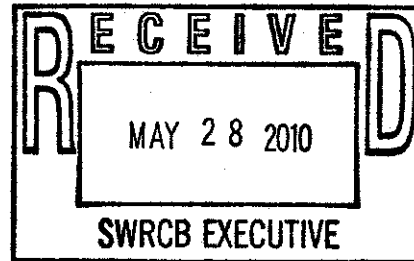


# NEWHALL LAND

28 May, 2010

Ms. Jeanine Townsend, Clerk to the Board  
California State Water Resources Control Board  
1001 I Street  
Sacramento, CA 95814



Attention: Ms. Jeanine Townsend

**Re:           Comments on the SWRCB's Draft Decisions on the 303(d) Listing for Benthic-Macroinvertebrate Bioassessment for Santa Clara River Reaches 5 (Decision ID 18003) and 6 (Decision ID 17217)**

Dear Ms. Townsend,

We appreciate the opportunity to comment on the Draft California 2010 Integrated Report 303(d) List (Draft List). The Newhall Land and Farming Company (Newhall) takes its responsibility to maintain and protect water quality very seriously, and works hard to meet its obligations. Our comments will focus on the proposed Benthic Macroinvertebrate Bioassessment listing for the upper Santa Clara River (SCR) Reaches 5 and 6.

We commend the State Water Resources Control Board (State Board) for making continued progress toward improving the clarity and objectivity of the 303(d) listing process through the development and implementation of the *Water Quality Control Policy for Developing California's Clean Water Act 303(d) List (Listing Policy)* (September 2004). We understand that the goal of the Listing Policy is to "establish a standardized approach for developing California's 303(d) list" and we support these efforts.

In general, we believe that several modifications should be made to the Draft List for the following purposes:

1. To base the listing on the standardized Biological Objectives, currently in development at the State level, in order to formally assess whether aquatic beneficial uses are supported;

2. To comply with Listing Policy Table 3.2, which specifies a minimum of 5 samples for the listing of conventional or other pollutants;
3. To allow for public review and comment at the local Regional Board level, rather than circumventing this process by adding a listing for SCR Reach 5 at the State level;
4. To avoid listing SCR Reaches 5 and 6 based on Southern California Index of Biotic Integrity (SCIBI) scores, developed without an appropriate level of transparency;
5. To take into account the fact that the SCIBI scoring system was predominantly based on high-gradient reference streams, not necessarily representative of the geomorphic conditions present along SCR Reaches 5 and 6;
6. To allow for the collection of biological data used to support the SCR listings using the most effective collection method available; and
7. To develop an SCIBI scoring system specific to low-gradient streams sensitive to variability in watershed-scale disturbance in low-gradient streams.

Newhall has collected bioassessment data on the SCR in the past and is familiar with and knowledgeable on SWAMP procedures and the SCIBI scoring system. Newhall has been tracking these issues and respectfully requests that SCR Reaches 5 and 6 not be listed for Benthic Macroinvertebrate Bioassessment based on the following points:

- **Listings for Benthic Macroinvertebrate Bioassessments should not be made while the State is in the process of developing Biological Objectives.** The State is currently in the process of developing Biological Objectives for freshwater streams and rivers in California. Biological objectives are intended to “help improve water quality in streams and rivers by providing the narrative or numeric benchmarks that describe conditions necessary to protect aquatic life beneficial uses” (State Board, 2010). It is the public position of the State Board that without formalized Biological Objectives it is not possible to assess whether aquatic life beneficial uses are supported; the sufficiency of chemical criteria to protect aquatic life cannot be determined; and methods for identifying impaired waterbodies are inconsistent<sup>1</sup>.

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<sup>1</sup> Source: [http://www.swrcb.ca.gov/plans\\_policies/docs/biological\\_objective/bio\\_objectives\\_ovrvw030910.pdf](http://www.swrcb.ca.gov/plans_policies/docs/biological_objective/bio_objectives_ovrvw030910.pdf)

It is the opinion of Newhall that as the State's effort to develop new biological objectives is ongoing, there is no basis for this new proposed listing. As the State is well aware, there can be no 303(d) listing without an applicable water quality standard. While the proposed listing does cite a narrative toxicity objective, this would not be an applicable standard as there is no documentation provided to support a linkage between IBI scores and the presence of toxic substances or observed toxicity. Once finalized, the State's Policy for Biological Objectives should provide more definitive guidance for such Benthic Macroinvertebrate-based 303(d) assessments, however prior to the finalization of this Policy any such listing would be premature.

- **There are an insufficient number of bioassessment samples referenced for SCR Reach 5.** While Draft Decision 18003 references Section 3.11 of the Listing Policy, Situation Specific Weight of Evidence Listing Factor, a total of four (4) samples and four (4) exceedances are referenced in the Line of Evidence (LOE) for Decision ID 18003. Listing Policy Table 3.2 lists five (5) as both the minimum sample size and number of exceedances to place a water segment on the Section 303(d) list for conventional or other pollutants. While the proposed listing cites a narrative toxicity objective, bioassessment, iron, and chloride measurements are specifically referenced as the lines of evidence used to determine that water quality standards are not being met, however none of these are reliable indicators of toxicity. And as noted previously there is no documentation provided to support a linkage between bioassessment/IBI scores and the presence of toxic substances (iron here is a drinking water supply issue and chloride is an agricultural supply issue) or observed toxicity (i.e., no toxicity impairment exists and toxicity measurements have not been cited). Therefore, if this proposed listing was properly classified as a "conventional or other" pollutant, a minimum of 5 samples would be necessary for a listing. Therefore, it is the opinion of Newhall that SCR Reach 5 should not be listed at this time due to an insufficient sample size.

Additionally, Newhall has agreed to work with Regional Board staff to carry out systematic bioassessments to develop a more site-appropriate, robust database. As described in the attached Work Order, Michael Lyons of the Regional Board is currently working with San Jose State University to implement a monitoring and assessment program with a goal of collecting samples from random sites in the Santa Clara Watershed so that "at the end of five years (i.e., after 2012) a total number of random sites per watershed will have been sampled to produce a statistically valid assessment." Therefore Regional Board staff also acknowledge the need for additional bioassessment

information in order to develop an accurate and robust basis for assessing the stream condition and major stressors to aquatic life in these reaches of the SCR.

- **The State Board listed SCR Reach 5 for Benthic Macroinvertebrate Bioassessment without providing a forum for discussion at the Regional Board level.** A listing at the State level circumvents the process that would traditionally allow for transparency and dialogue between the Regional Board and interested parties/stakeholders at the local level.
- **There is a lack of transparency associated with the raw data from the Index of Biotic Integrity (IBI) reference sites.** The Southern California Index of Biotic Integrity (SCIBI) was developed using data collected from numerous reference sites around California (Ode et al. 2005). These data are not readily available to the public via the State Board or California Department of Fish and Game web sites. The raw data supporting the SCIBI should be easily accessible to the general public to allow for independent analyses if the SCIBI is to be the basis for 303(d) Benthic Macroinvertebrate Bioassessment listings.
- **The SCIBI was developed using reference sites that were predominately high-gradient stream systems (slope > 1 percent) and is not representative of the unique characteristics of low-gradient streams such as found in SCR Reaches 5 and 6.** The SCR encompassing Reaches 5 and 6 is a low-gradient system differing in many ways from high-gradient streams. For example, low-gradient channels are typically complex (e.g., braided), subject to reformation due to frequent flooding, have shifting sandy substrates, and a scarcity of riffles and other microhabitats commonly targeted for bioassessment evaluations (Mazor et al. 2009, see attached). High gradient streams have more defined, consolidated channels supporting bed substrates of cobble, boulders, or bedrock (i.e., a diversity of microhabitats for benthic macroinvertebrates). In development of the SCIBI, Ode et al. (2005) noted that three IBI metrics scored substantially lower in chaparral reference sites (where low-gradient systems occur) than in mountain reference sites (EPT richness, percent collector gatherer + collector-filterer individuals, and percent intolerant individuals). To address this issue, the authors "adjusted" for the differences creating two scales; one for each ecoregion. However, given the clearly distinct differences in stream substrate material, channel morphology, and microhabitat diversity of low gradient streams in California (combined with the gravity of 303(d) impairment listings), mere adjustment of metric scoring values in this

manner (not clearly described in Ode et al. 2005) is inconsistent with the otherwise statistically rigorous analysis of the subject in Ode et al. (2005). Additionally, one reference site (Cattle Creek) with characteristics roughly geomorphically similar to SCR Reaches 5 and 6 (meandering alluvial channel) was found to have a 'poor' IBI score, potentially bringing into question the validity of this scoring system for such waterbody types.

