Methods Manual

Scale Consumers and Lotic Ecosystem Rates: SCALER

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Contents

1. Introduction

These protocols were designed and executed for the Scale Consumers and Lotic Ecosystem Rates (SCALER) project. They include site selection criteria, as well as guidelines for consumer manipulation, measures of ecosystem rates, and sampling for complimentary data. Information on data format and storage is also included. Section 2 describes the reasoning for selection of specific sites at three different spatial scales: the network, the reach, and the habitat. That section also includes instructions for the installation of consumer exclosures at the habitat and reach scales. Section 3 details the field methods for measuring metabolism and nutrient uptake used at the three scales of this project as well as additional measures taken during the experiment. Section 4 details the lab protocols for processing of field samples. Section 5 gives a timeline of the field sampling as well as the experiments with specific tasks conducted at each site. Section 6 details data management and use of field and electronic. Appendices give material lists (overall and by experimental days), datasheets, electronic datasheets, and other forms as well as reasoning for potential changes in methods over the course of the project.

This document represents a minimal set of measurements, made as consistently as possible in each biome. Researchers associated with each of the five core biomes as well as two additional biomes were encouraged to undertake additional studies and measurements to answer questions that might be site-specific or of special interest to the investigators (but such studies will not be detailed in this manual). Coordination among biomes in such studies was encouraged to examine more broadly applicable patterns. As detailed in the authorship agreement, such studies were detailed to the group.

Parts of this manual were taken from the LINX-II protocols led by Pat Mulholland. All LINX-II project participants are thanked for that text. More detailed information on those protocols can be found at http://andrewsforest.oregonstate.edu/data/abstract.cfm?dbcode=AN006.

2. Site selection at the network, reach, and habitat scale

The following biomes took part in the SCALER study: Luquillo LTER (LUQ), Coweeta LTER (CWT), Konza Prairie LTER (KNZ), Caribou/Poker Creeks (affiliated with Bonanza Creek LTER; CPC), and the Arctic LTER (ARC). Two additional sites were added: 1) HJ Andrews LTER (AND) were one intensive site was sampled in 2014 and three intensive sites in 2015 but without synoptic sampling, and 2) Litchfield National Park (AUS), Darwin, Australia, with sampling at three intensive sites and 12 synoptic sites in 2013. In the rest of the manual, biome will refer to stream networks in these five core locations, and in some cases also HJ Andrews and Darwin.

Within a stream network there were four types of sites. Habitat experiments occurred at about 0.5 - 1 m^2 spatial area and reach experiments at approximately 30-100 m stream lengths. Twenty synoptic sites were distributed across the stream network, with 6 intensive (more detailed sampling) and 14 extensive sites (basic sampling only). This was a target, but all sites did not accomplish sampling this many locations in every year due to drought or other sampling constraints.

Sampling was conducted at two different scales (reach and habitat). Linkages across these scales, and to the network scale, are accomplished with modeling. Table 1 describes some of the models of increasing complexity that could be used to scale metabolism and nutrient uptake from habitat to reach and from reach to network.

We assessed the ability to scale ecosystem rates (i.e., stream metabolism, ammonium uptake) and consumer effects on those rates across scales to an entire stream network. Models to scale ecosystem rates were calibrated using information from habitat-scale measures and the experimental reaches (three per year), and a subset of the synoptic sites at the network scale that were studied during baseflow conditions (if possible) in both the years 2013 and 2014. The habitats and experimental reaches spanned stream sizes ranging from roughly 1 to 500 L s^{-1} at baseflow across all biomes (Table 2).

Data from the extensive synoptic sampling sites were used to inform selection of representative experimental sites (habitat and reach) *when possible*; some biomes needed to select sites based on accessibility and water availability. The intensive synoptic survey data were used to test and validate model predictions at the network scale. If a model of a particular level of complexity (Table 1) was able to predict the synoptic ecosystem rate measurements, then we could have increased confidence that our network scale estimates were reasonable, and that results of model experiments were useful (e.g., quantifying metabolism of a stream network with and without consumers).

The extent of the watershed to be studied was selected based on the following criteria, while realizing that optimizing all of these would be difficult**: 1) watershed minimally influenced by humans, 2) watershed large enough to capture important gradients (range of stream sizes and features such as width or canopy as well as gradient in macro-consumer communities), and 3) as large a watershed as possible given constraints to be able to accomplish the experiments and measurements in the largest streams.** Following a 'suitability model' approach, synoptic and experimental reach selection occurred using a combination of available LiDAR and GIS data layers of each river network within a biome, and mainly informed by those investigators with intimate knowledge of each biome. Criteria for both synoptic and experimental reach selection were based on ranking of variables such as surficial geology, stream gradient, proximity to access, and discharge. While stream order was calculated for networks at each site, discharge was used to provide a comparative basis between sites with categories small, medium, and large (see Table 2).

Table 1. Approaches of increasing complexity for reach-to-network scaling of ecosystem rates. In each scenario, estimates of stream metabolism (gross primary production [GPP], ecosystem respiration [R]) and nutrient uptake from our experimental reaches [n=6, from three different stream sizes]) were used as the basis for making a network wide estimate of the same. We tested which scaling scenario (hypothesis) was most appropriate in each biome, but not necessarily all scenarios were used for final inter-biome comparison.

Scaling Scenario	Description	Test	Synoptic Data Used
Simple Linear	Assume mean/stdev from n=6 intensive sites can be applied to benthic area of entire network.	H0: Mean/SD of synoptic sites are not significantly different from intensive sites, residuals not a function of stream order.	GPP, R, and uptake from synoptic sites.
Stream-Order Stratified Linear	Apply mean from each size class (n=2) to total benthic area of each order.	H1: Mean/SD of synoptic sites of each order are not significantly different from intensive sites of that order	GPP, R, and uptake from synoptic sites. Hydraulic measurements for width vs. drainage area relationships.
Empirical Model (e.g. GIS and spatial modeling approach, such as geographically weighted regression)	a) Develop empirical model of GPP, R, and uptake from intensive sites as function of temperature, substrate, light to benthos, water depth, flow, nutrients (based on n=6 sites measured over time) and apply to network using synoptic relationships and GIS. b) same as (a), but considering consumers.	H2A: Model predicted GPP, R, and uptake consistent with synoptic measured GPP, R, and uptake. (P vs. O) H2B: Model requires accounting for the distribution of consumers.	Water temp., light, hydraulic dimensions, substrate, DOC, nutrients, consumers, GPP and R, and uptake from each synoptic site. Relationships as a function of river size.
Process Model (no consumers)	Dynamic model of GPP, R, and uptake that incorporates spatial heterogeneity of drivers (water temperature, light, DOC, nutrients), C:N:P stoichiometry and effects of upstream transformations (serial processing and advection). Consumer effects not important.	H3: Model predicted GPP, R, and uptake consistent with synoptic measurements, plus variability within the network is greater than indicated at synoptic sites alone.	Same as above plus a greater number of stations to characterize heterogeneity of drivers and inputs throughout river network.
Process Model (with consumers)	Same as previous model, but with effects of consumers parameterized as a control on processes.	H4: Model predictions better fit to observations than process model with no consumers.	Additional synoptic information on consumer abundance variability throughout the network.

Table 2. Categorized values of baseflow discharge at SCALER study sites estimated based on a normal water year. Synoptic and experimental reach locations attempt to capture the proportional number of small, medium, and large reaches based on availability within each watershed.

Site Specific Baseflow Discharge Range (L s^{-1}) by category			
Site	***Small***	***Medium***	***Large***
LUQ	1 - 10	10 - 20	20 - 50
CWT	0.25 - 20	20 - 75	75 - 600
KNZ	1 - 10	10 - 50	50 - 150
CPC	20 - 35	35 - 90	90 - 300
ARC	0.25- 50	50 - 150	150 - 500
AUS	0.25 - 40	40 - 140	140 - 400
AND	2 - 6	6 - 20	15 - 50

2.1. Network scale (synoptic) sampling

Within each river network, we conducted a synoptic survey of 20 sites (6 intensive, 14 extensive) that provided base data and validation for various approaches for scaling reach-scale process measurements to entire river networks. The site selection was accomplished during the initial stages of the first year of experiments by the postdocs, students, and PIs at each site. Intensive sites had a more thorough suite of measurements, conducted in both years of the study that would have been logistically unfeasible to conduct across the additional 14 extensive sites. The 14 extensive sites allowed more complete spatial coverage throughout the watershed. The primary difference between the intensive and extensive sites was that process rates were measured at the intensive sites (Table 3).

Wherever possible, sites were stratified by discharge and other characterizing metrics (e.g., stream order), and if necessary by major stream reach-scale variability. Ideally, reach heterogeneity was included when characterizing by stream size, but if broad-scale habitat differences occur (e.g., headwater streams with and without canopy cover; reaches with drastically different sediment types, etc.), then synoptic sites were sampled to account for this heterogeneity. The distribution of sites ideally had been weighted by benthic surface area, meaning that more headwater than higher order streams were included, with one site situated at the mouth of the basin. This required information on average stream width as a function of stream order. As metabolic activity and nutrient uptake are generally driven by benthic surface area, this approach weighted sampling more toward the processes of interest rather than simply relying upon stream length.

Synoptic sites were preferably un-impacted by water withdrawals and nutrient point sources, and had uniform land use (e.g., little or no agricultural or urban land uses). If impacts were unavoidable, estimates of water withdrawals, inputs, or point sources were provided to inform the modeling efforts. Some of this information was available from prior existing datasets. Sites needed to be accessible but avoided locations with potential effects on hydrology such as road crossings and other impacting factors. If possible, synoptic sites were at least 10 stream widths downstream from tributaries or lakes, to ensure complete mixing. Though junctions may be

hotspots of activity (Benda et al. 2004), the study of these was left to site-specific studies by the individual biomes. Site selection was based mainly on hydrology and basin characteristics and adjusted for reality by site knowledge of individuals, and, as a final reality-adjustment, selection was refined after field surveys.

Synoptic sampling was conducted at the same 20 sites in 2013 and 2014 (except if sites had to be changed due to external influences such as drought). We decided to revisit sites to account for potential interannual variation instead of expanding spatial coverage, considering that most watersheds would be hard pressed to find 40 sites to study. Repeating the same sites also helped put varying experimental results into context. The synoptic surveys were carried out (as much as possible) during the 40-day experimental period and thus the same conditions as the intensive reach-scale measurements (i.e., similar light regime, flow regime, season, air temperature). No substantial changes in discharge occurred in the basin in the week prior to sampling, and discharge conditions were relatively steady over the course of synoptic sampling *to the extent possible*. If a significant storm occurred in the middle of the synoptic sampling, we waited until flow had returned to near baseflow conditions, and re-sampled sites. If site conditions changed significantly as a result of a storm event, that site was resampled for physical characteristics (e.g., sediment size).

Synoptic surveys included a characterization of the channel geometry (e.g., depth and slope profiles), bed substrate, in-stream habitat, and adjacent land use. As these metrics are relatively invariant, they were only measured once, if possible before the 2013 field season (i.e., two weeks before the experiment started). Measurements during the experimental period at ***all synoptic sites*** included: water quality parameters (i.e., temperature, dissolved oxygen, conductivity and pH), velocity, discharge, and nutrient chemistry (NO_3, NH_4, PO_4, DOC, TDN, TDP, PP, PN, PC) (see Table 3). Not all these aspects were analyzed for every site, except for NH_4^+, but we collected large enough sample volumes to allow for measurement of these chemical parameters. At the 6 synoptic sites, termed ***intensive***, whole-stream metabolism, nutrient uptake, chlorophyll *a*, and benthic organic matter were also measured, both in 2013 and 2014.

The intensive synoptic survey enabled us to: 1) increase sample sizes on some key metrics above and beyond what was collected at experimental sites, to obtain sufficient data to develop and parameterize biological process models at the reach scale without huge increases in work load, 2) provide quantification of the input variables necessary to simulate network-scale metabolism and nutrient uptake, and 3) test predictions of the network-scale simulations at different locations within the river network (Table 1). Specifically, measurements from a subset of the intensive sites and information from the experimental reaches were used to develop and parameterize models of biologically-driven element flux and subsequent metabolism and nutrient uptake. Once models had been initially constructed (functional formulation and parameterization) using data from the experimental reaches, network-scale models that incorporated the reach-scale biological process models and hydrology were calibrated with a subset of the intensive synoptic measurements. The network-scale model predictions were

validated with intensive synoptic data not used to parameterize the reach-scale biological process models or as drivers in the network-scale models.

Table 3. Measures made at synoptic sites

Measure	Method	Replicates	Comments	Section
All synoptic sites				
a) Site characterization survey (2013 only, except if substantial changes between 2013 and 2014; if truly pre-synoptic include width/depth and discharge listed under b)				
Substrate characteristics	Transects, pebble counts	Min. 10 transects, evenly spaced along a reach of 1.5x min. determined reach length, 20 pebbles per transect	Done once at same time as depth	3.1.3.
Canopy cover	Spherical Densiometer	Min. 10 transects, evenly spaced along 1.5x min. determined reach length, at thalweg	repeat if leaf cover is developing or decreasing during experiment	3.1.6.
Slope	Entire reach, clinometer or hose, or survey equipment, or based off of DEMs		Use best method available at each site	3.1.7.
GPS location	GPS unit, map-based, or ArcGIS, bottom of reach in thalweg			3.1.1.
b) During the experiment (2013 and 2014)				
Water chemistry	Filtered and unfiltered samples, nutrients cooled on ice and then frozen	One filtered and one unfiltered, at time of synoptic sampling (within days)	~50 mL for nutrient analyses, depended upon capacity of laboratory and desired level of replication (noted if protocols of individual laboratories analyze particulate fraction, PC, PN and PP, then needed to filter before freezing and save filters but no unfiltered samples)	3.5.
Water quality	Conductivity, dissolved oxygen, temperature	Minimum once per site at beginning, downstream reach end	Using probes, snapshot measurements	3.1.4.
Mean width	Transects/reach	Min. 10 transects, evenly spaced	8-10 depth measurements across each	3.1.2.

and depth		along 1.5x min. reach length	transect, if width measurements were available, average depth could be calculated rather than measured	
Discharge	Flow meter or salt slug	Minimum once per site, constraint point for flow meter, downstream end for salt slug	Repeat if base flow substantially increases or decreases during experiment	3.1.6.
Intensive sites only (n=6, during the experiment)				
Metabolism	Single station (2013) and two-station (2014) diurnal change (DO, light) (Bott et al. 2006)	Minimum one 24 hour measure per site	logging every 10 min	3.7.
Aeration	Plateau method of SF_6 addition (Mulholland et al. 2001) or two station slug method	1 per site, in conjunction with initial travel time measurements or multiple to develop flow-aeration relationship	Needed to be done separately from nutrient release	3.4.3.
Ammonium uptake	Single station pulse method (Covino et al. 2010)	1 per site		3.8.
Mean width	transects	Whenever uptake or aeration measurement	Minimum of 10 widths	3.1.2.
Benthic organic matter	Divide reach into 10 sections	One sample each coarse (CBOM) and fine (FBOM) fraction per section	Weighted by habitat and substrate cover across sections	3.9.
Chlorophyll *a*	Divide reach into 10 sections	One sample per section	Weighted by habitat and substrate cover across sections	3.10.

2.2. Reach-scale experimental sites

Three experimental sites were selected in 2013 (Figure 1). In 2014, three new sites were selected if possible but could remain the same (e.g., drought condition limited selection of "large" sites in KNZ). Sites were chosen to represent a gradient of stream discharge with the different stream sizes of small, medium and large represented in each year of sampling. The initial survey of synoptic sites was used to ensure that experimental reach-scale sites were representative of the river network. Repeated survey of synoptic sites helped control for inter-annual variation. C*onsumers were not manipulated in 2014* as a mid-project reassessment of consumer densities and effects on stream ecosystem structure and function showed little effect of consumer exclusions (see Appendix "Consumer approach 2014" for rationale). The effort of consumer manipulation in 2014 was thus focused on experiments that were biome-specific and thus not detailed here. However, the scaling aspect of the project was continued at the "experimental" sites in 2014.

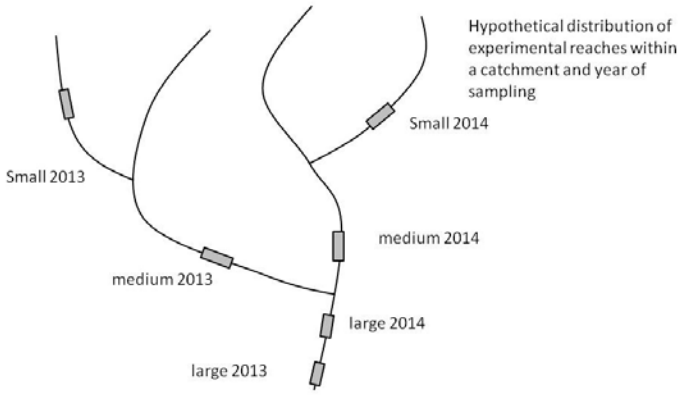

Figure 1. Experimental design at the network scale (1 biome)

Reaches were variable in length and set based on the minimum distance required to effectively measure metabolism and nutrient uptake. The shortest length was used because consumer removal in long reaches was difficult, particularly in larger streams. The measurement lengths depended on travel time and other physical characteristics of a reach site. As control and treatment reaches within each experimental site were compared, they had approximately the same length, similar travel times and roughly the same proportion of pools and riffles. For specific reach length calculation see sections on experimental installation (2.4.1. and 2.4.2.) though often reach selection and length was constrained by the presence of tributaries.

