

Assessment of Episodic Streams in the San Diego Region



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EXECUTIVE SUMMARY

Ephemeral streams and intermittent rivers are an important component of watersheds in arid regions, such as Southern California. They face increasing pressure from storm- or wastewater discharges, groundwater extraction, intensive recreation, and other human activities. Watershed managers need tools to assess and manage the impacts of these activities. To this end, the San Diego Regional Water Quality Control Board, the Southern California Coastal Water Research Project, and California State University at Monterey Bay completed a pilot project to develop and evaluate potential indicators of biological condition for use in ephemeral streams during their dry phase. When complete, these tools could support the integration of ephemeral streams into Water Board programs, such as headwater protection, identification of impaired beneficial uses, and evaluation of discharges or spills in these systems. Currently, such activities focus primarily on perennial or long-duration intermittent streams, which comprise only a small portion (~10%) of the San Diego Region’s watersheds.

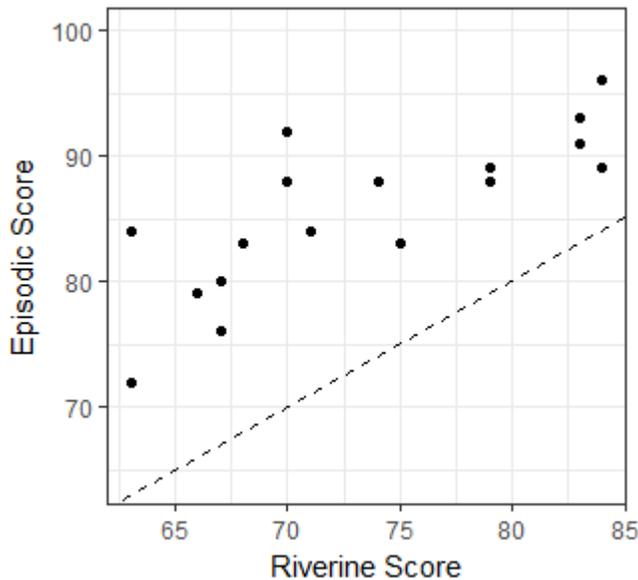


Cold Spring Creek (left), a minimally disturbed site, and Trabuco Creek (right), a highly disturbed site, represent the range of conditions evaluated in this study.

Following the EPA’s tiered approach towards wetland assessment, this study evaluated both “Level 2” (rapid, field-based) and “Level 3” (intensive) methods for assessing condition. We evaluated a newly developed “Episodic” module of the California Rapid Assessment Method (CRAM) for ephemeral streams, in comparison to the more traditional Riverine module for intermittent and perennial streams. In addition, we developed protocols and evaluated assessment metrics for two assemblages that inhabit dry streambeds: arthropods (e.g., spiders and insects) and bryophytes (e.g., mosses). Although caution is warranted when interpreting a study of limited size (39 sites, 22 reference sites), we demonstrate that these methods hold promise as tools to assess conditions in ephemeral streams.

Both CRAM modules provide similar information, but selecting a module requires more guidance

The Episodic CRAM module resulted in assessments that were up to 22 points higher (on a 100-point scale) than those realized through the traditional Riverine module, although module scores were typically within 11 points. The differences in scores largely driven by the Episodic module's lower expectations for biotic complexity. In most cases, the differences in scores were small enough that outcomes are unlikely to be influenced by the selection of a module. However, the choice of module could make the difference between a passing and failing score at moderately stressed sites. For regulatory applications, module selection requires transparent guidance that can be easily implemented and standardized among practitioners.



At most sites, the Episodic CRAM module yields higher scores than the traditional Riverine module, although scores were positively correlated with each other ($r=0.79$). The dashed line represents perfect agreement between the modules.

to human activity. Sampling effort is comparable to effort required to sample benthic macroinvertebrates in flowing streams, although arthropod sampling requires overnight deployment of traps, and therefore two consecutive site-visits. Capacity of labs to perform taxonomic analysis is likely high in the case of arthropods, but could be limited for bryophytes. However, molecular tools may be worth evaluating eventually as a means to obviate taxonomical expertise for that group.

Guidance in selecting between the Riverine and Episodic modules in the CRAM field books emphasizes map-based indicators, such as geographic location, stream order and mean annual rainfall. In contrast, we found that field-based indicators, such as dominant vegetation type and channel morphology, can be helpful. Moreover, we found that there are certain sites where either module may be appropriate. Guidance in the CRAM field books needs to be updated to help practitioners select an appropriate module.

Bioassessment in ephemeral streams is feasible, and likely to be successful

We developed sampling protocols for two potential bioindicators in ephemeral streams: terrestrial arthropods (such as insects and spiders) and bryophytes (mosses). For the two indicators, we calculated 130 metrics expected to respond

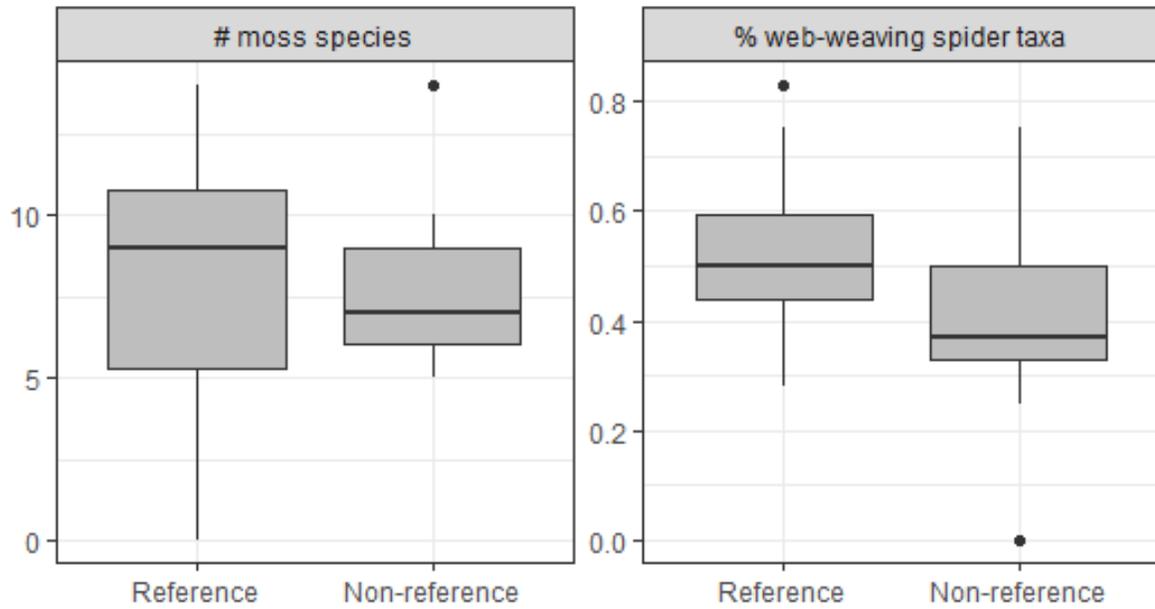


Arthropods are sampled by deploying “ramp” traps overnight (left), while bryophytes (right) are sampled through time-limited searches.

A number of metrics exhibited significant relationships with measures of human activity, suggesting that they could be used in an index of stream condition. Some metrics, such as the number of moss species, or the percent of web-weaving spider taxa, characterize sedentary components of the stream community, which may be more vulnerable to frequent physical disturbance (like active recreation or grazing). Others may reflect trophic structure or feeding strategies. For example, the relative numbers of predatory versus fungus-eating beetles may reflect a change in food sources associated with eutrophication or dumping of trash. Further investigation of the life histories of bryophyte and arthropod species could yield useful bioassessment metrics that provide insight into ecosystem function, and will be pursued through literature reviews in planned research projects.



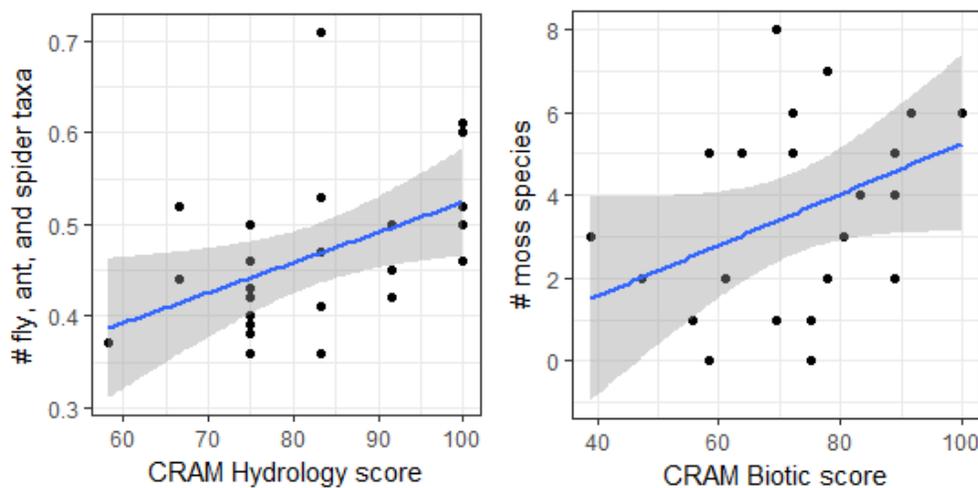
Samples are identified under a microscope to identify taxa, such as darkling beetles (left) and Fissidens moss (right).



Several metrics decline with increasing human activity. For example, the number of moss species, as well as the percent of web-weaving spiders, were both higher at reference sites (22 sites) than non-reference sites (17 sites).

Bioassessment metrics demonstrate the validity of rapid assessment methods

Many bioassessment metrics showed a strong relationship with the CRAM index and attribute scores, demonstrating the validity of these rapid methods. For example, the number of fly, ant, and spider taxa increased with CRAM scores, as well as measures of hydrologic and physical structure; similarly, the number of moss taxa in the streambed was positively correlated with the biotic CRAM attribute.



Both arthropod and bryophyte metrics are correlated to CRAM scores.

Recommendations for assessing conditions of ephemeral streams

This study demonstrates the feasibility of assessing ephemeral streams and including them in Water Board programs from which they are presently excluded. However, some additional steps may facilitate this integration. These steps may be beyond the scope of the Regional Board to pursue on its own; therefore, identifying collaborators with similar interests, both within the region (e.g., the Stormwater Monitoring Coalition [SMC]), in other parts of California (e.g., State Water Board and other Regional Water Boards), and in other states (e.g., regulatory agencies in Arizona or Nevada) should be a priority.

- *The Episodic CRAM module may be used now*, but additional guidance is necessary to help practitioners select between this and the Riverine module.
- *More work is needed to use arthropods or bryophytes as assessment tools*. Collect additional samples from both reference and stressed sites to validate results and assess temporal variability. Explore (and generate, if necessary) life history information to identify assessment metrics that provide meaningful insight into stream condition. Use these data to develop indices that provide a standardized, repeatable measure of biological condition.
- *Implement sampling protocols now* in programs or studies that need to assess the condition of ephemeral streams (e.g., the stream survey of the SMC). Although indices are not yet available, protocols are suitable for application to many monitoring programs.
- *Improve infrastructure* required to conduct assessments of ephemeral streams. In particular: conduct trainings and audits for practitioners in the region; refine quality assurance steps for both lab and field analyses; and create a standard taxonomic effort for both bryophyte and arthropod assemblages. Explore the utility of molecular methods to improve capacity to analyze bryophyte samples.

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SECTION 1: DEFINITION OF TARGET POPULATION

For the purpose of this study, the target population is streams that undergo prolonged (> 1 month) dry periods most years within the San Diego region. This population includes highly episodic channels that flow only after major storm events and might be dry for several years at a time, as well as streams that flow for several months a year in most years. This type of stream is common in headwaters, middle reaches, and lower portions of most watersheds in the San Diego region, as well as in deserts and in more mesic regions, such as coastal northern California.

SECTION 2: REVIEW OF BIOLOGICAL INDICATORS AND SAMPLING METHODS FOR ASSESSING ECOLOGICAL HEALTH OF DRY PHASE INTERMITTENT RIVERS AND EPHEMERAL STREAMS

Purpose

This chapter reviews the potential assemblages and collection methods we considered for assessing the ecological condition of dry intermittent rivers and ephemeral streams (IRES). We summarize the different assemblages and methods we assessed and the criteria we applied in selecting methods to incorporate into our protocols.

Candidate Indicator Assemblages

We evaluated eight potential biological assemblages as indicators of the ecological health of dry streams (Table 1), and chose terrestrial invertebrates and bryophytes as the assemblages most likely to respond to stress in a way that can be detected using low-cost sampling methods. To be useful in assessing the ecological condition of IRES, candidate indicators must have a few essential characteristics:

- 1) They are widely distributed throughout the region of interest.
- 2) They occur in IRES channel or riparian habitats during dry phases.
- 3) They conceivably respond to aquatic stressors (i.e., altered chemical, physical, or hydrological conditions) either by interacting with stream when it is flowing or with the dry streambed
- 4) They are minimally influenced by local upland conditions (i.e., have small home ranges) so that they reflect in-stream conditions, not in adjacent uplands.

In addition to these requirements, good candidate indicators share a number of desirable characteristics:

- 1) They have been successfully used as indicators in other regions or habitats.
- 2) Natural variability can be modeled from easily measured natural factors.
- 3) They have high diversity with a large capacity to respond to disturbance.
- 4) They are easy to sample and analyze.

We based our assessments on published literature, if available (summarized by assemblage, below), or best professional judgement.

Table 1. Indicators considered and the criteria applied in assessing their potential for assessing the ecological condition of IRES streams during dry phases. Criteria used are the 4 essential (in yellow) and 4 preferred requirements described in the text above. Y = Yes, indicator met criteria; N = No, indicator did not meet criteria; ? = conflicting or lack of evidence to assess criteria. Diversity and logistics assessed as low (L), medium (M) or high (H). Green text signifies that the criterion is supported, and red text indicates that the criterion is not supported. *Hyporheic invertebrates live in the saturated substrate of a streambed (e.g., tardigrades, nematodes, microcrustaceae, and small insect larvae).

Assemblage	Occur across ecoregions	Occur during dry phase	Respond to alteration in flowing or dry state	Minimally influenced by adjacent upland conditions	Previously used as indicator	Reference condition predictable	Diversity	Logistical requirements
Birds	Y	Y	?	N	Y	Y	L-H	H
Mammals	Y	Y	N	N	N	Y	L	H
Reptiles (lizards and snakes)	Y	Y	?	Y	N	Y	M	M
Terrestrial arthropods	Y	Y	Y	Y	Y	Y	H	L
Hyporheic Invertebrates*	Y	?	Y	Y	N	Y	H	H
Riparian vegetation	Y	Y	?	Y	Y	Y	H	M
Bryophytes	Y	Y	Y	Y	Y	Y	M	L
Diatoms	Y	?	Y	Y	Y	Y	H	L

Birds and mammals

Birds, and to lesser extent mammals, have some potential as indicators of ecosystem health but are not good candidates due to their poor connection to stream conditions and high logistical burdens. Birds were first suggested for use in bioassessments by Karr (1987), who showed that bird guilds can respond to the amount of human stress. Bird diversity ranges from relatively high to low in riparian zones in desert southwest (Austin 1970, Fleishman et al. 2003). However, there is little evidence that species found in IRES systems are likely to respond to changes in stream conditions and most species are more likely to respond to changes in uplands, which make up much of the available habitat. Because many birds migrate, the assemblage sampled during the dry phase may be significantly different from that which occurs when the stream is flowing. Sampling for birds would also require highly trained crews, making this option logistically

difficult. The connection between mammals and stream conditions is also largely unknown (although see Cross 1985). Mammals require relatively long trapping times (> 1 week) and return low diversity (< 3 species on average) (Ellison and Van Ripper 1998), making them unsuitable as indicator taxa. Unlike other terrestrial vertebrates, there is little evidence that birds rely on dry streambeds as transportation corridors.

Reptiles

Reptile assemblages are associated with dry streams, but sampling requirements and concerns about protected species of this assemblage make them less than ideal for use in bioassessment. Herbivorous and insectivorous lizards could conceivably respond to changes in arthropods and vegetation assemblages in response to human caused stressors, but this has not been demonstrated to date. They also have relatively small home ranges (< 40 m, Christian and Waldschmidt 1984), so lizards observed in channels would be primarily responding to channel and riparian conditions. They are also moderately diverse, with 10-30 species occurring in most areas of the desert southwest (Barrows et al. 2013). However, determining lizard species composition and abundance is difficult. Pitfall traps can result in death of the lizards through predation, and should be avoided to minimize the possibility of harming protected species (Conner and Holm 2006). Establishing pitfalls in dry cobble-bedded or bedrock streams would also require extensive effort and may not be practical in many places. Cover-boards are a good alternative to pitfall traps (Tietje and Vreeland 1997), but require a long deployment period (> 7 days). Methods that rely on direct observations (e.g., transect searches) require specialized training, and must be employed at a consistent time of the day (preferably during peak activity; California Department of Fish and Game 2004, Conner and Holm 2006) to eliminate the influences of daily variation. This constraint severely limits the number of sites that can be visited by a single crew in a day.

Terrestrial arthropods

Terrestrial arthropods meet all of our criteria, and have already been shown to respond to the ecological condition of dry streams in Queensland, Australia (Steward et al. 2016). Steward et al. (2016) showed decreased diversity or abundance with increasing stress for multiple taxa (beetles, ants, spiders). Arthropods have the greatest taxonomic richness of any assemblage in California (Howard et al. 2015), and most taxa have limited ranges, thus providing the greatest likelihood that composition or richness of this assemblage will respond to a wide range of stressor in dry streams that can be detected. Terrestrial arthropods can be easily collected using a variety of methods, making this assemblage logistically feasible.

Hyporheic invertebrates

Aquatic invertebrates in the hyporheic zone are probably the assemblage most directly associated with stream ecological health, but the logistic requirements (e.g., installment of collection wells, Del Rosario and Resh 2000) to collect them make them an unsuitable indicator for rapid assessment. Invertebrates from the hyporheic zone are the likely primary source of colonists of ephemeral streams when flow resumes (Vander Vorste et al. 2016). The aquatic invertebrate assemblage in flowing ephemeral streams is also sensitive to stream condition (Mazor et al. 2014), so the hyporheic assemblage is potentially the one most closely associated with the stream ecological condition. A meta-analysis of 75 studies of hyporheic invertebrates by Leigh et al.

(2013) supported the conclusion that this assemblage is likely to respond to anthropogenic disturbance in temporary rivers. However, sampling the invertebrates in the hyporheic zone requires extensive time and effort (e.g., Del Rosario and Resh 2000). There are also important questions that remain unanswered regarding sampling in dry streambeds, especially the depth and volume that need to be sampled to represent this assemblage adequately. These unknowns and the high logistical cost make hyporheic invertebrates unsuitable.

Riparian vegetation

Riparian vegetation has some of the characteristics needed to be useful in assessing stream condition, but it presents several difficulties that make it less suitable. Vegetation has been successfully used for bioassessments of wetlands (Cohen et al. 2004), and riparian vegetation generally has high diversity, facilitating robust indices. However, previous attempts at developing assessment tools using riparian vegetation were not successful due to their greater sensitivity to local hydrologic conditions than to human stressors (Wells 2005). Collection of data also requires botany expertise to allow plants to be identified in the field, increasing logistical costs. Although the California Rapid Assessment Method (CRAM, California Wetlands Monitoring Workgroup 2013), which is already used in routine biomonitoring at intermittent and ephemeral rivers, collects some botanical data, this protocol is focused on estimating richness of co-dominants and percent invasive species, and therefore produces taxonomic data that is inadequate for a Level-3 indicator.

Riparian vegetation does impact streams both directly (Cummins et al. 1989) and indirectly (King et al. 2005), and so it is an important input to stream health and is therefore assessed by CRAM. However, riparian vegetation does not necessarily respond to stressors or processes in streams (Belletti et al. 2015). Some species are sensitive to hydro-modification (e.g., cottonwood, Merritt and Cooper 2000), but this sensitivity may also only reflect local changes in water table due to log jams or other spatially isolated events and not overall stream condition (Wells 2005). This disconnect between riparian vegetation and stream processes can be especially problematic in urban areas (Imberger et al. 2014).

Bryophytes

Bryophytes (mosses) are known to be sensitive to stress and possess several of the desired characteristics for indicators of dry stream health. Aquatic bryophytes have long been known to respond to water quality (Vanderpoorten and Palm 1998, Gecheva and Yurukova 2014), and more recently have been shown to respond to physical and hydrological changes also (Vieira et al. 2012, Ceschin et al. 2012). Both semi-aquatic and aquatic bryophytes are commonly found in ephemeral streams during their dry phase (Vieira et al. 2016), and so have great potential as an indicator assemblage. Although bryophyte richness in Mediterranean streams ranges from relatively low (four species per site) to moderate (23 species per site; Vieira et al. 2012), the presence of cryptic species (e.g., *Grimmia laevigata*) and limited taxonomic understanding may artificially reduce these richness estimates (Malcolm et al. 2009). The potential for molecular identification is currently under exploration (S. Theroux, personal communication).