It is the opinion of Newhall that the development of a SCIBI specifically targeted to low-gradient systems is needed in California, and would be a more robust and representative approach for evaluating stream impairment for the purposes of 303(d) listings based on Benthic Macroinvertebrate Bioassessments. This is not a novel position; as it is our understanding that such an effort is underway or under consideration by the State Board as part of the Biological Objectives development process, and we encourage and offer our support in this regard. Until such an SCIBI and associated implementation guidance is developed and integrated with the Biological Objectives for the State, the 303(d) Benthic Macroinvertebrate Bioassessment listing for SCR Reaches 5 and 6 would be premature.

- The biological data used to support the SCR Benthic Macroinvertebrate Bioassessment listings may not have been collected using the most effective collection method. Mazor et al. (2009) conducted a comprehensive review of three benthic macroinvertebrate sampling methods and their applicability to low-gradient streams in California in the context of the SCIBI. The methods evaluated included the targeted riffle composite (TRC), reach-wide benthos (RWB), and modification to the RWB method termed the "margin-center-margin" (MCM) sampling method; which unlike the RWB method, samples the more biologically productive and stable vegetated stream margins. The authors concluded that the MCM method "greatly improved [organism collection] efficacy", and recommended the use of MCM in low-gradient streams in California as a substitute to the RWB method.

The current SWAMP Bioassessment Procedures manual (SWAMP 2007) specifies the use of the RWB method for low-gradient streams that do not have sufficient riffle habitat. However, at the time of publication (2007) there was a recognized lack of consensus regarding sampling methods, and RWB versus MCM comparison studies were underway. As a result "interim recommendations" were included in the Procedures manual directing investigators to collect both RWB and MCM samples in low-gradient streams until the

outcome of the comparison studies was known. We now know (Mazor et al. 2009) that the MCM method is a more effective method for collecting benthic macroinvertebrates in low-gradient streams and that the resulting benthic macroinvertebrate community composition is more similar to that associated with the TRC method than the RWB method. This infers that the consistent use of the MCM method in low-gradient streams will produce a greater number of organisms and more accurately reflect community structure than the historically recommended RWB method.

As such, potential implications are that 303(d) listing determinations based on Benthic Macroinvertebrate Bioassessments using the RWB collection method may not be an accurate representation of the impairment status where low-gradient streams are under review. Perhaps more seriously, the SCIBI itself may be flawed by the inclusion of data from low-gradient streams likely collected using the RWB method known now to be a potentially inadequate collection method for these unique systems.

Therefore, it is the opinion of Newhall that the 303(d) Benthic Macroinvertebrate Bioassessment listing for the low-gradient SCR Reaches 5 and 6 would be premature where such determination is based on macroinvertebrate data collected using the RWB method exclusively.

- **Other lines of evidence point to the potential inadequacy of the current SCIBI in properly characterizing the impairment status of low-gradient streams in California.** Mazor et al. (2009) calculated SCIBIs for 15 of the 21 low-gradient streams (slope  $\leq$  1 percent) they evaluated. Mean SCIBI scores were found to be well under the threshold score used to designate impairment (39). It is possible that all of these streams are impaired, but it is also possible that the SCIBI, based predominately on high-gradient stream systems, does not provide an appropriate representation of impairment status for low-gradient streams.

Specific to the SCR, it is reported in the Los Angeles County 1994-2005 Integrated Receiving Water Impacts Report<sup>2</sup> that of the six Watershed Management Areas in Los Angeles County, the SCR Watershed Management Area: (i) *“is the least developed and urbanized”*; (ii) *“has the lowest ratio of impervious land area”* (7 percent); and (iii) *“is*

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<sup>2</sup> Source: [http://www.waterboards.ca.gov/water\\_issues/programs/tmdl/records/region\\_4/2009/ref3248.pdf](http://www.waterboards.ca.gov/water_issues/programs/tmdl/records/region_4/2009/ref3248.pdf)

*the least populated*'. These attributes are not consistent with a determination of impairment for SCR Reaches 5 and 6 based on the current SCIBI. Mazor et al. (2009) reported that the SCIBI may not be sensitive to variability in watershed-scale disturbance in low-gradient streams. If this is indeed the case, (other factors notwithstanding) the current SCIBI may be overstating the impairment status for SCR Reaches 5 and 6.

Again, it is the opinion of Newhall that the 303(d) Benthic Macroinvertebrate Bioassessment listing for SCR Reaches 5 and 6 would be premature until such time consensus is reached on the suitability of the current SCIBI to low gradient stream systems.

Thank you for the opportunity to comment on the Draft List. Should none of the previous comments result in a removal of the proposed BMI listings for SCR reaches 5 and 6, we request that the SWRCB change the proposed listings from category 5 (requiring TMDL) to 4C (not requiring a TMDL), in acknowledgement of the fact that an individual pollutant is not causing the perceived impairment. We would be happy to discuss our comments in a follow-up meeting with SWQCB staff. Please contact me at 661-255-4069 to discuss our comments or address any questions you may have.

Sincerely,

The Newhall Land & Farming Company



Mark Subbotin

Vice President of Community Development

- att: (1) Mazor, R.D., Schiff, K., Ritter, K., Rehn, A., & P. Ode (2008) Bioassessment tools in novel habitats: an evaluation of indices and sampling methods in low-gradient streams in California. *Environmental Monitoring and Assessment*. DOI 10.1007/s10661-009-1033-3.
- (2) Lyons, M. (2007) SWAMP/SMC Field and Laboratory Analytical Services for RWQCB 4 for FY0910 Funds. Work Order No. SJSURF-09-4-001.

cc: Sam Unger, Interim LARWQCB Executive Officer  
Jon Bishop, SWRCB  
Jennifer Fordyce, SWRCB



**Work Order No. SJSURF-09-4-001**

**SWAMP Field and Laboratory Analytical Services for RWQCB 4 for FY0910 Funds**

1. **Work Order No.:** SJSURF-09-4-001 in support of SWRCB Contract No. 06-395-250-2.
2. **Work Order Title:** SWAMP/SMC Field and Laboratory Analytical Services for RWQCB 4 for FY0910 Funds
3. **Contractor:** San Jose State University Research Foundation.
4. **Contacts for this Work Order:**  
Regional Board 4: Michael Lyons 213-576-6718; Email: [mlyons@waterboards.ca.gov](mailto:mlyons@waterboards.ca.gov)  
MLML Data Team RB4 Liaison: Susan Mason, 831-771-4119;  
Email: [smason@mml.calstate.edu](mailto:smason@mml.calstate.edu)  
MLML QA Team RB4 Liaison: Will Hagan, 206-527-3313; Email: [whagan@mml.calstate.edu](mailto:whagan@mml.calstate.edu)
5. **Term of this Work Order:** 5/29/07 through 3/31/11.
6. **Maximum cost for this Work Order:** **\$208,183**

**\$208,183** is a portion of R4's FY0910 allocation in the SJSURF Master Contract.

7. **Signatures authorizing to proceed within this Work Order:** The signatures below indicate that the parties agree to the scope, deliverables, and budget specified in this Work Order. This Work Order may not be invoiced until the Project Director and the Contract Manager sign the Work Order. If the Contractor begins work prior to the signing of the Work Order, it is done so at the Contractor's own risk, should the Work Order not be executed for any reason. However, all work must be conducted within the term of this Work Order as shown above. If the services identified in this Work order cannot be completed for the budgeted amount, the Work Order must not be signed. Under no circumstances is any work to be completed in excess of the budgeted amount, or outside of the term of the Work Order, unless there is a formal written amendment to the Work Order.

**For Contractor:**

\_\_\_\_\_  
Signature  
Russell Fairey, SJSURF Project Director

\_\_\_\_\_  
Date

**For SWRCB:**

\_\_\_\_\_  
Signature  
Dawit Tadesse, SWRCB Contract Manager

\_\_\_\_\_  
Date

**8. WORK TO BE PERFORMED:**

**TASK 3: Coordination with SMC 2010**

**A. Purpose and Objectives of the Proposed Work:**

This Work Order implements a monitoring and assessment program under SWAMP for the RWQCB 4 (R4) using a portion of R4's FY 09/10 State WDPF funding allocation (\$374,000) in the SWRCB to SJSURF master contract No. 06-395-250-2. The services described in this Work Order will focus on monitoring numerous R4 streams in conjunction with the Stormwater Monitoring Coalition (SMC) and statewide SWAMP Perennial Streams Assessment (PSA) effort.

**SMC/SWAMP Perennial Streams Assessment**

Integrated regional watershed monitoring of Wadeable streams will be conducted in 2010 to implement designs developed by the Stormwater Monitoring Coalition (SMC) and the Surface Water Ambient Monitoring Program (SWAMP). The SMC is a coalition of stormwater management agencies and Regional Water Quality Control Boards (RWQCBs) from Ventura to San Diego. The SMC's mission is to cooperatively answer the technical questions that enable better environmental decision-making regarding stormwater management. The SWAMP is a statewide receiving water monitoring program administered by the State Water Resources Control Board (SWRCB). The two programs effectively cross paths in the area of Wadeable streams in southern California with the parallel objective of assess health of the region's aquatic resources. As such, the two programs have joined forces to create an integrated regional watershed monitoring program.

