup> percentile for all stream sites (Suplee et al. 2007, Herlihy and Sifneos 2008, Evan-White et al. 2013). Furthermore, in the Mountains region, the intersection of 75th percentiles of reference sites for TN and Becks3 was outside the FPL curve, indicating that reference sites do not fully represent the distribution of observed conditions in the region. Therefore, using a threshold point from the model that is derived from both reference and test sites is more empirical (i.e., better reflects the actual biological patterning observed in Montana).

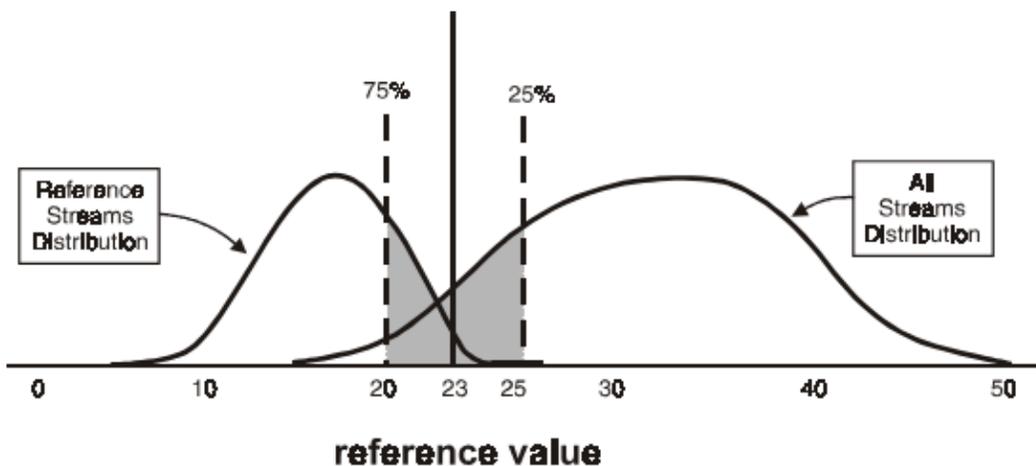


Figure 5. USEPA guidance for integrating the 75th percentile of reference site nutrient concentrations with the 25th percentile of test site nutrient concentrations. With this conceptual model, the reference site 75th percentile is lower than the test site 25th percentile. This approach has been critiqued in scientific literature (see text). From USEPA (2000).

Nevertheless, reference site distributions were still considered important barometers of nutrient concentrations and macroinvertebrate metric values in the Jonah Ventures models (Schulte and Craine 2023) to integrate USEPA guidance and multiple lines of evidence into Montana criteria. In both the Mountains and Low Valleys and Transitional regions, the 75th percentile of TN is between the initialization and the midpoints of the FPL regression, indicating that the initialization point is overprotective given the relatively large proportion of reference sites that would not meet these TN concentrations. Since the midpoint is the mathematically defined inflection point and is widely considered a critical threshold in ecological regime shift literature, the midpoint of the regression is the most defensible location for a nutrient criterion or, its corollary, a macroinvertebrate criterion.

Finally, in keeping with the weight-of-evidence approach of different models used by various states for establishing nutrient criteria with a biological response, we revisited potential thresholds from multiple piecewise regressions, generalized additive models (GAMs), and regression trees. For both regions, the 75th percentile of reference site TN exceeded the initialization point of change for all models (Figure 6). The midpoint of change for multiple piecewise regressions, GAMs, and regression trees were largely comparable, all within 0.022 mg/L of the FPL regression midpoint in each region (Table 2). In the Mountains, the FPL midpoint TN was lower (i.e., more protective) than that of all other models. In the Low Valleys and Transitional region, the FPL midpoint TN was between those of the multiple piecewise and regression tree models and that of the generalized additive model.