2.3. Habitat-scale locations

Metabolic rates, ammonium uptake, and the influence of consumers on those rates were measured for the dominant substrate types (e.g., cobble or sand) in representative habitats (e.g., riffle and pool). Survey of substrate type (see section 3.1.3.) and habitat type was necessary to determine placement of the experimental exclosures and proportion baskets among habitats in an approximate area-weighted fashion. The basic idea of the sampling was to capture the dominant types of substrate or cover all types at each of the experimental sites to allow extrapolation from baskets to habitat and from habitat to network. We classified substrate types via a modified Wentworth scale for inorganic particles and by type for organic materials as follows (Table 4).

For the purposes of this study, everything cobble size and larger was considered the same since we could not conduct chamber incubations on any substrates bigger than gravels (of course substrate classification was conducted on a finer scale). Sand was differentiated from silt as we

know that flow through sand can create very different biogeochemistry than much more restricted flow through silt or mud (Dodds et al. 1996).

Table 4. Modified Wentworth scale of substrate types

Substrate Type	Median Axis Length (mm)
Cobbles and Boulders	> 64.0
Pebbles and Gravels	4.0 – 64.0
Sands and Granules	0.063 – 4.0
Silts and Clays	< 0.063

Appropriation of sediment size was based on the following equation related to total area of a stream segment (A), and the n types of substrate and their areal cover (Sa_i):

$$A = \sum_{i=1}^{n} Sa_i \qquad \text{(Equation 1)}$$

We use this relationship to find the most dominant substrates, as defined by the i^{th} term that made up 80% of the areal cover of up to three substrate types. A set of decision rules is described in section 2.4.4. to deal with the best way to capture the majority of surface area biological activity. Apportioning these substrate types by experimental exclosures and in experimental reaches is covered in sections 2.4.5. The substrate representation in habitats was used for 1) basket selection in habitat-scale experimental exclosures, 2) basket selection in experimental reaches, and 3) sampling strategy for BOM and chlorophyll in synoptic sites.

2.4. Experimental installation at the habitat and reach scale

2.4.1. Calculation of experimental reach length

In general, travel times of 30 to 45 minutes are optimal but reach lengths were often constrained by other parameters such as tributaries or groundwater seeps. With shorter travel times, metabolism and nutrient uptake measurements were more difficult and with longer travel times, each measurement takes a prohibitively long time. More importantly, travel time could be used to empirically calculate re-aeration in some reaches (dependent on the geomorphic characteristics). If possible, prior estimates of ammonium uptake rates and metabolism rates provided the best way to estimate experimental reach length, but if not, background ammonium (measured or estimated) and measured physical variables (travel time and slope) were used to calculate minimum usable reach length.

A rough determination of reach length is based on the minimum length to get a discernible metabolism measure. Riley and Dodds (2013) found that the equation of Tsivoglou and Neal (1976) gave best estimates of measured aeration rates for small streams:

$$k = \frac{C * \Delta elev.}{t} \qquad \text{(Equation 2)}$$

where, C = 0.187 m^{-1}, Δ_{elev} is the change of elevation (m), t the travel time (s) and k the empirically derived re-aeration in units of per second. From this equation and the estimates of Reichert et al. 2009 for minimum practical reach length in a two-station measurement:

$$\Delta x = \frac{0.4v}{k} \quad \text{(Equation 3)}$$

where, v = velocity in m s^{-1} and *k* is aeration coefficient in s^{-1}, a minimum reach length can be calculated.

A second option is to use nutrient uptake length data from the LINX-I project (with consideration given to the fact that lengths and travel times are specific to different sites). The following information could provide some guidelines on time/distance it took to decrease the ammonium concentration from a 5-times background plateau addition:

A *30%* reduction of the addition took at most **30 minutes** or **80 m**.

A *20%* reduction of the addition took at most **20 minutes** or **52 meter**.

If an approximate uptake length (S_w) is known the distance in meters (x) can be calculated as follows:

$$x = S_w * \left(\frac{\ln(upstream\ conc.)}{\ln(desired\ downstream\ conc.)}\right) \quad \text{(Equation 4)}$$

though ***practically***, many sites were chosen to get as long a travel time as possible without intersecting tributaries or major groundwater input.

2.4.2. Measurement of travel times combined with site characteristics

An estimate of travel times can be conducted before setting each experimental reach site including the measurement of average width, average depth and discharge. If time permitted, travel time measurements were combined with aeration (see section 3.4.3.), otherwise travel times were measured with pulsed releases before reaches were established and aeration was done the same day as the nutrient releases. For pulse measurements of travel time, we determined the time for the peak to pass each potential point of a reach (i.e., top control, bottom control, top treatment and bottom treatment reach), starting at the top of the most upstream reach. If we used a plateau approach, then travel time was the time to reach half the concentration of tracer eventually obtained at plateau.

2.4.3. Reach-scale exclusion

Material list

Mesh fencing (3 or 4 ft high; 4 sections that are ~1.5 stream widths long for each experimental site (3); for mesh size see Table 5; recommended supplier: www.tepinc.com)
Wire cutters
Shovels
Rebar (~1 every 30 cm for the 12 fences) maybe t-posts if high flow
Zip ties (the more the better ☺, 1000s)
Mallet or sledgehammer

For reach-scale experimental manipulations, consumer manipulations were set up at three sites across the gradient of stream sizes (see reach-scale selection (2.2.) for further details, Figure 1). Each site contained three reaches: a) an upstream control reach with consumers removed and returned, b) an area with site-representative habitats, where small mechanical consumer exclusion/control patches were installed to remove large consumers (see habitat-scale exclusion below), and c) a consumer removal reach (Figure 2).

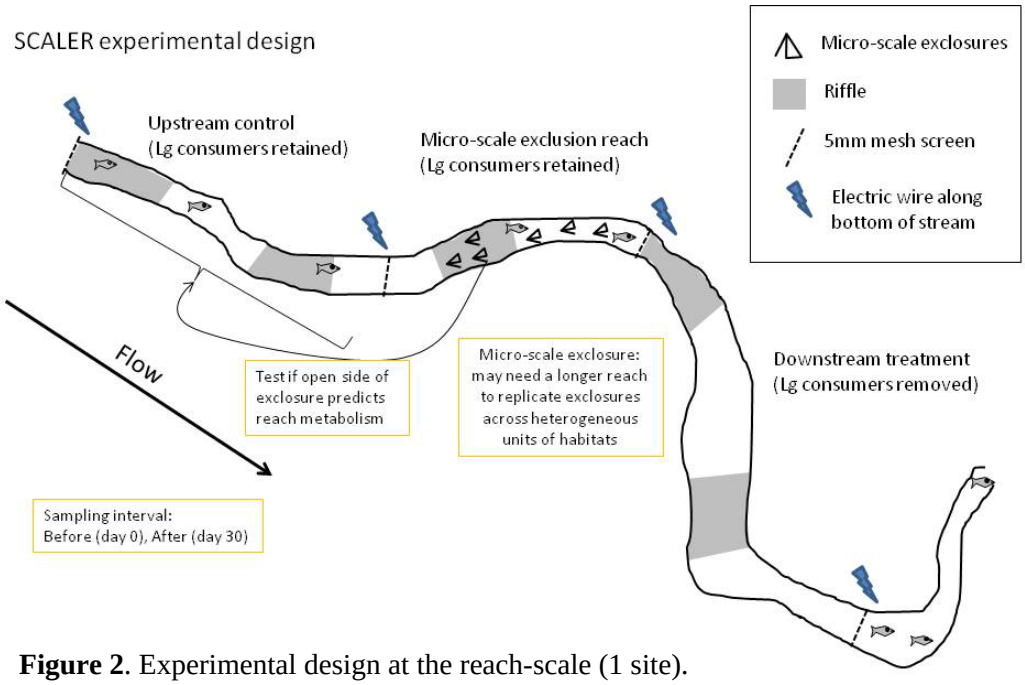

Figure 2. Experimental design at the reach-scale (1 site).

Metal fencing with biome-specific mesh size (Table 5) was used to delineate reaches, and exclude macro-consumers such as fish, crayfish, shrimp, and/or salamanders from the downstream treatment reach. Note, macro-consumers are defined based on mesh size, and mesh size was chosen to exclude the dominant large animal(s) in each biome. Macro-consumers are not defined based on functional group. Mesh size was determined for each biome, but within a biome mesh size will remain constant at all experimental sites. Mesh screen was placed upstream and downstream of control and treatment reaches (Figure 2). Constricted locations were chosen to make it easier to install fences and to facilitate nutrient uptake measurements. At the designated upstream and downstream locations, a trench (the deeper the better) was dug across the stream. Rebar (steel concrete reinforcement bar) was hammered into the sediments at the downstream end of the trench, spaced every 30 cm unless strong flow requires more support and thus tighter spacing. A piece of fence was cut to stretch the channel and up the banks, and a 20 cm lip bent at a 90° angle (bending on land allows for a clear angle). The fencing was then placed across the stream, with the lip flush on the sediments inside the trench and facing upstream and the remaining vertical part of the fencing placed against, and upstream of, the rebar. The stream sediments removed during trench digging were then shoveled onto the lip to bury the fencing. Zip ties were used to attach the fencing to the rebar. Additionally, wire was added to connect the rebar and provide added stability to the fence if deemed necessary. A wire

with electric current (e.g., using a fence charger) was run near the stream bed to inhibit consumers from burrowing through gravel into the exclosure (if deemed necessary based on site and macro-consumer characteristics). If particulate organic matter transport and/or flow velocities were high, a second fence with a larger mesh size was installed across the stream (without the burial) upstream of the "reach fences" to help prevent fences from being blown out by spreading the pressure across multiple fences. If multiple fences were installed they were spaced far enough apart to allow for cleaning off of organic material. It was necessary that fences were cleaned (long-handled scrub brushes work well) often (daily at some sites).

In 2014, due to low consumer densities at all sites and challenges in maintaining exclusion fences, reach-scale consumer manipulations were not conducted. Rather, each biome conducted its own smaller scale experiments on consumer effects which are not detailed in this manual. The experimental sites then were treated similar to intensive synoptic sites, except for inclusion of the scaling aspect from habitat- to reach-scale (see section 2.4.5. for more details).

Table 5. Mesh size for macro-consumer exclusion at reach- and habitat-scales.

Biome ID	Biome description	Selected mesh size	Reasoning
LUQ	Luquillo LTER	6 mm	Consumers relatively large and high organic matter transport makes fence maintenance difficult
CWT	Coweeta Hydrological Station	6 mm	Particulate organic matter transport high, macroconsumers medium-sized
KNZ	Konza Prairie LTER	3 mm	Particulate organic matter transport and flow velocity very low, macro-consumers small
CPC	Caribou/Poker Creeks LTER	6 mm (12mm later in experiment)	Consumers relatively large and high organic matter transport makes fence maintenance difficult
ARC	Arctic LTER	6 mm (LDPE Plastic mesh)	Consumers relatively large and high organic matter transport and high discharge makes fence maintenance difficult
AND	HJ Andrews LTER	3 mm	Juvenile macroconsumer small
AUS	Darwin, Australia (Litchfield Nat Park)	10mm	Particulate organic matter transport high, macroconsumers relatively large

2.4.4. Habitat-scale exclusion

Material list

Exclosure wood frame: wood pieces (W×H×L 0.5×0.5×19 in.) screwed together in a square with a 20.5 in. crosspiece across the diagonal for stability. 8 exclosures per experimental site (N=3) for a total of 24 20.5" pieces and 96 19" pieces. Size and shape may need to be modified slightly for the narrowest streams, but 5 baskets still need to fit into each side of the exclosure
Staple gun and staples
Fencing (3ft high, ~50ft long per site, mesh size biome-dependent, see Table 5)
Wire clippers or heavy duty scissors
Zip ties (~1000)
Rebar (24 pieces 4ft long 1/2in diameter)
Mallet or sledgehammer
Shovel
Strawberry baskets (W ×L×H 10×10×6 cm), 80 per experimental site for patches (total 240)
Liners for strawberry baskets if holes are too big to hold relevant substrate (these should be synthetic fabric of some sort, could be, for example, mosquito netting, window screen, or tulle material), *see section 2.4.5.*
Relevant stream substrate (mixed in bucket/bin)
Bucket to collect and mix substrates

Done in advance:
Build exclosure frames and line strawberry baskets (if necessary)
Frame construction: Wood pieces were cut into four 19-in long sections and one 20.5-in piece. A hole was drilled in one end of each 19-in piece perpendicular to length of piece, approximately 0.25-in from the edge. The wood screws were used to fix pieces together to create a square frame by placing the screw in the drilled holes and then screwing in into the next piece so the screw was parallel to the new piece. Screwing was repeated until all four sides were connected. The 20.5-in piece was placed along the diagonal and fix in place using a staple gun at the four corners, both from the top and bottom (Figure 3a).
Basket lining: If the dominant sediments or organic matter content in a habitat were too small to be held within the baskets (<1 cm), the baskets needed to be lined with nylon netting. Netting was placed on the inside and fixed at the top using zip ties or held in place by the sediment itself. The point of the mesh was to mimic the natural subsurface flow rates, too fine and flow would have been impeded, too coarse and they would have washed out.

To be done during experimental setup:
Construct and install exclosures

Construction: Smaller-scale exclusion was accomplished with mesh fencing around the 19 x 19 in frame. A square piece of mesh was stapled to the bottom of the wood frame (Figure 3b)

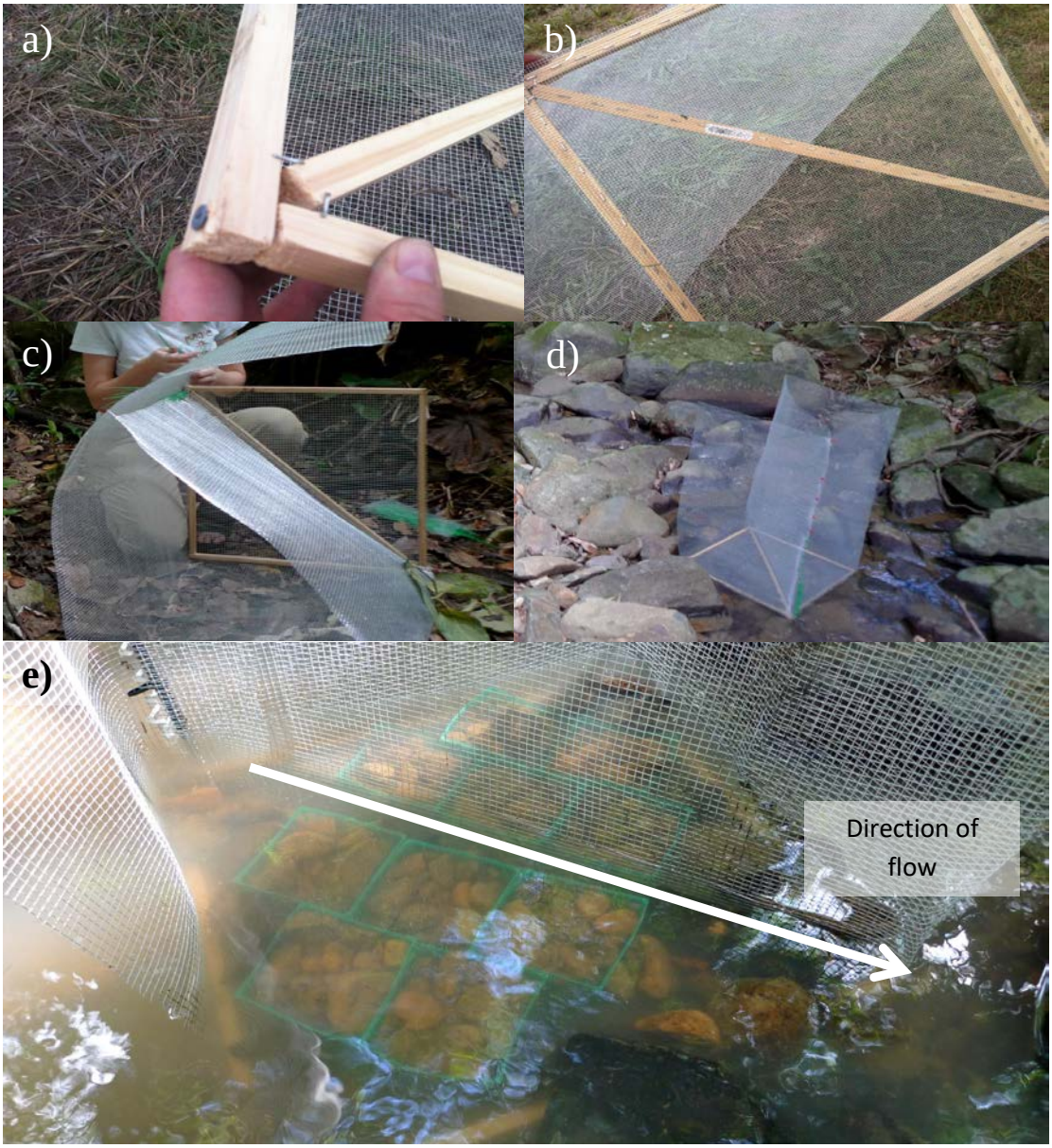

Figure 3. Construction and set up of patch exclosures: a) diagonal piece stapled to external square; b) mesh stapled to one side of square; c) Mesh stapled to side of diagonal and around three sides of the square and diagonal zip-tied to close off one side; d) finished exclosure; and e) exclosure embedded in sediment with strawberry baskets on both sides of the exclosure (flow is from left to right).

followed by a length of fence stapled along the diagonal and then three outsides. For ease of stapling, the frame was placed on a flat surface, then the first part of the fencing was stapled to the diagonal wood piece. Once finished, the fencing was bent at a 90° angle so it was standing perpendicular to the bottom frame. Then the fencing was bent around the outside of the frame and staple to two sides, meaning the fencing along the diagonal was reached. Leaving the last

side unstapled helped to have access to connect the diagonal piece to the one around the outside using zip ties (Figure 3c). Once the diagonal was zip tied to close off, the third side was stapled. To randomize the open side of the exclosure, the mesh was placed on alternate sides of the diagonal.