The Regional Watershed Monitoring Program addresses three questions of importance to regulated agencies, regulatory organizations, and public:

1. What is the condition of streams in Southern California?
2. What are the major stressors to aquatic life?
3. Are conditions in locations of special interest getting better or worse?

**B. Technical Approach:**

**i. Sampling Design**

**SMC/SWAMP**

Sample collection will be conducted by the MPSL-DFG SWAMP field sampling crew at sites selected within the Los Angeles Region via a randomized probabilistic design. The monitoring design includes sites within each of 2 watersheds [Santa Monica Bay (6) and Santa Clara River (14)] to be sampled during 2010. The goal is to continue to sample random sites per watershed each year, so that at the end of five years (i.e., after 2012) a total number of random sites per watershed will have been sampled to produce a statistically valid assessment. Sampling will include bioassessment of stream macroinvertebrates, assessment of the algal community, water toxicity testing and water chemistry analyses (nutrients, trace metals, pyrethroids) at each station.

**ii. Sample Collection and Transport**

For the SMC/SWAMP monitoring, the MPSL-DFG SWAMP field sampling crew will collect the samples, and will provide for storage of samples in proper containers and at proper temperature until return to the laboratory. MPSL-DFG SWAMP field crew staff shall enter data into the most current MLML SWAMP data management system, including latitude and longitude and GPS coordinates recorded during collection of samples at stations, and including digital cross-referenced photographs, directions to the site, and a site map shall be provided for the site for future reference. Other information collected in the field by MPSL-DFG SWAMP field crew staff should also be provided to MLML SWAMP data management staff, including field conditions and any other ancillary information, as requested/authorized and as site conditions allow. All of the field information requested shall be used in order to submit information for inclusion in the SWAMP database. Sample collection and subsequent processing and testing will be performed according to the SWAMP Quality Assurance Project Plan (QAPP) and SWAMP Laboratory SOPs.

**iii. Laboratory Analysis**

Chemistry analyses on water samples will be performed by DFG-WPCL in Rancho Cordova. Water toxicity tests will be performed by the Aquatic Toxicity Laboratory in Davis. Stream macroinvertebrate samples will be analyzed by DFG-ABL while soft algae samples will be analyzed by CSU San Marcos and diatom samples analyzed by the University of Colorado.

Actual analytical services that will be performed on each sample are shown on the attached table: "Table A - Services to be performed at each station/cost".

**iv. Data Analysis**

Chemistry data will include the analytical result, method detection limit, reporting limit, and relevant quality assurance (QA) information (or metadata information within the data report) on surrogate recovery (where applicable), duplicate relative percent difference (RPD), matrix spike percent recovery and RPD, blank spike percent recovery and RPD, and CRM percent recovery and spike concentration.

Any deviations from QA goals established in the QAPP will be noted. Data will be made available in electronic format unless otherwise requested.

**v. Data submission to SWAMP database/deliverable product for Work Order:**

Field crews will use the most recent SWAMP Field Data sheets for collecting field information and will enter the field data into the SWAMP 2.5 database.

The laboratory and any subcontracting laboratories must submit all analytical data, including applicable QA/QC data, in electronic format using the standard formats for the new 2.5 SWAMP database as specified by the SWAMP Information Management Plan, as outlined in the SWAMP QAMP and found at: <http://mpsl.mlmf.calstate.edu/swdataformats.htm>. Failure to provide electronic data, including QA/QC data, in the specified SWAMP format, will be ground for rejection of payment for respective analyses until such time that the data are submitted in the format requested.

**9. WORK BUDGET AND MAXIMUM WORK ORDER COST: \$208,183 (see Table A, attached).**

**\$208,183** is a portion of R4's FY0910 allocation in the SJSURF Master Contract No. 06-395-250-2.

## Bioassessment tools in novel habitats: an evaluation of indices and sampling methods in low-gradient streams in California

Raphael D. Mazor · Kenneth Schiff ·  
Kerry Ritter · Andy Rehn · Peter Ode

Received: 19 December 2008 / Accepted: 3 June 2009  
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**Abstract** Biomonitoring programs are often required to assess streams for which assessment tools have not been developed. For example, low-gradient streams (slope  $\leq 1\%$ ) comprise 20–30% of stream miles in California and are of particular interest to watershed managers, yet most sampling methods and bioassessment indices in the state were developed in high-gradient systems. This study evaluated the performance of three sampling methods [targeted riffle com-

posite (TRC), reach-wide benthos (RWB), and the margin-center-margin modification of RWB (MCM)] and two indices [the Southern California Index of Biotic Integrity (SCIBI) and the ratio of observed to expected taxa (*O/E*)] in low-gradient streams in California for application in this habitat type. Performance was evaluated in terms of efficacy (i.e., ability to collect enough individuals for index calculation), comparability (i.e., similarity of assemblages and index scores), sensitivity (i.e., responsiveness to disturbance), and precision (i.e., ability to detect small differences in index scores). The sampling methods varied in the degree to which they targeted macroinvertebrate-rich microhabitats, such as riffles and vegetated margins, which may be naturally scarce in low-gradient streams. The RWB method failed to collect sufficient numbers of individuals (i.e.,  $\geq 450$ ) to calculate the SCIBI in 28 of 45 samples and often collected fewer than 100 individuals, suggesting it is inappropriate for low-gradient streams in California; failures for the other methods were less common (TRC, 16 samples; MCM, 11 samples). Within-site precision, measured as the minimum detectable difference (MDD) was poor but similar across methods for the SCIBI (ranging from 19 to 22). However, RWB had the lowest MDD for *O/E* scores (0.20 versus 0.24 and 0.28 for MCM and TRC, respectively). Mantel correlations showed that assemblages were more similar within sites among methods than within

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methods among sites, suggesting that the sampling methods were collecting similar assemblages of organisms. Statistically significant disagreements among methods were not detected, although *O/E* scores were higher for RWB samples than TRC. Index scores suggested impairment at all sites in the study. Although index scores did not respond strongly to several measurements of disturbance in the watershed, percent agriculture showed a significant, negative relationship with *O/E* scores.

**Keywords** Low-gradient streams · Bioassessment · Multimetric indices · Multivariate indices · RIVPACS · Habitats · California · Methods comparison

## Introduction

Large-scale biomonitoring programs are often confronted with the need to assess habitat types for which assessment tools have not been developed. This problem is severe in large heterogeneous regions like California (Carter and Resh 2005). Developing and maintaining unique assessment tools for multiple habitat types may be prohibitively expensive and may impede comparisons of results from different regions. Therefore, assessing the applicability of tools in diverse habitat types is a critical need for large biomonitoring programs.

In Southern California, biomonitoring programs use tools like the Southern California Index of Biotic Integrity (SCIBI, Ode et al. 2005), which were developed using reference sites that were predominantly in high-gradient (i.e., >1% slope) streams. However, low-gradient streams are a major feature in alluvial plains of this region (Carter and Resh 2005). According to the National Hydrography Dataset (NHD Plus), approximately 20–30% of stream miles in California have slopes below 1% (US Environmental Protection Agency and US Geological Survey 2005). Several biomonitoring efforts in California specifically target low-gradient streams, as these habitats are subject to numerous impacts and alterations (e.g., Stormwater Monitoring Coalition Bioassessment Working Group 2007), even though the applicability of assessment tools created and validated in high-gradient streams has not been tested.

Low-gradient streams differ in many respects from high-gradient streams (Montgomery and Buffington 1997). For example, bed substrate is typically composed of fines and sands, rather than cobbles, boulders, or bedrock. In California and other semiarid climates, low-gradient channels are often complex, with ambiguous and dynamic bank structure. Frequent floods create new channels and cause streams to abandon old ones (Carter and Resh 2005). For bioassessment programs, an important distinction between high- and low-gradient streams is the scarcity of riffles and other microhabitats that are typically targeted by macroinvertebrate sampling protocols (e.g., Harrington 1999).

In this study, we evaluated application of three sampling methods and two bioassessment indices for use in low-gradient streams in California. We assessed sampling methods for efficacy (the ability to collect sufficient numbers of benthic macroinvertebrates), comparability (community similarity and agreement among assessment indices), sensitivity (responsiveness of the indices to watershed disturbance), and precision of the assessment indices.