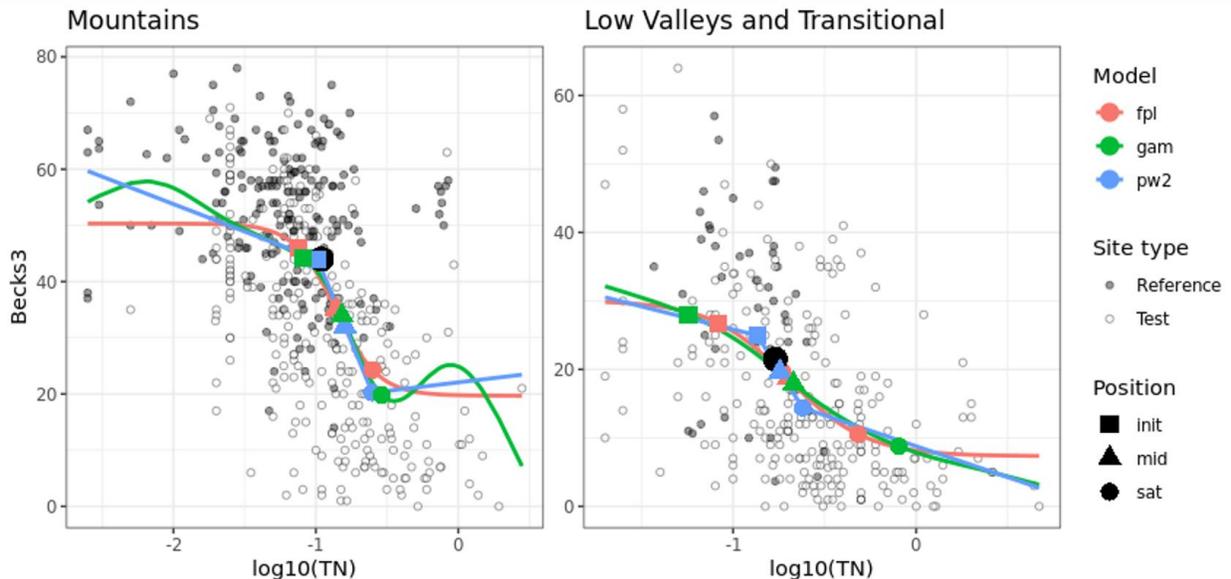


Figure 6. Change point models for each region, showing the original four parameter logistic (fpl), generalized additive model (gam), and multiple piecewise regression (pw2). The black circle represents the 75th percentile of reference site TN-by-Becks3. Position codes: init (initialization), mid (midpoint), sat (saturation).

Table 2. Midpoint total nitrogen (TN) concentrations (mg/L) for different change point analysis models for Becks3-TN relationships in Montana streams.

Region	Four parameter logistic	Multiple piecewise	Generalized additive model	Regression tree	75th reference percentile
Mountains	0.139	0.160	0.153	0.133	0.110
Low Valleys and Transitional	0.199	0.180	0.214	0.177	0.170

## 5.0 Summary

The midpoint of a logistic curve meets the criteria for a threshold value of a stressor (in this case, total nitrogen [TN]) that is protective of aquatic life given (a) its acceptance as a critical threshold in ecological regime shifts, (b) the mathematical inflection point in sigmoidal regressions, (c) its consistency with the range of change in biological condition across studies establishing nutrient criteria, and (d) its consistency with case studies in Montana and alternative models using long-term monitoring data from MDEQ. Nevertheless, there is uncertainty in defining tipping points in any stressor-response relationship. Interpreting uncertainty in environmental management is largely a policymaking decision associated with levels of risk tolerance. Mazor et al. (2022) show that varying levels of risk tolerance yield different nutrient thresholds for protecting aquatic life - each of which is ecologically and mathematically defensible but differing in the degree of protection. In a FPL regression, the concentration of TN between the onset of change (initialization) and the highest rate of change (midpoint) in biological condition can be interpreted as an interval of critical transition. For the most stringent protection, the initialization point could be used. Yet, this threshold may be overprotective, as communities have resilience to some change. Also, many reference sites would, consequently, not be considered to be supporting their beneficial uses, which is contradictory to their status as reference sites. Similarly, the 75th percentile of reference site TN could be used, but this value does not reflect the distribution of observed test sites and is inconsistent with case studies that show nutrient effects occur near the 86th percentile of reference site TN in Montana streams. Finally, the midpoint could be used. While less protective than the initialization or 75th reference percentile points because it allows some alteration in community structure up to the point of maximum change, the midpoint represents the most likely tipping point in regime shifts that follow an abrupt but smooth transition to a different stable equilibrium, is the mathematical inflection point for different states, incorporates the concept of resilience in ecological communities, is consistent with Montana case studies, and is more attainable for impacted systems under the premise of managed use.

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