Installation: In the stream, a 0.5 x 0.5 m plot was shoveled approximately 10 cm deep with a corner pointing upstream (i.e., the diagonal crossbar was parallel to stream flow). The exclosure was then placed in the excavated plot. Half the downstream edge was closed by mesh, while the other downstream-half is open to create a non-exclosure control for a paired design with equivalent hydrologic alteration (developed by Murdock et al. 2010, Figure 3d). Five strawberry baskets filled with substrate collected from the stream were placed in both the enclosed and open sides of the patch frame (Figure 3e). The sediment from the excavation were then placed around the baskets until the top of the baskets and the sediments are level with the stream bottom outside of the exclosure.

2.4.5. Basket design and installation at habitat-scale exclosures and experimental reaches

Material list

Native substrates, collected from within the reach based on substrate sizes (detailed below)
Strawberry baskets, 80 for patch exclosures, 32 for sampling experimental reaches, per experimental site per year (2013) OR 50 per experimental site (2014)
Mesh lining (if deemed necessary)

Plastic baskets (W×L×H = 10×10×6 cm, henceforth strawberry baskets) were modified to use as experimental units at the habitat scale and at the reach scale. Substrate used in baskets and basket mesh sizes were determined from sediment size structure analyses following the rules below:

1. At sites with streambeds consisting mostly of sand (2 mm) or smaller particles, baskets were lined to have wall and bottom mesh openings of 1 mm and 0.5 mm, respectively.
2. At sites with streambeds consisting of granules and have fine pebbles, baskets will be lined with finer mesh to contain the finer sediments (approximately 20th and 10th percentile of the particle size distribution). The 200-sample pebble count (Bevenger and King 1995; see Table 2) from the pre-synoptic sampling was used to quantify the sediment composition to determine percentiles.
3. In reaches where streambed sediments consist mostly of pebbles and gravel, the maximum mesh opening size (for both walls and bottoms) was 10 mm. At such sites, rocks were picked that will fit into the baskets. As mentioned before, cobbles, boulders and bedrock could not be accounted for.

To determine placement of patch exclosures and substrates used in exclosures, the entire experimental reach site was surveyed using pebble counts and average width as per method for the pre-synoptic sampling (section 3.1.). In addition, it was noted if each transect was a riffle or a pool. **Note, a run might be considered a riffle or a pool, but it did not matter where that line was drawn within a biome as we were relatively consistent within that biome. In this section riffle/run is referred to as riffle from this point forward**. Thus, transects were

separated into riffles and pools. We were interested in scaling up from area-specific rates in baskets to whole-stream rates and we thus needed the relative riffle and pool areas. The total area in riffles A_r is calculated as:

A_r = **average** width (m) of riffle transects* length (m) between transects* number of riffle transects. (Equation 5)

The total area in pools A_p is calculated the same way. The proportion of area in riffles is calculated as:

$$\text{Proportion of Riffle} = \frac{Ar}{(Ar + Ap)} \quad \text{(Equation 6)}$$

Then the patch exclosures are apportioned relative to the areas of pools and riffles (Table 6).

Table 6. How to determine the total number of exclosures to be placed in each habitat type.

Proportion Riffle	Riffle habitat exclosures	Pool habitat exclosures
0 – 20%	0	8
20 – 40%	2	6
40 – 60%	4	4
60 – 80%	6	2
80 – 100%	8	0

Within each habitat type exclosure (riffle or pool), we needed to apportion the baskets within exclosures based on the relative abundance of substrate types. Sediments used to fill the baskets were collected in a bucket per substrate type (Table 4; boulders/bedrock will be excluded due to size constraints of baskets) and baskets within one exclosure contained only one substrate size for all five baskets. If possible, the exclosures were placed in areas containing the same native substrate that filled the baskets. If 80% of the substrate was of type 1 (S1) then all of the exclosure baskets were filled with S1 in that habitat. If S1 and S2 together make up >80% of the surface cover, then two main options presented themselves (both sizes are approximately equal [left], or one dominates [right]):

Table 7. Exclosure apportionment with two dominate substrate types (S1 and S2).

Patch Exclosures (of a specific habitat)	S1 ≈ S2 >>S3		S1 ≈ 2*S2 >>S3	
	S1	**S2**	**S1**	**S2**
2	1	1	1	1
4	2	2	3	1
6	3	3	4	2
8	4	4	2	3

No biome had three substrate types making up 80% (considering the limited classes, Table 4).

Each side (open or enclosed) of the patch exclosure has 5 baskets buried flush with substrata (n=80 baskets). Additionally, pairs of baskets were buried flush with the ambient substrata in

pools and riffles of the control and treatment reach (see Figure 2). Locations of the baskets and substrates in the control (n=8 pairs) and treatment reach (n=8 pairs) followed the same method as for the exclosures (replace exclosure with pair in Table 6). Baskets in the patch exclosures were used for habitat metabolism and NH_4 uptake in chambers (3 baskets per exclosure, section 3..8.1.). The remaining two baskets and the pairs of baskets in the control and treatment reaches are used for invertebrate sampling (1 basket; see section 3.8.4.) and chlorophyll *a* and benthic organic matter (1 basket; see sections 3.8.2.)

In 2014, due to low consumer densities at all sites across biomes, consumer manipulation experiments were changed and conducted according to site-specific criteria. To allow for scaling of rates, a minimum of 10 locations were selected per site and 5 baskets installed at each location to allow for all measurements described for patch scale exclosures. The locations were weighted again based on habitat and substrate type.

At sites where the installation and upkeep of baskets was difficult (e.g., high flow) alternative approaches were used such as sampling of *in situ* sediment placed in baskets for benthic rate measurements. Sediment types then reflected the *in situ* distribution and were sampled according to habitat types.

3. Field sampling methods

A full list of material needed for the entire SCALER project for one biome and year can be found under Appendix “Field material list (one biome, one year)”. Appendix “ SCALER sampling-measurement effort” provides a list of the different samples collected as well as an estimation of the load likely to be generated, while Appendix “Scaler sample hold times” details the samples produced in one biome over one year indicating the sample load produced and the timing needed for analyses (see section 4).

3.1. Physical characteristics for (pre)-experimental synoptic site survey

3.1.1. Site locations

Material list

GPS

Rangefinder/ Tape Measure

All sample sites were surveyed using a GPS or points identified on paper maps and sent to the modeling group to ensure accurate spatial location on the river network. Synoptic sites were assessed prior to the experimental manipulation in 2013, *if possible*. Initial synoptic site surveys consisted of reaches that were 1.5x the minimum-determined reach length of the appropriate experimental stream size, and represented by a minimum of 10 transects perpendicular to the flow direction of the thalweg and spaced evenly along the reach. This sampling was also conducted at each experimental site before the experiment started to determine placement of exclosures and baskets (see section 2.4.3.). Every transect was classified as riffle or pool habitat. Again, runs and riffles can be combined, and it was not as important how they were defined as

long as the definition remained consistent across sites within each biome. For each transect, width, depth, substrate and canopy cover were surveyed (see below for details). Slope, water quality and discharge were measured for the reach (also see below). If possible, triplicate ammonium samples were taken at each synoptic site to help guide calculation of ammonium addition rates for ammonium uptake experiments.

Database submission files: sitecharacteristics_reach, sitecharacteristics_synoptic.

3.1.2. Stream width and depth

Material list

Rangefinder / Tape Measure
Meter stick

Stream width and depth were surveyed along at least 10 evenly spaced transects of the reach at all synoptic sites as well as the experimental sites moving in a downstream to upstream direction. Width was measured using an electronic distance measure or a measuring tape. Water depth was measured at a minimum of 10 locations approximately evenly spaced across the stream channel using a meter stick and relative to water surface. Note, water depth is difficult to measure accurately in shallow streams and we thus used 0.5 cm accuracy. Average velocity, average width, and discharge can be used to more accurately calculate average depth for a reach.

Database submission files: width_reach, width_synoptic, depthsubstrate_reach, depthsubstrate_synoptic

3.1.3. Substrate size distribution

Material list

Gravelometer or Ruler

At all synoptic and experimental sites, substrates were surveyed at all transects of the reach. A minimum of 200 particles needed to be surveyed in a reach, meaning the number of substrates per transect depended on the number of transects chosen (min. 10). The median axis of each particle was determined using a gravelometer or a ruler. For gravelometer, the lower edge of the distribution was used so a particle of a specific size class was between the listed size class and then next larger class. Size classes larger than the gravelometer were measured, and bedrock denoted using text.We did note organic matter only if it was prevalent across sites within a biome, such as wood in the forested areas or macrophytes in slower moving streams as these could dominate the biological activity.

Database submission files: depthsubstrate_reach, depthsubstrate_synoptic

3.1.4. Water quality

Material list

Multiparameter sonde (use YSI ProODO for temperature)

At all 20 synoptic sites, general physical parameters were measured at the downstream end of the reach at each site (DO, water temperature, barometric pressure, pH, conductivity). All metrics were measured during the timeframe of other (experimental) measurements (e.g., water chemistry).

Database submission files: physchem_synoptic (data available via web link)

3.1.5. Canopy cover

Material list

Spherical densiometer
Compass

Canopy cover was surveyed at all experimental and synoptic sites. A spherical densiometer (http://www.cspforestry.com/Spherical_Crown_Densiometer_p/densiometer.htm) was used to estimate canopy cover in the middle of the stream channel at each transect along the reach. The protocol was printed on the densiometer, and as follows: Measurements were taken in each cardinal direction (i.e., facing north, east, south, and west) or relative to flow (i.e., direction of channel from upstream to downstream at the point of measurement. Holding the densiometer at the same height and distance from body, the body moved around when changing positions. To take readings, the instrument was held level, 12" to 18" in front of the body and at elbow height. The bubble level was centered. Then, four equi-spaced dots were assumed in each square of the grid and all dots equivalent to open quarter-squares systematically counted. All sites followed the protocol regardless of species height and composition, but we acknowledge that at sites with low shrubs (<1 m) there may be an underestimation of cover. Percent cover was calculated according to the densiometer's manual: The count of open quarter-squares was multiplied by 104 to obtain percent of overhead area not occupied by canopy. The difference to a hundred was an estimation of percent overstory canopy.

Database submission files: canopy_reach, canopy_synoptic.

3.1.6. Discharge

Material list

Pulse release method

Salt or dye solution
Conductivity meter or fluorometer
Sample bottles (at least 20)

Plateau method
Metering pump and tubing
Battery
Graduated cylinder
Stopwatch
Salt or dye solution (can be mixed quantitatively in field with stream water)
Conductivity meter or fluorometer
Sample bottles (at least 7)

Flow meter method
Measuring tape
Flow meter

Discharge was measured with inert solute releases or velocity meters. For shallow streams or streams with highly variable substrata, inert solute releases were preferred. Details for all three methods below, though the flow meter method was used rarely.

Generally, pulse and plateau methods take the same amount of time, because a plateau is reached in the same amount of time as it takes the pulse to completely travel past a downstream point. The pulse method does not require a metered pump and battery, uses less mass of solute and the release solution does not need to be mixed volumetrically. *Sample burden is much greater with the pulse method as samples need to be taken and analyzed for every part of the pulse to accurately calculate the discharge.* However, if a conversion regression between specific conductance and chloride concentration is measured, field measurements with conductivity meters are relatively easy to obtain. Such a regression between specific conductance and chloride concentration can be developed by measuring the specific conductance (µs cm^{-1}) of an aliquot of stream water while adding increasing increments of pre-weighed NaCl (g). If the volume of the aliquot (contained in a bucket, for example) is known then the specific conductance is known across a range of Cl concentrations (g Cl L^{-1}). The slope and intercept of this relationship can then be applied to specific conductance data from the inert solute release.

Detailed discussion of both methods can be found at http://pubs.usgs.gov/twri/twri3-a16/pdf/TWRI_3-A16.pdf (Measurement of Discharge Using Tracers U.S. Geological Survey, Techniques of Water-Resources Investigations, Book 3, Chapter A16 By Frederick A. Kilpatrick and Ernest D. Cobb). This source was used as the method reference. In using inert solute releases to calculate streamflow discharge, we made two assumptions:

1. All of the injected NaCl mass is recovered at the downstream sampling point
2. The NaCl tracer is completely mixed across the channel at the downstream sampling point.

The appropriate reach length for measuring discharge with inert solute releases was chosen to accommodate these assumptions. Typically, the minimum reach length required to achieve

complete lateral mixing of the tracer was used (or approximated as 10-15x the channel width) to avoid loss of tracer by flow paths that bypass the downstream sampling point, which is more likely to occur with longer reach lengths.

As each stream and probe read slightly different a standard rating curve for specific conductance and NaCl concentration was created before or after each release. Specific conductance was logged at ≤5 second intervals at the downstream sampling point. This scale of temporal resolution was required to characterize the breakthrough curve (BTC) of the salt pulse. Once specific conductance was logging, the pre-weighed mass of tracer NaCl (determined as the mass required to raise background specific conductance by approximately 50-100%) was mixed with stream water in a bucket at the upstream sampling point until all NaCl was dissolved. The NaCl mass was recorded, and the NaCl-stream water injectate poured across the width of the stream in a smooth, rapid motion. Once the stream specific conductance at the downstream sampling point returned to ambient conditions, the data logger was removed and the breakthrough curve analyzed. For travel time, the release time, length between release and sampling point as well as the time of the peak of the pulse was noted.

Discharge was approximated by integrating the area under the background-corrected Cl concentration breakthrough curve:

$$Q\ (L\ s^{-1}) = \frac{Mass\ of\ injected\ Cl\ (g)}{\int_0^t Cl\ concentration\ (\frac{g}{L}) \times Time\ (seconds)} \qquad \text{Equation 7}$$

For measuring discharge using the plateau method, see the section on aeration (3.4.3.). Discharge was calculated as follows:

$$Q\ (L\ s^{-1}) = \frac{concentration\ of\ injectate\ solution}{concentration\ at\ plateau} * \frac{pump\ rate\ (min^{-1})}{60\ (s\ min^{-1})} \qquad \text{Equation 8}$$

For the flow meter method, discharge was measured at a constrained location of the stream using a flow meter. At a minimum of 15 points across the channel, flow velocity was measured at 0.6x depth, and flow, distance across the channel, and depth recorded. Discharge was then calculated as,

$$Q\ (L\ s^{-1}) = \sum_{i=1}^{n} d_i * w_i * v_i\ , \qquad \text{Equation 9}$$

where d is the depth of a measurement spot, w is the width of that segment (i.e., the distance from halfway between the previous and next measurement spots), and v is the velocity at a measurement spot.

Database submission files: SlugDischarge_reach, SlugDischarge_synoptic, SF6plateau_reach, SF6plateau_synoptic, Discharge_reach, Discharge_synoptic

3.1.7. Slope

Material list

Clinometer, hand level and pocket rod, or cell phone geo application (e.g., GeoCam)
Auto-level (24x) & Tripod

Slope was measured using a clinometer from the upstream to downstream end of a reach. One person stood at the upstream end with the other person at the downstream end. The clinometer was then aimed from one person to the other at the same height. Alternatively, especially when line of site was obscured, shorter distances were done and then converted to an overall slope. If available, a handheld level or auto-level and a stadia rod were used with standard surveying techniques. Note: most sites did not have accurate enough GIS and LIDAR to get good slopes over the length of experimental or synoptic reaches but measured slopes will be compared to GIS-generated slopes to assess the accuracy of modeled slopes and, if accurate, slopes from GIS were used.

Database submission files: sitecharacteristics_reach, sitecharacteristics_synoptic

3.2. Consumer survey at experimental reaches and intensive synoptic sites

Material list

Notebook, pencil
Backpack electroshocker
Dip nets
Buckets
Aerators/bubblers
Measuring boards
Seine
Minnow traps and bait
Waders
Nalgene bottles (for voucher specimen)
Formalin
Label paper

Consumer surveys were conducted in control and treatment reaches of experimental sites as well as in the patch reach and the intensive synoptic sites, if feasible. Appropriate sampling method(s) were selected for each biome and reach site. The goal was to remove as many consumers (species and individuals) as possible, and each biome needed to determine the best methods to achieve this goal. The use of multiple types of gear (e.g., seines and electrofishing) was often the most effective way of removing different species. Independent of gear, control and treatment reaches were sampled in multiple passes for a depletion sampling. A minimum of three passes with diminishing catch were conducted to allow for population estimates though more passes

were preferable as the first pass was often not useable for depletion calculation. For each pass, individuals were identified and lengths measured to use with published weight-length relationships (e.g., nose/rostrum to fork/fin or carapace length depending on species and how published relationships were developed). The reach containing the patch exclosures was walked through the same number of passes as exclosure reaches to replicate disturbance. Selection of holding containers was made to minimize consumer mortality (e.g., flow through containers or frequent water exchange) in control reach surveys while individuals from treatment reaches were released below the reach after measurements as the reaches were meant to be depleted in consumer biomass while individuals in the control reach needed to be returned to the same reach.