## Methods

### Study areas

Twenty-one low-gradient sites were sampled in several regions across California (Table 1, Fig. 1). Most sites were in heavily altered rivers, although a few had protected watersheds. Slopes were estimated from the National Hydrography Dataset Plus (NHD+, US Environmental Protection Agency and US Geological Survey 2005) or from digital elevation models (for Jack Slough, Wadsworth Canal, and the Santa Ana River, which lacked associated data in the NHD+). All sites were on reaches defined in the NHD+ that had slopes of 1% or less.

### Sampling

At each site, three sampling methods were used to collect benthic macroinvertebrates: targeted riffle composite (TRC), reach-wide benthos (RWB),

**Table 1** Low-gradient sites included in the study

Site	Watershed	County	Watershed size (km <sup>2</sup> )	Stream order	% Developed		% Agricultural		% Open space	
					Shed	Local	Shed	Local	Shed	Local
Within Central and Southern California										
Central Coast										
S	Aptos Creek <sup>a</sup>	Santa Cruz	200	3	18	92	0	0	82	8
S	Salinas River 1	Monterey	10,940	6	14	71	0	1	86	28
S	Salinas River 2 <sup>a</sup>	Monterey	10,666	7	5	28	7	61	88	11
S	Salinas River 3	Monterey	9,141	7	5	13	4	27	90	60
S	San Lorenzo River	Santa Cruz	378	4	5	7	6	56	88	37
S	Santa Maria River <sup>a</sup>	Santa Barbara	1,844	6	4	4	6	0	91	96
South Coast										
S	Agua Hedionda Creek <sup>a</sup>	San Diego	80	3	76	77	0	0	24	23
S	Las Virgenes Creek <sup>a</sup>	Los Angeles	63	3	19	29	0	0	81	71
S	Rio Hondo <sup>a</sup>	Los Angeles	325	3	70	83	0	0	30	17
S	Santa Ana River	Riverside	1,965	6	25	78	1	0	74	22
S	Santa Clara River 1	Los Angeles	817	4	14	68	0	0	86	32
S	Santa Clara River 2	Los Angeles	1,107	5	16	76	0	1	84	23
S	Santa Clara River 3	Los Angeles	1,107	5	16	75	0	5	84	20
S	Santa Margarita River 1 <sup>a</sup>	San Diego	1,856	6	13	48	3	0	84	52
S	Santa Margarita River 2 <sup>a</sup>	San Diego	1,888	6	14	24	3	0	83	76
Outside Central and Southern California										
Bay Area										
X	Butano Creek	San Mateo	234	3	11	34	0	0	89	66
X	Redwood Creek <sup>a</sup>	Marin	44	2	4	10	2	24	94	67
Central Valley										
X	Jack Slough	Yuba	Unclear	3		7		91		2
X	Morrison Creek <sup>a</sup>	Sacramento	114	3	40	100	4	0	56	0
X	Pleasant Grove Creek	Placer	40	3	69	34	3	16	28	50
X	Wadsworth Canal	Sutter	Unclear	Unclear		12		87		1

Two sites in the Central Valley (Jack Slough and Wadsworth Canal) had ambiguous watersheds which could not be delineated. In addition, Wadsworth Canal had an ambiguous stream network, and stream order could not be determined. These ambiguities are in cells marked "Unclear"

S Assessed with the Southern California Index of Biotic Integrity, X not assessed with an index of biotic integrity

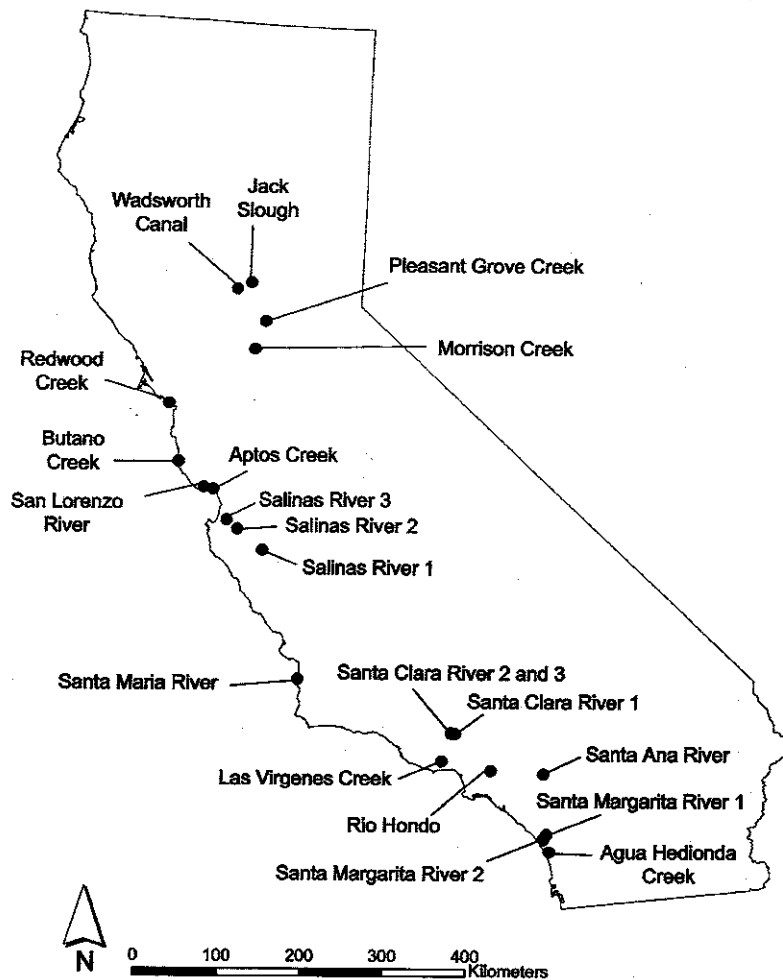
<sup>a</sup>Triplicate samples collected

and the margin-center-margin modification of RWB (MCM). The three sampling methods differed in the degree to which they targeted the richest microhabitats (e.g., riffles or vegetated margins). The TRC and RWB samples are similar to methods used in the nationwide Environmental Monitoring and Assessment Program (EMAP, Peck et al. 2006), and both methods are currently used in California's bioassessment programs (Ode 2007). MCM, a modification of RWB, is intended to capture marginal habitats not sampled by RWB and has been adopted for use in low-gradient streams in California (Ode and van Buuren 2008). Samples were displaced up- or downstream by 1 m when necessary to avoid interference among

different methods. At 12 sites, triplicate samples were collected for each method (Table 1).

For the TRC method, 11 equidistant transects were established along the 150-m reach, and three 1-ft<sup>2</sup> areas of streambed were sampled at three randomly selected transects. At each transect, field crews targeted the richest microhabitats, and a total of 9 ft<sup>2</sup> of streambed in three riffles were sampled. This method is similar to the targeted riffle composite method used by EMAP, which sampled a total of 8 ft<sup>2</sup> of streambed from four to eight riffles (Peck et al. 2006). A second difference was the fixed reach length of 150 m, in contrast to EMAP, which had a variable reach length set at 40 times the wetted width.

**Fig. 1** Location of study sites



In contrast to TRC, which allowed the field crew to sample the richest microhabitats within transects, the RWB method distributed sampling locations systematically. For RWB, 11 equidistant transects were established along the 150-m reach, and one sample was collected with a D-frame kicknet along each transect at 25%, 50%, or 75% of the stream width (with the position changing at each transect). A total of 11 ft<sup>2</sup> of streambed was sampled. This method is similar to the Reach-Wide Benthos method used by EMAP, except that EMAP used variable reach length set to 40 times the wetted width (Peck et al. 2006).

The MCM method was identical to RWB with minor modification. Instead of collecting samples at 25%, 50%, and 75% of stream width, samples were collected at 0%, 50%, and 100%. Unlike RWB, MCM samples from margins, which in low-

gradient streams often contain the richest, most stable microhabitats (e.g., vegetated margins). As with RWB, 11 ft<sup>2</sup> of streambed were sampled.

Benthic macroinvertebrates were sorted and identified to the Standard Taxonomic Effort Level 1 (i.e., most taxa to genus, with Chironomidae left at family) established by the Southwestern Association of Freshwater Invertebrate Taxonomists (Richards and Rogers 2006). When possible, at least 500 individuals were identified in each sample.

#### Data analysis

Bioassessment metrics and indices were calculated for each sample and analyzed to evaluate the efficacy, comparability, sensitivity, and precision of the three sampling methods.

### Calculation of indices and metrics

The Southern California Index of Biotic Integrity (Ode et al. 2005) was calculated for 15 sites located on coastal drainages from Santa Cruz to San Diego Counties. No indexes of biotic integrity (IBIs) were calculated for the two sites in the Bay Area and the four sites in the Central Valley because no IBIs for these regions were available at the time of the study. Furthermore, small sample sizes in these regions and unknown comparability of IBIs for different regions would limit the utility of including these sites. In order to calculate the SCIBI, benthic macroinvertebrate data were processed according to the requirements of the index. For example, samples containing more than 500 individuals were randomly subsampled with replacement to obtain 500 individuals per sample.

