To: State Water Resources Control Board of California, c/o Gerald W. Bowes, Ph.D.

From: Wendy Monk, Ph.D., Environment and Climate Change Canada @ Canadian Rivers Institute, Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada, E3B 5A3, and Faculty of Forestry and Environmental Management, University of New Brunswick, Fredericton, New Brunswick, Canada, E3B 5A3.

Subject: Scientific review and comments on **Conclusion 1 and the Big Picture** for the Draft Biological Objectives report based on the San Diego Regional Water Quality Control Board Biological Objectives for the San Diego Region.

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Reviewer's research background: My interdisciplinary research focuses on the interactions between environmental factors, particularly flow/water level, to help quantify the variability in structural and functional composition in aquatic assemblages. Part of my research explores the development of bioassessment approaches for freshwater systems, particularly streams, rivers and wetlands, with the development of national-scale diagnostic tools and regional reference condition-based assessment models using benthic macroinvertebrates, including the application of genomics-based tools.

This review focuses on **Conclusion 1** for the Draft Biological Objectives report based on the San Diego Regional Water Quality Control Board Biological Objectives for the San Diego Region (referred to as Draft Report in the remainder of the review). Broader comments on the overall document are provided.

The proposed amendments to develop and incorporate biological objectives into the broader Basin Plan reflects the wider scientific consensus that comprehensive watercourse assessments should involve more than individual ecosystem components. This is particularly important within multiple stressor environments because of highly variable spatial and temporal responses of different measured ecosystem components (e.g., water quality vs. benthic algae vs. benthic macroinvertebrates) to potential natural and anthropogenic stressors. The authors of the Draft Report clearly acknowledge that watercourse assessment objectives should move away from a solely bottom-up view (e.g., using water quality alone) and directly incorporate biological objectives in a structured design that involves multiple attributes for a more complete ecosystem assessment. The approach, methods and proposed amendments for implementation presented within the Draft Report are scientifically sound and use the best-available scientific and data methods, where the use of potentially-subjective scientific judgement is relatively limited, with the exception of individual re-assessments of reference sites outside the 10th percentile although these are also supported by data-based methods and consensus approaches. However, further clarification and additional details are needed to support some of the statements and analyses within the Draft Report and these are outlined in the review below.

Based on my research expertise, the focus of my review is **Conclusion 1: Numeric biological objective derivation for perennial and seasonal streams** for the following statement: "*The underlying method for deriving the numeric biological objective for streams is scientifically sound and protective of Beneficial Uses.*" The basis for the proposed amendment to the Basin Plan is the incorporation of a reference-based predictive index using benthic macroinvertebrates and the identification of a percentile-based threshold for the index. The review of **Conclusion 1** explores **three key components** outlined in the provided review documents:

a) *Use of benthic macroinvertebrates and the California Stream Condition Index – The underlying method for using benthic macroinvertebrates and the California Stream Condition Index is scientifically sound and will protect and restore the biological integrity associated with perennial and seasonal stream systems.*

   The State of California has developed an extensive monitoring program for both benthic macroinvertebrate and soft algae and diatoms. The authors of the Draft Report highlight the importance of applying a complete ecosystem approach for site assessment moving beyond water chemistry and incorporating biological objectives. The adoption of a top-down approach, which is well supported within the wider scientific literature, underlies the methods and assessment development within the Draft Report. The use of benthic macroinvertebrate sampling paired with other sampling of other ecosystem components (e.g. benthic algae, water chemistry, habitat surveys, etc.) is a scientifically-sound approach for a bioassessment program.