The treatment reaches were sampled at the beginning and end of the experiment. Additional passes at approximately day 11 and 22 of the consumer removal experiment were used to test for exclusion of large consumers as well as removal of consumers that had outgrown the selected mesh size of the fence. As patch exclosures were disturbed during installation there should be no consumers in the closed side, but a quick check (e.g., with an electroshocker) may be warranted. Consumers in the removal reach were released below the downstream fence (to avoid inflating the number of consumers in the middle reach where the small exclosures are) or above the top most fence if consumers had an upstream migratory behavior. The control reaches were sampled at the beginning and end of the experiment also, but individuals returned into the reach following the final survey pass. During day 11 and 22 the same disturbance from the treatment reach were imparted on the control and patch reaches.

Intensive synoptic sites were surveyed for macroconsumers using a single pass to gain some understanding of the diversity and abundance of macroconsumers in other parts of the network. These surveys were not done at all sites.

Species were given a specific identification code (e.g., three letters of genus and species), which was listed in the species information file, including type of consumer (e.g., fish, crustacean), its general trophic position (e.g., herbivore, omnivore),family, genus, species, common name, as well as parameter for length to weight conversions and the citations for the origin of such conversions.

Copies of field notes from consumer exclusion were included in a zip file with this manual and should include: sampling gear and methods used to collect specimens, collection permit information, and other information collected at the time specimens were captured (e.g., weather, water quality). Voucher specimens representing large excluded consumer species were not collected during this project due to IACUC and site-specific constraints, but may be available as part of previous collections at each LTER site.

Database submission files: consumer_reach, consumer_synoptic, Macroconsumer_species

3.3. Water chemistry sampling

Material list

Sample bottles, labeled (~110 125 mL acid washed Nalgene bottles, per biome per year; note minimum requirement for acid washing is 1 h soak in 0.1 molar HCl followed by rinsing with DI water)
Syringe and filter apparatus
Filters (GFF from Zefon, Whatmann or similar)
Forceps
Cooler with ice

Water samples were collected at the bottom of each experimental reach, at least at the end of the experiment. In 2013, when consumers were manipulated, water chemistry was sampled at the beginning and end of the experiment and at the top and bottom of each experimental reach. At each location, 125 mL of filtered and unfiltered stream water each were collected and placed in the cooler and frozen immediately upon return to the laboratory. A known volume of water was filtered in the field for particulates (or unfiltered water collected to be filtered in the lab) and filters saved for particulate analyses. Additionally, water chemistry samples were collected once at the bottom of the reach at all 20 synoptic sites during the experimental period.

Database submission files: waterchem_reach, waterchem_synoptic

3.4. Metabolism

3.4.1. Calibration of sensors for reach and chamber measurements

PAR sensors

- Initially, PAR sensors were all compared to a standard probe calibrated at NEON (National Ecological Observatory Network in Boulder, CO). This was done at the beginning of each year at Kansas State University (KSU) or NEON. A calibration coefficient of the Odyssey Irradiance measures to PAR was calculated based on the defined 10 min logging intervals. As Odyssey probes measure irradiance by summing intensity over the course of the set interval, coefficients needed to be adjusted for different intervals. However, as the relationship was linear, coefficients were converted using the following formula: coefficient * sampling interval (min)/10 min.
  Database submission file: CalibrateLight, LightSlopes
- Prior to work at each site, all sensors were run simultaneously over the course of a day to include the full range of light conditions. Absolute readings differed but the run allowed to check for differences in patterns should a sensor have been damaged in transit. If readings varied, the malfunctioning probe was returned for immediate re-calibration or repair to the manufacturer (Odyssey Data Recording Systems; www.odysseeydatarecording.com).
  Database submission file: CalibrateLightSite

Oxygen probes

- Prior to work at each site, all probes were run simultaneously over the course of a minimum of 2 hours to test for issues due to shipping. The below listed calibrations were considered. If readings varied, the malfunctioning probe was returned for immediate re-calibration or repair to the manufacturer (YSI Systems; www.ysi.com).
    - Temperature sensors on the oxygen meters were calibrated against a known standardized thermometer at two points at each site. An ice water bath and room temperature were standards (http://www.astm.org/Standards/temperature-measurement-standards.html). Fisherbrand* Red-Spirit* General-Purpose Laboratory Thermometers meet or exceed NIST* and ANSI*/SAMA tolerances for accuracy15-041-5A and do not have problems with mercury contamination if broken. Comparing probes against each other was a secondary check as 20 probes were used. If a probe was not at the correct temperature, the divergence was noted. If it was more than 0.2 ^{0}C off, the probe was swapped with a meter that was working to determine if the probe or the meter were malfunctioning. Malfunctioning meter or probe were immediately sent in for repair.
      Database submission file: CalibrateTempSite
    - Atmospheric pressure values were calibrated against the closest active weather station. If no weather station was available, an average reading from all 20 probes was used as a substitute and all meters set to that standardized reading by entering into the probes directly. The probes were then run simultaneously to ensure that the barometric pressure readings were similar across all probes.
      Database submission files: CalibrateBaroSite
- Dissolved oxygen was calibrated for each use and thus a bit different. It was absolutely essential that O_2 probes be calibrated and ensured to all be stable. Each day before deployment, all meters were calibrated together in the field (if this was not possible or outdoor temperatures were above 30°C, then calibrated in the lab immediately before traveling to the field site(s)). First, it was ensured that all the barometric readings were the same, and set to be the same if not. The sensors were then calibrated in moist air (plastic cover with a wet sponge). Probes needed to be stabilized for temperature and read the same temperature across probes for this step. It was best to place all probes and meters in the shade for these steps. After water-saturated-air calibration, all meters were placed in the stream or in a bucket with stream water and constant mixing from an air pump (the bucket is preferred as we cannot be certain to have all probes in one place in a stream with exactly the same O_2; if aeration in the bucket is not possible a bucket can still be used as LDO technology does not consume oxygen as membrane-based probes do) and allowed to log for a minimum of 30 minutes at 5-minute intervals. Temperature was attempted to be kept constant by placing all probes in the same location in flowing water or in a bucket that was shaded and placed in flowing water. O_2 reading were compared

across meters and calibration repeated until all meters gave the same results (within 0.5 mg/L of each other) before deployment. All meters to be deployed at one experimental reach site (n=4) and for synoptic sites (n=6 in 2013 or n=12 in 2014) were calibrated as a group each time they were used. At the end of deployment, meters were again placed together at one location (or in an aerated bucket if necessary) for a minimum of 30 minutes, logging at 5-minute intervals. This allowed for correction assuming a linear drift in calibration over the period of measurement if meters did not read the same value post-deployment. However, such meters were not to be used again until stability of the meter was determined. To trouble shoot drifting probes, probes were switched to a meter known to be working, as well as a working probe with the problem meter. Thus, the problem could be isolated. The problem meter or probe was immediately sent for repair to have equipment ready for the next set of measurements. This meant contacting YSI repair and Fedex-ing the equipment to them.

3.4.2. Whole-stream metabolism at experimental and synoptic sites

Material list

Mallet
Bull's eye level
Zip ties (minimum four times the numbers of rebar, see below)
Experimental sites:
4 4ft ½ in rebar (2013; only 2 in 2014)
4 YSI ProODO meters with extended battery compartment (2013; only 2 in 2014) (Hydrolab sondes for AUS)
4 Odyssey irradiance meters (2013; only 2 in 2014)
Intensive synoptic sites:
6 4ft, ½ in rebar (12 in 2014)
6 YSI ProODO meters with extended battery compartment (12 in 2014)
6 Odyssey irradiance meters (12 in 2014)

Whole-stream metabolism at the *experimental sites* was measured using a two station method at the beginning and end of the exclosure experiment for at least a few days (up to a week). Dissolved oxygen (DO) probes and PAR meters were placed below the upstream and above the downstream exclosure fences of both the control and the treatment reach outside of potential influence from the exclosure fences. Whole-stream metabolism at *intensive synoptic sites* was measured using a single station method in 2013 and a two-station method in 2014 to better constrain reaches. Dissolved oxygen (DO) probes and PAR meter were placed at the top (2014 only) and bottom of the surveyed, intensive synoptic reaches. All 6 intensive synoptic sites were measured on the same dates during the experimental window, for a minimum of 36 hours but preferably longer. The DO and PAR meters were calibrated by methods detailed above (3.4.1.), especially pre- and post-calibration runs of DO to establish drift. A rebar was hammered into the

sediment and the PAR meter zip tied to the top and adjusted with a bullseye level. The DO meter was zip tied to be parallel to stream flow and in the water column without touching the sediment.

The PAR meters needed to be initiated with a computer before departing for the field and the location was noted with corresponding logger number once placed at a site. The DO meters were programmed in the field and carried the designations specified in section 6.1. Both loggers were set to log at 10 minute intervals (staring on a 10 minute to have light and DO measurements synced, e.g., XX:20h) and remain in the field for a minimum of 36 hours but preferably for a few days. This allowed comparison across reaches. The exact times of probe placements were recorded to establish which data needed to be discarded from PAR and DO probes as they were started before placement in the stream.

Aeration rates and travel times were determined during the deployment using SF6 tracer releases (see section 3.4.3.). If timing did not allow for aeration measurements during the measurement period, multiple measurements at different discharges were used to estimate aeration retroactively across the network or at minimum provide enough data to constrain modeled aeration rates. Widths were measured at a minimum of 10 evenly spaced transects at the end of the plateau tracer addition or in junction with whole-stream nutrient uptake (section 3.5.). Downloaded data was separated into metabolism data and calibration runs for oxygen sensors

Database submission files: DO_synoptic, DO_reach, DO_synoptic_cal, DO_reach_cal, Light_synoptic, Light_reach, widths_synoptic, widths_reach

3.4.3. Aeration

Material list

Plateau method

Injection pump (battery operated, capable of constant mL/min delivery for at least 5 hours)
Battery
Tubing
Stopwatch
Graduated cylinder, 20 to 250 ml
Thermometer
Meter tape, 50 to 100 m; alternatively, an electronic distance measure unit or range finder
Injection carboy (40 L) or gas bag (5L), with graduation marks to monitor fluid level
Injection solution (pre-measured amounts of conservative tracers (NaCl, NaBr or rhodamine)
Conductivity meter, ion-specific probe, or fluorometer
SF_6 gas

For water sampling (gas extraction in lab):

40mL glass scintillation vial (10 per plateau); ChemGlass: Item number—CG-4909-05

For gas sampling (gas extraction in the field):

30 12mL pre-evacuated Exetainers (838W)
3 140mL Piston Syringes (Covidien Monject™ Item Number 22-257-152)
1 60mL Piston Syringe (BD™ Item Number 13-6879-8)

25mm GFF Filter and Holder
40 50mL Conical Centrifuge Tube (Falcon™ Item Number 14-432-22)
3 BD 305109 27G Luer Lock Syringe Sharps
3 Two-Way Luer-Lock Stop Cocks

Pulse method
Timing pulse and solution prep:
Salt
Bucket or large trashcan (depending on volume of solution needed)
2 conductivity probes
Release:
Multiple airtight solution bags fitted with tubing and stopcock ranging in volume from 10 to 80 L
Funnel
SF_6 gas
Sampling:
88 vacutainers/exetainers –pre-labeled (per site)
4 BD vacutainer needle holders
2 test tube racks for organizing vacutainers/exetainers
2 conductivity probes
Stopwatch
Discharge slugs
Meter tape

Done in advance:
Plateau release: Conservative tracer was added to stream water (distilled if stream water not available), and the solution then filled into the gasbag. As much air as possible was squeezed from the bag. The solubility of SF_6 is 0.007 vol/vol at 20ºC and 1 bar. 40 mL of SF_6 gas per liter of release solution was added for a saturated SF_6 solution. The solution was then left to equilibrate over night before injection to ensure enough SF_6 dissolved for a saturated solution.
Pulse release: One day prior to reaeration measurement, a timing pulse release was conducted along the metabolism reach. One conductivity probe was placed at the location of the upstream DO probe/sampling location and the second conductivity probe at the downstream DO probe/sampling location. The timing pulse solution was released at a distance upstream that was approximately equal to the metabolism reach length. Thus, the breakthrough curve was allowed to spread out enough at the top station to allow sampling of the breakthrough curve at two stations. The top station needed to be far enough downstream from the release point to allow ~8-10 samples to be collected on the rising limb at a minimum of 15 second intervals. If timing indicates that this was not possible, reach length should be increased.

The SF_6 solution was prepared the day prior to release either in the lab or field depending on the volume needed. The salt solution was mixed in a bucket and then transfered to bag using a funnel. As much air as possible was removed from the bag. SF_6 was injected using 60 mL

syringes into the stopcock attached to the bag's tubing. A minimum of 70 mL SF_6 per 10L solution was needed. Salt to be added was calculated based on a target Cl concentration of 10-15 mg L^{-1} at the downstream station. Mass of NaCl needed to achieve the target Cl concentration was calculated based on the timing pulse. For larger sites, the reaeration solution was prepared in the field on the same day of the timing pulse, and NaCl mass to add calculated based on the mass added for a previous site and discharge difference between the two sites. A minimum of 3 hours is needed to give SF_6 enough time to saturation the solution, but overnight is preferable.

Done before release and release:

Plateau Release: We used a plateau release for aeration because of concerns about SF_6 sample load with a pulse mass method, concerns about analyzing SF_6 concentrations in concentrated stock solutions, inability to rapidly sample for SF_6 at the top pulse station, and current lack of computational methods to measure aeration with two pulse stations. However, sites with high discharge needed to use a pulse method as the amount of release solution necessary became too large to transport and handle. If that was the case, a method comparison at a site small enough to allow for the plateau method was warranted.

To accomplish the plateau, a continuous release of dye or tracer solution amended with SF_6 was released at the top of the reach (for experimental sites, lower treatment reach first due to downstream flow, see section 2.4.3.). The release point was chosen far enough above the start of the reach (i.e., fence placement) to allow complete horizontal mixing. The top of a reach should already be at a constricted site for experimental reaches which will facilitate good mixing.

When it was time to start the release, the release solution was first sampled and saved for later analysis of solute concentrations (stock solution concentration was necessary for calculating discharge). At this time, a background water chemistry sample was also taken. The gas bag was carefully opened, as much of the SF_6 as possible pressed out, and the tubing attached allowing as little headspace in the bag as possible. The tubing was then attached to the pump and the rest of the headspace pumped out. Once the tubing was filled with release solution, pump rate was measured. The tube was placed in the thalweg of the stream at approximately half the total depth of the water column of the selected release point (i.e., make sure the tube wasn`t pumping into the substrate). The start time of the release and the pump rate were recorded.

The conductivity/ion concentration/fluorescence was monitored at the most downstream site. Measurements were noted or logged at an appropriate frequency (5-10 seconds logging frequency or adjust according to the actual velocity). The travel time to that station was when the concentration had reached half the plateau concentration, so frequent sampling was needed along the rising limb. When the concentration stopped increasing substantially (less than 1% change per minute) at the bottom station, sampling for gas and solute started. Note that solute concentrations will continue to rise, even for days, as the deeper subsurface zones gradually come to equilibrium with the water column concentrations, but we will ignore these very slow processes. The stream with the added solutes was sampled in the thalweg from the bottom station, the estimated end point of the reach, up towards the top. As we were most interested in

the aeration across the entire reach, sampling for gas and inert solutes was concentrated along the continuum. Using a meter tape along the reach, about 8 locations were sampled along the reach, as evenly spaced as possible. The meter marking for reach location was recorded, and the stream water carefully sampled (gas will evade if the surface is disturbed). See below for sampling protocols.

Once all the samples were taken, the pump rate was again measured. Stream water was pumped to rinse out the solution before the pump was turned off. Also, width was measured at a minimum of 10 evenly spaced transects. The temperature at the top and bottom of the reach as well as time were noted.

Pulse release: Prior to releasing of the SF_6 solution, extra SF_6 headspace was removed from the solution bag using a syringe. The SF_6 solution the released as instantaneously and as close to the stream surface as possible. The SF_6 and Cl breakthrough curve were sampled at two stations. One person sampled the breakthrough curve at the downstream station, and the other person released the SF_6 solution and ran to the upstream station before the breakthrough curve arrived. Sampling times were predetermined based on the shape of the timing pulse breakthrough curve at each station. Samples were collected along the breakthrough curve at 20 time points, with 8-10 samples occurring on the rising limb and the remainder spread out along the falling limb with the first and final samples collected at background conductivity. For each time point, two replicate vacutainer samples were collected. Thus 40 vacutainers were needed at each sampling station.

Discharge was measured using the pulse method at the upstream and downstream ends of the reach. Widths were measured at a minimum of 10 transects along the reach.

Sample collection:

Plateau release: Collecting gas samples: Before the solute concentration at the downstream monitoring point reaches plateau, 7-10 sampling sites were chosen along the longitudinal profile of the stream. Sampling stations were evenly spaced at GPSed/metered transects from the release to the monitoring site. At each sampling station, the necessary bottles were were placed which included 3 sets of 12 mL Vacutainers encapsulated in a 50mL Falcon™ tube and an additional 50mL Falcon Tube for conservative solute sampling or one 40mL scint vial.