### Calculation of *O/E* scores

*O/E* scores were calculated for all sites using a predictive model developed for the state of California (Charles P. Hawkins, personal communication, Western Center for Monitoring and Assessment. Accessed online March 30, 2007: <http://129.123.10.240/wmcportal/DesktopDefault.aspx>). These scores are the ratio of observed to expected taxa and are based on only those taxa with a probability of occurrence  $\geq 50\%$ . The original identifications were converted to operational taxonomic unit (OTU) names used in the models, and ambiguous taxa (i.e., those that could not be assigned to an OTU and those that could not be adequately identified, such as early instars), as well as all Chironomidae larvae, were eliminated. The resulting sample counts were reduced to 300, if more than 300 individuals remained after removal of ambiguous taxa. Sites were assigned to the appropriate submodel based on climate (i.e., low mean annual precipitation and high mean monthly temperature), which were used to predict expected taxa occurrence (*E*) using longitude, percent sedimentary geology in the watershed, and log mean annual precipitation. Climatic data were obtained from the Oregon Climate Center (accessed online March 30, 2007: <http://www.ocs.orst.edu/prism>), and geological

data were obtained from a generalized geologic map of the USA (accessed online March 30, 2007: <http://pubs.usgs.gov/atlas/geologic>). Details of these predictive models can be found in Ode et al. (2008).

The two sites in the Central Valley were located in streams with ambiguous watersheds and therefore required that percent sedimentary geology be estimated, rather than calculated by geographic information system (GIS). For this study, percent sedimentary geology was estimated at 100%. Using other values of percent sedimentary geology (i.e., 0%, 20%, 40%, 60%, and 80%) had little effect on *O/E* scores (i.e., coefficient of variation of scores within each sample at the two Central Valley sites  $< 2\%$ , data not shown), perhaps as a result of the low numbers of observed taxa at these sites.

### Evaluation of sampling methods and indices

**Efficacy** To assess the efficacy of the sampling methods, we calculated the percent of samples for each method that collected at least 450 individuals (within 10% of the minimum number for calculating the SCIBI) or at least 270 individuals (within 10% of the minimum number for calculating *O/E*, counting only unambiguous taxa). In bioassessment applications, smaller samples would be rejected and represent wasted resources. In order to minimize the effects of pseudoreplication, the percent of samples containing an adequate number of individuals was calculated for each site, then averaged across all 21 sites. This rate estimated the likelihood of collecting adequate samples from the population of sites in the study. McNemar's test was used to test differences between methods (paired within sites) for statistical significance (Zar 1999; Stokes et al. 2000). Because McNemar's test required binary data, within-site rates were rounded to 1 or 0 at replicated sites. A Bonferroni correction was used to account for multiple tests across methods (i.e.,  $\alpha = 0.05/3 = 0.017$ ).

**Comparability** To see if the different sampling methods collected similar types of organisms, we compared community structure between sampling methods using a Mantel test (Mantel 1967). Mantel tests provide a measure of correlation (Mantel's *R*) between two sampling methods.



Sorensen distance was used as a dissimilarity measure. For sites where multiple samples were collected, mean distances were used; that is, matrices comprised mean or observed distances between pairs of sites, not samples. All samples were included in this analysis, regardless of the number of individuals collected. Significance was tested against correlation values for 999 runs with randomized data. A Bonferroni correction was used to account for multiple tests across methods (i.e.,  $\alpha = 0.05/3 = 0.017$ ). PC-ORD [Version 5.12] was used to run Mantel tests (McCune and Mefford 2006).

To determine the relative influence of sampling method on assessment indices, a variance components analysis was used to determine how much of the variability was explained by differences among sites, sampling methods, and their interaction. Restricted maximum likelihood (REML) was used to calculate variance components because of the unbalanced design. SAS was used for all calculations (using PROC VARCOMP method = REML, SAS Institute Inc. 2004). Unlike the mean-square method of estimating variance components, REML ensures that all components are greater than or equal to zero (Larsen et al. 2001). Because sites were a fixed factor and not a random factor, the variance component attributable to site must be considered a finite, or pseudo variance (Courbois and Urquhart 2004). Only sites where all three sampling methods were represented (after excluding samples containing inadequate numbers of organisms) were used in this analysis.

To assess agreement among the sampling methods, mean SCIBI and *O/E* values were calculated and regressed for each pair of methods. Slopes were tested against 1 and intercepts to 0 ( $\alpha = 0.05$ ); Theil's test for consistency and agreement, which is based on differences between sampling methods, was used as an additional test of comparability (Theil 1958). Pairwise differences between mean SCIBI and *O/E* scores were regressed against log watershed area and stream order to see if these gradients contributed to the observed disagreements. A Bonferroni correction was not used for either analysis in order to increase the ability to detect disagreements. Bias was not explicitly assessed because none of the methods

could be assumed to represent a true value. Only samples with adequate numbers of individuals were used in this analysis.

**Sensitivity** The sensitivity of the assessment indices to watershed alteration was assessed by correlating mean SCIBI and *O/E* scores against land cover metrics, including percent open, developed, and agricultural land within the watershed (for all sites with unambiguous watersheds; Table 1). This analysis assumed that the biology of the streams respond to these alterations of the watershed. Open water was excluded from all calculations. Land cover data was obtained from the National Land Cover Database (US Geological Survey 2003). Relationships were assessed by calculating the Spearman rank correlation, which is robust to non-normal distributions and extreme values in land cover metrics (Zar 1999). Only samples with the minimum number of individuals for each index were used in this analysis. Data from each sampling method were analyzed independently. A Bonferroni correction was used to account for multiple comparisons ( $\alpha = 0.05/6 = 0.008$ ) across two indices and three land cover classes within each method.

**Precision** Precision was evaluated by calculating the minimum detectable difference (MDD) of each sampling method for SCIBI and *O/E* scores (Zar 1999; Fore et al. 2001). The MDD was calculated using the mean-squared error (MSE) from a nested analysis of variance (replicates within site) as an estimate for average within-site variance. Because within-site, within-method replication was required, we only used site-by-method treatments where at least two samples had adequate numbers of individuals. These estimated variabilities were applied to a two-sample *t* test ( $\alpha = 0.05$ ,  $\beta = 0.10$ ) with three replicates in each sample. Additionally, we calculated the coefficient of variation (CV) of the indices for each method, averaged across sites.

## Results

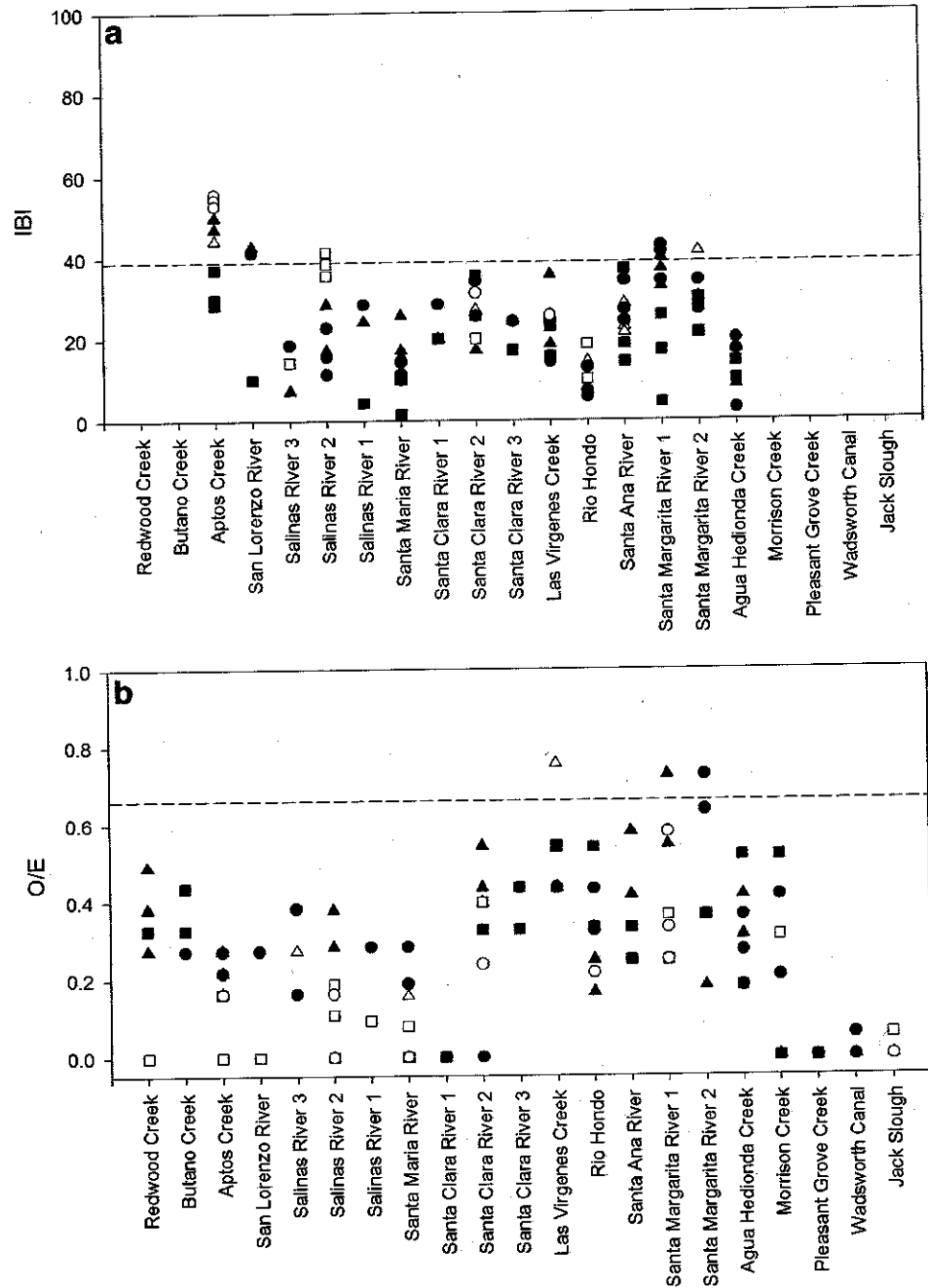
A total of 135 samples were collected at 21 sites throughout the state, of which 15 were in Southern and Central Coastal California. All three methods