Bryophytes can be easily sampled and stored for later identification, similar to invertebrates. Bryophyte species composition can be predicted from environmental characteristics like climate and geology (Vieira et al. 2016), so the development of a predictive index similar to indices used

for benthic macroinvertebrates should be possible (e.g., Mazor et al. 2016). Bryophytes also respond physiologically to chemical and physical stress, showing detectable reductions in chlorophyll fluorescence after exposure to high nutrient levels, even after desiccation and rewetting (L. Stark, personal communication, 2016). Because bryophytes bioaccumulate contaminants in the water column, they have also long been used as *in situ* biomonitors for metals (Gecheva and Yurukova 2014). The ability to measure responses of individual plants to stressors provides the potential for these measurements to be included as metrics in a multi-metric index, which could potentially be sensitive to multiple forms of stress. Although the capacity of labs in California to provide taxonomic analyses of bryophytes is not known, keys are available (e.g., Malcolm et al. 2009).

Diatoms

Diatoms have been extensively used in bioassessments of flowing streams (Stevenson et al. 1999, Fetscher et al. 2014). Diatoms have also been used to infer historic environmental conditions in dry lakes (Smol 1992), and the potential for collecting and identifying valves in the dried biofilm crusts sometimes found in dry streams was pointed out by Rosen (1995). All published uses of diatoms to assess dried streams have used those found in undisturbed sediments (Stevenson et al. 1999) in deltas or floodplains. Diatom valves are typically found only in depositional habitats of a dried streambed (e.g., pools, Carvalho et al. 2002), which may be absent from some sampling reaches. However, Robson et al. (2008) established that benthic diatoms found in ephemeral streams after flow resumes were derived from a combination of upstream perennial pools, dried biofilms, and leaf-packs that serve as refugia for algae when the stream is dry. Therefore, diatoms collected from dried biofilm represent a subset of those observed under flowing conditions, and may not reflect the broader conditions of the stream reach. Steward et al. (2012) argue that sampling refugia may also be affected by stochastic founder effects, and strong biotic interactions, complicating their use as indicators. Pilot efforts by the San Diego Water Quality Control Board have had limited success in characterizing diatom communities from rehydrated sediments (B. Fetscher, personal communication).

Description of Recommended Sampling Methods

We selected sampling methods for terrestrial invertebrates and bryophytes that maximized efficiency of estimating richness, minimized logistics, and allowed effort to be standardized among sites based on effort levels used in other studies (e.g., Steward et al. 2016) and personal experience. We chose to sample both invertebrates on the ground using ramp-traps and in vegetation using a beating technique, and to sample bryophytes using a modified floristic habitat survey (Newmaster et al. 2005).

Terrestrial invertebrates sampled with ramp-fall traps

Pitfall traps have long been used to sample terrestrial invertebrate assemblages (Southwood and Henderson 2000), but have several drawbacks. For example, they need to be set at least a day before collection to allow for sufficient number of animals to enter the trap, thus requiring two visits, which impose a logistical burden. Digging pitfall traps would be impossible in areas where the streambed is composed of bedrock or a cement-lined channel and very difficult where the channel is comprised of loose or embedded cobble. Pitfall samples can also be biased by ‘digging-in effects’ (Greenslade 1973) (for example, newly deployed pitfall traps may

preferentially attract or repel species influenced by the scent of disturbed soil). Observational methods (i.e., using transects or quadrats) are an alternative that provides an invertebrate sample in a single visit, but in a comparison with pitfall traps, Corti et al. (2013) found that pitfall trap samples contained 3.5 times more species than taxa collected using quadrats in dry streambeds. Uetz and Unzicker (1976) also showed that pitfall traps give better estimates of wandering-spider diversity than quadrats, so despite the preference for sampling methods that can be completed in a single day, we rejected observational methods because of their limited ability of to measure diversity.



Figure 1. Ramp traps. Left shows adding sand/soil to ensure good contact between ramp and ground; the ramp leads to a small opening near the top of the container. Middle shows adding ~200 mL propylene glycol and a couple of drops of dish soap. Right shows securing lid on trap.

Ramp-fall traps (Bouchard et al. 2000) are increasingly popular and avoid the other difficulties and bias associated with digging in of pitfall traps (see Figure 1). Because ramp-traps do not require digging, they minimize ground disturbance, which could otherwise affect capture rates, and they can be applied to substrates that are difficult or impossible to dig. Tests of ramp-fall traps vs. pitfall traps show that ramp-falls catch equal or slightly greater numbers of invertebrate species (Pearce et al. 2005, Patrick and Hanson 2013) and also reduce the amount of vertebrate by-catch (Pearce et al. 2005). Ramp-traps are also inexpensive and require little time to emplace (Bouchard et al. 2000). For all of these reasons, we selected ramp-traps as our method for sampling terrestrial invertebrates. A comprehensive description of trap construction and deployment is provided in Section 3, but is summarized below.

Ramp trap deployment: Location and number of traps

There are many options for how traps can be operated, including location, spacing, number of traps, season, duration of trapping, and type of kill agent/preservative used. We followed the method used by Steward et al. (2011) with the deviations noted below. We limited trapping to the dry channel because the invertebrate assemblages in these areas have been shown to be distinct from the riparian assemblages (Steward et al. 2011), and these channel assemblages should be more closely related to stream health than the adjacent riparian. Steward et al. (2011) used six replicate traps, but we increased this to eight to allow us to better determine the optimal number of traps following Uetz and Unzicker (1976). Eight traps are expected to capture approximately 70-80% of the taxa (Corti et al. 2013). We placed traps in the channel using a stratified random design, with one trap randomly positioned in each 20-m segment of the sample reach (Figure 2).

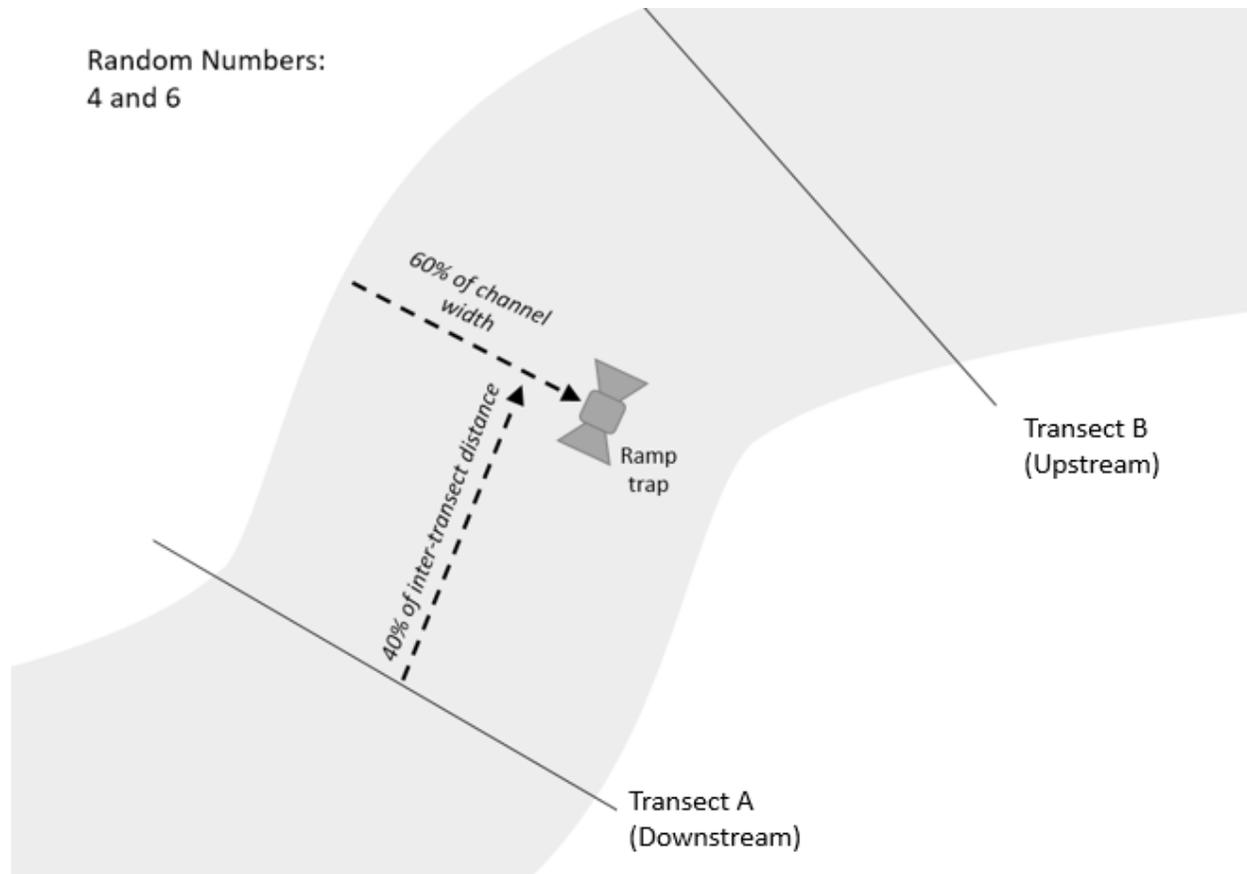


Figure 2. Example of random positioning of ramp traps. Two random integers are selected (between 0 and 10). The first number indicates the proportional longitudinal distance between the two transects, and the second number indicates the proportional lateral distance between the edges of the channel.

Ramp trap deployment: Season and duration of deployment

In this study, the season for arthropod sampling was constrained to late Summer by the time available for fieldwork, although additional studies are required to determine the influence of season on sampling arthropods in dry streambeds in southern California. Longcore (1999) found only a small (17%) decline in arthropod diversity in coastal sage scrub when using pitfall traps, suggesting that the influence may be small.

Trap deployment duration followed Steward et al. (2011), who deployed traps for 24 hours. To capture the full range of diel variation in invertebrate movement, traps should be left in place for close to 24 hours to ensure that each of the primary periods of movement (late afternoon, night, early and mid-morning) are sampled. Deployments of over 10 days are considered ideal for approximating relative abundance (Woodcock 2005), although shorter deployments still provide useful presence-absence data. Multi-day deployments are logistically difficult, and can increase the likelihood of predation of trapped invertebrates and drying or dilution of killing agent, and of traps being disturbed by animals, people, or floods. Schirmel et al. (2010) found that for long sampling periods, increased frequency of emptying is needed for accurate estimation of abundance. Many studies used sampling periods < 48 hours (Goehring et al. 2002, Borgelt &

New 2006, Sánchez-Montoya et al. 2016, and see review by Skvarla et al. 2014). An approximately 24-hour deployment period allows a single field crew to emplace and collect traps without excessive travel time. Therefore, these short deployments are preferred, even if they do not provide optimal information about relative abundance.

Both pitfalls and ramp-traps have higher capture efficiencies if a kill agent/preservative is added to the bottom. Propylene glycol has equal capture efficiencies as ethylene glycol, and significantly better than water or no-kill (dry) traps (Weeks and McIntyre 1997). Ethanol was used by Steward et al. (2011) as a killing agent/preservative, but Schmidt et al. (2006) found that it had lower capture efficiencies and invertebrates were not as well preserved. We chose propylene glycol as our agent/preservative because of its superior capture and preservation efficiency, relatively low cost, low toxicity (Skvarla et al. 2014).

Terrestrial invertebrates sampled from riparian vegetation

It is desirable to explore sampling protocols that, unlike ramp-fall traps, require only a single site-visit, so we decided to also sample terrestrial invertebrates in adjacent riparian vegetation to test its potential as an indicator. The composition of invertebrates on vegetation will be at least partly associated with the vegetation type, and thus be affected by the same variation that was discussed above when considering using vegetation as an indicator assemblage. However, invertebrates collected from vegetation close to the channel could potentially reflect stream condition while offering a much lower logistical cost than traps. Sampling invertebrates on vegetation can be carried out variously by observation/collection, brushing, washing, and physical, chemical or thermal knock-down (Southwood and Henderson 2000). Of these methods, physical knockdown with a beating sheet has the greatest collection efficacy for the least logistical cost.

In the beating sheet method, riparian vegetation is wrapped in a durable cloth sheet, such as canvas, that has been sewn on two ends together on two ends (similar to a pillow case, Figure 3). The wrapped vegetation is lightly “beat” with a stick (such as PVC pipe, a measuring stick, or the handle of a D-frame net) to dislodge arthropods from the vegetation into the sheet. After a set amount of time or set number of “beats”, the sheet is removed from the vegetation. All invertebrates found inside the sheet are removed by hand (with an aspirator or forceps, if necessary) and placed into a collection jar.



Figure 3: An example of the beating sheet technique to collect arthropods from mulefat.

The beating sheet technique provides a > 84% capture efficiency (McCaffrey et al. 1984, Ehmann 1994, Southwood and Henderson 2000, Heikkinen and MacMahon 2004). The beating sheet technique also produces better estimates of species composition than using funnel or vacuum methods (Costello and Daane 1997). This technique has been widely applied to sampling individual shrubs (Ehmann 1994, Hatley and MacMahon 1980). Often this technique is applied to an entire shrub surrounded by sheets, with the amount and density of the shrub estimated to allow for variation among shrubs to be controlled for statistically. However, riparian vegetation is often quite dense, and separating individual shrubs is not always possible. A variation of this technique is also applied to individual limbs of fruit trees (McCaffrey et al. 1984). Knutson (2010) tested multiple techniques for sampling individual limbs of riparian vegetation (salt cedar and willows), and found that beating sheets often under-sampled leafhoppers. We modified the beating sheet technique to use a cloth bag instead of a sheet. This approach provides some degree of standardization with regards to amount of vegetation sampled. The bucket technique (Knutson 2010) is a possible alternative to using a bag to sample vegetation, but a bag is considered easier to transport and potentially have a longer life span.

Bryophyte sampling

The recently developed floristic habitat sampling approach of Newmaster et al. (2005) was selected for sampling bryophytes. Vegetation and bryophyte samples are traditionally collected using randomly placed plots (such as quadrats), and all species within a quadrat are collected or identified in the field. In theory, each microhabitat (e.g., sand deposit, boulders, tree roots) are sampled in proportion to its abundance in the assessment area. But unless sampling intensity is high, this approach may miss or under-represent rare microhabitat types, which can greatly influence estimates of biodiversity. Newmaster et al. (2005) demonstrated that the floristic

habitat sampling approach more efficiently estimates bryophyte diversity than a plot-based technique.

In this method, each mesohabitat (specifically, the channel bottom and each bank) is searched to identify different microhabitats (e.g., sand deposit, tree root). These microhabitats are then sampled, providing the maximum amount of environmental diversity being sampled at a site in a minimum amount of time. The total amount of time searching each microhabitat is allocated to maximize efficiency in detecting species within the assessment area.

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SECTION 3: FIELD PROTOCOL FOR ASSESSING ECOLOGICAL HEALTH OF DRY PHASE INTERMITTENT RIVERS AND EPHEMERAL STREAMS

Introduction to the sampling protocol

This section describes the site selection, measurements, and sample collection methods developed to provide data to characterize the terrestrial invertebrates (both streambed and adjacent riparian) and bryophyte assemblages for assessing the ecological health of IRES during their dry phase. The goals of this protocol are to provide:

- a list of tasks and necessary equipment to collect samples and data
- instructions on how each task should be completed
- the recommended order of tasks to maximize efficiency of the field crew and limit time at each site.

Modularity

This protocol includes several modules: three modules focused on biological sampling (i.e., terrestrial streambed invertebrates, terrestrial invertebrates on riparian vegetation, and bryophytes), and three focused on characterizing the habitat in sampling reach (i.e., physical habitat measurements, stream characterization, and final walkthrough). As written, this protocol assumes that all modules are implemented at a site. If collection of only certain biological assemblages is desired, some modules may be excluded. However, we recommend that the modules focused on habitat characterization be implemented at every sampling event.

Time and effort required

This protocol is designed to be completed over two consecutive days to allow traps to be set for approximately 24 hours. By working on tasks concurrently, a two- or three-person crew should be able to complete day-2 tasks in 3-4 hours. This will allow up to 2 sites to be sampled by a crew of 2-3 people in a single day if they are near one another. The order of day-2 tasks can be adjusted, as needed, so that ramp traps are retrieved approximately 24 hours after emplacement. Vegetation should also be sampled for invertebrates at the beginning of day 2, before other activities (i.e., physical habitat measurements) on site disturb the vegetation. By deploying traps over a 24-hour period, this protocol can collect arthropods that are active at different times of the day.

Day 1	
Task	Estimated time
Sample Site Selection and Marking	30 minutes
Ramp Traps Deployment	30 minutes

Day 2	
Task	Estimated time
Invertebrate Vegetation Sampling	60 minutes
Bryophyte Sampling	120 minutes

Physical Habitat Measurements	60 minutes
Ramp Traps Retrieval	30 minutes
Stream Characterization	15 minutes
Final Walkthrough/Data Sheet Check	10 minutes

Field Equipment Checklist

General and Multipurpose:

- Rugged Tablet (Samsung Galaxy Tab Active or similar, serves as GPS, map, camera, and data sheet) or paper data sheets
- Aluminum clipboard (Tatum)
- Field notebook
- Black permanent (alcohol-safe) markers
- Pencils
- 100-m tape measure
- Aluminum stakes
- Garden clippers
- Leather gloves
- 2 m PVC pipe (in two 1-m long sections with connector, marked every 5 cm)
- 40+ Flags
- Electrical tape (to seal sample containers) and packing tape (to protect exterior labeling on sample containers)
- Packs/bags for transporting equipment to and from site (1 bag for general equipment, 1 for ramp traps, 1 for samples) and cargo boxes for storage in truck
- 20 plastic sample containers (350 mL, 8 for ramp traps, 8 for vegetation samples, 4 extras)
- 16 Sample interior labels
- 2 Spray bottles of tap water

Ground Invertebrate Sampling:

- 8 Ramp traps (each constructed from one Rubbermaid 5-cup food storage container or similar and two ramps constructed from aluminum; Figure 1)

- 2 L of propylene glycol (Peak Sierra Antifreeze, or similar)
- Dish detergent (<200 mL)

Vegetation Invertebrate Sampling:

- Sample bag (1 m², white duck cloth, sewn on 2 sides, Velcro closure on 3rd side, drawstring closure on 4th side)
- Sample labels
- Forceps
- 1-2 L 70% ethanol

Bryophyte Sampling:

- 100+ specimen envelopes - Both coin (~6x9cm) and small (~9x16cm) envelopes
- 25 + microhabitat envelopes (~15x23cm) with data sheet
- 5 site envelopes (~25x38cm) with data sheet
- Compass
- 10x or 20x hand lens
- Knife, spoon, and forceps for scraping/collecting bryophytes
- Timer

Physical Habitat Measurements:

- Clinometer or autolevel
- Gravelometer

Day 1 Protocol

Select and Mark Site

This protocol is designed to be applicable to a wide range of streams and rivers when dry. Sites should not be sampled using this protocol if they are:

1. Unsafe
2. Inaccessible
3. Wetted over 50% of the sampling reach (sites with damp ground or a few isolated pools can be sampled)
4. Lacking a discernible channel

A standard sampling reach is 160 m long. The lateral extent of the channel is defined by the banks. Look for indicators of bankflow (e.g., topographic change, evidence of erosion or transportation of sediment, change in perennial vegetation) when determining the lateral extent of the assessment area. By convention, the most downstream portion of the sampling reach should coincide with target latitude and longitude coordinates.

Designate and mark the sampling reach:

1. **Visually Survey** the general characteristics of the channel focusing on the following:
 - a. Dominant stream habitats (riffle, run, pool)
 - b. Vegetation types and cover
 - c. Substrate types
 - d. Channel confluences

The intent is to generate a general mental impression of the channel, rather than to formally begin data collection
2. **Identify the downstream end of the 160-m reach** that includes the common characteristics, and is the best representation of the stream as a whole, but that does not include other stream confluences > than 25% of main bankfull-width. If the channel is multi-threaded, then whichever channel appears to carry the most flow will be designated the sample reach and the other side-channels will be ignored (i.e., no data collected).
3. **Mark** sampling reach by placing flags in center of channel to mark the beginning and end of the sampling reach. Place additional flags at 20-m intervals along the side of the channel to designate 8 sampling areas for collecting arthropods and physical habitat data (bryophyte sampling is not linked to these transects). Although the transition from main channel to bank is conspicuous in many streams, it may be helpful to **mark the lateral extent** of the channel as well at some sites.

Deploy Ramp Traps

Each individual trap will include the following:

- 1 plastic food container (e.g., Rubbermaid 5-cup food storage container or similar, with a 2cm x 10cm slot cut into opposite sides 4 cm from bottom – see Fig. 1.)
- 1 lid (Marked with “Do not disturb, research in progress” and point-of-contact information)
- 2 aluminum ramps (30 cm long, with 25-cm long bottom opening, 10-cm top opening with lip, 2 cm sides, and coated with spray-on adhesive (e.g., 3M General Purpose 45 Spray Adhesive) covered in dirt to provide traction and camouflage – see Fig 1.)
- Approximately 200 ml propylene glycol
- Few drops of dish soap

Ramp traps are set during the initial visit to each site. Each site has eight ramp traps, one located randomly within each 20-m segment defined by the transects. Traps should be numbered corresponding to their location in the channel, with the downstream-most trap designated as 1, and the furthest upstream trap as 8. Traps can be emplaced at any time of the day, but need to be active at each site for approximately 24 hours so that all of the periods of invertebrate activity (early evening, night, early- and mid- morning) are sampled.