Once the conservative solute concentrations at the most downstream location had reached plateau, sampling began at the most downstream point and proceeded in the upstream direction. Triplicate field gas samples were collected simultaneously at each sampling site using 60 or 140 mL syringes as follows: 1) A syringe was affixed with a the two-way stop cock in the “on” position; 2) The piston plunger is pulled out of the syringe and the syringe and the plunger triple rinsed in the flowing stream water; 3) the syringe body was filled with stream water and the plunger carefully placed back into the syringe body; 4) the stop-cock was turned to the “off” position before removing it from the water and the syringe inverted to check for air bubbles; 5) the stop-cock was turned back to the “on” position and water slowly expelled until there was only 40 or 100 mL of bubble free volume remaining in the syringe; 6) once the desired water volume was reached, 20 or 40 mL of air was drawn in, bringing the full 60 or 140 mL syringe to

a 40:20 mL or 100:40 mL water to air ratio and the stop-cock turned back to the "off" position; 7) after each syringe was filled, gases were equilibrated for 3 minutes by rigorously shaking the syringes underwater (to maintain stream water temperature) and 15mL of air injected into the 12 mL Exetainer (overpressurizing by 3mL); 8) the Exetainer was then placed in a 50mL Falcon™ tube and fill with water for storage (zero-headspace), and 9) a surface water sample taken with 50mL Falcon™ tube if conservative tracer concentration was determined from grabbed sample.
Collecting water samples: When plateau was reached, sampling began. The glass vials were filled under water by inserting the vial carefully as to not degas the sample during collection, and closed without any gas bubble inside the vial. The sample provided both the gas as well as the conservative tracer solution which allowed for calculation of discharge at each of the sampling stations as well as correcting the SF_6 samples for dilution. The dilution also provided important information for metabolism.
Pulse release: Before release of the SF_6 solution, two replicate background samples were collected at 4 stations along the metabolism reach. Samples were collected in pre-evacuated vacutainers by holding needle holders and vacutainers underwater and piercing the septum for ~2 seconds allowing the vacutainer to fill with water to ~50% capacity. Exact volume of water was calculated later by subtracting the mass of empty vacutainers from the mass of the sample vacutainers. Samples were refrigerated until analysis.

Database submission files: SF6plateau_reach and SF6plateau_synoptic, SF6pulse_reach and SF6pulse_synoptic (data available via web link), widths_reach, widths_synoptic, traveltime_reach, traveltime_synoptic

3.5. Whole-stream nutrient uptake

Material list
Meter tape or range finder
Open-topped bucket/garbage can (note: the injection volume may depend on how much NaCl/NaBr is needed; a larger carboy/release container will allow more NaCl or NaBr to be dissolved)
Injection solution (i.e., appropriate amounts of NH_4Cl and conservative tracer – Cl or Br)
Filter apparatus (GFF, Zefon type filters)
Sampling bottles (~30 60mL acid washed Nalgene per pulse, pre-labeled, minimum acid washing requirement, 1 h soak in 0.1 molar HCl followed by at least 6 DI rinses; sample bottles should also be rinsed with a small amount of sample water at least once before filling)
Conductivity meter or Bromide meter
GPS
Notebook
Optional: Sampling bottles (250 mL) for collecting unfiltered samples in the field with same label numbers as sample bottles for later filtering
Salt slug (for discharge calculation; see section 3.1.6.)

The NH_4 pulse (slug) addition experiment consisted of an instantaneous addition of NH_4 (NH_4Cl) together with a conservative tracer (e.g. NaCl or NaBr) and sampling of water at various times during the "breakthrough" of the pulse at one location downstream (based the TASCC method; Covino et al. 2011). The idea was to capture the entire concentration-time curve for both tracers at the sampling site. The injection solution consists of stream water and the conservative and active tracers.

Done in advance:
The masses of solutes needed was carefully calculated, based on a recent discharge measurement (it was ideal to measure discharge the morning of the experiment, but acceptable to have measured discharge the day before so long as flow did not receive new inputs, if the SF_6/ discharge/ travel time measurement was done the day before as planned this gave discharge). All solutes were weighed out to 0.1% accuracy and store them in labeled Ziploc bags or bottles/vials. It was critical to know the exact mass added, but it did not need to be the exact mass targeted in the prior calculations. The masses of all solutes were noted in the field note book.

An upstream injection site was identified. This site was well-mixed (best at a constriction and engineered to push most flow through a narrow area). When the nutrient addition experiment occured at a site, the injection location was upstream of the experimental reach's top fence for experimental reaches or at or upstream of reaches for intensive synoptic sites. **For experimental sites, we put the release site a meter or two above the upper fence** (Figure 2). While this did not allow for complete lateral mixing at the very top of the experimental reach, it obviated the need for two pulse sampling stations. The lower sampling station was also at a constriction. **For the intensive synoptic sites, sampling sites were selected based on the previously stablished reaches.** Once the stations were located, the injection location and the sampling station were GPS located or at minimum the distance between the two points was measured. Results were noted in the field note book.

During the experiment:
The dry solute masses was mixed into the specified volume of stream water in the bucket or garbage can. Wash solute residue from the bags into the container. Generally, we used containers with a completely open top to allow a truly "instantaneous" injection of the solute solution. High concentrations of salt took a substantial amount of time to dissolve dissolved properly; mechanical shaking/stirring of the solution was used to speed this process. A small aliquot of the injection solute was set aside for later analysis. This sample needed to be diluted prior to analysis, so a scintillation vial (available volume for analysis) sufficed. It was important to keep this highly-concentrated stock solution separated from other samples to prevent contamination. Alternatively, we used the specific amount of water to dissolve the dry solutes to calculate the stock solution. Thus, we recorded the volume of water in the notebook. Triplicate background

samples (dissolved nutrients and conductivity) were collected at the sampling station prior to starting the experiment.

A conductivity (or bromide) meter was installed at the sampling station in order to monitor the arrival and change of the added solute pulse to ensure they are in the water column and to follow the break-through curve (BTC). Any offsets of the conductivity meter's time and the recorder's watch was noted. If a sonde or HOBO conductivity logger was available, conductivity was logged at short intervals (as short as 1 s, no more than 1 min) for later analysis/interpretation. It was likely (especially for smaller streams) that the breakthrough curve for a pulse addition would happen too quickly (sampling every 30s – 1 min) to allow for filtration in the field. In this case, it was better to use a different set of field sampling bottles (e.g., wide-mouth, 250 mL Nalgene HDPE bottles). Bottles were numbered sequentially and the bottle number recorded in the field notebook with both the exact time and conductivity (note time in 24-h format). It was easiest to fill the temporary field sample bottles in numerical sequence from *1* to *n*. These temporary field bottles were filtered in the field after the BTC has passed, or returned to the lab to filter into final sample bottles (60 mL). The method described below assumed that temporary field sample bottles were used, but if there was sufficient time for filtering in the field during the pulse release (i.e., one person pulls up stream water in syringe while the other person filters water into bottles) it was preferable.

The well-mixed, completely-dissolved solution was poured into the stream, in one very quick motion, at the pre-determined mixing site. However, care was taken to not pour the solution so rapidly that tracer was forced into interstitial spaces in sediments. Also, while most of the solution was poured into the thalweg, we attempted to roughly apportion the rest into the less rapidly flowing regions (i.e., mixed across the stream with the bulk of the solution going into the thalweg). The release container was the quickly rinsed with stream water back into the stream. **The release time was recorded in the field notebook**. At the sampling site, the conductivity using the conductivity meter/bromide probe was monitored, noting the rising limb, peak, and falling limb of the BTC.

In all cases, samples were taken in a well-mixed area (thalweg) facing upstream or standing out of the channel. In fact, on the day of nutrient measurements, as little disturbance as possible to the streambed was critical. In general, at least two people were assigned to the station, with one taking samples and the other recording time, conductivity, and supplying sample bottles.

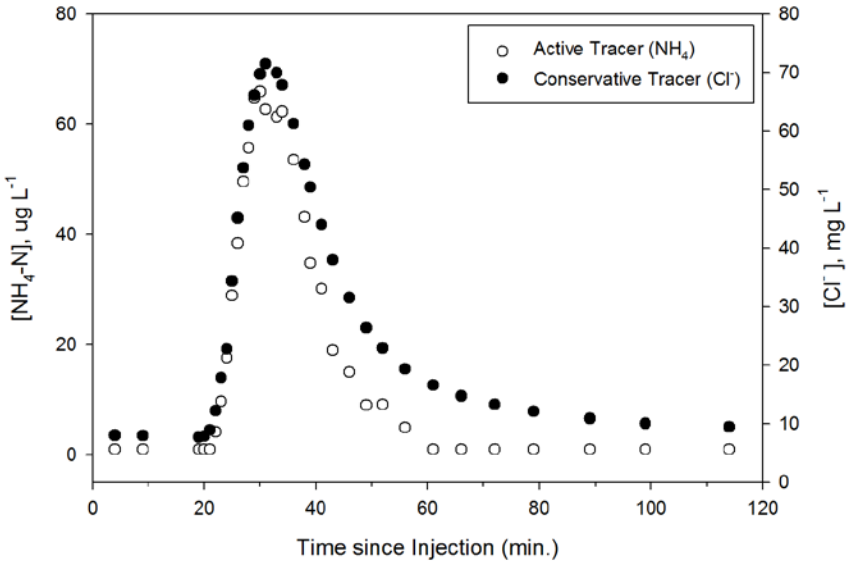

Figure 4. Example breakthrough curve (BTC) based on real data.

More samples over time were better than replicate samples at any individual time, statistically. Samples needed to be taken most quickly when concentration was changing rapidly, so sampling happened very quickly when the pulse reached the sampling station as it often rose to peak rapidly. The real-time data provided in Figure 4, adjusted to each stream's travel time, helped to guide the collection of grab samples to ensure characterization of the entire BTC for the biologically-active tracer as well as the conservative tracer.

At least 20-25 samples were collected during the BTC. Background sampling before the pulse started to hit the station were sparse, but we ensured to collect 3 baseline samples. As the conductivity began to rise, the most intense sampling occurred. A good measure of travel time required getting a good fix on when the peak came through, so careful attention to sample through this period was critical. Sampling ceased after characterization of the falling limb of the breakthrough curve, when conductivity had returned to baseline. However, we ensured to no stop sampling prematurely at the tail end of the experiment. Measuring the tail was the only way to get a good estimate of nutrient uptake. We reserve at least 2-3 samples for the long tail of the BTC.

Samples were taken by either dipping the temporary field sample bottle in the stream to "grab" a sample or with a syringe. **Sample number, date, time, and conductivity (or bromide) needed to be recorded for each sample taken**. Stream widths of at least 10 transects within the reach were measured and recorded after the release was completed.

Once the experiment was completed (or during the experiment if time or extra hands allowed), all of the water samples were filtered. Ideally, filtering in the field was best, or filtering in a clean lab as soon as possible after the uptake experiment was complete. Atmospheric ammonium contamination by cleaning products, cigarette smoke, and mowing lawns were avoided. Also, bottles were capped quickly to avoid any exchange with the atmosphere. If filtering occurred later, all the temporary field sample bottles were arranged in two sequences: one sequence for the rising limb and one for the falling limb of the BTC. Samples from the two limbs were filtered separately, **always working from the lower concentrations to the higher concentrations near the peak.** Each sample was filtered from the temporary field bottle with a 60-mL syringe through a GFF filter into a 60-mL bottle. Since samples along a time series were closer in concentration to the next sample, there was no need to rinse and change filters between samples, but 5 mL extra were forced that through the syringe and filter before collecting the filtrate of a sample. The first 5 mL were also used to rinse the sample container. The same filter was never used for a lower concentration sample or for a sample from another limb of the BTC. If samples were not analyzed immediately, they were frozen (allowing for enough headspace for the bottles to expand as they freeze).

Database submission files: ammuptake_reach, ammuptake_synoptic, widths_reach, widths_synotic

3.6. Algae chlorophyll *a* and benthic organic matter (BOM) sampling (synoptic)

Material list

Loeb sampler with brush (4.9 cm^2 area) for bedrock and substrates with large planar area (Velcro dot sampler (8.0 cm^2 for AUS; Garcia et al. 2015)
Neoprene template (4.9 cm^2 area) and toothbrush for smaller substrates
Small PVC cylinder (4.9 cm^2 area) and a thin, stainless steel standard non-slotted spatula (the kind you use for a frying pan, not the kind for spreading icing or cleaning out jars) for sampling soft substrates (small petri dishes for AUS; Garcia et al. 2015)
Bottomless 5 Gallon Bucket
White tray
Stainless steel brushes
Whirlpacks with labels
Squirt bottle for rinsing
Cylindrical sampling templates (several pipe-corers see below)
Meter stick
Aquarium net
Sample bags for leaves and wood (10 per reach, 1 per sample)
Sample bottles for FBOM subsamples, approx. 250 mL size (10 per reach, 1 per sample)
Small plastic ruler
Cooler

Sampling in the synoptic reaches used a random approach by dividing each intensive synoptic reach into 10 blocks (i.e., 10 m per block if reach length is 100 m). We used random numbers (e.g., seconds on a watch) to decide the perpendicular stream transect location within a block (1-X m for meter markings, and 1-10 for location across the selected transect). Within the randomly selected transect and the random location across the stream, the dominant substrata type was sampled for both biofilm and benthic organic matter (BOM). Sampling method depended on substrate type (see below). The assignment of regular blocks and random transects insured that the habitats are sample weightedly. Additionally, the habitat of each sample was noted.

For **chlorophyll *a*** sampling there were three main methods:

1) *On large rocks and bedrock*, the Loeb sampler (4.9 cm^2 area) was used. The neoprene was pressed firmly against the substrate, then the brush was used to vigorously scrape the substrate. While maintaining contact with the substrate, the brush handle was pulled up, so that the water/biofilm slurry entered the Loeb sampler tube. The hole at the base of the sampler was covered to maintain suction and the spatula slid between the neoprene and the rock. The Loeb sampler was held to the spatula with the other hand and then lifted from the substrate and above the water and inverted. If more than $1/5^{th}$ of the sample was clearly lost, the sample was discarded and started again. Otherwise, the contents of the Loeb were emptied into a Whirlpack. Because of the small area sampled by a Loeb

sampler, at least three sub-samples were taken at each transect location and combined for a single sample in a Whirlpack bag. The number of subsamples taken were recorded (e.g., n=3, total area sampled 14.7 cm^2).

2) *For medium sized rocks* that were easily removable from the stream but are too large for whole-rock extraction, the substrate was placed in the sampling tray and either an area the size of the template or the entire rock scrape/brushed. The material was rinsed into a whirlpack. The size of the template or the entire rock was recorded.
3) *For small rocks* (less than 10 cm^2) two to three rocks were simply remove and place in a whirlpack bag for later whole-rock extraction. For smaller gravel substrata or fine sediment, a template was placed on the stream bottom and a) small gravel collected or b) a PVC tube pressed to a depth of 2 cm and, using the spatula to seal the sample, transfer it to the sampling whirlpack.

Sampling primary producer biomass has several constraints. Scraping misses 20-50% of attached chlorophyll depending upon the roughness of the substrata sampled (Murdock and Dodds 2007). However, large rocks were difficult or impossible to sample with whole rock extraction while it was the only option for fine substrata such as silt. In sites where most rocks were sampled using the extraction method (e.g., there were few large rocks/bedrock), only whole-rock extraction was used. If possible, whole-substrata extraction was the preferred method. While scraping may have simulated what herbivores were capable of, this project was centrally concerned with scaling metabolism as influenced by consumers, and only sampling the portion of the primary producer community that was susceptible to grazing could substantially over estimate the effects of grazers while seriously underestimating the total producer biomass.

Upon return to the laboratory, rocks and gravel were frozen. Fine sediments were settled if excess water was present, the water decanted, and sediments frozen. Liquid samples from scrapes were well mixed and filtered. If not the entire biofilm-water slurry could be filtered, the slurry was thoroughly mixed, then 20-40 mL subsampled through a GFF filter (does not need to be pre-combusted or weighed) and filtered and total volume recorded. Each filter was wrapped in a small square of aluminum foil, labelled, and frozen until analysis. The remaining sample was used for nutrient ratio analysis (see section 4.5.2.). The settled material and/or filter are to be kept frozen until analysis. Analysis for chlorophyll should be completed within one month if at all possible. Data showed 5-10% loss of chlorophyll over 6 weeks of freezing (Hallegraeff and Jeffrey 1985).

For **benthic organic matter** (BOM) sampling, we used sampling cylinders (e.g., 5 gallon bucket with bottom sawed off). Given the method, the cylinder were no longer than 10 cm shorter than the longest arm in the group. Although long cylinders were necessary for sampling deeper pools, they were unwieldy and we also used a shorter cylinder (say 16”). Cylinders had a diameter of at least 8” but not much wider than 12”. The diameter of the cylinder used was noted in the field note book. At CPC in year 2, stream depth and velocity were too great to sample with cores. As

an alternative, sediment were scooped from the stream bottom using a large PVC cup and the sediment placed in a strawberry basket to measure out equal volumes to 2013 samples.

As the sampling methods needed a relatively homogeneous sampling area and did not work with large rocks, the randomly selected sites were used if possible but otherwise a spot within a block that was possible to sample was selected. For each sample, the cylinder was placed as deeply into the sediments as possible. Five depths (four cardinal directions plus the middle) were measured and recorded in the field notebook. All coarse benthic organic matter (CBOM, > 1 mm, leaves and wood) was removed from the stream surface within the cylinder using hands and place in a durable plastic bag (e.g., pollination bag or large Whirlpack). An aquarium net (1 mm mesh size) was used to get suspended CBOM. The sediment was stirred to a depth of 6 cm and then, while swirling, a sample of the suspension for fine benthic organic matter (FBOM, < 1mm) was quickly collected in a bottle and placed in the cooler. The area and depths were used to calculate water volume. For these samples, transect location within the reach, habitat, diameter of cylinder, and 5 depths for each sample taken were recorded. (Due to flow constrains in 2014, some sites used different methods to collect a representative sample, by using PVC cups filled with sediments.)

To compare the chlorophyll and BOM measurements to the patches (i.e., basket measurement see section 3.8.2.), the synoptic style sampling was also conducted in the control reaches of the experimental sites close to the installed baskets.