were used at each site, and a total of 196 taxa were identified. SCIBI and *O/E* scores were low at most sites for all sampling methods (Fig. 2). Apart from one site (Aptos Creek), mean SCIBI scores were well under 39 (the threshold for impairment designation). *O/E* scores indicated impairment in nearly every sample, as all scores were below the impairment threshold of 0.66 in all but three samples.

Efficacy

Efficacy was low for all methods, and many samples contained fewer than the required number of individuals. Although each sample was supposed to contain at least 500 individuals, only 46 of 135 samples met this target. Another 34 samples had at least 450 individuals, the minimum required for calculation of the SCIBI. However,

**Fig. 2** SCIBI (a) and *O/E* (b) scores by site and method. Each point represents an individual sample. Triangles represent MCM samples. Squares represent RWB samples. Circles represent TRC samples. Black symbols are samples containing sufficient individuals for index calculation, and white symbols are samples containing insufficient individuals for index calculation. Dashed lines represent the threshold for identifying impairment with each index (i.e., 39 for the SCIBI, and 0.66 for the *O/E*)



55 samples had fewer than 450 individuals, meaning that IBIs calculated for these samples may not be valid. Furthermore, 55 samples had fewer than 270 unambiguously identified individuals, meaning that *O/E* scores may not be valid for these samples.

Several samples had extremely low counts (e.g., four individuals; Table 2). Most of these samples were collected by the RWB sampling method. Nearly half (22 out of 45) of RWB samples had fewer than 450 individuals. In contrast, only two MCM samples and six TRC samples had fewer than 450 individuals. The adjusted efficacy rate for the MCM method (54%) was twice that of RWB (27%) for collecting at least 450 individuals, and TRC was nearly as high (46%). However, these differences fell short of statistical significance once Bonferroni corrections were applied (i.e.,  $p > 0.017$ ). The rates were slightly higher for collecting 270 individuals (i.e., 67%, 32%, and 67% for MCM, RWB, and TRC, respectively), and these differences were statistically significant (McNemar's test  $p = 0.0039$ ).

### Comparability

Comparability of sampling methods was good, both in terms of multivariate community structure and in terms of index scores. Mantel's test showed significant correlations among benthic macroinvertebrate communities collected by all three sampling methods (Table 3). However, the RWB method had weaker correlations with both TRC (0.40) and MCM (0.45) compared to the higher correlation observed between TRC and MCM (0.69). In all cases, the correlations were significant ( $p < 0.002$ ).

Variance components analysis showed that the methods were highly comparable and that site accounted for nearly all of the explained variance

**Table 3** Mantel correlations between sampling methods

Method 1	Method 2	Mantel's <i>r</i>	<i>P</i>
RWB	MCM	0.45	0.001*
RWB	TRC	0.40	0.002*
MCM	TRC	0.69	0.001*

\* $p < 0.017$  statistical significance

in both indices. The analysis of SCIBI scores included seven sites and 26 samples, and the analysis of *O/E* scores included ten sites and 52 samples. Site accounted for 100% of the explained variance in SCIBI scores and 95% in *O/E* scores. Method and the interaction of site and method explained none or negligible components of the variance in these indices (0–5%).

Significant disagreements between pairs of sampling methods were not observed for either index (Table 4, Fig. 3). Slopes for all three comparisons were not significantly different from 1, and no intercepts were significantly different from 0. Consistency among SCIBI scores was best (i.e., slope closest to 1) between the MCM and TRC methods (slope 0.96) and worst for the MCM and RWB methods (0.62). In contrast, consistency among *O/E* scores was best between the MCM and RWB methods (slope 0.97) and worst for the RWB and TRC methods (slope 0.72). Their's test confirmed the lack of significant disagreements among IBI and *O/E* scores between pairs of methods. No differences between sampling methods were significantly related to log watershed area or stream order (regression slope and intercept  $p > 0.05$ ).

### Sensitivity

Sensitivity of both indices to gradients in land cover was poor, although to some extent, the relationships were affected by sampling method, spe-

**Table 2** Number of organisms collected by each sampling method

Method	Total		≥450 organisms			≥270 organisms		
	Samples	Sites	# Samples	Rate	# Samples	Rate	Rate	
MCM	45	21	34	76%	54%	32	71%	67%
RWB	45	21	17	38%	27%	14	31%	32%
TRC	45	21	29	64%	46%	30	67%	67%

Rate Site-adjusted estimate of sampling success rate

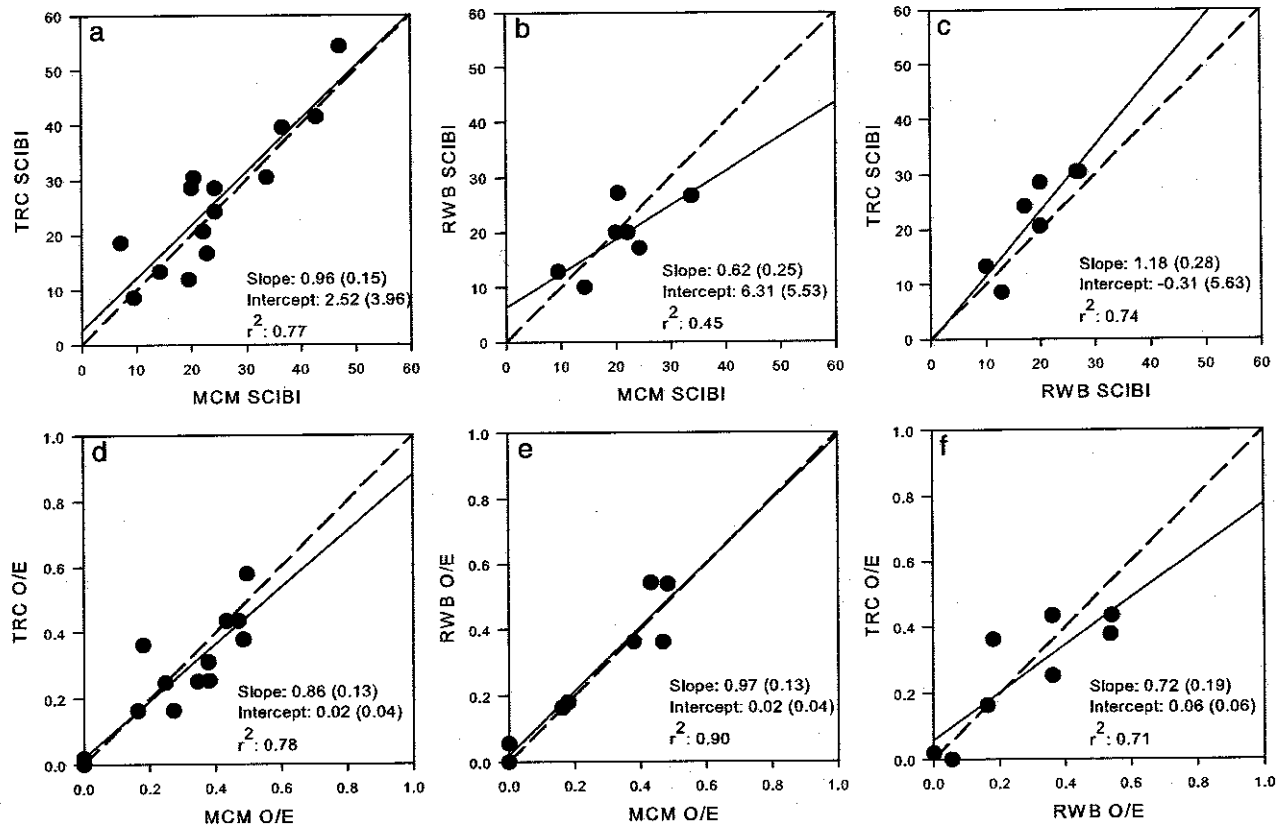
**Table 4** Regressions of mean IBIs and *O/E* scores for each method