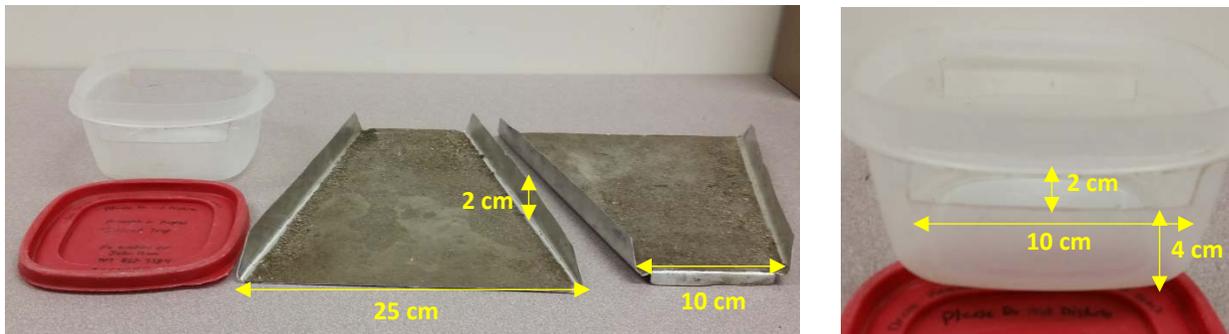


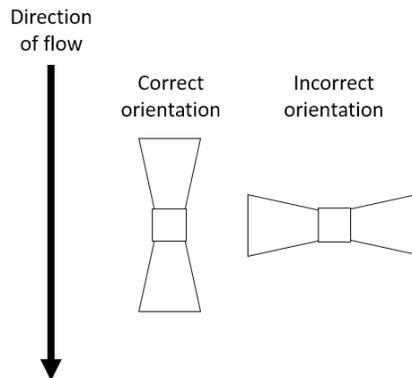
Figure 1. Ramp trap design.

Set one ramp trap within each of the eight 20-m sections between transects.

a) Select a random location within the stream section by generating a two-digit random number. The first digit times 10 designates how far upstream (in terms of percent of entire length of the section) the trap should be placed from that section’s downstream transect. The second digit designates how far (percent) across the channel the trap will be placed from the left bank, when facing upstream. The trap should be positioned in a place that is level and can be made flat by removing cobbles or debris, and should be as close as possible (within approximately 1-m radius) to the random location. *Note: If the first number is 0, place the trap 1 m upstream of the transect to avoid interference with physical habitat measures that are collected on the transect line, such as substrate particle size.*

Example: 45 is selected as a random number, so the trap is placed 40% of the way up the 20-m section from the downstream flag (i.e., 8 m), and 50% across the width of the channel.

b) Place the plastic container at the center of the stable, flat spot, with the entry holes facing up- and downstream. The trap should be oriented parallel to the inferred direction of flow during the wet phase:



c) Lay the ramps so the bent tab hangs over the cut edge of the container. Remove any cobbles or debris to ensure the ramp edge is flat to the ground. Minimize unnecessary disturbance of the soil and vegetation at the trap location.

d) Ensure that the base of the ramp is in full contact with the ground by adding soil or sand. Move at least 3 m away from trap when collecting the soil in order to minimize soil disturbance immediately around the trap. Use a trowel to place soil to minimize contact with your hands (See Fig 2).

e) Add about 200 mL of propylene glycol to the bottom of the container.



Figure 2. Trap emplacement. Left shows adding sand/soil to ensure good contact between ramp and ground. Middle shows adding ~200 ml propylene glycol and a couple of drops of dish soap. Right shows securing lid on trap.

f) Add 2-3 drops of dish soap to the propylene glycol to reduce surface tension and increase trapping efficiency.

g) Attach lid and place a flat rock on top of the trap to keep the trap stable and minimize the chance of it being disturbed.

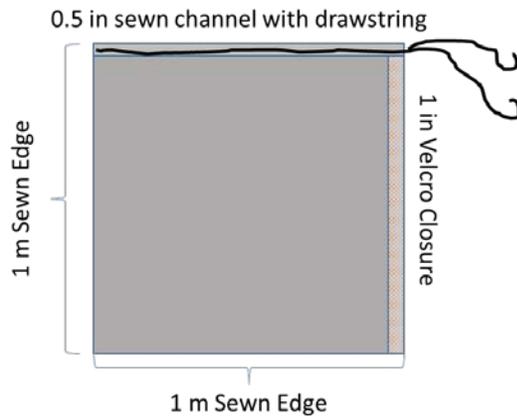
h) Note the location of the trap on a stream diagram drawn in the field notebook.

Day 2 Protocol

Vegetation Sampling for Invertebrates

The following materials are needed for sampling vegetation:

- Sample bag (1 m², white duck cloth, sewn on 2 sides, Velcro closure on 3rd side, drawstring closure on 4th side)



- 1-m 2" PVC pipe or similar beating device
- Forceps for collecting arthropods
- 1 L 70% ethanol
- 8 or more plastic sample containers (350 ml clear plastic recommended) (note: If samples are composited, a smaller number of larger containers may be sufficient)
- 8 or more sample interior labels (see example below)
- Permanent marker (alcohol-safe)
- Bag to carry samples and equipment around site

Recommended sample label for riparian vegetation arthropod samples:

Riparian vegetation arthropod sample
Station code: _____
Site name: _____
Collector(s): _____
Sample Date: _____ Time: _____
Transect: _____ Jar _____ of _____.
Plant species: _____

Vegetation should be sampled for invertebrates at the beginning of the day-2 visit, before other activities in and along the sampling reach can disturb the vegetation. One sample will be collected in each of the eight sections. Samples will be numbered same as the ramp trap samples were numbered, with sample 1 coming from the most downstream section and sample 8 from the most upstream section. For each of the eight 20-m sections:

1) **Survey** the channel, channel banks, and immediate riparian zone within a 20-m section and choose the plant that provides good invertebrate habitat closest to the channel. This plant should be a specimen that provides the best structure and nutrition, exhibiting robust growth of green leaves. The plant should be either in the channel, overhanging the channel, on the banks of the channel, or in the immediate riparian zone (i.e., the plant should be in contact with water during typical flooding events). Healthy, foliage-dense, perennial plants should be chosen over grasses or annuals, and actively growing plants should be chosen over dormant plants. Native and non-native plants alike should be considered for sampling. It is more important to choose a plant specimen that provides good arthropod habitat than it is to choose a specimen that represents typical conditions in the transect.

2) **Sample** chosen plant.

a) Record plant species, and photograph for later identification (if identity is uncertain).

b) Place a portion of the plant in the sampling bag. This portion can be a single limb, several limbs, or the entire plant, depending on the plant size. Select a portion that is close to the channel (less than 1 m from the ground), avoiding high-growing portions that are unlikely to be in contact with the water during typical floods. To the extent practical, exclude portions of neighboring plants, particularly if they represent different species. Open the Velcro closure, hold the bag with the drawstring side toward the plant, wrap the bag around the portion of the plant to be sampled, and close the Velcro. Cinch and hold the drawstring tight around the stem(s).

c) Beat closed bag with the PVC pipe vigorously 30 times (10 times on each side, 5 times on top and bottom).

d) Loosen drawstrings slightly and pull bag from the vegetation, brushing and shaking the plant as you do so, to trap any clinging invertebrates.

e) Open the drawstrings all the way and shake all plant detritus and invertebrates into the sewn corner (not the corner with Velcro). Working down from the top, open the bag gradually, shaking and brushing down any invertebrates clinging to the sides of the bag. Be careful to prevent flying or jumping insects from escaping. The goal is to gather arthropods into a corner of the bag. Large pieces of plant material can be discarded after visual inspection to make sure all arthropods have been removed, but small pieces should be collected along with the arthropods.

f) Once the sample is concentrated into the sewn corner, place a plastic sample container into the bottom of the bag, hold the bag tight around the container on the outside, and shake the sample from the corner into the container. Repeat this process to get all of the material from the bag into the container.

g) Open the Velcro closure and inspect the bag for any clinging invertebrates. Remove and place in the sample container using fingers or forceps.

h) Add approximately 100 mL ethanol to the sample container to kill the invertebrates and prevent predation. Put completed interior label in container, seal, and mark the lid with site code, date, sample number, and vegetation type (species, or common name).

3) **Sample Storage.** Once back at the vehicle, fill each container so all material is covered by at least 70% ethanol. Seal each container using electrical tape (wrapped clockwise). Mark sides of container with site code, date, sample number, and vegetation type and cover with packing tape to prevent it from coming off.

Bryophyte Sampling

The following materials are needed for sampling bryophytes:

- 25+ flags for marking microhabitats
- Spray bottle with tap water
- 100+ specimen envelopes - Both coin (~6x9 cm) and small (~9x16 cm) envelopes
- 25 + microhabitat envelopes (~15x23 cm) with microhabitat data sheet (see example below)
- Compass
- 10x or 20x hand lens
- Knife, spoon, and forceps for scraping/collecting bryophytes
- Camera
- Timer
- Bag to carry equipment and samples around site

Bryophyte sampling is conducted during the second visit to each site. This sampling protocol is designed to maximize the richness detected at each site and is adapted from the floristic habitat sampling approach of Newmaster et al. (2005), which focuses on 3 different “mesohabitat” types, as described below.

1) **Identify boundaries of 3 mesohabitats** within the entire 160-m sampling reach:

Mesohabitat	Description
Channel	Between indicators of bankfull flow (evidence of erosion or transport of sediment, less perennial vegetation)
Left and Right Riparian Zones	Left and right designated looking downstream. Riparian zone extends from bankfull flow indicator to break in slope, where inundated by extreme flood events, or 15 m (whichever is closer).

2) **Survey each mesohabitat and identify microhabitats** containing bryophytes for 20 minutes. Microhabitats can be any place providing necessary stability and moisture, but are commonly large rocks, wood, soil, and seeps. Stable, shaded areas at the base of large shrubs (e.g., mulefat) are often productive areas for bryophytes. Although the channel mesohabitat may lack bryophytes at some sites, it is very rare that bryophytes cannot be found on either bank. Typically, one bank (often the north-facing bank) is more productive than the other, and the channel mesohabitat is often the least productive.

Rarely, bryophytes are entirely absent from a sampling reach. This situation occurs where sites are very frequently disturbed throughout the entire reach (e.g., flood control channels with frequent vegetation removal). If no bryophytes are observed after the 60-minute survey, make notes in the data sheet explaining why a sample could not be collected.

Place a flag at each location where bryophytes are found, and mark a tally on the site data sheet for the appropriate microhabitat type (rock, soil, log, etc.) within the appropriate mesohabitat. All portions of a microhabitat need not be contiguous (e.g., a group of rocks can be designated a microhabitat). Microhabitat sampling patches are not restricted by size but are typically about 1 m², and should be able to be seen while standing in one spot. Flagging should preferentially target microhabitats with the highest density and richness of bryophytes determined by brief visual assessment made within 1 m of the microhabitat (e.g. squatting/crouching close enough to observe differences in richness and density). At this distance, non-specialists can detect differences in bryophyte colors and textures, which can distinguish different species. Differences among microhabitat types likely to influence bryophyte diversity and colonization potential should be separated into different categories. For example, rocks with contrasting chemical or physical surfaces (i.e., smooth granites vs. craggy conglomerates) should be recorded as different microhabitat types, living wood separated from dead wood, and seeping soil/rock distinguished from dry soil/rock.

3) **Allocate sampling effort** among microhabitats. Each of the three mesohabitats will be sampled for 12 minutes, for a total of 36 minutes of bryophyte sampling time per site. The total number of each microhabitat type detected during the survey (and recorded on the site data sheet) should be used to allocate sampling effort such that each microhabitat type is sampled at least once. If less than 5 microhabitats types are present, a microhabitat type may be sampled more than once (at different locations), based on its abundance within the mesohabitat. Time

should be allocated based on size and complexity of microhabitats, allocating more time to species-rich and/or complex microhabitats (e.g., craggy rocks), and less time to simpler ones (e.g., soils). Larger and more species-rich microhabitats should be given more sampling time than those with fewer species and smaller size.

Example:

Microhabitat	Rocks	Logs	Soil
Number of occurrences			
# of areas to sampled	2	1	2
# of minutes per sample	3	3	1.5

[2 rocks sampled for 3 min (= 6 min) + 1 log for 3 min + 2 soil patches for 1.5 min (= 3 min) = 12 minutes]

Often, microhabitats within a mesohabitat will all be similar, so time can be apportioned evenly across microhabitats (i.e., 5 microhabitats can be sampled in 2.4 minutes each).

4) **Record microhabitat data.** These data will be recorded on the microhabitat data sheet (provided below) included with the Medium envelopes:

- a) Mark the mesohabitat type.
- b) Mark the microhabitat type.
- c) Visually estimate size of microhabitat being sampled to nearest 1 m².
- d) Visually estimate shade cover directly above the microhabitat to nearest 10% (i.e., how much shade at mid-day).
- e) Visually estimate the relative cover to the nearest 10% of all bryophytes occurring in 1 m², centered on the microhabitat.
- f) Using the compass, record the direction (aspect) the microhabitat is facing.
- g) Record the time allocated for sampling the microhabitat.

5) **Sample the microhabitat.**

- a) Once all collection envelopes and tools are ready, lightly spray water over the entire microhabitat to aide identifying differences among bryophytes and collecting a variety. The bryophytes will quickly hydrate, making their colors more vibrant.
- b) Start a countdown timer set to the amount of time allocated to that microhabitat. Stop collecting when the allocated time for that microhabitat is reached.
- c) Collect a sample of each bryophyte species/functional-type (i.e., morphologically/chromatically distinct) and place it in a specimen envelope. A hand lens and a knife/spoon may be needed to distinguish species/types and dislodge samples. Entire clumps of bryophytes should be taken to increase species capture rates, which may result in several species placed in the same envelope. When possible, collections should contain enough tissue to facilitate microscope identification (>2 cm³), but should not exceed the size of the specimen envelope (the size of your palm).

- d) If a bryophyte species/functional-type appears to occur in multiple places in the microhabitat, multiple samples may be placed in the same envelope.
- e) If a bryophyte is found to occur on a substrate different from its characterized microhabitat type, note this difference on the specimen envelope. For example, if a rock microhabitat is being sampled, but a bryophyte is collected from soil occurring on or abutting the rock, note “soil” on specimen envelope.
- f) Once each bryophyte species/functional-type has been collected once, focus the remaining time on collecting additional samples of species/types already collected (aggregating species/functional-types in a single envelope when possible).
- g) If it appears all bryophyte species/functional-types have been collected from a microhabitat before the end of the allotted time, look for bryophyte specimens more than 1 m (but less within 1.5 m) from the initial central point of the microhabitat patch. Note this expansion of search area on the data sheet.

6) Sample storage.

- a) After the individual specimens have been collected, close, but do not seal, the specimen envelopes, and place inside the respective microhabitat envelope along with the datasheet for that microhabitat.
- b) All microhabitat envelopes are placed into one or more site envelopes (~25x38 cm), and label:

Bryophyte microhabitat sample						
Station code: _____						
Mesohabitat:						
Left Riparian	Channel	Right Riparian				
Microhabitat type:						
Rock1	Rock 2	Soil 1	Soil 2	Wood	Seep	Other _____
Estimated abundance/cover over 1 m²						
<1%	1-5%	5-10%	10-25%	25-50%	>50%	
% Shade: _____ % Aspect: N NE E SE S SW W NW						
Comments						

- c) Place the site envelopes in a box with no lid, facing upright and organized by date. This storage will allow the samples to dry to prevent growth of mold/fungus.

d) If samples are moist (due to rainfall or collection from seeps), *immediately* upon returning from the field, lay out each sample on top of their envelopes and allow them to dry overnight for at least two nights or until the samples are sufficiently dry.

e) Samples should be stored in a cool, dry location before being shipped to the laboratory for taxonomic identification.

Site Evaluation

The site evaluation consists of 3 steps:

- 1) Stream Characterization
- 2) Stream Condition
- 3) Physical Habitat Measurements

All data during this stage will be entered into a [digital data form in Excel](#) using a Samsung Galaxy Tablet, or on paper data sheets (printable hardcopy versions provided in appendix). The first two steps will be carried out by making estimates based on observations. This evaluation will be used to characterize the local natural characteristics of the sampling reach and levels of human stress impinging upon it, in order to supplement watershed characterizations based on spatial data. Physical habitat measurements should be made last, to minimize disturbance to the site before invertebrate and bryophyte samples are collected. Stream characterization and condition can be observed and recorded at any time however, even during day 1.

Stream Characterization

1) Enter the **Site ID, Site Name, and Dates and Times of the beginning and end of each sample collection.**

2) Estimate the percent cover of each **habitat type** present in the channel (Riffle, Run, Pool, Steps/Cascade, Percent wetted) by selecting the appropriate option from the drop-down menu:

Not Present
<5%
5%-25%
25%-50%
50%-75%
>75%

3) Estimate the percent of **vegetation types** present in channel (Grasses in Channel, Non-Woody in Channel, Woody in Channel).

4) Estimate the percent of **vegetation types** present in the immediate riparian zone (Grasses in Riparian, Non-Woody in Riparian, Woody in Riparian)

5) Estimate the percent of **vegetation cover** present in channel by selecting the appropriate option from the drop-down menu:

Open

Light Cover
Medium Cover
Heavy Cover
Full Cover

Site condition

Site condition will be assessed by scoring 5 attributes. These attributes are categorized with higher resolution in the data sheet. Each stressor is to be assessed by estimation using best judgement of the site after a visual inspection of the entire 160-m reach and surrounding riparian zone. If a stressor is absent, it should be left blank on the data sheet.

- 1) Land use Effects
- 2) Chemical Stressors
- 3) Hydrologic Stressors
- 4) Physical Stressors
- 5) Biological Stressors

The 5 stressor attributes will be scored based on three categories:

1) **Proximity**- determined by the distance of the stressor from the center of the channel. Select the appropriate option from the drop-down menu:

Proximity(m)
In Channel
1 to 5
5 to 50
50 to 100
100 to 250

2) **Extent**- The relative amount of area that this stressor covers within a site, including the riparian zone.

3) **Intensity**- The relative intensity of the impacts on the stream. For example, a narrow, dirt, walking trail would have lower intensity than a concrete running path. Extent and Intensity are characterized with the following scale:

1	Low extent or intensity
2	Medium extent or intensity
3	High extent or intensity

Physical Habitat Measurements

At top/bottom of each of the eight sections (i.e., at each of the 9 flags dividing the sections), measure and record the following:

1) **Channel width**. Estimate where the top of bank, where the average (roughly 2.5-year return interval) flood reaches. Generally, this will be the “green line” where vegetation changes from

absent or annuals to perennials. Signs of active erosion or deposition will also indicate where top of bank is. Measure channel width from top of bank to top of bank across the channel to the nearest 5 cm using tape measure or marked PVC pipe.

2) **Measure channel depth.** With the width measurement still in place, measure the depth of the channel at 25%, 50%, and 75% of the way across it. The PVC pipe should be placed on the channel bed and the depth reading should be taken at the point the tape touches the PVC pipe to the nearest 5 cm.

Note: If the channel is too wide for the rope, run it from one bank to the center. Try to get the rope as level as possible. Take the first two measurements and move the rope to the other half of the channel to take the last depth measurement.

3) **Pebble Count.** Without looking at the streambed, reach with a finger and pick up (if possible) the first substrate particle touched at the 0% (i.e., left bank), 25%, 50%, 75%, and 100% (i.e., right bank). If the substrate is buried underneath organic detritus, dig through until non-organic substrate is reached. Determine the particle size using the gravelometer, recording the size of the largest hole it will not go through.

3) **Measure channel slope.** A clinometer and the 2-m PVC pipe (i.e., the two 1-m sections attached to each other) are used to measure stream slope between transects. One team member will stand at the lowest portion of bottom of the section (at the downstream transect) with the clinometer and the other team member will stand at the lowest portion the top of the section (at the upstream transect) with the 2-m PVC pipe. The upstream team member will mark the eye height of the downstream member on the pipe with their hand. The downstream team member then estimates percent slope by aligning the cross hair of the clinometer with the hand of the upstream team member and reading the right-hand scale on the clinometer. If the stream characteristics make a top-to-bottom reading impossible (due to blocked line of view, etc.), break the section into 2 or more equal length sections and take separate readings. Take the average of these readings to characterize the entire study site. Record the clinometer reading to the nearest 0.5%.

If desired, an autolevel may be used to measure slope instead of a clinometer. For slopes < 1%, the precision provided by an autolevel may be preferable.

Retrieving Ramp Traps

The following materials will be needed when collecting ramp traps:

- Spray bottle of tap water
- 8 or more plastic sample containers (350 ml clear plastic recommended)
- 8 or more sample interior labels
- Permanent marker
- Bag(s) to carry samples and equipment around site

An example sample label is provided below:

Ramp trap arthropod sample	
Station code:	_____
Site name:	_____
Collector(s):	_____
Sample Date:	_____ Time: _____
Transect:	_____ Jar _____ of _____.

1) **Ramp Trap Retrieval.** The trap samples are collected as close to 24 hours after deployment as possible.