Database submission files: Chla_synoptic, BOM_synoptic, Chla_reach_synstyle, BOM_reach_synstyle

3.7. Biofilm and organic matter stoichiometry

Material list

Whirlpacks or pollination bags (10 per synoptic, 60 total)
60 mL Nalgene bottle (10 per synoptic and experimental site, 60 total)
Turkey baster

Benthic stoichiometry samples were collected and samples analyzed for C, N, and P but were not sampled quantitatively (i.e., did not need to quantify surface area). Specifically, samples were taken from three intensive synoptic sites (n = 3) and experimental control reaches (n = 3) in 2013. Each compartment (CBOM, FBOM, biofilm) was collected according to and at the same time as the methods for regular synoptic sampling (section 3.6. and 3.8.2.). All compartments were sampled in at least 4 of the 10 locations and 4 of the 8 exclosures (see section 3.8.3.) where they were collected for algal biomass and organic matter quantification (Section 3.6. and 3.8.2.). Total samples collected were thus 6 reaches × 3 compartments × 4 samples for a total of 72 samples per year.

For **biofilm**, an additional scraping or rocks for later scraping in the lab were collected for biofilm C:N:P. Scraped material was poured into a 60 mL Nalgene bottle with a minimal amount of water. Alternately, additional whole rocks were placed in a Whirlpack or pollination bag. For **FBOM**, a 60 mL Nalgene was filled from the BOM cylinder, after taking the suspended FBOM sample, for the FBOM C:N:P. Alternately, a turkey baster was used to directly collect surface FBOM from within the transect block. For **CBOM**, a representative sample/handful of submerged leaves/wood near the synoptic BOM sample location was collected (Section 3.6.). Sample (e.g., stuff leaves) was placed in a large Whirlpack bag. (Note: It was necessary to do this extra grab because ALL of the CBOM was being quantified in the BOM cylinder – whereas the FBOM was subsampled, so there was additional sample volume that could taken from the BOM cylinder.) After sample collection, all samples were frozen or freeze-dried, and frozen or freeze-dried samples were shipped to University of Georgia for processing and analyses.

Database submission files: Stoich_reach, Stoich_synoptic (data available via web link)

3.8. Basket (patch scale) measurements

3.8.1. Metabolism and nutrient uptake

Material list

7 chambers (one as reserve/backup) (AUS used 9 dome chambers see Garcia et al. 2015)
7 fabric chamber covers (one as reserve/backup)
7 electrical boxes (one as reserve/backup)
12v battery
7 YSI ProODO meters (one as reserve/backup)
7 Odyssey irradiance meters (one as reserve/backup)
Chamber tools and spare parts (Allen wrenches, screw drivers, duct tape, assortment of screws etc., supplied with chamber)
1L volumetric cylinder, bucket with 7L mark
Funnel
Ammonium stock solution calculated so that 3 mL will take 10 L to 3x background concentration or 25 ug/L NH4-N, whichever is higher
Sample bottles (85, at least 30 mL bottle size, acid wash Nalgene)
Sample syringe and filter apparatus (GFF Zefon)
5 pieces of black window screen large enough to cover a chamber

Motor box assembly:

Before beginning assembly of motor housing, videos on the use of the chambers were reviewed (Supplemental Video S5 Rüegg et al. 2015) and checked that all parts were available from shaft assembly diagram (Figure 5). In order to simplify the assembly process, certain pieces were pre-assembled. This included: connecting the propeller to the propeller connecter, placing the PVC

tubing sleeve and rubber stopper on the brass sleeve, and attaching the motor connector to one side of the brass drive shaft.

Assembly began by attaching the post to the top of the motor box. The bottom bolt, metal washer and plastic washer were removed from the post, leaving one metal washer and bolt attached. The post was pushed through the smaller of the two holes on the top of the motor housing box. Next, the plastic and metal washers and bolt were re-attached. The bottom bolt was tightened with a 7/16 wrench until snug (over tightening may crack plastic), making sure that the loop at the top of the post was parallel with the long side of the chamber. Next, the brass sleeve (H) with the attached rubber stopper (L) was put into the larger hole on the motor box. It was easiest to attach stoppers by simultaneously twisting and pushing down. The pink PVC tubing (G) needed to be in line with the white set screws in the plastic shaft. The brass sleeve was moved up or down within the rubber stopper to adjust placement accordingly. Next, the motor connecter (J) was removed from the brass drive shaft (F) and brass drive shaft slipped into the brass sleeve. The motor box was turned on its back, and the propeller connecter (D) attached to the brass drive shaft by tightening the set screw (E). Enough space was left on the top of the shaft for the nylon spacer and the motor connecter to attach. Also, the set screw on the propeller connecter attached to the notched area of the brass shaft. While the motor box was still on its back, the nylon spacer (I) was slid on and the motor connecter attached to the other side of the brass shaft. One of the set screws on the motor connector was tightened to keep the brass shaft from taking up too much space within the connecter (i.e., this ensured there was enough space to attach the motor). Again, the set screw was attached to the notched area of the brass shaft. The motor box was placed upright. The white set screws were tightened (or loosened) until the shaft was held in place, while the propeller was also spinning freely within the PVC elbow. The top of the propeller needed to be flush with the top of the PVC elbow (this may have vary slightly, but most of the propeller was contained within the elbow). **At this point, it was vital that the propeller not made contact with the PVC elbow when spinning the brass shaft**. The motor (K) was attached to the motor connector by loosening the top set screw, inserting the flattened edge of the motor shaft, and re-tightening the set screw. Finally, the rubber band

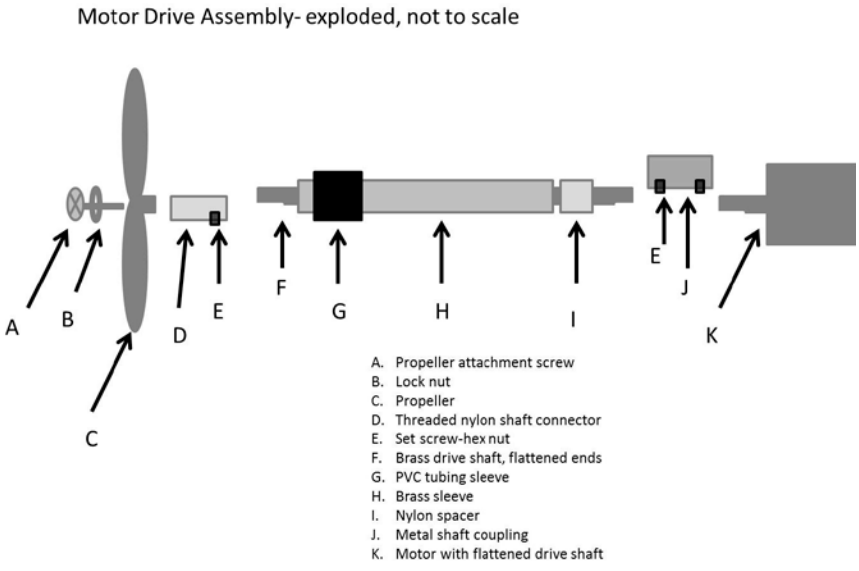

Figure 5. Motor shaft parts and assembly drawing for chambers.

(attached through the loop of the post) was looped over the motor. The rubber band loosely held up the motor; keeping the motor in place, while still allowing some wiggle room during operation. Once more, the propeller needed to spin freely as the motor sometimes pulled the shaft slightly out of place.

Filling and starting the chamber runs:
Videos on the use of the chambers were reviewed (Supplemental Video S6 Rüegg et al. 2015). The inner box was filled with water, the hole stoppered, and the box placed place it on stilts inside the chamber with the stopper facing down and towards the back corner of the chamber. It needed to slide under the two holders on the sides of the chamber. Next, the table that held the baskets was slid under the lip of the box making certain it fit flat on the stilts and was held in place by the two holders on the motor side. The entire motor housing assembly was placed into the chamber and the link locks tightened. The chamber drain was stoppered. The brown cover (if cover was of the style that encloses the entire chamber) was laid down and the chamber placed on top with the motor end of the chamber slightly elevated relative to the other end of the chamber. The portal for the stopper needed to be at the highest point of the chamber. Seven L of stream water were filled into the chamber.

The power supply was attached to a 12v battery. **THE RED (Positive lead) NEEDS TO BE ATTACHED TO THE POSITIVE TERMINAL OR THE POWER SUPPLY WILL SHORT OUT**. The wires were plugged into the power box then the other wires clipped onto the motor (red to plus) making certain the power supply was in the off position. The power was slowly turned up as to not strip the shaft connectors. Free movement of the propeller was tested. The water movement should be down through the PVC elbow to the far end of the chamber and flowing back across the top toward the motor. If the propeller was running backwards the connections from the power supply to the motor were switched (but never at the battery). The motor needed to be running quietly at this point. If the motor was noisy, the rubber band was adjusted; if this did not help the free movement of the propeller was tested and the nylon screws adjusted if necessary. The motor was then turned off again.

As gently as possible, the three baskets were collected from an exclosure using the chamber lid or a small plastic box as a transporting device (the other two baskets were used for FBOM, chlorophyll (section 3.8.2.), and invertebrates (section 3.8.3). The sediment baskets to be measured were gently lowered onto the table between the box and the motor assembly. The material that fell from the baskets was rinsed into the chamber while measuring the volume of water used to rinse. Stream water was added until the edge of the chamber was reached; making sure to keep track of the volume. The lid was then attached to the chamber, starting with the link locks connecting the motor assembly to the lid, followed by the link locks moving along the length of the chamber toward the end opposite the motor housing. More water was added through the portal for the oxygen probe using a volumetric cylinder and noting the volume until the chamber was nearly full. The oxygen probe was then inserted and twisted in place. The temperature sensor for the probe needed to be shaded inside the chamber or it would have given

a bad measure. Using a funnel, the rest of the chamber was filled through the stopper portal and closed with the stopper when full. The motor was turned on to ensure that any air bubbles were loosened. If the flow dislodged more bubbles, they accumulated at the stopper portal and more water was added to try and remove most bubbles, though a few small ones did not matter.

Once the chamber was filled without bubbles and the motor running, velocity was set by attaching a multimeter with leads plugged in to measure amperage. The ground lead was clipped to the positive red lead from the power source, and the red text lead to the plus poll on the motor. The power was set to read 0.5 amps and thus an approximate velocity in all chambers of 7 cm/s. This value also let us know what type of battery we needed as 12V lawn mower and scooter batteries have 18 amp hours while modest size deep cycle marine batteries have 40 amp hours. If we ran 6 chambers for 8 hours at 0.5 amps, we would have need 24 amp hours, so 2 lawn mower batteries charged per day ran the chambers. Also, as batteries drained their voltage dropped, so every couple of hours velocities needed to be reset by checking the amps, because the amperage dropped as voltage dropped.

Preparation for metabolism measurement (one day in advance, one time only):
These measurements were done in full midday light when possible, as we tested light attenuation after testing for linearity of changes in DO. Spare baskets (which had been incubated for the experimental time, or alternatively had been filled with similar substrate from the treatment reach) or some substrate collected from the stream were used for this method test. Continuous measurement of PAR (using an Odyssey irradiance meter) was measured to allow for normalization of differences due to diurnal metabolic characteristics. The cover was closed over the chamber and the light logger and dissolved oxygen set to log at 1 minute intervals (or shorter). Starting time and DO concentration were recorded and logged until dissolved oxygen has changed by 1 mg L^{-1} from the initial concentration or for 2 hours whichever came first. Alternatively, data were plotted on the fly and linearity of the decrease determined. The cover was removed and the time noted. The same amount of time required to get a significant DO drop in the dark was recorded in the light (if GPP = ER then actual DO might not change at all in this light incubation).

Data for ER was plotted using the dark measures and NEP using the light measures to determine how long of a measurement was required to get a consistent change in O_2 over time. The R^2 needed to be larger than 0.95. The point was to use the smallest amount of time per measurement to get a statistically significant result. The longer the time used to take the measurements the more the stream water in the chamber deviated in temperature and nutrient content from the stream. The determination was made using ER because if NEP was close to zero in the light, there were no detectable change in O_2 concentration. If changes in dissolved oxygen were linear, we will use at least 10 minutes even if a good relationship was achieved over over less time.

Once the time needed to measure a discernable change in DO (e.g., 10 min for linear) was determined, the photosynthesis irradiance curve needed to be determined. This curve was

required to account for changing light levels when the full set of baskets was measured throughout the next day. Logging light while making these measurements was critical. Both the chamber and the loggers were covered with 5 layers of window screen and light and O_2 logged for the amount of time determined as the minimum time required for DO changes. One layer at a time was removed and the procedure repeated up to full light (we generated rates for dark (initial), 5, 4, 3, 2, 1, and 0 layers of screen).

At some sites we also ran blanks, meaning empty baskets were run to test for potential effects on dissolved oxygen independent of temperature.

Database submission files: DO_patch_linearitylight, Light_patch_linearitylight, DO_patch_blank

Experimental measurements:
Baskets were carefully removed from the exclosures, placed on chamber lid (or in a plastic container) to transport, and placed in chambers following the instructions listed under the section above (pre-sampling metabolism measurements). Using the time determined by the linearity test, DO and PAR were logged in both the dark (under cover; ER measurements) and the light (no cover; NEP measurements). Site names for logging files were created based on naming convention (see section 6.1.).

Database submission files: DO_patch, Light_patch

Uptake measurements:
For nutrient uptake measurements, 3 mL of the nutrient stock solution were added after the dark/light incubations for metabolism were completed. Ammonium was added to increase concentrations to 3-5x background. This was close to background but high enough to allow analyses. If concentrations were so low that confidence in detection was low, 25 µg NH_4^+-N L^{-1} were aimed for. The first sample was taken after 1 minute and the rest of the four-seven samples spaced over the course of 40 minutes (e.g., 1, 4, 10, 20, 40 min). The removed water was not replaced and some aerated headspace did develop. Water samples (30mL) were removed, filtered immediately into acid washed bottles, and placed in a cooler. Water volume wis replaced with air. Samples were frozen upon return to the laboratory.

Database submission files: ammuptake_patch

Emptying chamber:
At the end of the run, power supply was turned off and unplug from battery. The lid was opened and some of the water ran out over top. The baskets were gently removed and emptied out. The drain plug was pulled since lifting a full chamber ran the risk of dropping and cracking the whole chamber. The motor assembly was removed and rinsed, as was the chamber, before being

refilled with fresh stream water. Tightness of all set screws was checked and the motor assembly replaced into the chamber, making the chamber ready for the next filling and sampling. Amperage of power supply was checked when starting each new run.
(Some sites used chamber baskets to measure chlorophyll and organic matter, by using the water from the chambers and following the protocols detailed in section 3.8.2. in 2014.)

3.8.2. Algae chlorophyll *a* and benthic organic matter sampling of patch exclosures and experimental reaches

Material list
Whirl packs (50), labeled
Cooler with ice
Nalgene bottles (50 500 ml), labeled
Bucket with 5 L mark
100% Formalin

For **chlorophyll *a*** whole substrates were collected from one strawberry basket in each habitat exclosure as well as from the baskets placed in the pools and riffles of the control and treatment reaches and placed in Whirlpacks. The rocks were generally picked after placing the basket in the bucket for organic matter sampling (see below). The surface area of the substrate pieces collected covered at least 5 cm^2 of the basket. Up to three rocks were removed from each basket depending on substrate sizes. The selected rocks were returned to laboratory in a cooler and frozen as soon as possible. Analysis for chlorophyll *a* was completed within one month.

For **benthic organic matter** a bucket was filled with 5 L of stream water. One of the two remaining strawberry basket in exclosures or one of the pair from the control and treatment reaches, from which the chlorophyll *a* rocks were/will be removed, was placed in the bucket. The basket was emptied and the substrate agitated to suspend benthic organic matter and a 500 mL sample taken (for fine benthic organic matter only). Samples were transported back to the lab and refrigerated if not processed immediately. If the sample couldn't be processed within two days, 40 mL of 100% formalin (37% saturated formaldehyde) were added to create an 8%-formalin solution. Processing should be done within a week. Note, if there was time to process BOM materials that evening, formalin was not necessary for this step.

Database submission files: Chla_reach_patch, BOM_reach_patch

In addition, control and treatment reaches were sampled for chlorophyll *a* and BOM as described for the synoptic sites (see section 3.6.). Reaches will be divided into 10 blocks, and the stream length of a block will be noted. These samples should be taken to cross calibrate basket sampling methods with methods used in synoptic sampling.

Database submission files: Chla_reach_synstyle, BOM_reach_synstyle

3.8.3. Biofilm and benthic organic stoichiometry of sample baskets

Material list

Whirlpacks or pollination bags (12 per experimental site, 36 total per year)
60 mL Nalgene bottle (24 per experimental site, 72 total per year)

Samples were collected from at least 4 (preferably all 8 if time allowed) of the locations within each reach (control, patch, and treatment reach) at the end of the experimental exclusion period. In the patch reach, samples were collected from the exclosure (closed) side of the patch exclosure. Thus, the total basket sample load was (6 reaches + 3 patch reaches) × 3 compartments × 4 samples each = 108 samples (see also section 3.7.).

For **biofilm**, rocks were removed from the "macroinvertebrate" basket (see section 3.8.4.). After placing the basket in a tray, rocks were taken from the basket surface, and gently rinsed with water to remove attached invertebrates (see section 3.8.4.). Then, rocks were scrapped to remove attached biofilm (~30 seconds total effort). Scraped material was place, with a minimal amount of water, into a 60 mL Nalgene bottle. Alternatively, rocks were placed in a whirlpack.