Index	Method 1 (x)	Method 2 (y)	n	r <sup>2</sup>	Slope	SE	p	Intercept	SE	p
SCIBI	MCM	TRC	14	0.77	0.96	0.15	0.803	2.52	3.96	0.537
	MCM	RWB	7	0.45	0.62	0.25	0.194	6.31	5.53	0.305
	MH	TRC	7	0.74	1.18	0.28	0.540	-0.30	5.63	0.959
<i>O/E</i>	MCM	TRC	14	0.78	0.86	0.13	0.284	0.02	0.04	0.633
	MCM	RWB	8	0.90	0.97	0.13	0.816	0.02	0.04	0.653
	RWB	TRC	8	0.71	0.72	0.19	0.185	0.06	0.06	0.401

Slopes were tested against 1 and intercepts were tested against 0  
SE Standard error

cific cover type, and geographic scale (Table 5, Fig. 4). For example, *O/E* scores were strongly and negatively correlated with agricultural land cover in the watershed (Spearman's Rho ranged from -0.46 to -0.89 across sampling methods). However, most relationships between index scores and land cover metrics were not statistically significant (i.e.,  $p < 0.008$ ). Only the rela-

tionship between *O/E* scores from RWB samples were significantly correlated with agricultural land use in the watershed (Rho = -0.89,  $p = 0.003$ ). Although the direction of correlation often met expectations (e.g., percent open space in the watershed versus SCIBI, Fig. 4c), a few showed no clear relationship (e.g., percent developed land in the watershed vs. *O/E*, Fig. 4d).



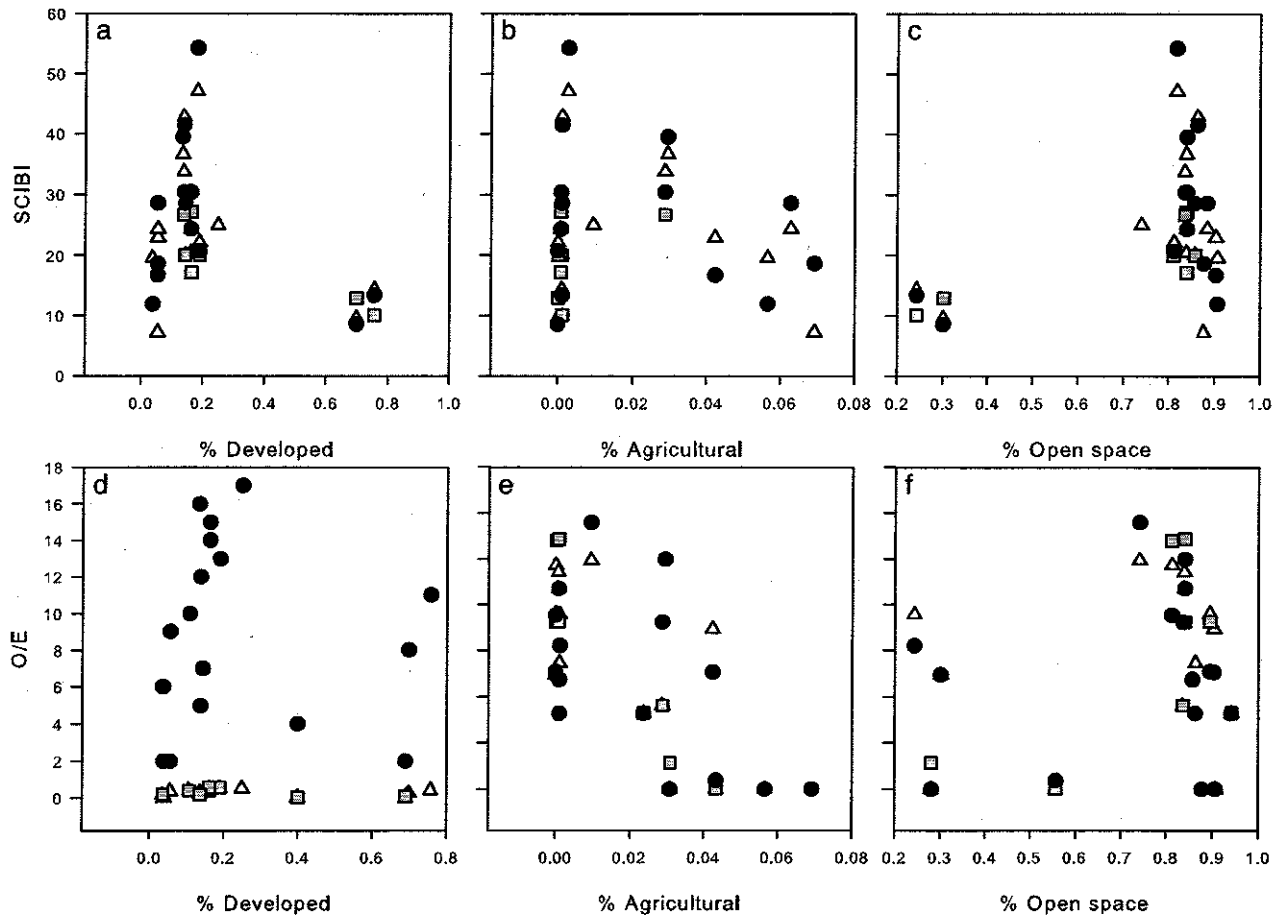
**Fig. 3** Agreement between the sampling methods for the SCIBI (a-c) and *O/E* scores (d-f). Each point represents the mean index score at a site. Solid lines represent linear regressions, and dashed lines represent perfect 1:1 relation-

ships. Numbers in parentheses are standard errors. Slopes were tested against 1, and intercepts were tested against 0. \* $p < 0.005$  indicates significant results

**Table 5** Spearman rank correlations (Rho) between bioassessment indices and landscape metrics

Index	Land cover	Method	Watershed			1-km radius		
			<i>n</i>	Rho	<i>p</i>	<i>n</i>	Rho	<i>p</i>
SCIBI	% Developed	MCM	15	-0.08	0.783	15	0.11	0.685
		RWB	7	-0.75	0.054	7	-0.59	0.159
		TRC	14	-0.32	0.914	14	0.20	0.487
	% Open	MCM	15	-0.04	0.892	15	0.09	0.742
		RWB	7	0.62	0.139	7	0.67	0.102
		TRC	14	-0.04	0.890	14	-0.08	0.782
	% Agricultural	MCM	15	0.06	0.842	15	-0.11	0.689
		RWB	7	0.12	0.799	7	0.22	0.628
		TRC	14	0.00	0.991	14	-0.02	0.954
O/E	% Developed	MCM	15	0.14	0.640	15	0.35	0.202
		RWB	8	-0.28	0.509	8	-0.07	0.866
		TRC	17	0.23	0.370	17	0.31	0.222
	% Open	MCM	15	-0.05	0.857	15	0.01	0.980
		RWB	8	0.40	0.333	8	0.17	0.693
		TRC	17	-0.24	0.355	17	0.02	0.948
	% Agricultural	MCM	15	-0.67	0.009	15	-0.24	0.388
		RWB	8	-0.89	0.003*	8	-0.15	0.719
		TRC	17	-0.46	0.064	17	-0.31	0.220

\**p* < 0.008 statistical significance



**Fig. 4** Index scores versus landcover metrics. Each point represents the mean of all samples collected by one method at each site. Gray triangles represent MCM samples. Black

squares represent RWB samples. White circles represent TRC samples

**Table 6** Within-site variability (expressed as mean square error, MSE) and minimum detectable difference (from a two-sample, two-tailed *t* test with  $n = 30$ ,  $\alpha = 0.05$ , and  $\beta = 0.1$ ) for each of the sampling methods

Index	Method		<i>df</i>	<i>SS</i>	<i>MSE</i>	<i>F</i>	<i>p</i>	<i>MDD</i>
SCIBI	TRC	Sites	7	2,507	358	12.5	>0.0001	19
		Residuals	15	430	29			
	RWB	Sites	3	403	134	3.7	0.0701	22
		Residuals	7	254	36			
MCM	Sites	8	1,745	218	8.0	0.0002	19	
	Residuals	16	437	27				
O/E	TRC	Sites	8	0.625	0.078	12.7	>0.0001	0.28
		Residuals	13	0.074	0.006			
	RWB	Sites	3	0.115	0.038	14.5	0.0037	0.20
		Residuals	6	0.016	0.003			
	MCM	Sites	9	0.860	0.096	20.9	>0.0001	0.24
		Residuals	17	0.078	0.005			

*df* degrees of freedom, *SS* sum of squares, *MSE* mean square error, *MDD* mean detectable difference

**Precision**

Sampling method affected the precision of both the SCIBI and *O/E* scores (Table 6). For example, the RWB sampling method had the largest MDD for the SCIBI (i.e., 22 versus 19 for the other two methods). However, RWB provided the lowest MDD when *O/E* scores were used (i.e., 0.20 versus 0.28 for TRC and 0.24 for MCM). CVs showed similar trends, with similar variability in the SCIBI among methods (ranging from 22% to 27%), and lower CVs for RWB when *O/E* scores were used (i.e., 12% versus 20% for MCM and 45% for TRC).