- a) Locate each pitfall trap and take note of any disturbance or movement of the trap. If the trap has been overturned, attempt to collect any remaining sample, if possible.
- b) Remove the covering rock and ramps.
- c) Without removing the lid, tilt the container to drain the arthropod samples into sample containers through one of the slots cut in the side.
- d) Remove lid and use the spray bottle of water to wash any remaining sample from the bottom, sides, and lid of the trap into the sample container.
- e) Put completed interior label in container, seal, and mark the lid with site code, date, sample number, and amount of cover to the nearest 10%.
- f) Stack the containers and lids, and ramps and place in their travel bag.

2) **Sample Storage.** Once back at the vehicle, seal each container using electrical tape (wrapped clockwise). Mark sides of container with site code, date, sample number, and vegetation type and cover with packing tape to prevent it from coming off.

Final Walkthrough / Data Sheet Check

This should be the last step before leaving a site on the second day. The length of the transect will be walked, including the riparian area where vegetation invertebrate sampling had occurred. This time will be spent looking for materials or tools left behind (papers, flags, jars, etc.). The data sheet on the tablet should be inspected for completeness by two crew members, and a back-up copy of the data sheet saved. Once this has been done, the tasks for the site have been complete.

SECTION 4: EVALUATING THE BIOLOGICAL CONDITION FOR THE DRY PHASE OF INTERMITTENT AND EPHEMERAL STREAMS: AN EVALUATION OF RAPID (LEVEL-2) AND INTENSIVE (LEVEL-3) INDICATORS IN SAN DIEGO STREAMS

Introduction

Ephemeral streams and intermittent rivers are an important component of watersheds in arid regions, such as Southern California (Solek and Stein 2011, Mazor et al. 2014). They face increasing pressure from storm- or wastewater discharges, groundwater extraction, intensive recreation, and other human activities (Acuña et al. 2017, Chiu et al. 2017). Watershed managers need tools to assess and manage the impacts of these activities. To this end, the San Diego Regional Water Quality Control Board, the Southern California Coastal Water Research Project, and California State University at Monterey Bay completed a pilot project to develop and evaluate potential indicators of biological condition for use in ephemeral streams during their dry phase. When complete, these tools could support the integration of ephemeral streams into Water Board programs, such as headwater protection, identification of impaired beneficial uses, and evaluation of discharges or spills in these systems. Currently, such activities focus primarily on perennial or long-duration intermittent streams, which comprise only a small portion (~10%) of San Diego's watersheds (Mazor et al. 2014, Mazor 2015).

Following the EPA's tiered strategy towards wetland monitoring, we evaluated both "Level 2" and "Level 3" indicators of ephemeral streams (USEPA 2002, Stein et al. 2009). Level-2 methods are rapid and based on visual observation and field measurements, such as the California Rapid Assessment Method (CRAM, CWMW 2013). A CRAM module for perennial and intermittent streams has been in use in California for over 10 years as part of ambient assessment programs (e.g., the stream survey of the Stormwater Monitoring Coalition [SMC], Mazor 2015), and assessing wetland mitigation projects and permit compliance. This module (henceforth referred to as the "Riverine" module) was known to underestimate condition of highly ephemeral streams, where naturally sparse vegetation and distinct geomorphological processes erroneously signal degradation. As a result, an "Episodic" module was developed, recalibrating several metrics to account for the natural conditions in highly ephemeral streams (CWMW 2015). Although the draft module contains preliminary guidance on selecting which module is most appropriate for the type of stream system at hand, this guidance was developed primarily with desert regions in mind, and it is unknown if it works well in coastal southern California. This study represents an opportunity to improve guidance on selecting modules, and to validate the Episodic CRAM with more intensive data about biological condition, such as biological assemblage structure.

Level-3 assessments are based on more intensive data collection, providing more direct measures of ecosystem structure or function than Level-2 assessments provide (Stein et al. 2009). Level-3 assessments may include measures of biodiversity, based on standardized sampling methods focused on key indicators, such as benthic macroinvertebrates in flowing streams. Based on a literature review (Section 2), we selected arthropods and bryophytes (i.e., mosses) as indicators that could be effectively sampled, while also providing valuable information about stream condition. Arthropods have been used in ephemeral stream assessments in Australia (Steward et al. 2012), and bryophytes are known to be highly responsive to activities that degrade ephemeral streams (Malcolm et al. 2009, Vieira et al. 2013, Vieira et al. 2016).

We intend to demonstrate the feasibility of using Level-3 data to assess conditions of ephemeral streams in the San Diego region. In this study, we investigated: 1) the comparability of Riverine and Episodic CRAM modules; 2) the relationship of arthropod- and bryophyte-based bioassessment metrics to both natural and stressor gradients; and 3) the validity of the rapid field measures of condition in the Episodic CRAM module, based on more intensive measures.

Methods

Site selection

Thirty-nine sites in the San Diego region representing a range of natural and disturbed conditions were sampled for bryophytes and terrestrial arthropods, following the protocols in Section 3 (Table 1, Figure 1). Geographic data were derived from the StreamCat database (Hill et al. 2016), which contains catchment landcover and similar environmental data associated with every stream segment in the National Hydrography Dataset-Plus (McKay et al. 2012). Sites with no signs of local disturbance and upstream development less than 5% were classified as high-quality “reference” sites. “Disturbed” sites were affected by a range of stressors, including grazing, urban runoff, trash dumping, and intensive recreation. Reference sites were screened based on watershed landcover alteration following Ode et al. (2016).

Table 1a. Reference (undisturbed) sites sampled in 2016. Superscripts: E: Ephemeral CRAM module was implemented; R: Riverine CRAM module was implemented.

Site Code	Site Name	Latitude	Longitude	Date Sampled
901AUDFOX ^E	Fox Canyon	33.59874	-117.56467	7/15/2016
901AUDCRW ^E	Crow Canyon	33.58576	-117.56319	7/16/2016
901BELOLV	Bell Canyon	33.64158	-117.55241	7/18/2016
901NP9MRC	Morrell Canyon	33.62658	-117.38848	7/19/2016
902SMAS2x ^E	Tributary to Arroyo Seco	33.45641	-116.97191	7/21/2016
902NP9CWC	Cottonwood (Temecula Cr trib)	33.41900	-116.86100	7/22/2016
903ACPCT1 ^{ER}	Agua Caliente Creek at Pacific Crest Trail	33.29600	-116.63900	7/23/2016
903NP9SLR ^{ER}	San Luis Rey at Indian Flats	33.35192	-116.66522	7/25/2016
907SYCAM ^E	Sycamore Creek	32.92859	-116.98161	7/26/2016
907NP9OSU ^{ER}	Oak Spring Canyon Upstream	32.85510	-117.05190	7/27/2016
910NP9CCN ^{ER}	Cedar Canyon	32.64149	-116.83598	7/29/2016
910NP9ARP ^{ER}	De Luz Tributary at Airport	32.63001	-116.88292	7/29/2016
911NP9HTC	Horsethief Canyon	32.75520	-116.66199	8/2/2016
911NP9EPC	Espinosa Creek	32.74431	-116.64791	8/2/2016
911S01142	Pine Valley Creek	32.73729	-116.65398	8/3/2016
911TJKC1x ^{ER}	Kitchen Creek (Downstream)	32.76072	-116.45148	8/4/2016
911NP9UCW ^{ER}	Upper Cottonwood Creek (Tijuana trib)	32.81992	-116.49137	8/5/2016
911TJPC2x ^{ER}	Pine Valley Creek at Noble Canyon Trailhead	32.85372	-116.52251	8/5/2016
905SDBDN9	Boden Canyon	33.09154	-116.89716	8/8/2016
903NP9PRC	Prisoner Creek	33.26036	-116.80925	8/9/2016
905DGCC2x ^{ER}	Tributary to Carney Canyon	33.18890	-116.82746	8/10/2016
905DGCC1x ^{ER}	Carney Canyon	33.15908	-116.84042	8/10/2016
901NP9CSC ^{ER}	Cold Spring Canyon	33.59088	-117.52132	8/13/2016
901NP9LCC	Ortega Canyon	33.62750	-117.42862	8/14/2016

Table 1b. Disturbed sites sampled in 2016.

Site Code	Site Name	Latitude	Longitude	Date Sampled	Observed stressors
901SJLANV	San Juan Creek at La Novia	33.50156	-117.64946	7/14/2016	Urban runoff, recreation (hiking, equestrian)
902PECHNG ^E	Pechanga Creek Santa Margarita River Arroyo Seco above Dripping Springs Campground	33.45977	-117.11800	7/20/2016	Urban runoff, Golf Course/Park/Sportsfields, Excessive Human Visitation, Walking path, & ATVs
902SMAS1x ^E	Canada Verde at Pacific Crest	33.45574	-116.96974	7/21/2016	Excessive Human Visitation, groundwater extraction
903CVPCT ^{ER}	Trail Oak Spring Canyon	33.26799	-116.63880	7/23/2016	Grazing, Walking path
907NP9OSD ^{ER}	Downstream	32.84798	-117.05018	7/27/2016	Excessive Human Visitation, Walking path
910SYCAM	Sycamore_Otay	32.64566	-116.80611	8/1/2016	
907NP9KLC	Kelly Creek	32.99088	-116.69268	8/6/2016	Minor hydromodification, light grazing
907SRSD2x ^{ER}	San Diego River Headwaters above Highway 79	33.10938	-116.65748	8/7/2016	Grazing, Unpaved Road
905DGSY1x ^{ER}	Santa Ysabel at Highway 79	33.12778	-116.67761	8/8/2016	Grazing, Paved Roads
903SLFRCx ^{ER}	Fry Creek San Juan Creek above Ortega	33.34400	-116.88000	8/12/2016	Hydromodification, Golf Course/Park/Sports fields, Excessive Human Visitation
901SJOF1x ^{ER}	Falls San Juan Creek mainstem below canyon	33.61645	-117.42656	8/14/2016	Rural development
901SJMS1x ^{ER}	Santiago Canyon above education house	33.58246	-117.52364	8/15/2016	Rural development
801SANT1x		33.70866	-117.61543	8/16/2016	Trash, rip-rap, Unpaved Road
910NP9RJT	Rancho Jamul Trabuco Creek at Trabuco	32.69870	-116.86973	7/28/2016	Urban runoff
901TCTCR ^{ER}	Canyon Road	33.66066	-117.58454	8/16/2016	Urban runoff, rip-rap, trash, Excessive Human Visitation, Paved Roads, Parking lot/Pavement

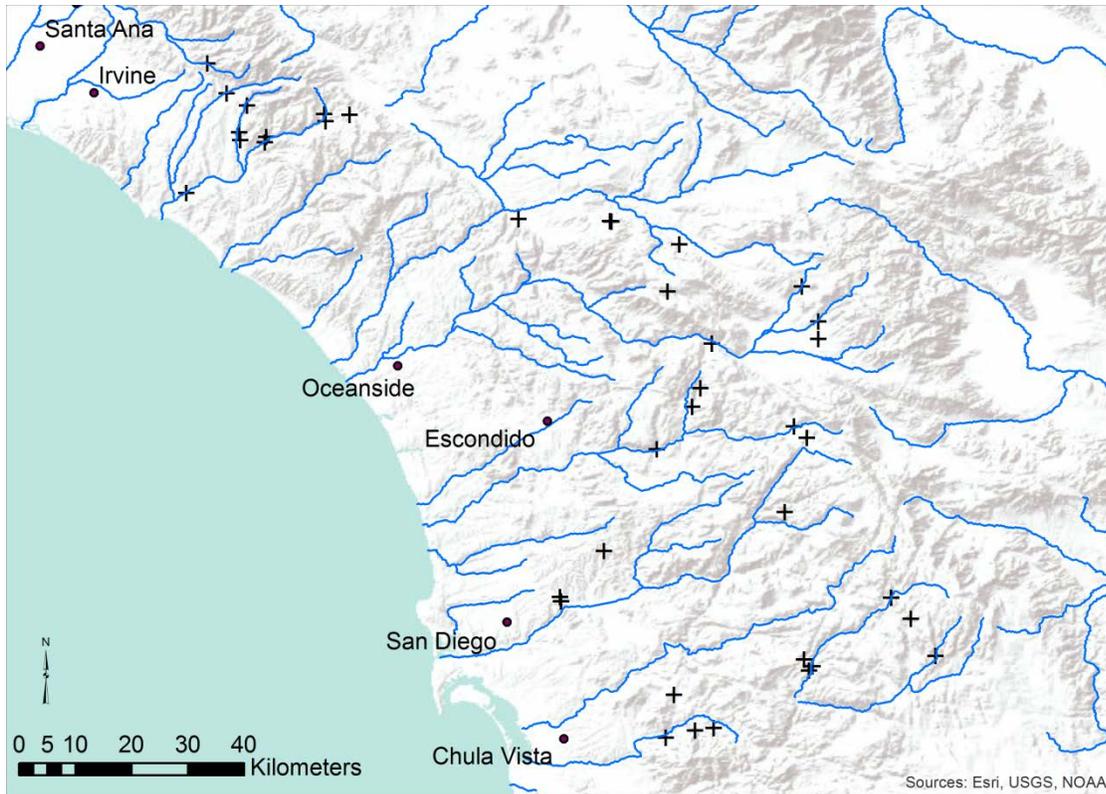


Figure 1. Locations of sites where bryophytes and terrestrial arthropods were collected.

CRAM data collection

The two CRAM modules were applied at 18 sites in Southern California during the summer of 2016; data from the episodic module, alone, were available at 6 additional sites that had been sampled with the Episodic module alone under previous studies (24 sites with CRAM data, total). Crews noted which module was considered most appropriate based on characteristics described in the Episodic module field guide (CWMW 2015), as well as additional indicators observed in the field, such as channel morphology and riparian vegetation. These additional indicators are specific for the San Diego region, and may not apply elsewhere (Table 2). Unless otherwise specified, results for the preferred module were used for further analyses; in cases where either module was equally acceptable, results from the Episodic module were used.

Table 2. Indicators for selecting CRAM modules in the San Diego region

Indicators that the Episodic module is appropriate	Indicators that the Riverine module is appropriate	Indicators that both modules are appropriate
<p>From CWMW (2015)</p> <ul style="list-style-type: none"> • 0 or 1st order channels with limited drainage areas, sandy substrate (no bedrock), and no apparent seeps/springs • Located in interior desert areas with <5 in annual rainfall • Located in areas with 5 to 10 in annual rainfall, plus sandy substrate (no clay or bedrock), usually with a multi-thread or compound channel 	<p>From CWMW (2015)</p> <ul style="list-style-type: none"> • Streams that do not meet criteria for Episodic module • Well-defined channels with a vegetated riparian zone 	<p>From CWMW (2015)</p> <ul style="list-style-type: none"> • None
<p>Additional indicators (present study)</p> <ul style="list-style-type: none"> • Vegetation dominated by sage scrub and chaparral species (e.g., sugar bush, scale broom, buckwheat, sagebrush, chamise, cactus, and yucca). • Perennial vegetation grows inside main channel • Physical indicators vary by position in watershed: <ul style="list-style-type: none"> ○ For sites in upper watershed, reaches are typically steep, single-thread, and often underlain by bedrock. ○ For sites in lower watershed, reaches are typically low-gradient, sandy, braided or multi-thread, with poorly defined banks. 	<p>Additional indicators (present study)</p> <ul style="list-style-type: none"> • Riparian zone dominated by plants that tolerate prolonged inundation, such as mulefat, sycamore, and willow. • Banks tend to be well formed (though possibly eroding) in both lower-watershed and upper-watershed sites. 	<p>Additional indicators (present study)</p> <ul style="list-style-type: none"> • Vegetation typical of both riverine and episodic streams interspersed in patches. • Banks may be shallow in lower portions of the watershed; well-formed in parts, and indistinct in other portions of the reach.

Sample collection

At each site, using the methods described in Section 2, 160-m long sampling reaches were established, and bryophytes were collected through time-constrained searches (30 minutes maximum) along the banks and main channel; terrestrial arthropods were collected in ramp traps deployed overnight at random locations within 8 transects along the reach. Bryophytes were collected as dry specimens, and arthropods were collected in a fixative containing propylene glycol. Samples were sorted under a dissecting scope into "morphospecies" (i.e., morphologically distinct specimens), with taxonomic names applied where possible (generally,

genus- or family level for beetles and spiders). Henceforth, the term “taxa” is used to refer to both morphospecies and properly identified taxa.

Data analysis

Comparison of CRAM modules

CRAM index and attribute scores were compared at sites where both the Episodic and Riverine modules were implemented. In addition, Pearson correlation coefficients between the modules were calculated for the index, attributes, and attribute metrics.

Evaluation of bioassessment metrics

A suite of 130 potential bioassessment metrics were calculated for bryophyte and arthropod assemblages separately (Table 3). Metrics for both assemblages were calculated for different taxonomic or functional groups, and expressed in terms of richness (i.e., counts of taxa) or relative richness; arthropod metrics were also expressed in terms of abundance (i.e., counts of individuals) or relative abundance. Because the collection methods for bryophytes were aimed at detecting species, rather than quantifying abundance (Newmaster et al. 2005), abundance metrics were not considered; the value of abundance metrics based on bryophytes remains to be investigated. For all metrics, we assumed that disturbance could result in an increase or decrease in metric values; that is, we did not assume *a priori* a direction of response for each metric.

Metrics were screened based on a number of properties that contribute to good bioassessment index performance (Stoddard et al. 2008), such as range, bias from natural gradients, and responsiveness to disturbance. Several metrics were unsuitable for analysis because they had poor ranges of values within the dataset. Metrics were screened for further analysis if they met the following criteria, based on the data at hand for metric development: 1) At least 5 unique numeric values; 2) No more than 25% zero-values; and 3) a median value > 3 (for richness metrics), > 0 (for abundance metrics), or between 0.1 and 0.9 (for relative richness or relative abundance metrics). Means and standard deviations of metric values at reference and nonreference sites were also calculated.

In order to evaluate the relationship between selected natural gradients and biological assemblages, a random forest model was calibrated to predict metric values at reference sites from natural watershed characteristics. If the percent of variance explained by the model (i.e., the pseudo- R^2) was greater than 0.2, then a model residual (i.e., the deviation from reference expectations) was used in subsequent analyses; otherwise, the raw metric value was used. Unless otherwise stated, the term “metric value” is henceforth used to refer to both raw values and residuals from modeled values.

In order to test responsiveness of metrics to stress, means of metric values (or residuals) at reference and nonreference were compared with a t-test using pooled variance. In addition, responsiveness was assessed by calibrating a random forest model to predict metric values (or residuals) based on stressor variables from the StreamCat database (specifically: imperviousness, urban land cover, and agricultural land cover at the watershed, catchment, and riparian scales; Hill et al. 2016). From these analyses, responsive metrics were those identified as having a t-statistic > 2 or a pseudo $R^2 > 0.2$.

Table 3. Bioassessment metrics evaluated in the study; only those that met selection criteria are shown. The full list is included in the appendix.