For **FBOM**, a 60 mL Nalgene was filled after resuspending the sample from which FBOM was collected from (see section 3.8.2.) for the C:N:P. For **CBOM**, a sample/handful of submerged leaves/wood was grabbed and placed in a large Whirlpack bag. If control reach was sampled for *in situ* stoichiometry at the same time, one CBOM sample sufficed (see section 3.7.). After sample collection, all samples were frozen and frozen samples shipped to University of Georgia for processing and analysis.

Database submission files: Stoich_reach_patch (data available via web link)

3.8.4. Invertebrate sampling of patch exclosures and experimental reaches

Material list

White pan
250µm sieve (2)
Water squirt bottle
Forceps
Whirl packs (32) labeled outside and in
Bottle with 100% formalin (diluted with stream water)

One strawberry basket from each side of each habitat exclosure was designated for invertebrate sampling (n=16). Additionally, one strawberry baskets from the pools and riffles of the control (n=8) and treatment (n=8) reaches was also for invertebrate sampling. Baskets were collected at the end of the experiment by carefully removing them from the stream while holding a 250 µm mesh bag or sieve just downstream of the basket. The basket was then lifted through the water column with the mesh/sieve around it. Baskets were placed in a white pan, along with contents of the sieve. All sediment particles of the pan were rinsed thoroughly and then removed. The

remaining organic material was rinsed into the 250 µm sieve. Contents of the sieve were then condensed in one area of the sieve and rinsed with a squirt bottle into a whirlpack. The final sample only had enough fluid to keep the contents submerged, and enough 100% formalin was added to make the liquid 8% formalin. Each sample was double labelled: the internal label was a plasticized paper label (e.g., write in the rain paper) and the same information was written in permanent ink on the outside of the whirl-pack. Organic matter from these samples was used to determine coarse benthic organic matter.

Database submission files: Inverts_reach, Inverts_species, BOM_patchreach

4. Laboratory procedures

4.1. SF_6 samples

Samples that were not extracted for gas in the field (water samples and vacutainers) were processed in the lab to extract the gas. The *vacutainers* were processed as soon as they are returned to the laboratory, within a week if possible. On the day prior to analysis, the samples were allowed warm to room temperature and samples equilibrated with the atmosphere by inserting a needle into the septum for 5 seconds. The mass of all vacutainers was measured to calculate the volume of water sampled. If the mass of vacutainers was consistent, five empty vials were weighed and the mean calculated. If the mass of vacutainers was not consistent, the mass of each vacutainer was measured prior to sampling. After analysis of SF_6, enough water was retained from the vacutainer for later Cl/Br analysis. As a week for all sampling was often not feasible, a control vial with a known volume of SF_6 was prepared to estimate loss through septa. The *water samples* were also processed quickly. Using a needle as an outlet through the septa, 20 mL of N_2 gas were inserted with a syringe while holding the vial upside down. The 20 mL of fluid that were removed were retained for determination of conservative tracer. The vials were then shaken for three minutes to equilibrate SF_6 with the headspace.

The SF_6 was measured from the headspace in vacutainers or from the water sample vials, or directly from the vials of gas samples collected in the field using a gas chromatograph with electron capture detector. The concentration of SF_6 in vials was checked prior to analysis: if SF_6 concentration was high, samples were diluted before injecting to avoid swamping the detector. This dilution was accomplished using a sealed flask and injecting sample with a high accuracy glass syringe (e.g., Hamilton glass syringe). Care was taken to avoid carry over as high concentrations of SF_6 tend to bleed into subsequent sample injections. The conservative tracer concentration was measured *in situ* using conductivity or bromide meters, from the water removed from the water sample scint vials or from water samples collected in the field. Used vacutainers were discarded as SF_6 binds to the septa of vials and contaminates subsequent analyses if vials were reused.

Laboratories at LUQ (University of New Hampshire, McDowell), KNZ (Kansas State University, Dodds), CWT (University of Georgia, Ballantyne), and CPC (University of Alaska Fairbanks, Jones) processed samples and used their standard operating procedures. Since relative

values were key for analyses, direct comparison among laboratories was not required. If own samples could not be analyzed, a trade was negotiated with another laboratory to analyze them. Generally, the approach was to offer to analyze some of other types of samples in return (e.g., did BOM samples for them).

4.2. Stream water nutrient and ammonium uptake samples

Stream water samples were thawed and analyzed for soluble reactive phosphorous, ammonium, nitrate, total dissolved phosphorous, total dissolved nitrogen, and dissolved organic carbon as well as particulate nitrogen, phosphorous, and carbon (though not all parameters were measured at all sites in all years). Samples for total N and P were determined via whole-sample digestion of filtered samples and adding of values of particulate N and P from the filters that were analyzed for N and P content. Thus, a whole sample digestion was still required. The benefit was that particulate and dissolved N and P could be indicated. Samples for whole-stream and chamber nutrient uptake were analyzed for ammonium and the conservative tracer if an ion chromatograph was available (Cl^- – specific conductance or Br^- – mV relationships can be used alternatively).

Each biome and laboratory analyzes samples based on their standard operating procedures. To compare values across biomes, laboratories generally participated in the USGS round robin (http://bqs.usgs.gov/srs/) to verify accuracy. The check samples were available two times per year, and checks were run at least once per year (if possible), though twice would have been better, preferably run before unknown project samples so any problems could be fixed before sample analyses.

4.3. Benthic organic matter (BOM)

Materials needed

Pre-ashed, pre-weighed 47 mm GFF filters
Pre-weighed aluminum tins
Filter apparatus
Graduated cylinder
Forceps
Paper bags

Numbers were inscribed into the weighing aluminum tins and tins pre-ashed. Enough tins were pre-weighed to contain all BOM samples. Tins and filters were pre-ashed at 450°C for a minimum of 3 hours. Pre-weigh filters were put into numbered tins on scale, tared, and weight and tin number recorded on a lab sheet. Ashed tins or filters were never handled by hand but rather with clean forceps.

Basket samples:

Coarse BOM (CBOM; > 1 mm; most CBOM samples were derived from the invertebrate basket, see section 4.6.): A volume of 250 mL of the benthic organic matter samples was filtered through a 1 mm mesh. The mesh was then rinsed into a pre-weighed aluminum tin.
Fine BOM (FBOM; 0.45µm – 1.0 mm): The filtrate from the CBOM sample was used to filter a known volume through a pre-ashed and pre-weighed GFF filter, until the filter clogged. The filtrate volume was recorded. The filter was then transferred into its pre-weighed aluminum tin. An additional set of samples on filters were saved if PP, PC, and PN analyses were desired.
All tins (CBOM and FBOM) were dried at 60° C for a minimum of 48 h, and then weighed. Tins were subsequently ashed at 450°C for a minimum of 3 hours, and re-weighed. Dry mass, ashed mass, and ash-free dry mass (AFDM) were calculated based on the differences in weights, and converted to per area basis (0.1 m^2 surface area of basket).

Corer samples:
CBOM: If possible wood was separated from leaves and organic matter place in small paper bags or aluminum tins, and dried at 60°C for a minimum of 48 h. All dry weights were recorded, followed by combustion a subsample (recorded how much subsampled) of each sample for a minimum of 3 hours at 450°C. Weights after ashing were again recorded.
FBOM: Sample bottle were shaken thoroughly, and a subsample poured into a graduated cylinder. The volume in the cylinder was noted and filtered through a pre-combusted, pre-weighed GFF filter until filter clogged. Filters were dried at 60°C, weighed, and combusted at 450°C for a minimum of 3 hours before re-weighing to calculate ash-free dry mass and ashed mass. All weights were recorded. To calculate FBOM standing stocks for each field sample the following equation was used:

$$\text{FBOM (mg AFDM m-2)} = \frac{\frac{Subsample\ AFDM\ (g)}{Subsample\ Volume\ Filtered\ (mL)} \times Total\ Cylinder\ Volume\ (mL)}{Cylinder\ Surface\ Area\ (m^2)} \quad \text{Equation 10}$$

Where $Subsample\ AFDM\ (g) = Preash\ subsample\ dry\ mass\ (g) - Postash\ subsample\ mass\ (g)$.

4.4. Chlorophyll *a*

Material needed.
GFF filters
Clavies® Autoclavable Bags 10X10 Bel art (131821010)
50mL test tubes labeled with scratched numbers
95% Ethanol (~ 5 gal drum per lab) (AUS used acetone instead of ethanol)

Chlorophyll *a* was determined by hot ethanol extraction using the method of Sartory and Grobbelaar (1984). Hot ethanol extraction is far more efficient than acetone, similar to methanol, but much less toxic. Samples were kept in the dark at all times and at maximum in low light

when working on them. If a sample is kept in the dark, only 1.3% of the chlorophyll degrades in the 5 min. hot extraction and 24-hour storage.

All samples: The filter were placed in a test tube (50 mL centrifuge tubes work well), the fine sediments in containers, and whole rocks in autoclave bags (alternatively the collection whirlpacks were used while ensuring that they had no leaks). A known volume of 95% ethanol was added and said volume noted on a datasheet. The location of the meniscus on the side of the tube, container, or bag was marked with sharpie. If using tube or container, a loose cap/lid was placed on top of the tube/container, if using bags the top was folded over, but none were sealed. The tube, container, or bag was heated in a 79°C water bath for 5 minutes, then shake and keep cool (5-15°C) for 24 h in the dark. Tubes, containers, and bags were sealed once cooled. As ethanol spills on the side could wipe out sample markings, care was taken when handling the samples. After extraction, additional 95% ethanol was used to bring liquid back up to the mark on the side of tube if ethanol had evaporated, then samples were shaken. Samples were cleared by centrifugation, filtration, or settling if necessary. Rocks in autoclave bags were shaken and allowed to settle for an hour before analysis (note, whirl packs are cheaper and can withstand the heat and ethanol).

There were three methods of analysis used depending on the site. As all methods were calibrated against the spectrophotometric method, they worked equally well. However, since most sites had a spectrophotometer, it was the preferred method. 1) Spectrophotometric with acidification correction for phaeophytin, 2) fluorometric with acidification correction for phaeophytin or 3) fluorometric with specific lamp and filter combinations to avoid phaeophytin.

1) Spectrophotometric method: A sample was analyzed with spectrophotometric analysis at 665 and 750 nm using a 1 cm spectrophotometer cuvette (APHA 2005). If absorption was over 1.5 absorbance units, the sample was diluted. 0.1 mL of 0.1 M HCl was added for each 10 mL of extractant after the first reading and sample left for 90 s to covert all chlorophyll *a* to phaeophytin before the second reading. A correction factor was needed for this acidification step, as 90 s was too short a post-acidification step (Parker et al. 2016). As stated by Parker et al (2016); for future analyses we recommend acidifying ethanol-extracted algal samples to 0.008 mol HCl L^{-1} and allowing samples to react for 30–60 min to ensure accurate and consistent results. The amount of acid was important as too much causes precipitates. The APHA (2005) method was modified with absorption coefficients from Sartory and Grobbbelaar (1984). Calculations were made as follows:

$$\text{Chlorophyll } a \text{ (mg m}^{-2}) = (28.78(665_0\text{-}665a)*v/(A*l)$$

$$\text{Phaeophytin (mg m}^{-2}) = 28.78\ [1.72(665a)\text{-}665_0]*v/(A*l)$$

Where, 665_0 = absorption at 665 before acid addition with absorption at 750 nm subtracted out, 665_a = absorption at 665 nm after acid addition with absorption at 750 nm subtracted out, v = volume of extractant used (L), A = area of benthos sampled (m^2) and l = path length of cell (cm) (usually 1 cm).

Chlorophyll analyses were corrected for the acidification step based on Parker et al (2016) (see Appendix “Chlorophyll corrections”).

2) Fluorometric method: A Fluorometer needed filters and lamps appropriate for chlorophyll *a* analysis and to be pre-calibrated with chlorophyll solutions of known concentration. Spinach leaves extracted in 95 % ethanol could be used as a chlorophyll source. Known samples were diluted and the above method (the spectrophotometric method) was used to measure and calculate concentration of the diluted samples. The diluted samples were then placed in the florometer and fluorescence units read. 0.1 mL of 0.1 N HCl was added for each 10 mL of extractant after the first reading and sample left for 90 s. The difference in the fluorescence units was regressed against the calculated chlorophyll concentration of each solution to create a calibration curve for the fluorometer. Then, the unknown samples were read, acidified, and re-read. Flourometers generally hold calibration for months, but the calibration samples will degrade rapidly, so to check calibration fresh standards needed to be prepared.
3) Alternate fluorometric method: With the appropriate filters, chlorophyll was measured on a fluorometer without the acidification step (Welschmeyer 1995, APHA 2005). However, a set of known diluted chlorophyll standards was still needed to calibrate the machine. This method does not need a correction for acidification.

Determination of area sampled:
If a Loeb sampler was used, or known area was scrubbed, surface was known. For whole rocks, different methods were available to determine surface area. The rock were directly scanned on leaf area measuring equipment, rocks traced onto a paper with pencil then scanned the area later, or using photographs of rocks and software (e.g., Image J) to determine area later. Areas based on such scans were converted to whole-rock area as up/down was hard to determine after the extraction. Alternatively, rocks were wrapped in aluminum foil and a weight-to-area relationship applied to foil weights (see Bergey et al. 2006). Some sites used water displacement which were converted to surface area using the foil-relationship determined for a subset of the rocks. To get chlorophyll *a* per unit area of sediment (we do not try to account for every nook and cranny of rock surface but decided to use whole rock aera), calculate the total amount of chlorophyll *a* in the extractant based on its volume considering any dilution, extrapolate absolute amount if subsampling occurred and divide by area sampled as determined above.

4.5. Nutrient ratio analysis (C:N:P)

4.5.1. Benthic organic matter C:N:P

The four samples of BOM were analyzed from the blocks sampled or the baskets (see section 3.7. and 3.8.3.). For CBOM, an approximately 2 g representative subsample of oven-dried material was used (60° C, minimum 48 h). Wearing latex gloves, leaf litter was crushed by hand inside the paper bag, then place placed into a ball mill and ground into a fine powder (consistency of talcum powder; approx. 3-5 min). Wood subsamples were cut up into small wood fragments using PVC pipe cutters. Wood fragments were also placed into a ball mill and ground into a fine powder (approx. 5-10 min). For FBOM, volume (approximately 60 mL) was placed

into a labeled plastic scintillation vial, frozen at -20°C, freeze dried, and stored at room temperature until C:N:P analysis.

4.5.2. Biofilm C:N:P

The four biofilm samples from each synoptic site were analyzed for C, N, and P content from blocks and baskets sampled in the stream reach (see section 3.7. and 3.8.3.). The biofilm slurry was placed into a labeled plastic scintillation vial, frozen at -20°C, freeze dried, and store at room temperature until C:N:P analysis. If rocks were collected, rocks were placed on a white pan, and scrubbed with firm, short strokes using an old toothbrush or similar short-bristled brush to remove biofilm from the rock surface. Biofilm slurry was then poured into a labeled plastic scintillation vial, frozen at -20°C, freeze dried, and stored at room temperature until analysis.

4.5.3. Analytical analysis

Carbon, N, and P content were determined using standard operating procedures from the UGA laboratory. Analytical methods by nutrient and substrate, along with approximate mass of dried sample needed for each analysis, are shown in Table 8.

Table 8. Analytical methods and sample mass needed for C, N, and P analysis of freeze-dried, ground samples.

Substrate	Carbon & Nitrogen	Phosphorus
CBOM	Micro-Dumas combustion; 2 mg	Aqua regia extraction; 100 mg
FBOM	Micro-Dumas combustion; 2 mg	Persulfate digest; 25 mg
Biofilm	Micro-Dumas combustion; 2 mg	Aqua regia extraction; 100 mg

4.6. Invertebrate processing

All samples were rinsed with tap water through a 125 µm sieve. All invertebrates were then removed from samples under a dissecting microscope with fiber optic light. Invertebrates were identified to the lowest practical taxonomic level (usually genus), measured for length, and preserved in 8% formalin in vials with poly seal caps. After all invertebrates have been removed, remaining materials were processed to estimate CBOM as ash-free dry mass (AFDM). Materials were placed in aluminum pans, labeled, and placed in a drying oven at 50°C for at least 48 hours. After 48 hours, the material with the pan is cooled in a dessicator and weighed. Pans with samples were then placed in a muffle furnace at 500°C for at least 2 hours. After combustion, samples were cooled in a desiccator, re-wetted with DI water, and then put back into the drying oven for at least another 48 hours. Pans with samples were then weighed, samples are discarded, and the pans re-weighed. Ash-free dry mass ass then estimated as the difference between dry mass and ash mass and converted to areal mass by surface area of the baskets (0.1 m^2).

5. Timeline of measurements

5.1. Project timeline of field measurements (among years and across biomes)

Biomes were sampled consecutively (Figure 6, Table 9). In 2013, sampling started at the Luquillo (PR) LTER in January/February, followed by Coweeta Hydrological Station in March/April, the Konza LTER in May/June, and the two Alaska biomes with Caribou/Poker Creeks LTER in June-August and the Arctic LTER in July/August. Caribou/Poker Creek (CPC) and the Arctic (ARC) were sampled with overlap, meaning the setup and initial sampling was conducted in CPC, followed by set up and initial sampling at the ARC site, before returning to and finishing measurements in CPC followed by ARC. In 2014, general sampling times were similar in most biomes, except for Coweeta, where sampling was conducted Aug-September (Table 9).