   The Draft Report provides a summary of the sampling and laboratory methods for both benthic macroinvertebrates and soft algae and diatoms. However, further detail could be provided to support the understanding of both the benthic macroinvertebrate and benthic algae data including additional laboratory information (e.g., taxonomic resolution for benthic algae and diatoms, clarification that SAFIT II and IIa taxonomy are at the genus-/species-level with Chironomid reported at the subfamily level (IIa), laboratory subsampling procedures for both methods, high-level summary of QA/QC procedures). Also, additional information could be provided to describe how the duplicate paired benthic macroinvertebrate and benthic algae samples from the 10% of sites are used in assessment of the sampling program. The description of the physical habitat surveys could also be expanded, for example how often are physical habitat surveys conducted outside of a benthic sampling event? Depending on the stream type, hydrological variability (including flashiness), sediment mobility and the length of the delay then this could affect the site-level habitat assessment.

Other bioassessment metrics are discussed within the Draft Report (e.g., benthic algae) that can be linked to the Stream Biological Objective. Benthic algae is further discussed as a bioassessment indicator because it is routinely collected and is also part of the duplicate sampling at 10% of study sites. However, it must be remembered that benthic macroinvertebrates and benthic algae have differing temporal responses given their turnover. This should be considered when quantifying site status (both in terms of impact and also in terms of reference state).

Further, the use of water chemistry data to support a site assessment is a routine measure but can be problematic depending on frequency of samples, parameters assessed and field procedures to maintain high quality samples. Importantly, the authors highlight these potential sources of error and emphasise that multiple water chemistry samples are needed before a site is assigned, and combined with additional evidence as necessary.

The discussion for the inclusion of both perennial and regularly seasonal intermittent streams within the assessment is well supported by direct evidence from several peer-reviewed scientific publications. However, the authors only briefly discuss assessments outside of these two stream types – do these findings also hold true in ephemeral streams or is that part of the ongoing research (reference to Loflen, unpublished data)? Or are ephemeral streams more formally included in the definition of seasonal intermittent streams as suggested in Section 4.5 but with caveats because of sampling limitations? Are the distribution of these stream types changing given changing climatic conditions (short- and long-term) and the ongoing statewide drought?

The authors of the Draft Report highlight the recommendations of Mazor et al. 2015 with typical sampling periods depending on stream types and whereby a minimum of a four-week sampling delay from the start of stream flow/last storm resetting event is used prior to sampling to allow for recolonization and stabilized community composition. However, later in Section 4.5, the authors refer to a "two, and preferably, three week" delay following a storm event so clarification is needed here. Further paragraph 2 on page 62 mostly duplicates information from earlier paragraphs within Section 4.5 and could be incorporated with that earlier text.

The CRAM method is presented in a high-level summary but additional detail could be provided to explain how metric scores are aggregated across the four categories. Are these categories equally weighted? How are they combined? How are the different potential stressors tallied?

b) *Use of a reference approach – The assumptions and methods used to identify and define "reference" as a biological integrity benchmark are scientifically sound and will protect and restore the biological integrity associated with perennial and seasonal stream systems.*

The adoption of a reference approach for assessment is a widely-accepted method within the scientific literature to assess freshwater systems through both models and index calculations. The approach to identify site types and representative reference samples allows for the identification of tailored reference communities for use in an observed vs. expected assessment.

The objective of the Clean Water Act is to "… restore and maintain the chemical, physical, and biological integrity of the Nation's waters" (page 23) but there is no accepted definition of the term integrity in the CWA, although the authors of the Draft Report note that it was discussed extensively in committee. However, it is unclear if the authors of the Draft Report accept the presented definition by Karr (1999), which is widely cited and generally accepted within the broader scientific community in the field, and if this is the basis for the development of the reference approach and subsequent assessment.

The definition of reference is difficult and often subjectively defined depending on the focus of the assessment. Here a reference site is defined as "one that is exposed to very low or no anthropogenic stress" (page 24 of the Draft Report) allowing minimal disturbance sites to serve as reference. However, the authors should provide spatial and temporal bounds on this definition. How often are reference sites re-evaluated? What is the spatial frame of the reference site? How far upstream? Lateral connection?