Metric Code	Metric Description	# unique values	Frequency of zeroes	Median value
<i>Bryophyte metrics</i>				
Site_RICH	Bryophyte site richness	18	0.03	11
Site_FamRICH	Bryophyte Family site richness	10	0.03	4
Site_GenusRICH	Bryophyte Genera site richness	13	0.03	8
Channel_RICH	Channel Bryophyte richness	13	0.13	4
Channel_FamRICH	Channel Bryophyte family site richness	6	0.13	3
Channel_GenusRICH	Channel Bryophyte Genera site richness	10	0.13	4
Bank_RICH	Bank Bryophyte richness	18	0.03	9
Bank_FamRICH	Bank Bryophyte Family site richness	8	0.03	4
Bank_GenusRICH	Bank Bryophyte Genera site richness	14	0.03	7
Pottiaceae_RICH	Pottiaceae site richness	12	0.08	4
Pottiaceae_BankRICH	Bank Pottiaceae richness	10	0.08	4
Bryaceae_RICH	Bryaceae site richness	6	0.05	3
Bryaceae_BankRICH	Bank Bryaceae richness	6	0.10	2
Acrocarp_RICH	Acrocarp richness	19	0.03	10
Acrocarp_ChanRICH	Channel Acrocarp site richness	13	0.13	4
Acrocarp_BankRICH	Bank Acrocarp richness	15	0.03	9
<i>Arthropod metrics</i>				
Tot_Rich	Total site richness	24	0.00	35
Tot_Abund	total site abundance	35	0.00	192
Coleopt_Rich	Coleoptera richness	14	0.03	3
Dip_Rich	Diptera richness	11	0.00	6
Aranae_Rich	Spider richness	8	0.00	6
Ant_Rich	Ant richness	11	0.00	4
dipRICH_siteRICH	Diptera richness relative to site richness	20	0.00	0.16
aranaeRich_SiteRich	Spider richness relative to site richness	18	0.00	0.18
antRICH_siteRICH	Ant richness relative to site richness	16	0.00	0.11

coldipRICH	Coleoptera and Diptera combined richness	17	0.00	10
coldipRICH_siteRICH	Coleoptera and Diptera combined richness relative to site richness	22	0.00	0.25
colaranaeRICH	Coleoptera and Spider combined richness	18	0.00	9
colaranaeRICH_siteRICH	Coleoptera and Spider combined richness relative to site richness	24	0.00	0.28
DipandAranaeRICH	Diptera and Spider richness	14	0.00	12
dipandaranaeRICH_siteRICH	Diptera and Spider richness relative to site richness	20	0.00	0.32
antcolRICH	Ant and Coleoptera combined richness	15	0.00	8
antcolRICH_siteRICH	Ant and Coleoptera combined richness relative to site richness	23	0.00	0.23
antdipRICH	Ant and Diptera combined richness	15	0.00	10
antdipRICH_siteRICH	Ant and Diptera combined richness relative to site richness	22	0.00	0.27
antaranaeRICH	Ant and Spider combined richness	12	0.00	11
antaranaeRICH_siteRICH	Ant and Spider combined richness relative to site richness	21	0.00	0.29
ColDipArRICH	Coleoptera, Diptera, and Spider combined richness	17	0.00	15
ColDipArRICH_siteRICH	Coleoptera, Diptera, and Spider combined richness relative to site richness	21	0.00	0.44
ColDipAntRICH	Coleoptera, Diptera, and Ant combined richness	18	0.00	13
ColDipAntRICH_siteRICH	Coleoptera, Diptera, and Ant combined richness relative to site richness	21	0.00	0.40
ColAranAntRICH	Coleoptera, Spider, and Ant combined richness	20	0.00	14
ColAranAntRICH_siteRICH	Coleoptera, Spider, and Ant combined richness relative to site richness	20	0.00	0.40
DipAranAntRICH	Diptera, Spider, and Ant combined richness	16	0.00	15
DipAranAntRICH_siteRICH	Diptera, Spider, and Ant combined richness relative to site richness	21	0.00	0.45
DipAranAntColRICH	Diptera, Spider, and Ant combined richness	21	0.00	20

DipAranAntColRICH_siteRICH	Diptera, Spider, and Ant combined richness relative to site richness	20	0.00	0.55
Spider_SiteABD	Spider abundance	20	0.00	10
SpiderGround_ABD	Ground Spider abundance	19	0.00	6
SpiderGround_RICH	Ground Spider richness	6	0.00	3
SpiderGround_RelSp_ABD	Ground Spider abundance relative to spider abundance	27	0.00	0.66
SpiderGround_RelSp_RICH	Ground Spider richness relative to spider richness	14	0.00	0.50
SpiderWeb_ABD	Web spider abundance	9	0.03	4
SpiderWeb_RICH	Web spider richness	7	0.03	3
SpiderWeb_RelSp_ABD	Web spider abundance relative to spider abundance	23	0.03	0.30
SpiderWeb_RelSp_RICH	Web spider richness relative to spider richness	14	0.03	0.50
Spiderling_ABD	Spiderling abundance	16	0.00	6
Spiderling_RICH	Spiderling richness	7	0.00	4
Spiderling_RelSp_ABD	Spiderling abundance relative to spider abundance	29	0.00	0.60
Spiderling_RelSp_RICH	Spiderling richness relative to spider richness	16	0.00	0.57
SpiderGHunter_ABD	Ground Hunter spider abundance	19	0.00	6
SpiderGHunter_RICH	Ground Hunter spider richness	5	0.00	3
SpiderGHunter_RelSp_ABD	Ground Hunter spider abundance relative to spider abundance	29	0.00	0.60
SpiderGHunter_RelSp_RICH	Ground Hunter spider richness relative to spider richness	15	0.00	0.42
SpiderOtherHunter_ABD	Other Hunter spider abundance	7	0.21	2
SpiderOtherHunter_RelSpABD	Other Hunter spider abundance relative to spider abundance	20	0.21	0.11
SpiderOtherHunter_RelSpRICH	Other Hunter spider richness relative to spider richness	14	0.21	0.25
Lycosidae_ABD	Lycosidae abundance	13	0.23	2
Lycosidae_RelSp_ABD	Lycosidae abundance relative to spider abundance	21	0.23	0.16

Lycosidae_RelSp_RICH	Lycosidae richness relative to spider richness	11	0.23	0.16
SiteColABD	Coleoptera abundance	20	0.03	5
ColFDWDe_ABD	Fungivore, Dead Wood, and Detritivore Coleoptera abundance	12	0.15	2
ColFDWDe_RICH	Fungivore, Dead Wood, and Detritivore Coleoptera richness	7	0.15	2
ColFDWDe_RelABD	Fungivore, Dead Wood, and Detritivore Coleoptera abundance relative to Coleoptera abundance	18	0.15	0.54
ColFDWDe_RelRICH	Fungivore, Dead Wood, and Detritivore Coleoptera richness relative to Coleoptera richness	14	0.15	0.50

Validation of CRAM

CRAM and its attributes were validated first by comparing index and attribute score means at reference and nonreference sites in a t-test and in boxplots. These analyses were conducted on the full dataset using the recommended module on a site-by-site basis, plus on the two modules independently (regardless of which module was preferred). CRAM was further validated by evaluating the strength of relationships with bioassessment metrics. Spearman rank correlation coefficients (ρ) were calculated between metric values and scores for the CRAM index and attributes. Selected metrics were then plotted for visual analysis, based on the rho-square between metric values and results from the preferred CRAM module; specifically, metrics with rho-square greater than 0.1 were selected for plotting (up to 9 metrics total per CRAM variable). Linear regression lines were plotted for the preferred module, as well as for each module separately (using only data from sites where the module was recommended).

Results

Comparison of CRAM modules

Of the 24 sites where CRAM was implemented, the Episodic module was recommended at 7 sites, the Riverine module at 13 sites, and both modules were recommended at 4 sites (at these sites, data from the Episodic module was used for further evaluations). Index and attribute scores from the Episodic module were usually higher than those for the Riverine module (Figure 2). The median differences, expressed as Episodic score minus Riverine score, were: 11 points for the overall CRAM score, 22 points for the Biotic Structure attribute, and 13 points higher for the Physical Structure attribute; median differences were smaller for the Buffer and Hydrology attributes. In general, the bias was stronger for low-scoring sites than for high-scoring sites (Figure 3). Correlations between the two modules were generally high (median Pearson's r : 0.79; Table 4). However, the hydrology attribute was weakly correlated (r : 0.38), largely driven by the hydrologic connectivity metric; among the 18 sites with data for both modules, only one site scored below a 12 (i.e., the highest possible score) for this metric with the Episodic module, whereas 11 sites did so with the Riverine module. The metric's lack of variability within the Episodic module leads to an apparent negative correlation between the modules.

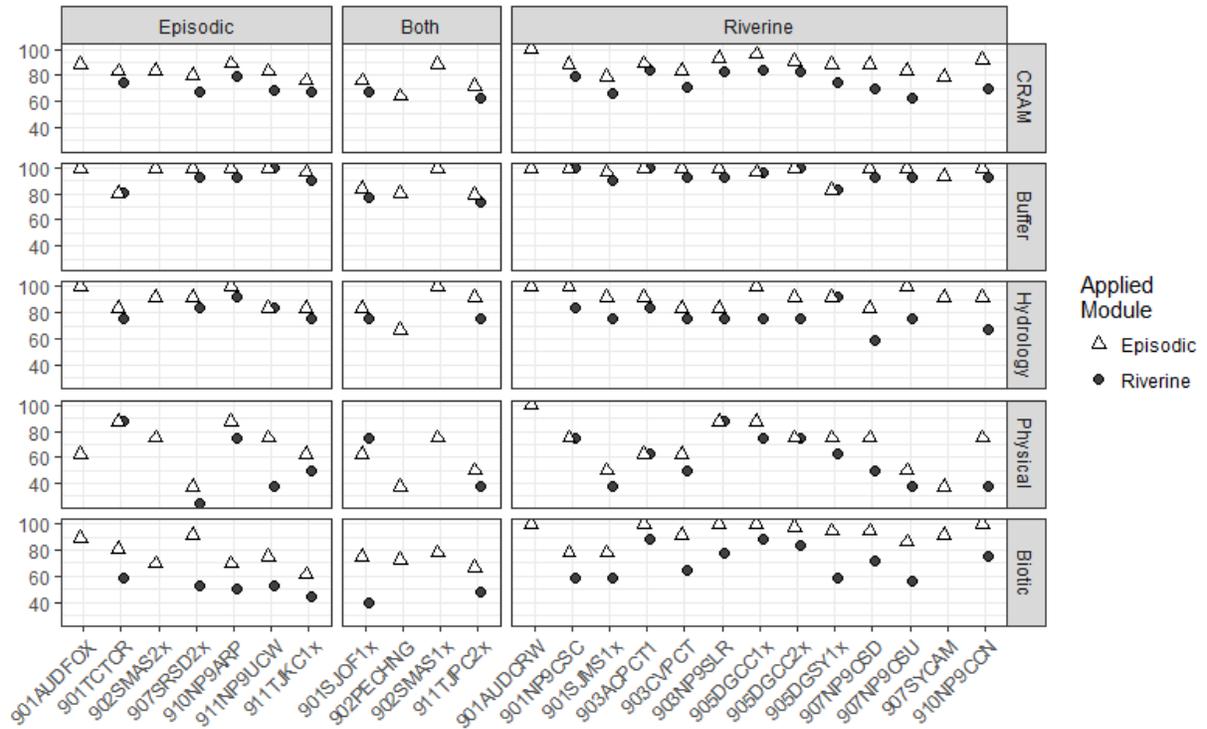


Figure 2. Comparison of scores for the episodic and riverine CRAM modules. Each column represents sites where the Episodic (left), Riverine (right), or both (center) modules were recommended by field crews. Each row represents scores for the overall CRAM index and each attribute.

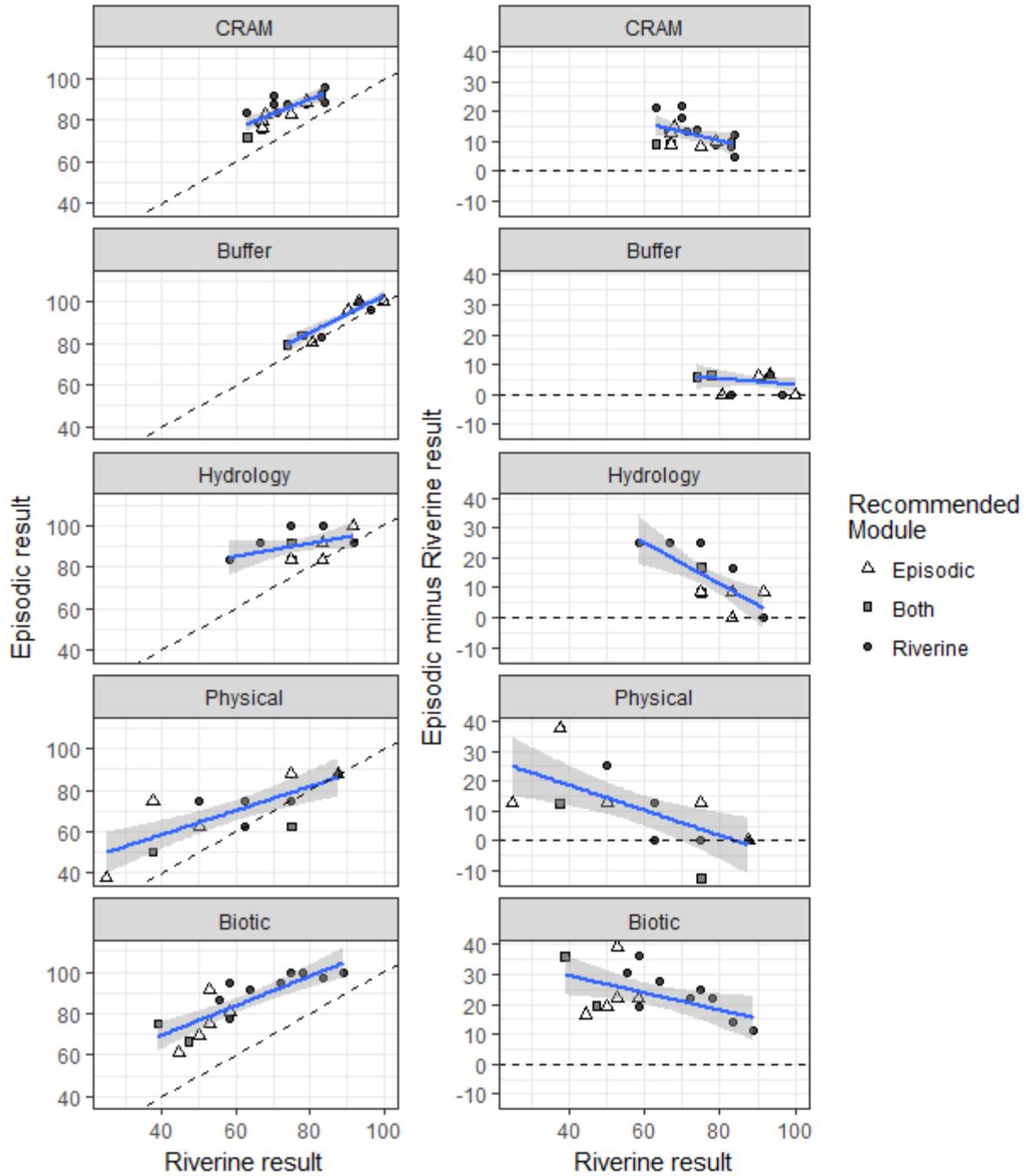


Figure 3: CRAM index and attribute scores for (left column) Episodic versus Riverine modules, and (right column) the difference between the two modules versus the Riverine module. Blue lines indicate a linear fit, and the gray ribbon indicates one standard error around the fit. The dashed lines represent perfect agreement between the two modules.

Table 4: Correlations between index, attribute, and metric scores for the Riverine and Episodic modules.

CRAM variable	Pearson's r
CRAM Index	0.79
Buffer and landscape	0.91
Average buffer width	1.00
Buffer condition	0.60
Landscape connectivity	1.00
Stream corridor continuity	1.00
Hydrology	0.38
Hydrologic connectivity	-0.27
Sediment transport	0.77
Water source	1.00
Physical	0.76
Structural patch richness	0.74
Topographic complexity	0.56
Biotic	0.84
Number of codominant species	0.72
Number of plant layers	0.95
Percent invasion	1.00
Horizontal interspersion	0.58
Vertical biotic structure	0.85

Evaluation of bioassessment metrics

Of the 130 metrics evaluated, 67 met all criteria for further analysis (Table 3). The most restrictive criterion was the frequency of zero values, which eliminated 51 metrics. Richness and abundance metrics were considered suitable more frequently (58% and 63%, respectively) than their relativized counterparts (44% for both). Overall, 13 of 39 bryophyte metrics and 54 of 91 arthropod metrics were considered suitable for analysis.

Random forest models typically explained a low proportion of metric variance at reference sites, as indicated by pseudo- R^2 values < 0.2 (Table 5). For six metrics, the pseudo- R^2 was high enough to justify analyzing metric residuals instead of raw metric values. Five of these metrics were related to arthropod assemblages, with just one bryophyte metric (richness of bryophyte genera across the site; Site_GenusRICH) requiring modeling. The highest pseudo- R^2 (0.43) was observed for the richness of ants and flies (antdipRICH). For several metrics, elevation and soil properties (% clay, % sand, % organic matter, and permeability) were the most important predictors. For the relative abundance of spiders with hunting strategies other than webs or ground search (SpiderOtherHunter_RelSpABD), climatic variables (e.g., mean precipitation, mean and max temperature) and watershed area, as well as depth to bedrock were the most important predictors (Table 6).

Of the 67 metrics evaluated, 10 could discriminate between reference and nonreference sites, as indicated by significance in a t-test (Table 5; Figure 4). These metrics included one bryophyte

metric (Site_GenusRICH), and 9 arthropod metrics, many of which were related to spider assemblages (e.g., spider richness relative to site arthropod richness, aranaeRich_SiteRich). In general, mean values were higher at reference sites than at nonreference, suggesting that most metrics decrease in response to stress. Only one metric (relative richness of ant and beetle taxa, antcolRICH_siteRICH) showed a strong relationship with land use variables in the StreamCat dataset (random forest pseudo- R^2 0.27; Table 5). Therefore, watershed-scale disturbance may be less important to these indicators than local disturbance.

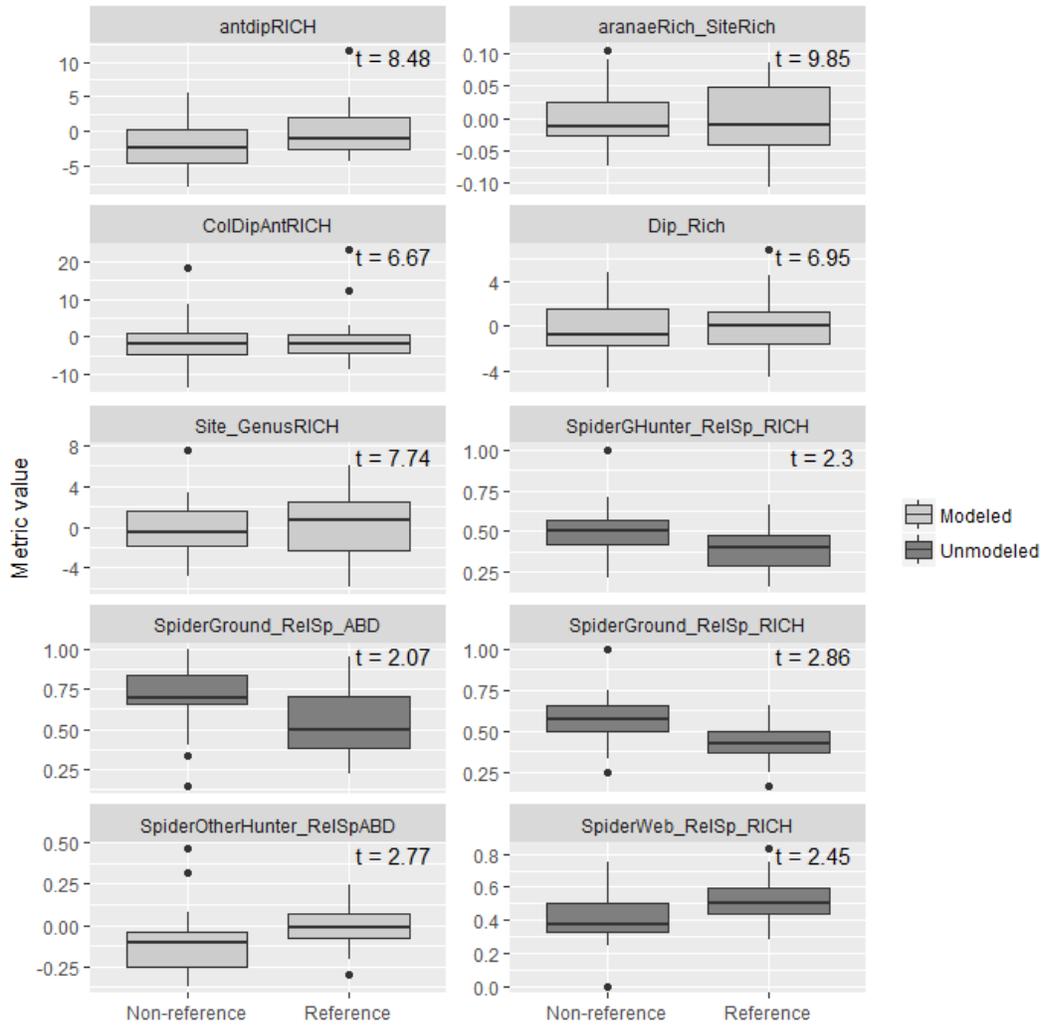


Figure 4. Scores of selected metrics at reference and nonreference sites with significant ($p < 0.05$) t-test results.

Table 5. Performance of selected bioassessment metrics. Pseudo R²: pseudo-R² from random forest models explaining metric values from natural factors at reference sites, or from stressors at both reference and nonreference sites. t: t-statistic comparing means at reference and nonreference sites. p: p-value associated with the t-test (yellow highlight indicates p < 0, and blue highlight indicates that p < . SD: standard deviation. Metric codes are described in Table 3. Asterisks (*) indicate metrics where deviations from modeled reference values were used.