The low number of samples containing adequate numbers of individuals meant that estimates of within-site variance were sometimes based on very small samples. For example, only four sites in the region of the SCIBI had multiple samples with sufficient numbers of organisms collected by the RWB method. This problem was less severe for estimates based on *O/E* scores because fewer individuals per sample are required for index calculation and because sites in the Central Valley and Bay Area could also be used.

**Discussion**

Low-gradient streams are distinct from other streams in many aspects, such as substrate material, bed morphology, and the distribution of microhabitats (Montgomery and Buffington 1997).

As a consequence of these differences, traditional bioassessment approaches in California that were developed in high-gradient streams with diverse microhabitats have limited applications in low-gradient reaches. The sampling methods evaluated in this study differed in the extent to which they targeted the richest microhabitats (such as riffles or vegetated margins). For example, the TRC method allows field crews to select the richest microhabitats specifically. In contrast, the RWB method may systematically undersample or miss these habitats entirely, as the richest areas in low-gradient streams are typically found at the margins (Montgomery and Buffington 1997). The MCM method, a modification of the RWB method, was designed so that these margins could be targeted.

Caution should be used when applying sampling methods or assessment tools that were calibrated for specific habitat types (e.g., high-gradient streams) to new habitats (e.g., low-gradient streams). Our evaluation of assessment tools unveiled a number of shortcomings that weaken application of these tools in low-gradient streams, including the inability to collect adequate numbers of organisms, poor sensitivity of assessments, and low precision of the sampling methods. Significant disagreements among the methods were not detected, although power was low because of the low number of samples. The inability of the RWB sampling method to collect an adequate number of individuals in nearly half of all samples makes it unsuitable for low-gradient

streams, even though this method is widely used by bioassessment programs in California (Ode 2007) and across the USA (Peck et al. 2006). Although biomonitoring programs must assess a diverse range of habitat types with the tools they have available, we recommend that these programs invest in evaluating tools in novel habitats where monitoring activities occur.

Variance components analysis of assessment indices showed that differences among sites explained more of the variance in index scores than differences among sampling methods, suggesting that similar types of benthic macroinvertebrates are collected by the different methods. However, analysis of disagreements among the methods indicated that some samples collected by RWB were distinct from those collected by TRC, and samples collected by MCM were intermediate between the other two. For example, samples collected by TRC had lower *O/E* scores than samples collected by MCM, which in turn were lower than those collected by RWB. However, differences among these methods did not reach statistical significance.

Other studies comparing single, targeted habitat sampling methods (e.g., TRC) to multi-habitat sampling methods (e.g., RWB) have shown similar results. For example, MDDs reported in other studies (or calculated from reported variabilities) were comparable to those reported here, although generally larger (Rehn et al. 2007; Blocksom et al. 2008). However, these studies found that multi-habitat sampling reduced variability in multimetric indices, whereas we found that variability was lower for the single-habitat method (i.e., TRC; Table 7). As in Rehn et al. (2007), we found that TRC samples had higher *O/E* scores than RWB samples but that the strength of disagreement was inconsistent in the largest watersheds.

The generally weak response of the indices to landcover metrics suggests that the SCIBI and *O/E* may not be sensitive to variability in watershed-scale disturbance in low-gradient streams. This conclusion is tempered by small sample sizes that limited power, and sensitivity to reach-scale degradation was not explored in this study for lack of data. Several studies have shown the strong impact of reach-scale factors on benthic macroinvertebrates, which may exceed the influence of watershed-scale stressors (e.g., Hickey and Doran 2004; Sandin and Johnson 2004). Furthermore, most of the watersheds in the study were highly altered, particularly those in the region of the SCIBI, and we may not have adequately sampled portions of the disturbance gradient to which these indices are more sensitive. Several studies have found that biota responds to disturbance gradients  $\leq 10\%$  development in a watershed, but responses above this gradient are muted (e.g., Hatt et al. 2004; Walsh et al. 2007). Agricultural land cover, which was low in most watersheds ( $<10\%$ ) showed strong responses with the indices, suggesting that the study was able to capture portions of this gradient to which both the SCIBI and *O/E* were sensitive.

The low numbers of organisms collected from the low-gradient streams in the study may reflect the naturally low population densities of benthic macroinvertebrates in these reaches. The River Continuum Concept predicts that higher order streams with larger watersheds have a lower energy base because of reduced allochthonous input as well as depressed autochthonous productivity (Vannote et al. 1980). This lower energy base would be expected to support reduced biomass. However, observation of the sites in this study suggests that the lack of stable microhabitats (e.g., riffles and vegetated margins) may account for the

**Table 7** Minimum detectable differences in multimetric indices

Index type	Method	Present study	Rehn et al. (2007)	Blocksom et al. (2008)	
Multimetric index	Single-habitat	19.2 (SCIBI)	19.7 (SCIBI+NCIBI)	19.88 (VSCI)	29.79 (MBII)
	Multi-habitat	22.6 (SCIBI)	15.5 (SCIBI+NCIBI)	17.37 (VSCI)	17.91 (MBII)
Predictive model	Single-habitat	0.28 ( <i>O/E</i> )	0.22 ( <i>O/E</i> )	nt	nt
	Multi-habitat	0.20 ( <i>O/E</i> )	0.19 ( <i>O/E</i> )	nt	nt

SCIBI Southern California Index of Biotic Integrity, NCIBI Northern California Index of Biotic Integrity, VSCI Virginia Stream Condition Index, MBII Macroinvertebrate Biotic Integrity Index, *O/E* California *O/E* Index, *nt* not tested

reduced numbers of macroinvertebrates, as few species are adapted to the shifting sandy substrate found in most low gradient streams in California. A well-known but extreme example of the impact of shifting sandy substrates on maintaining low densities of benthic macroinvertebrates is the migrating submerged dunes in the lower Amazon River (Sioli 1975; Lewis et al. 2005). Although very high productivity of Chironomidae and other benthic macroinvertebrates has been observed in low-gradient sandy rivers of the southeastern USA, this productivity was attributed to snags and other stable microhabitats, more than to the shifting sandy substrate (Benke 1998). Thus, the vast majority of the macroinvertebrate activity in a large reach of river was found in small areas containing snags (Wallace and Benke 1984). Snag microhabitats are arguably less common in streams of the arid Southwest, which lack dense riparian forests to contribute snag-forming woody debris and may be less likely to be sampled using a systematic sampling method like RWB.

Bioassessment programs are often required to make do with available tools to fulfill regulatory mandates, yet they lack resources to evaluate the tools for applications in all habitats of concern. Although all sampling methods in this study suffered from poor efficiency in collecting organisms, the MCM method greatly improved efficacy and reduced the frequency of rejected samples. Furthermore, the lack of significant disagreements and inconsistencies suggests that the MCM method produced results that were comparable to the other methods already in use in California, which may facilitate integration of historical data sets (Cao et al. 2005; Rehn et al. 2007). Therefore, we recommend the use of MCM in low-gradient streams in California as a substitute for the currently preferred method (i.e., RWB). In conclusion, bioassessment programs can improve data quality and avoid unnecessary expenses by explicitly evaluating assessment tools when assessing novel habitat types.

**Acknowledgements** We thank the Aquatic Bioassessment Laboratory of the California Department of Fish and Game for laboratory processing and identification; Aquatic Bioassay and Consulting, Weston Solutions, Inc., and California Regional Water Quality Control Boards for

field sampling; and Chuck Hawkins for assistance with predictive models. This project was partially supported by the Stormwater Monitoring Coalition of Southern California.

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