The reference stream approach was developed through the Reference Condition Management Program (RCMP). The program has data from 750 reference sites around the State. These reference sites are reported to represent natural gradients across different environmental variables. These sites are filtered by geospatial variables to assess their level of disturbance and can be assess by "on-the-ground post-hoc validation". How confident are the authors of the Draft Report that the pool of reference sites within the RCMP are fully representative of the range of variability in the State of California? How many reference sites are within each site type? Are they spatially and temporally replicated? The authors indicate that these reference pool sites will be re-evaluated over time but with caveats of feasibility and necessity. This is important, particularly given the potential temporal delays to updates for geospatial assessment layers (i.e. outdated layers), the sometimes rapidly changing land use and land cover (including potential illegal activities) and evolving assessment methods. However, it would be advisable to identify a core set of reference sites or a small percentage of rotating sites that should be reassessed on a regular rotation beyond the general temporal updates. These focused assessments would provide a structured approach to assess temporal change across the range of reference sites and highlight areas of change. If a shift is observed within a site or if assessments highlight a general change in conditions of a group of sites, how will the RCMP respond to these changes? How will new and more representative reference sites be added to the program?

Resistance and resilience to disturbances varies across different habitats and ecosystems. The authors explore the concept of resilience in the setup of reference sites focusing on the importance of interplay across different levels (e.g., population, community, species/individual). It might also help to discuss the concept of resistance to disturbance given its significance in understanding the integrity of an ecosystem and its key links with resilience. Note that there is a very strong reliance in the Oliver et al. 2015 citation and it would be beneficial to include other references that explore the concept including links to recovery from disturbance (e.g., Hodgson et al. 2015 https://doi.org/10.1016/j.tree.2015.06.010)

c) *Setting of index score threshold – The assumptions and methods to set the water quality objective as a percentile of reference using the California Stream Condition Index is scientifically sound, incorporates a margin of safety, and will identify sites that have a degraded biological condition. The allowance of site-specific scientific information on the physical, chemical, and biological condition of specific sites to prevent false positive identifications of impairment is scientifically sound.*

The SWAMP data led to the development of the California Stream Condition Index (CSCI) in 2015 using available benthic macroinvertebrate data. The use of the single 10th percentile threshold for the CSCI is typical within bioassessment frameworks (see RIVPACS program developed by the Environment Agency of England and Wales and the Australian AUSRIVAS program documentation and scientifically peer-reviewed publications as examples for their identification of reference condition). Indeed as described by the authors of the Draft Report, sites below the 10th percentile threshold are likely to be altered by single or multiple disturbances. The 10th percentile also balances the risk of Type I and Type II errors, thereby minimising risk. However, the authors should also consider the potential for higher CSCI scores at reference sites. Within other national (e.g., RIVPACS and AUSRIVAS programs) and regional bioassessment programs, reference sites are identified in the band between the 10th and 90th percentiles of reference samples to provide the most conservative group of reference sites. Sites with reference samples >90th percentile represent sites with potential organic enrichment or a possible biological hotspot that warrants further investigation. Further, there should be an assessment of the potential uncertainty around a calculated CSCI value, for example adding confidence intervals around the proposed reference values?

The approach adopted for the CSCI that allows for reference sites within similar site types based on physical (non-anthropogenic) factors forms the base for calculation of variables from other scientifically-defensible reference condition approaches at both regional and national scales (e.g., AUSRIVAS, RIVPACS, etc.) Importantly the authors of the Draft Report discuss potential limitations relating to the calculation of CSCI scores. However, while the calculation of the CSCI allows for the prediction of the expected community in terms of compositional structure, it is unclear how this approach can assess the "functional organization of the community" as proposed by the authors of the Draft Report and this could be expanded within the Draft Report.

The additional assessment of sites outside the 10th percentile of the reference distribution is important and the approach to consider additional measures for site status makes sense. However, further detail could be provided to formalise this assessment, for example how do the other methods of assessment align with the biological assessment? At what point is a site considered satisfactory by the San Diego Water Board? What if conflicts are observed across the different components? Are these sites reassessed over time?