<i>Bryophyte metrics</i>	Pseudo R ²		t	p	Reference		Disturbed	
	Natural	Stressors			Mean	SD	Mean	SD
Site_RICH	0.09	-0.32	0.6	0.586	10.2	6.0	11.1	4.1
Site_FamRICH	0.05	-0.41	0.3	0.740	4.6	2.3	4.4	1.9
Site_GenusRICH*	0.21	-0.49	7.7	0.000	7.9	4.0	7.8	2.7
Channel_RICH	-0.10	-0.28	0.9	0.377	4.0	3.9	5.1	3.2
Channel_GenusRICH	0.01	-0.40	0.8	0.422	3.4	2.9	4.1	2.1
Bank_RICH	0.10	-0.35	0.3	0.759	9.0	5.3	9.5	4.5
Bank_FamRICH	0.04	-0.34	0.4	0.677	4.3	2.0	4.1	1.9
Bank_GenusRICH	0.11	-0.36	0.2	0.864	7.3	3.8	7.1	3.4
Pottiaceae_RICH	0.09	-0.05	1.1	0.297	4.2	3.0	5.1	2.5
Pottiaceae_BankRICH	0.03	-0.01	0.9	0.356	3.8	2.7	4.5	2.0
Acrocarp_RICH	0.06	-0.31	0.5	0.642	9.6	5.8	10.4	3.8
Acrocarp_ChanRICH	-0.18	-0.32	0.7	0.498	3.9	3.9	4.6	3.2
Acrocarp_BankRICH	0.08	-0.26	0.3	0.730	8.5	5.1	9.0	3.9
<i>Arthropod metrics</i>								
Tot_Rich	0.04	-0.22	0.4	0.692	38.5	13.9	36.8	11.6
Tot_Abund	0.11	-0.03	0.9	0.375	193.7	108.1	251.8	246.7
Dip_Rich*	0.26	-0.34	7.0	0.000	6.4	3.2	5.9	2.4
Aranae_Rich	-0.45	-0.31	0.4	0.714	6.0	2.1	6.3	2.1
Ant_Rich	0.20	0.00	2.0	0.055	5.3	2.5	3.9	1.7
dipRICH_siteRICH	-0.19	-0.17	0.0	0.995	0.16	0.06	0.16	0.06
aranaeRich_SiteRich*	0.21	-0.34	9.9	0.000	0.16	0.06	0.17	0.05
antRICH_siteRICH	-0.16	-0.04	1.9	0.059	0.14	0.06	0.10	0.05
coldipRICH	0.00	-0.24	0.1	0.908	10.6	6.4	10.9	6.6
coldipRICH_siteRICH	-0.15	-0.15	0.8	0.439	0.26	0.08	0.28	0.09
colaranaeRICH	-0.19	-0.19	0.5	0.605	10.3	5.5	11.3	6.0
colaranaeRICH_siteRICH	-0.24	-0.12	1.2	0.238	0.26	0.09	0.29	0.08
DipandAranaeRICH	-0.35	-0.19	0.2	0.839	12.4	3.9	12.2	3.2
dipandaranaeRICH_siteRICH	-0.22	-0.32	0.3	0.730	0.33	0.07	0.33	0.07
antcolRICH	0.07	-0.09	0.3	0.740	9.5	5.3	8.9	5.8
antcolRICH_siteRICH	0.15	0.27	0.4	0.690	0.24	0.06	0.23	0.08
antdipRICH*	0.43	-0.20	8.5	0.000	11.6	5.0	9.8	3.4
antdipRICH_siteRICH	-0.11	-0.19	1.3	0.217	0.30	0.09	0.27	0.07
antaranaeRICH	-0.19	0.05	1.2	0.251	11.3	3.1	10.2	2.7
antaranaeRICH_siteRICH	0.03	-0.13	1.2	0.254	0.30	0.07	0.28	0.06
ColDipArRICH	-0.21	-0.25	0.2	0.836	16.7	7.4	17.2	7.4
ColDipArRICH_siteRICH	-0.52	-0.22	1.2	0.245	0.42	0.08	0.45	0.08

ColDipAntRICH*	0.22	-0.23	6.7	0.000	15.9	7.8	14.8	7.4
ColDipAntRICH_siteRICH	0.11	0.16	0.4	0.700	0.40	0.08	0.39	0.09
ColAranAntRICH	-0.18	-0.10	0.2	0.867	15.6	6.3	15.2	6.7
ColAranAntRICH_siteRICH	-0.03	0.14	0.0	0.989	0.40	0.07	0.40	0.07
DipAranAntRICH	0.12	-0.07	1.0	0.304	17.7	5.4	16.1	4.0
DipAranAntRICH_siteRICH	-0.10	-0.22	1.0	0.325	0.47	0.09	0.44	0.07
DipAranAntColRICH	-0.01	-0.15	0.3	0.756	22.0	8.5	21.1	8.1
DipAranAntColRICH_siteRICH	-0.55	0.08	0.1	0.959	0.57	0.07	0.56	0.07
Spider_SiteABD	-0.21	-0.29	0.7	0.472	16.3	20.5	23.9	38.7
SpiderGround_ABD	-0.10	-0.31	0.8	0.412	11.6	20.1	20.1	37.9
SpiderGround_RelSp_ABD	0.06	-0.29	2.1	0.047	0.54	0.21	0.69	0.22
SpiderGround_RelSp_RICH	0.09	-0.38	2.9	0.008	0.43	0.11	0.58	0.19
SpiderWeb_ABD	-0.30	-0.23	0.7	0.464	4.1	2.0	3.6	2.1
SpiderWeb_RelSp_ABD	-0.11	-0.27	1.6	0.110	0.40	0.19	0.29	0.22
SpiderWeb_RelSp_RICH	0.05	-0.46	2.5	0.021	0.52	0.13	0.40	0.18
Spiderling_ABD	-0.14	-0.27	0.8	0.428	9.2	14.2	14.2	22.3
Spiderling_RICH	-0.51	-0.28	0.4	0.710	3.6	1.4	3.5	1.4
Spiderling_RelSp_ABD	-0.30	-0.45	0.1	0.888	0.62	0.24	0.61	0.22
Spiderling_RelSp_RICH	-0.32	-0.40	0.7	0.462	0.62	0.19	0.58	0.19
SpiderGHunter_ABD	-0.11	-0.33	0.8	0.436	11.3	20.2	19.4	38.1
SpiderGHunter_RelSp_ABD	0.07	-0.21	1.6	0.122	0.51	0.23	0.63	0.24
SpiderGHunter_RelSp_RICH	0.10	-0.37	2.3	0.029	0.39	0.13	0.51	0.19
SpiderOtherHuunter_ABD	-0.10	-0.27	0.1	0.898	2.3	1.7	2.2	2.2
SpiderOtherHunter_RelSpABD*	0.32	-0.02	2.8	0.010	0.21	0.17	0.15	0.20
SpiderOtherHunter_RelSpRICH	-0.01	-0.29	1.2	0.255	0.26	0.17	0.20	0.17
Lycosidae_ABD	-0.15	-0.25	0.6	0.536	8.3	19.9	14.5	36.8
Lycosidae_RelSp_ABD	0.05	-0.10	0.7	0.493	0.23	0.28	0.29	0.28
Lycosidae_RelSp_RICH	0.05	-0.26	0.8	0.458	0.17	0.15	0.21	0.14
SiteColABD	-0.22	-0.30	0.4	0.728	9.4	11.8	10.9	14.5
ColFDWDe_ABD	-0.11	-0.36	0.6	0.546	3.5	4.6	4.4	5.0
ColFDWDe_RelABD	-0.12	-0.12	0.2	0.879	0.55	0.38	0.57	0.35
ColFDWDe_RelRICH	-0.17	-0.08	0.4	0.719	0.55	0.38	0.59	0.33

Table 6. Importance of natural predictor variables for predicting metric values at reference sites, measured as increase in mean-squared error. Cell shading indicates rank importance for each model: blue cells indicate relatively important predictors, and red cells indicate relatively unimportant predictors. The final row indicates the pseudo-R² of the model. Only models where pseudo-R² > 0.20 are shown.

Predictor	Description	antDipRICH	araneaeRich_SiteRich	ColDipAntRICH	Dip_Rich	Site_GenusRICH	SpiderOtherHunter_RelSpABD
BFIWs	Base-flow index	0.33	0.00003	1.28	0.02	0.69	-0.00035
ClayWs	Soil % clay	4.57	0.00033	16.06	1.35	0.03	0.00069
ElevCat	Elevation	9.48	0.00027	13.15	1.53	1.47	0.00031
OmWs	Soil % organic mater	1.22	0.00025	2.07	0.59	0.22	0.00117
PermWs	Soil permeability	5.45	0.00047	9.50	1.94	0.01	-0.00001
Precip8110Ws	Mean annual precipitation	-0.08	-0.00004	1.31	0.08	1.22	0.00287
RckdepWs	Depth to bedrock	0.26	0.00019	1.74	0.60	0.50	0.00178
RunoffWs	NHD Plus-derived annual runoff	0.82	0.00001	4.17	0.31	0.36	-0.00054
SandWs	Soil % sand	6.85	0.00021	12.57	1.17	0.25	0.00002
Tmax8110Ws	Annual max temperature	-0.02	-0.00008	-0.01	0.10	-0.12	0.00380
Tmean8110Ws	Annual mean temperature	0.28	0.00001	0.46	-0.05	0.40	0.00079
Wet	Watershed % wetlands	0.42	0.00033	-0.46	0.19	0.23	0.00068
WsAreaSqKm	Watershed area	0.32	0.00001	0.21	0.20	1.02	0.00475
WtDepWs	Depth to water	1.12	0.00012	0.26	-0.19	1.05	0.00215
Pseudo-R²		0.43	0.21	0.22	0.26	0.21	0.32

Validation of CRAM

Means of CRAM index and attribute scores were generally higher at reference sites, but these differences usually fell short of statistical significance (Table 7, Figure 5). In general, differences were larger when the recommended module was analyzed, and the largest differences were observed for the index and for the buffer and landscape attribute.

Of the 67 metrics evaluated, 26 had a strong (rho-squared > 0.1) relationship with CRAM index or attribute scores (Table 8; Figure 6). Many bryophyte metrics had strong, positive relationships with the CRAM index, and four or five did so with the hydrology or biotic attributes, but none did so with the buffer and landscape or the physical attribute. In contrast, arthropod metrics frequently had strong relationships with the buffer and landscape metric, and at least a few did so with all other CRAM attributes. The strongest relationship was between the Hydrology attribute and the richness of flies, spiders, and ants (rho: 0.49). Visual inspection of the plots showed that

the direction of the relationships were consistent, regardless of which module was analyzed (Figure 7a-e).

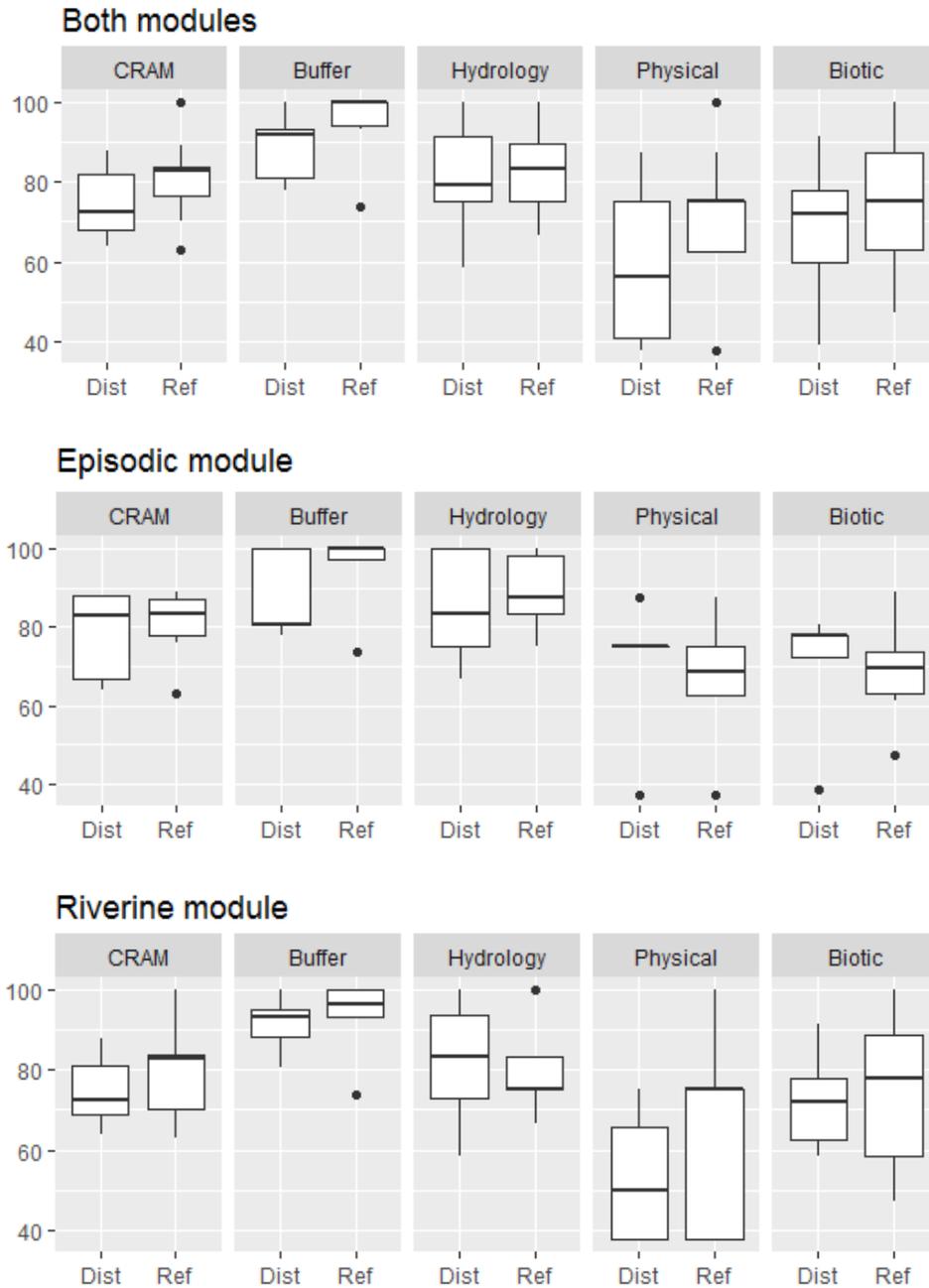


Figure 5. CRAM index and attribute scores at reference (Ref) and disturbed (Dist) sites. Top panel: results for the recommended module (n = 14 reference, 10 disturbed). Middle row: results for the Episodic module, where recommended (n = 6 reference, 5 disturbed). Bottom row: results for the Riverine module, where recommended (n = 9 reference, 8 disturbed).

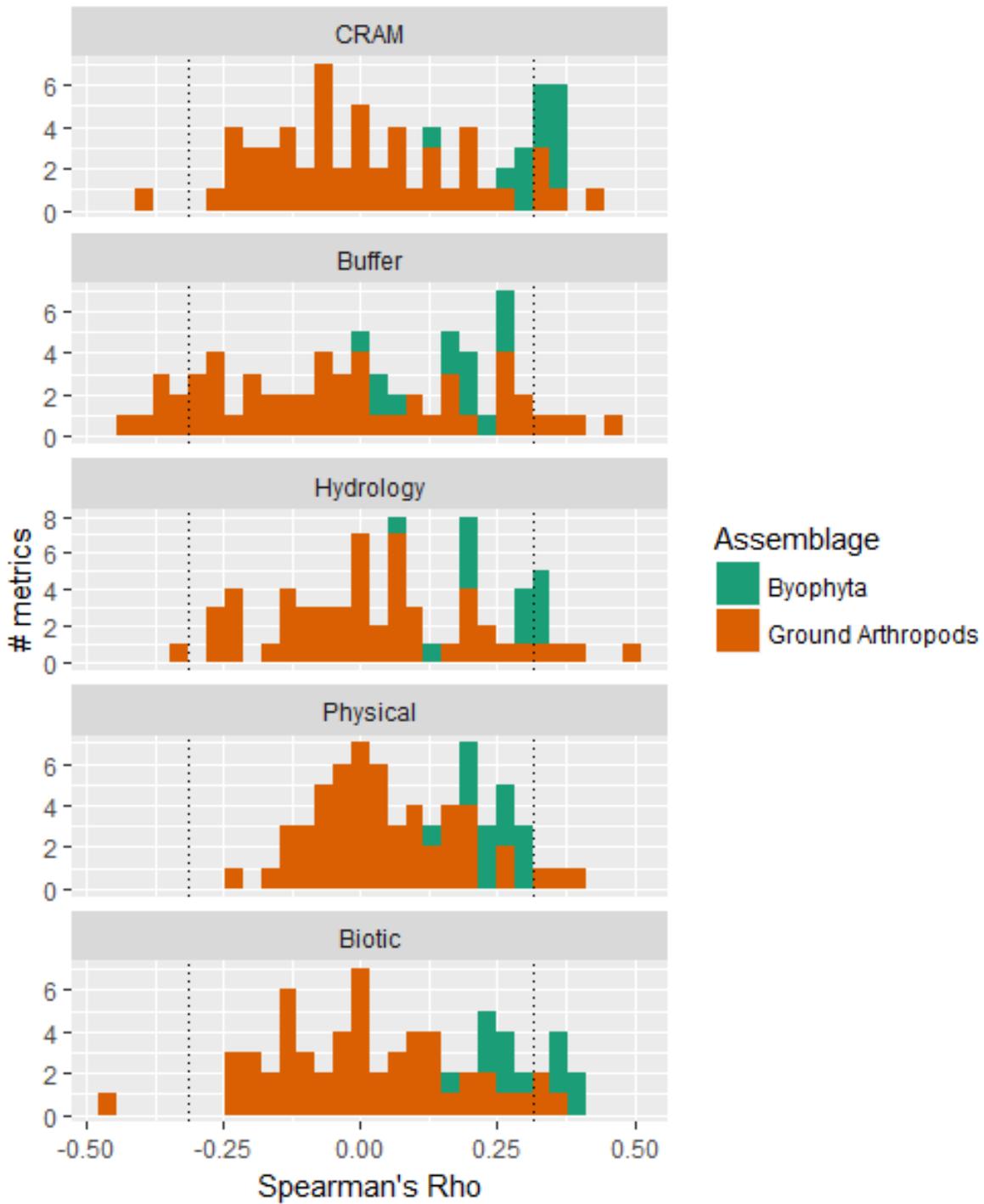


Figure 6. Distribution of Spearman's rho values between bioassessment metrics and CRAM index or attribute scores. The dotted lines represent thresholds for strong relationships ($\rho^2 > 0.1$).

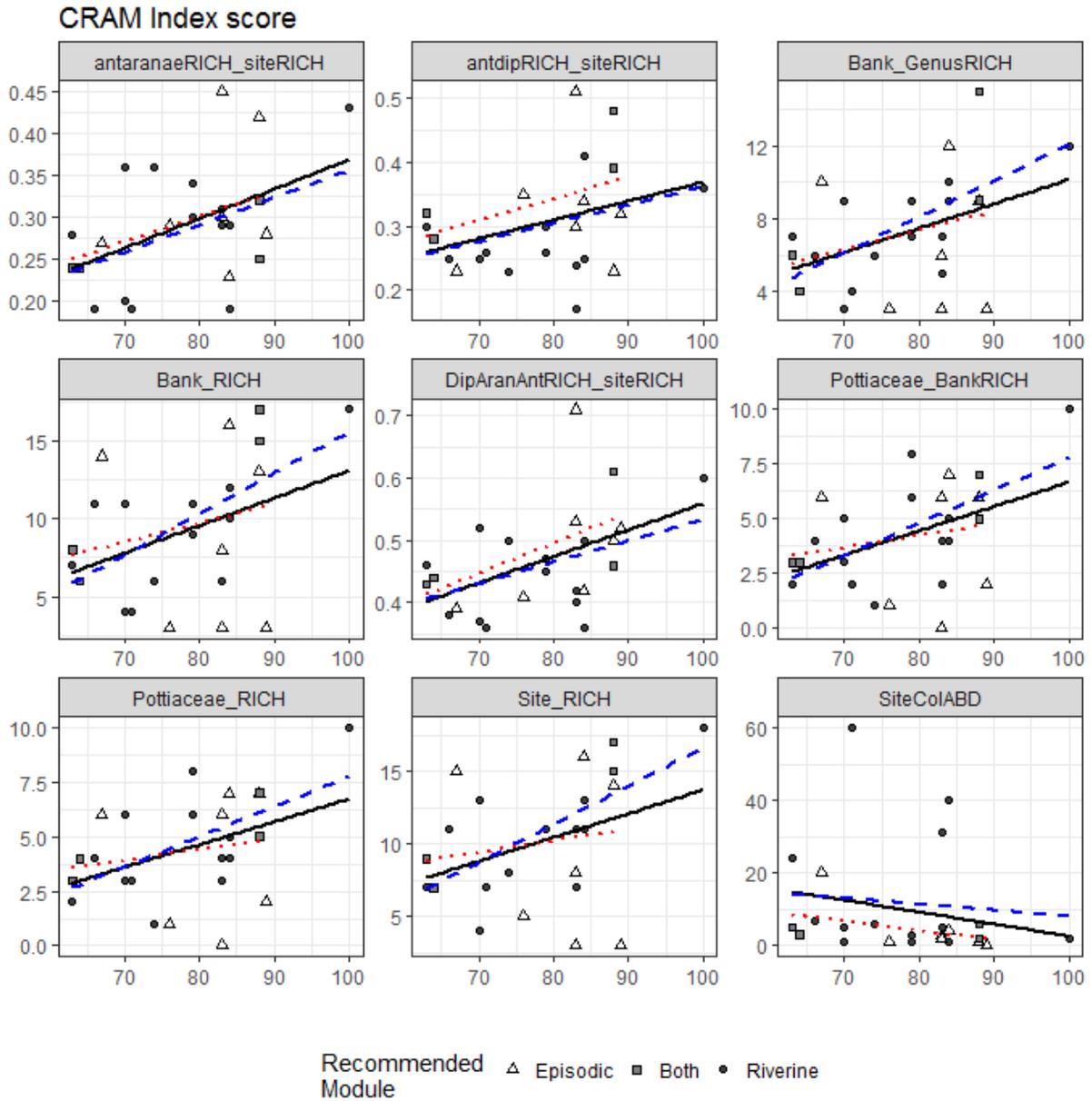


Figure 7a. Relationships between bioassessment metrics and CRAM index scores. Solid line: Linear regression of the recommended module. Dotted red line: linear regression of the Episodic module, where recommended. Dashed blue line: linear regression of the Riverine module, where recommended. Metrics with the highest rho-squared with the preferred module are shown.

CRAM Buffer and Landscape attribute score

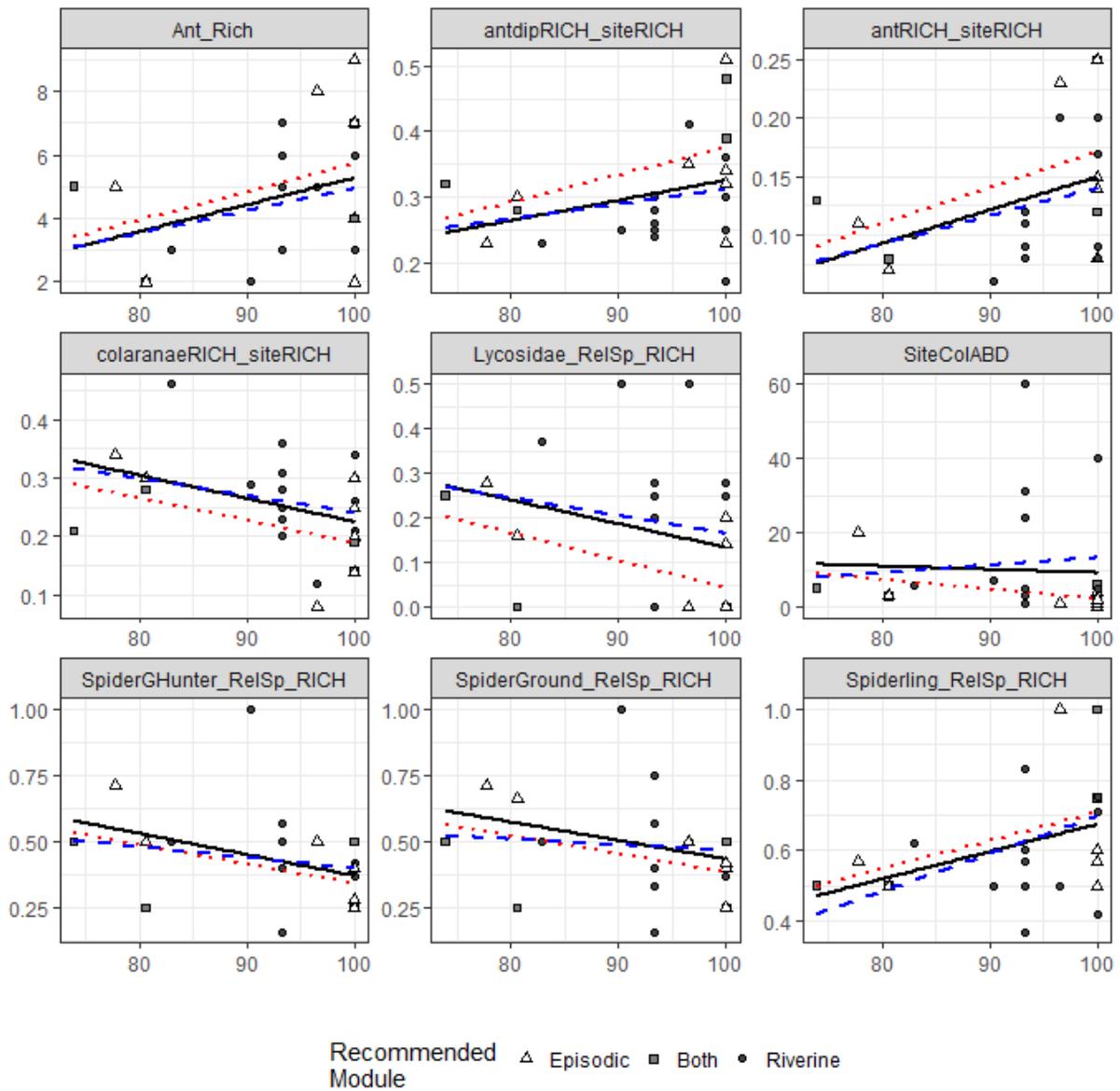


Figure 7b. Relationships between bioassessment metrics and CRAM buffer and landscape attribute scores. Solid line: Linear regression of the recommended module. Dotted red line: linear regression of the Episodic module, where recommended. Dashed blue line: linear regression of the Riverine module, where recommended. Metrics with the highest rho-squared with the preferred module are shown.

CRAM Hydrology attribute score

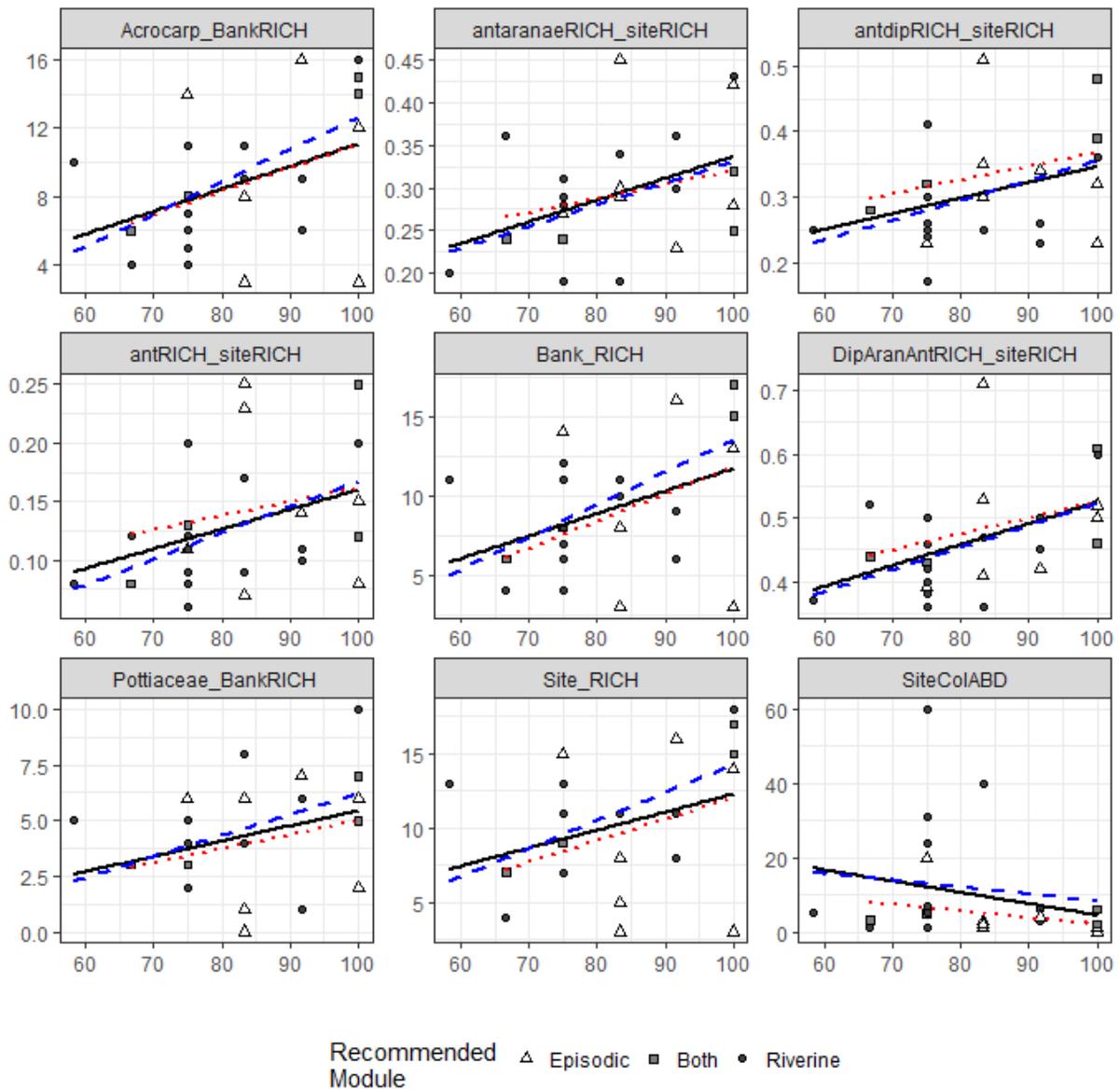


Figure 7c. Relationships between bioassessment metrics and CRAM hydrology attribute scores. Solid line: Linear regression of the recommended module. Dotted red line: linear regression of the Episodic module, where recommended. Dashed blue line: linear regression of the Riverine module, where recommended. Metrics with the highest rho-squared with the preferred module are shown.

CRAM Physical attribute score

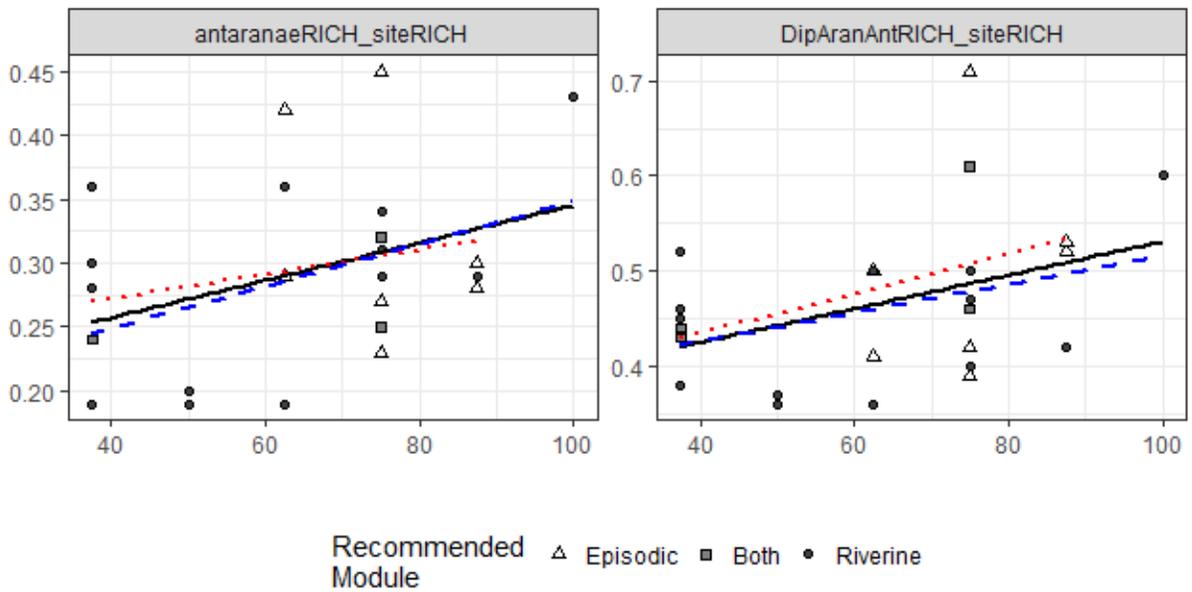


Figure 7d. Relationships between bioassessment metrics and CRAM physical attribute scores. Solid line: Linear regression of the recommended module. Dotted red line: linear regression of the Episodic module, where recommended. Dashed blue line: linear regression of the Riverine module, where recommended. Metrics with rho-squared with the preferred module > 0.1 are shown.

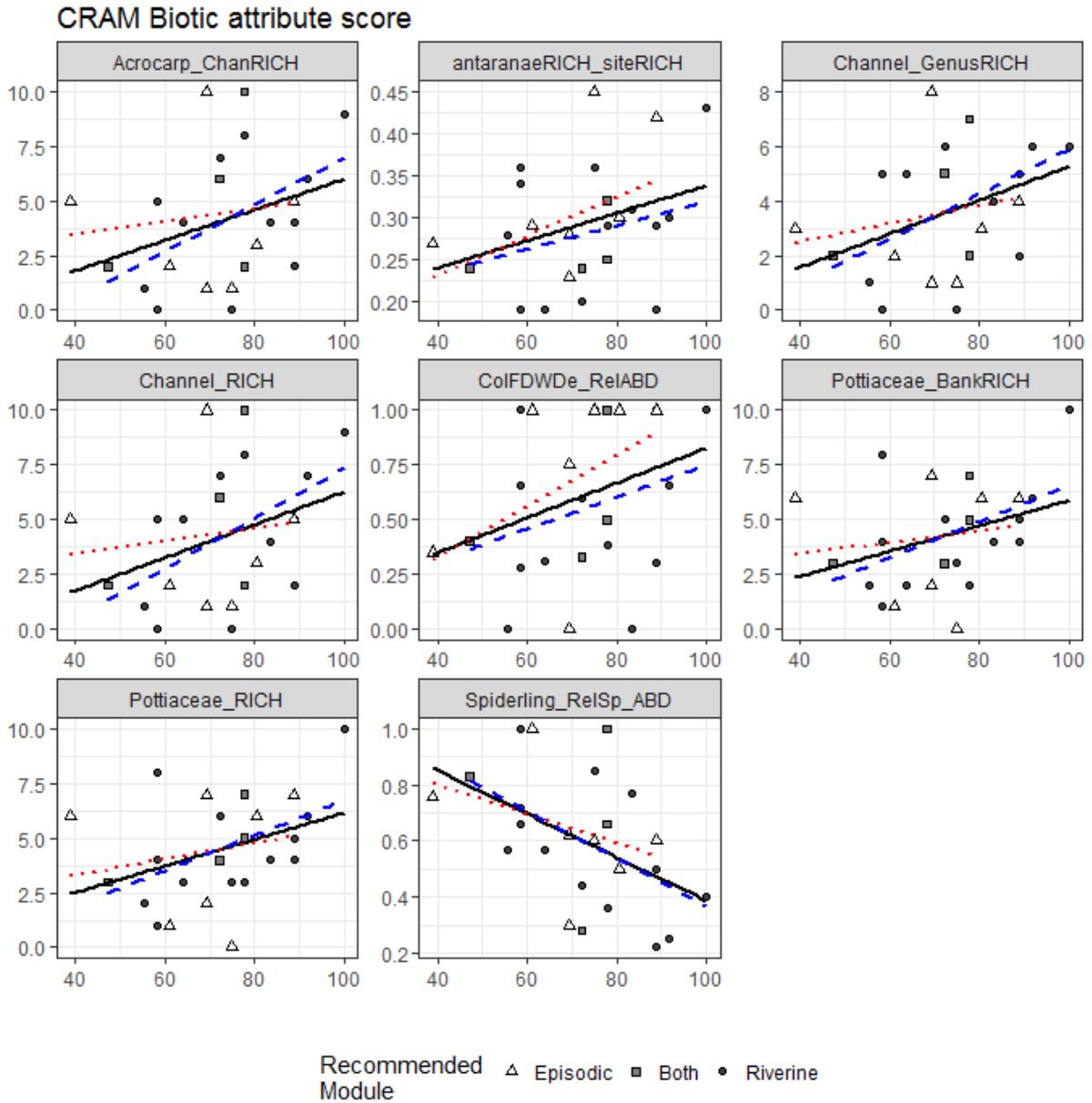


Figure 7e. Relationships between bioassessment metrics and CRAM biotic attribute scores. Solid line: Linear regression of the recommended module. Dotted red line: linear regression of the Episodic module, where recommended. Dashed blue line: linear regression of the Riverine module, where recommended. Metrics with rho-squared with the preferred module > 0.1 are shown.

Table 7. Comparison of mean CRAM index and attribute scores at reference and nonreference sites. SD: Standard deviation. t: t-statistic. p: p-value associated with t-test. Results for

Recommended and Episodic results are for all sites where CRAM was measured (n = 24); Riverine results are for those sites where the Riverine module was implemented (n = 18).

Variable	Reference		Nonreference		Recommended		Episodic		Riverine	
	Mean	SD	Mean	SD	t	p	t	P	t	p
Index	80.6	10.0	75.0	9.0	1.44	0.16	0.38	0.71	0.74	0.47
Buffer & Landscape	96.2	7.0	89.2	8.2	2.19	0.04	1.11	0.30	0.74	0.47
Hydrology	83.3	10.8	81.7	14.1	0.31	0.76	0.50	0.64	-0.56	0.59
Physical	67.9	19.4	58.8	18.7	1.16	0.26	-0.30	0.77	1.27	0.23
Biotic	74.2	15.1	69.2	14.8	0.82	0.42	-0.10	0.93	0.49	0.63

Table 8. Spearman correlation coefficients between bioassessment metrics and CRAM index or attribute scores. Dashes indicate that the correlation was weak (rho-square < 0.1).

Metric	Index	Buffer and landscape	Hydrology	Physical	Biotic
<i>Bryophyte metrics</i>					
Site_RICH	0.38	--	0.34	--	--
Site_FamRICH	0.32	--	--	--	--
Channel_RICH	--	--	--	--	0.38
Channel_GenusRICH	--	--	--	--	0.39
Bank_RICH	0.36	--	0.33	--	--
Bank_GenusRICH	0.38	--	--	--	--
Pottiaceae_RICH	0.38	--	--	--	0.38
Pottiaceae_BankRICH	0.38	--	0.32	--	0.36
Acrocarp_RICH	0.34	--	--	--	--
Acrocarp_ChanRICH	--	--	--	--	0.35
Acrocarp_BankRICH	0.33	--	0.34	--	--
<i>Arthropod metrics</i>					
Ant_Rich	--	0.34	--	--	--
antRICH_siteRICH	--	0.45	0.37	--	--
coldipRICH_siteRICH	--	-0.33	--	--	--
colaranaeRICH_siteRICH	--	-0.36	--	--	--
antdipRICH_siteRICH	0.34	0.35	0.34	--	--
antaranaeRICH_siteRICH	0.35	--	0.38	0.35	0.35
CoLDipArRICH_siteRICH	--	-0.33	--	--	--
DipAranAntRICH_siteRICH	0.44	--	0.49	0.39	--
SpiderGround_RelSp_RICH	--	-0.40	--	--	--
Spiderling_RelSp_ABD	--	--	--	--	-0.47
Spiderling_RelSp_RICH	--	0.38	--	--	--
SpiderGHunter_RelSp_RICH	--	-0.44	--	--	--
Lycosidae_RelSp_RICH	--	-0.38	--	--	--
SiteColABD	-0.38	-0.37	-0.32	--	--
ColFDWDe_RelABD	0.33	--	--	--	0.32

Discussion

This study demonstrates the feasibility of assessing ephemeral streams, or intermittent streams during the dry phase. The CRAM modules evaluated here provide a rapid way to assess ephemeral stream condition, and the more intensive measures are practical to implement, and show promise as potential bioassessment tools. These tools open the door to integrating ephemeral streams into monitoring programs alongside perennial streams, paving the way for comprehensive watershed management.

Both the Riverine and Episodic CRAM modules produced comparable results, although the higher scores associated with the Episodic module means that module selection has at least some undesirable potential to influence outcomes. Guidance in the current version of the Episodic CRAM field book (CWMW 2015) emphasizes map-based indicators (such as stream order or mean annual precipitation) that could be determined at a desktop, prior to any site visits. In the course of this study, we found this guidance to be insufficient, and generally biased practitioners towards the Riverine module across the San Diego region, even for reference sites that would clearly score poorly with this module. Field-based indicators (primarily, riparian vegetation) could enhance this guidance. However, incorporation of field indicators should be done in a transparent and standardized fashion to limit the potential of selecting a module that produces a favored outcome. Thus, in the interim, caution should be exercised when determining the appropriate module for regulatory purposes (e.g. mitigation).

The protocols evaluated in this study show that sampling dry streams is practical, with effort levels that are comparable to those required for benthic macroinvertebrates in flowing streams. Because dry-stream sampling is not dependent on flow conditions, there is relatively little uncertainty in whether or not a site visit will be successful, compared to traditional stream bioassessment protocols. Incorporating these methods into probabilistic programs, such as the SMC's stream survey (Mazor 2015) could reduce the high rejection rates associated with flow conditions. It should be noted, however, that these protocols might result in higher costs than traditional bioassessment, as one of the assemblages (arthropods) requires overnight deployment of traps, and therefore two site-visits. Additional research is needed to determine an appropriate sampling index period, or if a stream must be dry for a certain duration before sampling can occur.

Despite the low sample size and high variability, we observed in bioassessment metrics, we were able to identify several that could be used to measure ephemeral stream condition. Many of these metrics reflected relatively sedentary components of the stream community, like web-weaving spiders (which are less mobile than spiders that hunt by foraging prey on the ground) or mosses. Physical disturbance, like cattle grazing or human recreation, may have a greater impact on these species than on species that can actively avoid hooves or all-terrain vehicles. Given the diverse life histories of arthropods and bryophytes (Malcolm et al. 2009, Steward et al. 2017), metrics that reflect the different ways that these organisms interact with their environment may provide a better understanding of the ways human activities affect ephemeral streams. The observation that bryophytes and arthropods showed a differential response to watershed versus local disturbance suggests that both provide complementary information about stream condition, best used in tandem.

Several metrics showed influence from natural factors, suggesting that a predictive approach, similar to that used in the California Stream Condition Index (Mazor et al. 2016), may be necessary to disentangle human impacts from natural variability. In this study, we were able to use random forest models to calculate reference expectations for 6 metrics, and use deviations from reference as a measure of condition instead of raw metric values; in general, these “modeled” metrics were much more responsive than unmodeled metrics, consistent with patterns seen with benthic macroinvertebrates (Cao et al. 2007, Vander Laan and Hawkins 2014, Mazor et al. 2016). While the present data set included 22 reference sites, and this was sufficient for modeling the influence of natural factors for a handful of metrics, a larger data set representing a wider array of environmental settings is preferable to produce a predictive index (Ode et al. 2016).

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ACRONYMS

BMI: Benthic macroinvertebrates

CRAM: California Rapid Assessment Method

CSCI: California Stream Condition Index

CSUMB: California State University at Monterey Bay

CWMW: California Wetlands Monitoring Workgroup

DRI: Desert Research Institute at the University of Nevada, Las Vegas

EPA: Environmental Protection Agency

GIS: Geographic Information System

GPS: Global Positioning System

IRES: Intermittent rivers and ephemeral streams

NHD: National Hydrography Dataset

PSA: Perennial Streams Assessment

PVC: Polyvinyl chloride

RWQCB: Regional Water Quality Control Board

SCCWRP: Southern California Coastal Water Research Project

SMC: Stormwater Monitoring Coalition

SWRCB: State Water Resources Control Board

USEPA: United States Environmental Protection Agency

APPENDIX

Bioassessment metrics evaluated in the study. Highlighted cells indicate a failed criterion for further analysis; only metrics without any highlighted cells were selected for further analysis (Table 3).

Metric Code	Metric Description	# unique values	Frequency of zeroes	Median value
<i>Bryophyte metrics</i>				
Site_RICH	Bryophyte site richness	18	0.03	11
Site_FamRICH	Bryophyte Family site richness	10	0.03	4
Site_GenusRICH	Bryophyte Genera site richness	13	0.03	8
Channel_RICH	Channel Bryophyte richness	13	0.13	4
ChannelRICH_RelSiteRICH	Channel Bryophyte richness relative to site richness	1	1.00	0
Channel_FamRICH	Channel Bryophyte family site richness	6	0.13	3
Channel_RelFamRICH	Channel Bryophyte family richness relative to Bryophyte family site richness	2	0.82	0
Channel_GenusRICH	Channel Bryophyte Genera site richness	10	0.13	4
Channel_RelGenusRICH	Channel Bryophyte Genera richness relative to Bryophyte Genera site richness	2	0.97	0
Bank_RICH	Bank Bryophyte richness	18	0.03	9
BankRICH_RelSiteRICH	Bank Bryophyte richness relative to site richness	2	0.69	0
Bank_FamRICH	Bank Bryophyte Family site richness	8	0.03	4
Bank_RelFamRICH	Bank Bryophyte Family richness relative to Bryophyte Family site richness	2	0.33	1
Bank_GenusRICH	Bank Bryophyte Genera site richness	14	0.03	7
Bank_RelGenusRICH	Bank Bryophyte Genera richness relative to Bryophyte Genera site richness	2	0.51	0
Pottiaceae_RICH	Pottiaceae site richness	12	0.08	4
PottiaceaeRICH_RelSiteRICH	Pottiaceae richness relative to site richness	1	1.00	0
Pottiaceae_ChanRICH	Channel Pottiaceae richness	9	0.41	1
Pottiaceae_RelChanRICH	Channel Pottiaceae richness relative to Bryophyte Channel richness	1	1.00	0

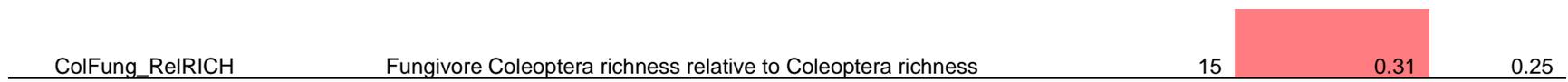
Pottiaceae_BankRICH	Bank Pottiaceae richness	10	0.08	4
Pottiaceae_RelBankRICH	Bank Pottiaceae richness relative to Bryophyte bank richness	1	1.00	0
Bryaceae_RICH	Bryaceae site richness	6	0.05	3
BryaceaeRICH_RelSiteRICH	Bryaceae richness relative to site richness	2	0.97	0
Bryaceae_ChannelRICH	Channel Bryaceae richness	4	0.18	1
Bryaceae_RelChannelRICH	Channel Bryaceae richness relative to Bryophyte Channel richness	2	0.82	0
Bryaceae_BankRICH	Bank Bryaceae richness	6	0.10	2
Bryaceae_RelBankRICH	Bank Bryaceae richness relative to Bryophyte bank richness	2	0.97	0
Acrocarp_RICH	Acrocarp richness	19	0.03	10
AcrocarpRICH_SiteRICH	Acrocarp richness relative to site richness	2	0.46	1
Acrocarp_ChanRICH	Channel Acrocarp site richness	13	0.13	4
Acrocarp_ChanRelRICH	Channel Acrocarp richness relative to Channel richness	2	0.31	1
Acrocarp_BankRICH	Bank Acrocarp richness	15	0.03	9
Acrocarp_BankRelRICH	Bank Acrocarp richness relative to Bryophyte bank richness	2	0.41	1
Pluerocarp_RICH	Pluerocarp richness	4	0.56	0
Pluerocarp_ChanRICH	Channel Pluerocarp richness	4	0.82	0
Pluerocarp_RelChanRICH	Channel Pluerocarp richness relative to Bryophyte channel richness	1	1.00	0
Pluerocarp_BankRICH	Bank Pluerocarp richness	5	0.62	0
Pluerocarp_RelBankRICH	Bank Pluerocarp richness relative to Bryophyte bank richness	1	1.00	0
PluerocarpRICH_RelSiteRICH	Pluerocarp richness relative to Bryophyte richness	1	1.00	0
<i>Arthropod metrics</i>				
Tot_Rich	Total site richness	24	0.00	35
Tot_Abund	total site abundance	35	0.00	192
Coleopt_Rich	Coleoptera richness	14	0.03	3

Dip_Rich	Diptera richness	11	0.00	6
Aranae_Rich	Spider richness	8	0.00	6
Ant_Rich	Ant richness	11	0.00	4
colRICH_siteRICH	Coleoptera richness relative site richness	20	0.03	0.07
dipRICH_siteRICH	Diptera richness relative to site richness	20	0.00	0.16
aranaeRich_SiteRich	Spider richness relative to site richness	18	0.00	0.18
antRICH_siteRICH	Ant richness relative to site richness	16	0.00	0.11
coldipRICH	Coleoptera and Diptera combined richness	17	0.00	10
coldipRICH_siteRICH	Coleoptera and Diptera combined richness relative to site richness	22	0.00	0.25
colaranaeRICH	Coleoptera and Spider combined richness	18	0.00	9
colaranaeRICH_siteRICH	Coleoptera and Spider combined richness relative to site richness	24	0.00	0.28
DipandAranaeRICH	Diptera and Spider richness	14	0.00	12
dipandaranaeRICH_siteRICH	Diptera and Spider richness relative to site richness	20	0.00	0.32
antcolRICH	Ant and Coleoptera combined richness	15	0.00	8
antcolRICH_siteRICH	Ant and Coleoptera combined richness relative to site richness	23	0.00	0.23
antdipRICH	Ant and Diptera combined richness	15	0.00	10
antdipRICH_siteRICH	Ant and Diptera combined richness relative to site richness	22	0.00	0.27
antaranaeRICH	Ant and Spider combined richness	12	0.00	11
antaranaeRICH_siteRICH	Ant and Spider combined richness relative to site richness	21	0.00	0.29
ColDipArRICH	Coleoptera, Diptera, and Spider combined richness	17	0.00	15
ColDipArRICH_siteRICH	Coleoptera, Diptera, and Spider combined richness relative to site richness	21	0.00	0.44
ColDipAntRICH	Coleoptera, Diptera, and Ant combined richness	18	0.00	13
ColDipAntRICH_siteRICH	Coleoptera, Diptera, and Ant combined richness relative to site richness	21	0.00	0.40

ColAranAntRICH	Coleoptera, Spider, and Ant combined richness	20	0.00	14
ColAranAntRICH_siteRICH	Coleoptera, Spider, and Ant combined richness relative to site richness	20	0.00	0.40
DipAranAntRICH	Diptera, Spider, and Ant combined richness	16	0.00	15
DipAranAntRICH_siteRICH	Diptera, Spider, and Ant combined richness relative to site richness	21	0.00	0.45
DipAranAntColRICH	Diptera, Spider, and Ant combined richness	21	0.00	20
DipAranAntColRICH_siteRICH	Diptera, Spider, and Ant combined richness relative to site richness	20	0.00	0.55
Spider_SiteABD	Spider abundance	20	0.00	10
SpiderGround_ABD	Ground Spider abundance	19	0.00	6
SpiderGround_RICH	Ground Spider richness	6	0.00	3
SpiderGround_RelSp_ABD	Ground Spider abundance relative to spider abundance	27	0.00	0.66
SpiderGround_RelSp_RICH	Ground Spider richness relative to spider richness	14	0.00	0.50
SpiderWeb_ABD	Web spider abundance	9	0.03	4
SpiderWeb_RICH	Web spider richness	7	0.03	3
SpiderWeb_RelSp_ABD	Web spider abundance relative to spider abundance	23	0.03	0.30
SpiderWeb_RelSp_RICH	Web spider richness relative to spider richness	14	0.03	0.50
Spiderling_ABD	Spiderling abundance	16	0.00	6
Spiderling_RICH	Spiderling richness	7	0.00	4
Spiderling_RelSp_ABD	Spiderling abundance relative to spider abundance	29	0.00	0.60
Spiderling_RelSp_RICH	Spiderling richness relative to spider richness	16	0.00	0.57
SpiderGHunter_ABD	Ground Hunter spider abundance	19	0.00	6
SpiderGHunter_RICH	Ground Hunter spider richness	5	0.00	3
SpiderGHunter_RelSp_ABD	Ground Hunter spider abundance relative to spider abundance	29	0.00	0.60
SpiderGHunter_RelSp_RICH	Ground Hunter spider richness relative to spider richness	15	0.00	0.42
SpiderOtherHuunter_ABD	Other Hunter spider abundance	7	0.21	2

SpiderOtherHunter_RICH	Other Hunter spider richness	4	0.21	2
SpiderOtherHunter_RelSpABD	Other Hunter spider abundance relative to spider abundance	20	0.21	0.11
SpiderOtherHunter_RelSpRICH	Other Hunter spider richness relative to spider richness	14	0.21	0.25
Lycosidae_ABD	Lycosidae abundance	13	0.23	2
Lycosidae_Rich	Lycosidae richness	4	0.23	1
Lycosidae_RelSp_ABD	Lycosidae abundance relative to spider abundance	21	0.23	0.16
Lycosidae_RelSp_RICH	Lycosidae richness relative to spider richness	11	0.23	0.16
Carab_Rich	Carabidae richness	7	0.41	1
Staphy_Rich	Staphylinidae richness	5	0.67	0
Carab_Staph_Rich	Carabidae and Staphylinidae combined richness	9	0.36	1
CarabRich_ColRich	Carabidae richness relative to Coleoptera richness	13	0.41	0.20
StaphyRich_ColRich	Staphylinidae richness relative to Coleoptera richness	12	0.67	0
CarabABD	Carabidae abundance	16	0.41	1
StaphyABD	Staphylinidae abundance	6	0.67	0
SiteColABD	Coleoptera abundance	20	0.03	5
StaphyABD_siteABD	Staphylinidae abundance relative to site abundance	12	0.67	0
CarabABD_siteABD	Carabidae abundance relative to site abundance	13	0.41	0.01
CarabABD_staphyABD	Carabidae and Staphylinidae combined abundance	17	0.36	1
CarabStaphyABD_SiteABD	Carabidae and Staphylinidae combined abundance relative to site abundance	16	0.36	0.01
CarabStaphRich_SiteRich	Carabidae and Staphylinidae combined richness relative to site abundance	14	0.36	0.02
CarabRich_SiteRich	Carabidae richness relative to site richness	11	0.41	0.02
StaphyRich_siteRich	Staphylinidae richness relative to site richness	6	0.67	0

CarabABD_ColABD	Carabidae abundance relative to Coleoptera abundance	16	0.41	0.20
StaphyABD_ColABD	Staphylinidae abundance relative to site abundance	11	0.67	0
StaphyCarabABD_ColABD	Staphylinidae and Carabidae combined abundance relative to Coleoptera abundance	19	0.36	0.33
ColPredABD	Predator Coleoptera abundance	16	0.33	3
ColPredRICH	Predator Coleoptera richness	10	0.33	1
ColPred_RelABD	Predator Coleoptera abundance relative to Coleoptera abundance	18	0.33	0.57
ColPred_RelRich	Predator Coleoptera richness relative to Coleoptera richness	16	0.33	0.50
ColHerbABD	Herbivorous Coleoptera abundance	6	0.56	0
ColHerbRICH	Herbivorous Coleoptera richness	4	0.56	0
ColHerb_RelABD	Herbivorous Coleoptera abundance relative to Coleoptera abundance	12	0.56	0
ColHerb_RelRich	Herbivorous Coleoptera richness relative to Coleoptera richness	13	0.56	0
ColFDWDe_ABD	Fungivore, Dead Wood, and Detritivore Coleoptera abundance	12	0.15	2
ColFDWDe_RICH	Fungivore, Dead Wood, and Detritivore Coleoptera richness	7	0.15	2
ColFDWDe_RelABD	Fungivore, Dead Wood, and Detritivore Coleoptera abundance relative to Coleoptera abundance	18	0.15	0.54
ColFDWDe_RelRICH	Fungivore, Dead Wood, and Detritivore Coleoptera richness relative to Coleoptera richness	14	0.15	0.50
ColFung_ABD	Fungivore Coleoptera abundance	9	0.31	1
ColFung_RICH	Fungivore Coleoptera richness	7	0.31	1
ColFung_RelABD	Fungivore Coleoptera abundance relative to Coleoptera abundance	17	0.31	0.20



FIELD DATA SHEETS

Below are printable hardcopy versions of field data sheets. A digital version, suitable for use with a Samsung Galaxy “Tab Active” Tablet with Microsoft Excel installed, can be downloaded here:

ftp://ftp.sccwrp.org/pub/download/TMP/RaphaelMazor/EphemeralStreams_DataSheet2017_v1.0.zip

Digital data entry is recommended over paper forms whenever possible.

Ephemeral stream assessment data sheet

Version 1.0

SiteID: _____ Site Name: _____

Location and timing of sample collection

Datum: NAD83 / WGS84 / _____

	Latitude (decimal degrees):	Longitude (decimal degrees):
Upper		
Mid		
Lower		

	Date (mm/dd/yyyy)	Time (hh:mm)
Deployment		
Recovery		

Stream characterization

Check one box for each row

	Not present	<5%	5 to 25%	25 to 50%	50 to 75%	>75%
Riffle	<input type="checkbox"/>					
Run	<input type="checkbox"/>					
Pool	<input type="checkbox"/>					
Steps/Cascade	<input type="checkbox"/>					
Wetted habitat	<input type="checkbox"/>					
Grasses in channel	<input type="checkbox"/>					
Non-woody in channel	<input type="checkbox"/>					
Woody in channel	<input type="checkbox"/>					
Grasses in riparian	<input type="checkbox"/>					
Non-woody in riparian	<input type="checkbox"/>					
Woody in riparian	<input type="checkbox"/>					
Vegetation cover	<input type="checkbox"/>					

Biological stressors

Vegetation management

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Fire breaks	Y/N	<input type="checkbox"/>										
Mowing or cutting	Y/N	<input type="checkbox"/>										
Burns	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Miscellaneous biological stressors

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Cattle grazing	Y/N	<input type="checkbox"/>										
Invasive plants	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Land use effects

Heavy urban

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Industrial	Y/N	<input type="checkbox"/>										
Landfill	Y/N	<input type="checkbox"/>										
Mining	Y/N	<input type="checkbox"/>										
Military land	Y/N	<input type="checkbox"/>										
Urban commercial	Y/N	<input type="checkbox"/>										
Urban residential	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Light urban

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Suburban residential	Y/N	<input type="checkbox"/>										
Rural residential	Y/N	<input type="checkbox"/>										
Golf course, park or sportsfield	Y/N	<input type="checkbox"/>										
Excessive human visitation	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Agricultural

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Crops—Irrigated	Y/N	<input type="checkbox"/>										
Crops—Non-irrigated	Y/N	<input type="checkbox"/>										
Vineyards	Y/N	<input type="checkbox"/>										
Timber harvest	Y/N	<input type="checkbox"/>										
Orchards	Y/N	<input type="checkbox"/>										
Hay	Y/N	<input type="checkbox"/>										
Fallow fields	Y/N	<input type="checkbox"/>										
Dairies	Y/N	<input type="checkbox"/>										
CAFOs	Y/N	<input type="checkbox"/>										
Pasture	Y/N	<input type="checkbox"/>										
Rangeland	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Transportation

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Highways (more than 2 lanes)	Y/N	<input type="checkbox"/>										
Paved roads	Y/N	<input type="checkbox"/>										
Unpaved roads	Y/N	<input type="checkbox"/>										
Parking lot or pavement	Y/N	<input type="checkbox"/>										
Railroad	Y/N	<input type="checkbox"/>										
Air traffic	Y/N	<input type="checkbox"/>										
Walking path	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Chemical stressors

Industrial water quality

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Point-source discharge	Y/N	<input type="checkbox"/>										
Acid mine drainage	Y/N	<input type="checkbox"/>										
Noxious chemical odors	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Urban water quality

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Nonpoint-source (stormwater)	Y/N	<input type="checkbox"/>										
Trash or dumping	Y/N	<input type="checkbox"/>										
Vector control	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Agricultural water quality

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Agricultural runoff	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Nutrient-related water quality impacts

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Algal surface mats or benthic algal growth	Y/N	<input type="checkbox"/>										
Direct septic or sewage discharge	Y/N	<input type="checkbox"/>										
Excessive animal waste	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Miscellaneous water quality impacts

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
High concentration of salts	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Hydrologic stressors

Water control actions

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Flow diversions	Y/N	<input type="checkbox"/>										
Groundwater extraction	Y/N	<input type="checkbox"/>										
Unnatural flows	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Water control features

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Dike or levee	Y/N	<input type="checkbox"/>										
Ditches or canals	Y/N	<input type="checkbox"/>										
Dam	Y/N	<input type="checkbox"/>										
Spring boxes	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Physical stressors

Sediment disturbance

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
ATVs	Y/N	<input type="checkbox"/>										
Mountain bikes	Y/N	<input type="checkbox"/>										
Horses	Y/N	<input type="checkbox"/>										
Excavation	Y/N	<input type="checkbox"/>										
Grading or compaction	Y/N	<input type="checkbox"/>										
Feral pig disturbance	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Excessive sediment input

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Passive input (construction or erosion)	Y/N	<input type="checkbox"/>										
Debris lines or silt-laden vegetation	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Hardened features

Stressor	Present?	In channel	Proximity				Extent			Intensity		
			1-5	5-50	50-100	100-200	1	2	3	1	2	3
Rip-rap, armoring of channel bed or bank	Y/N	<input type="checkbox"/>										
Obstructions	Y/N	<input type="checkbox"/>										
Other:	Y/N	<input type="checkbox"/>										

Transect measurements

Transect 1

Channel width (m): _____

Channel depth (m): _____

Left	Middle	Right

Pebble count:

Slope to upstream transect (%): _____

Pebble #	Size (mm)	Embeddedness (%, round to 10)
1		
2		
3		
4		
5		

Transect 2

Channel width (m): _____

Channel depth (m): _____

Left	Middle	Right

Pebble count:

Slope to upstream transect (%): _____

Pebble #	Size (mm)	Embeddedness (%, round to 10)
1		
2		
3		
4		
5		

Transect 3

Channel width (m): _____

Channel depth (m): _____

Left	Middle	Right

Pebble count:

Slope to upstream transect (%): _____

Pebble #	Size (mm)	Embeddedness (%, round to 10)
1		
2		
3		
4		
5		

Transect 4

Channel width (m): _____

Channel depth (m): _____

Left	Middle	Right

Pebble count:

Slope to upstream transect (%): _____

Pebble #	Size (mm)	Embeddedness (%, round to 10)
1		
2		
3		
4		
5		

Transect 5

Channel width (m): _____

Channel depth (m): _____

Left	Middle	Right

Pebble count:

Slope to upstream transect (%): _____

Pebble #	Size (mm)	Embeddedness (%, round to 10)
1		
2		
3		
4		
5		

Transect 6

Channel width (m): _____

Channel depth (m): _____

Left	Middle	Right

Pebble count:

Slope to upstream transect (%): _____

Pebble #	Size (mm)	Embeddedness (%, round to 10)
1		
2		
3		
4		
5		

Transect 7

Channel width (m): _____

Channel depth (m): _____

Left	Middle	Right

Pebble count:

Slope to upstream transect (%): _____

Pebble #	Size (mm)	Embeddedness (%, round to 10)
1		
2		
3		
4		
5		

Transect 8

Channel width (m): _____

Channel depth (m): _____

Left	Middle	Right

Pebble count:

Pebble #	Size (mm)	Embeddedness (%, round to 10)
1		
2		
3		
4		
5		