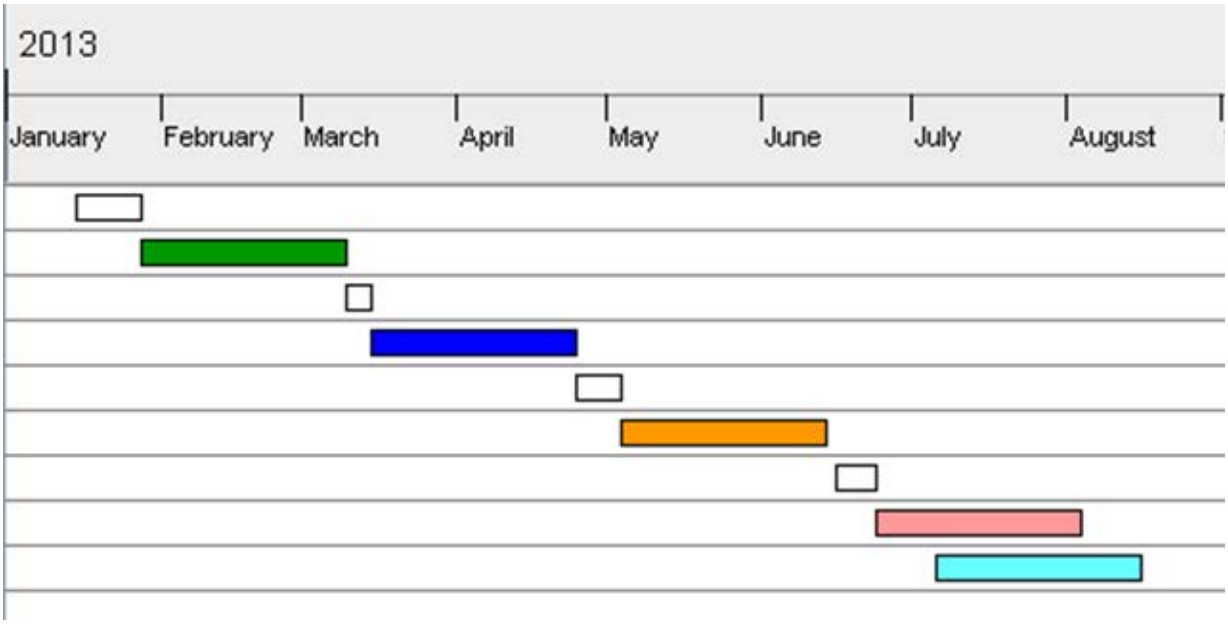

Figure 6. Approximate timeline across biomes. White squares represent preparation/travel times, green is LUQ, blue CWT, orange KNZ, salmon CPC and turquois ARC. See Table 9 for exact dates.

Table 9. Experimental schedule across all years of the SCALER project by site. Dates given refer to the beginning and end of the 41 days of experiment (including installation, see section 5.2.)

Site	***2013***		***2014***		***2015***	
	Begin	***End***	***Begin***	***End***	***Begin***	***End***
LUQ	28-Jan	9-Mar	6-Mar	16-Mar		
CWT	12-Mar	23-Apr	29-Aug	16-Sep		
KNZ	3-May	11-June	20-Mar	4-June		
CPC*	26-Jun	14-Aug	11-Jun	13-Sept		

ARC	11-Jul	11-Aug	8-Jul	24-Jul		
AUS	23-Jul	1-Sep				
AND			23-Aug	27-Sep	6-Jul	27-Aug

*In 2014 (year2), no basket installations due to flow restrictions, thus dates reflect period of data collection.

In each biome, three experimental sites were sampled in 2013 and a *new* set of three sites in 2014 *if possible* (e.g., drought conditions in KNZ prevented selection of all new experimental sites as streams were not flowing). The 20 synoptic sites remained the same in 2013 and 2014 to account for inter-annual variation. Six synoptic sites were designated as intensive, meaning that whole-stream metabolism and nutrient uptake were measured at those sites. The experimental duration was 30 days, for a total of 41 field days to account for installation time across experimental reach sites (see section 5.2.). All synoptic sampling occurred during the experimental window, as close together in time as possible except for pre-synoptic sampling.

5.2. 2013 Experimental timeline (1 year and biome)

The detailed timeline is provided in the table below (Table 10). Different colors represent the three experimental sites (labeled E1, E2, and E3 for simplicity) sampled in one year at each biome. Each site required a three-day window for installation and initial measurements. All sites also had a three-day window for final measurements.

Pre-experimental preparation included the following:

1) Selection of sites: three experimental sites and 20 synoptic sites (with 6 designated as intensive)
2) Marking locations for fences (2.2.) and habitat patches (2.3.)
3) Build wood frames for patch exclosures (2.4.1.)
4) Prepare materials for exclosure and sampling (including strawberry baskets, sampling bottles, whirlpacks, nutrients, fencing, rebar, …)

Table 10. Schedule of tasks over the SCALER field sampling period.

Day 1 E1 – Fence installation (2.4.3.), consumer survey (treatment reach; 3.2.)	Day 2 E1 – Consumer survey (treatment and control reach; 3.2.), patch exclosures (2.4.4./2.4.5.)	Day 3 E1 – Water chemistry (3.3.), WS nutrient uptake (3.5.), start DO and light (3.4.)	Day 4 E2 – Fence installation (2.4.3.), consumer survey (treatment reach; 3.2.)	Day 5 E2 – Consumer survey (treatment and control reach; 3.2.), patch exclosures (2.4.4., 2.4.5.)
Day 6 E2 – Water chemistry (3.3.), WS nutrient uptake (3.5.), start DO and light (3.4.)	Day 7 E3 – Fence installation (2.4.3.), consumer survey (treatment reach; 3.2.)	Day 8 E3 – Consumer survey (treatment and control reach; 3.2.), patch exclosures (2.4.4., 2.4.5.)	Day 9 E3 – Water chemistry (3.3.), WS nutrient uptake (3.5.), start DO and light (3.4.)	Day 10 E1 – collect DO and light probes (3.4.)
Day 11	Day 12 E2 – collect DO and light probes (3.4.)	Day 13 E1 – Water chemistry (3.3.), consumer survey treatment reach disturb treatment reach (day 11; 3.2.)	Day 14	Day 15 E3 – collect DO and light probes (3.4.)

Day 16 E2 – Water chemistry (3.3.), consumer survey treatment reach (day 11; 3.2.)	Day 17	Day 18	Day 19 E3 – Water chemistry (3.3.), consumer survey treatment reach (day 11; 3.2.)	Day 20
Day 21	Day 22	Day 23	Day 24 E1 – Water chemistry (3.3.), consumer survey treatment reach (day 22; 3.2.)	Day 25
Day 26 E1 – start DO and light (3.4.)	Day 27 E2 – Water chemistry (3.3.), consumer survey treatment reach (day 22; 3.2.)	Day 28	Day 29 E2 – start DO and light (3.4.)	Day 30 E3 – Water chemistry (3.3.), consumer survey treatment reach (day 22; 3.2.)
Day 31	Day 32 E3 – start DO and light (3.4.)	Day 33 E1 – Water chemistry (3.3.), WS nutrient uptake (3.5.), end DO and light (3.4.)	Day 34 E1 – Baskets: DO, nutrient uptake, chla, BOM, inverts (3.8.)	Day 35 E1 – Consumer survey all reaches (3.2.)
Day 36 E2 – Water chemistry (3.3.), WS nutrient uptake (3.5.), end DO and light (3.4.)	Day 37 E1 – Baskets: DO, nutrient uptake, chla, BOM, inverts (3.8.)	Day 38 E2 – Consumer survey all reaches (3.2.)	Day 39 E3 – Water chemistry (3.3.), WS nutrient uptake (3.5.), end DO and light (3.4.)	Day 40 E3 – Baskets: DO, nutrient uptake, chla, BOM, inverts (3.8.)
Day 41 E3 – Consumer survey all reaches (3.2.)	• Solid background colors indicate different experimental sites, 4-person crew (3 only day 13-30) • Gray background designated for synoptic sampling • Checking of exclosure fence should be conducted as often as needed and possible. • Post-experiment clean-up: remove all exclosures and fencing after Day 41			

See Appendix SCALER Experimental Prep Schedule, an excel file that can be more easily adjusted (Example given is CPC 2013).

6. Data procedures – data manager

6.1. Labeling protocol

6.1.1. Site labeling convention

Biome and site names in conjunction with date were unique identifiers for most data. The naming convention was as follows:

1) Three letter code for biomes: Luquillo (LUQ), Coweeta (CWT), Konza (KNZ), Caribou-Poker Creeks (CPC), arctic (ARC), Darwin (AUS) and HJ Andrews (AND).
2) One letter code for experimental (E) or synoptic (S) sites
3) Number for experimental (***1-6***) or synoptic (***1-20***) identifier, the designation of which was up to each biome, based on their preference (e.g., up-to-downstream, based on timeline). Generally, E1 – E3 was used for 2013.
4) In case of experimental sites, one letter code for reach type: ***C***ontrol (C), ***P***atch (P), and ***T***reatment (T).
5) For reach-scale experimental measures, ***top*** for below the upstream fence, and ***bottom*** for above the downstream fence, if applicable.
6) For baskets in the control and treatment reaches, the designation is Location, and numbers 1-8 assigned, downstream to upstream was suggested but actual use detailed in the site characteristics.
7) For patch scale measures, e**X**closure (X) was used to indicate the exclosure and the numbers ***1 to 8*** to indicate which exclosure it was, suggested labelling was again downstream (1) to upstream (8). The habitat designation of each exclosure was noted separately of these labels.
8) For eXclosures, enclosed sides are called *IN* and open sides are called *OUT*.
9) Date used three letter codes for months to avoid confusion on labels.

For Example:
KNZ_E2_C_top OR KNZ_E2_P_X1_IN
30-Apr-2013

Electronic sample labels are available for:
Water chemistry (waterchem_2013.docx)
Ammonium uptake (WSNH4uptake_2013.docx; basketuptake_2013.docx)
Chl*a* and BOM (baskets_2013.docx; chlaBOM_synoptic.docx)
Either used Waterproof, sticky labels (e.g., http://www.staples.com/Avery-5520-White-WeatherProof-Address-Labels-1-inch-X-2-5-8/product_440728?externalize=certona) or paper labels put on with clear packing tape.

6.2. Field data sheets

Data collected in the field was noted in notebooks. Such data included notes on weather and stream conditions, goals for the day, people also present in the field. In terms of data collection, they may have include placement of loggers and meters, macro-consumer surveys (species,

lengths), and stream widths depending on what the method sections call for. All field notebooks and field sheets were write-in-the-rain type paper.

6.3. Electronic data entry sheets

6.3.1. File naming protocols

The table below lists all the file names and a description of their content as well as the suggested interval for submitting (e.g., timing). The first part of each file name is the biome abbreviation followed by the current date in the format of YYMMDD. The second part of the file name indicates the metric contained in the file followed by the scale at which the data were collected. All files are available in the templates folder under Data in the SCALER all dropbox. Please use these templates since they are the basis for the database incorporation of the data.

Table 11. Data submission file names, grouped by main variables (alphabetical). Submission files were dependent on the scale of study as those determined the level of site description details (see Table 13). Submission generally occurred once per biome and year of study (except some site characteristics, e.g., depth, substrate). Letters AAA were replaced with biome abbreviation (Table 5) and 999999 with the date of data submission (yymmdd).

Main variable	Submission file	Data collection/ analysis methods
Aeration	AAA999999_SF6plateau_reach AAA999999_SF6plateau_synoptic SF6pulse (available via web link)	Sections 3.4.3., 4.1.
Benthic organic matter	AAA999999_BOM_reach_patch	Sections 3.6, 3.8.2., 3.8.4, 4.3., 4.6.
	AAA999999_BOM_synoptic AAA999999_BOM_reach_synstyle	Sections 3.6, 4.3.
Canopy cover	AAA999999_canopy_reach AAA999999_canopy_synoptic	Section 3.1.5.
Chlorophyll *a*	AAA999999_Chla_reach_patch AAA999999_Chla_reach_patch AAA999999_Chla_synoptic AAA999999_Chla_reach_synstyle	Sections 3.6., 3.8.2., 4.4.
CNP stoich	Available via weblink	
Depth (+Substrate)	AAA999999_depthsubstrate_reach AAA999999_depthsubstrate_synoptic	Sections 3.1.2.
Discharge	AAA999999_SlugDischarge_reach AAA999999_SlugDischarge_synoptic AAA999999_discharge_reach AAA999999_discharge_synoptic	Sections 3.1.6., 3.4.3.

Invertebrates	AAA999999_Inverts_reach_patch	Section 3.8.4, 4.6.
Invertebrate species	AAA999999_Inverts_species	Section 4.6.
Light	AAA999999_Light_patch AAA999999_Light_patch_linearitylight	Section 3.8.1.
	AAA999999_Light_reach AAA999999_Light_synoptic AAA999999_Light_synoptic(year2)	Section 3.4.2.
Macro-consumers	AAA999999_consumer_reach AAA999999_consumer_synoptic	Section 3.2.
Macro-consumer species	AAA999999_Macroconsumer_species	Section 3.2.
Nutrient uptake	AAA999999_ammuntake_patch	Sections 3.8.1, 4.2.
	AAA999999_ammuntake_reach AAA999999_ammuntake_synoptic	Sections 3.5., 4.2.
Oxygen	AAA999999_DO_patch AAA999999_DO_patch_blk AAA999999_DO_patch_linearitylight	Section 3.8.1.
	AAA999999_DO_reach AAA999999_DO_reach_cal AAA999999_DO_synoptic; AAA999999_DO_synoptic(year2) AAA999999_DO_synoptic_cal; AAA999999_DO_synoptic_cal(year2)	Section 3.4.2.
Physical-chemical characteristics	Available via web link	Section 3.1.4.
Site characteristics	AAA999999_sitecharacteristics_reach AAA999999_sitecharacteristics_synoptic	Sections 3.1.1., 3.1.7.
Substrate (+Depth)	AAA999999_depthsubstrate_reach AAA999999_depthsubstrate_synoptic	Section 3.1.3.
Travel time	AAA999999_traveltime_reach AAA999999_traveltime_synoptic	Sections 3.4.3., 4.1.
Water chemistry	AAA999999_waterchem_reach AAA999999_waterchem_synoptic	Sections 3.3., 4.2.
Width	AAA999999_widths_reach AAA999999_widths_synoptic	Sections 3.1.2.

6.3.2. Database structure

Data were entered into a MySLQ database. Data tables within the database follow a similar structure to the entry files detailed in Table 11, meaning they are grouped by topic (Table 12) and scale (Table 13). Table names are built out of the topic abbreviation, the specific variable within that topic (Table 12) and the scale of measurement (Table 13), for example METB22 = Metabolism data, specifically light data, collected at the reach scale. All database tables are summarized in Table 14, including details about variable units.

Table 12. Abbreviations of database tables based on their data topics. Topics are abbreviated by 3-4 letters, followed by a number to describe the specific type of data contained (variable code). *Some data are available via links on the SCALER webpage.

Abbreviation	Topic	Main variable(s)	Data collection and analysis methods
CalibrateBaro CalibrateLight CalibrateTemp	Calibration runs of sensors against NEON standard	NA (one column per sensor)	Section 3.4.1.
CalibrateBaroSite CalibrateLightSite CalibrateTempSite	Calibration runs of sensors after shipping	NA (one column per sensor)	Section 3.4.1.
BOMS	Benthic organic matter	1 = Benthic organic matter	Sections 3.6, 3.8.2., 3.8.4, 4.3., 4.6.
CHEM	Water chemistry	1 = Dissolved and particulate nutrients	Section 3.3., 4.2.
CNP*	Stoichiometry	1 = Carbon, nitrogen and phosphorous content	Sections 3.7., 3.8.3., 4.5.
CONS	Consumers	2 = Invertebrates	Sections 3.8.4, 4.6.
		3 = Macroconsumers	Section 3.2.
METB	Metabolism	1 = Oxygen	Sections 3.4.2., 3.8.1.
		2 = Light	Sections 3.4.2., 3.8.1.
		3 = Travel time	Sections 3.4.3., 4.1.
		4 = Discharge	Section 3.1.6.
		5 = Aeration (*)	Sections 3.4.3., 4.1.
NUTU	Nutrient uptake	1 = Ammonium	Sections 3.5., 3.8.1, 4.2.
PBIO	Primary producer biomass	1 = Chlorophyll *a*	Sections 3.6., 3.8.2., 4.4.
SITE	Site characteristics	1 = Site characteristics	Sections 3.1.1., 3.1.7.
		2 = Depth and substrate	Sections 3.1.2., 3.1.3.
		3 = Canopy cover	Section 3.1.5.
		4 = Discharge	Sections 3.1.6., 3.4.3.
		5 = Widths	Section 3.1.2.
		6 = Physico-chemical characteristics*	Section 3.1.4.
SlopeR2	Lux to PAR conversion	NA	Section 3.4.1.

Species	Macroconsumer species	NA	Section 3.2.
InvertSpecies	Macroinvertebrate species	NA	Section 4.6.

Table 13. Data within the topics were place in different tables based on the scale of data collection as the different scales required different site identifications. For some patch scale metrics data were only collected in 2013 due to the change in the consumer experiment (i.e., BOM, chl*a*, or invertebrates have same methodology at patch and reach scale, thus the removal of the patch scale manipulation reduced the data to the scale of reach)

Scale	Description	Site identification
Patch (=1)	Data collected at dm^2 scales, associated with patch measurements and in 2013 the consumer manipulations	Sample location ID by 5 columns: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ see section 2, Table 5 for biome abbreviations) 2. Site type: E = Experimental 3. Site number: 1-6 4. Patch number: 1-8 (2013), 1-10 (2014) 5. Patch treatment: IN (consumers reduced), OUT (consumers ambient); NA (2014)
Reach (=2)	Data collected at 10-100s m^2, associated within reach scale measurements and in 2013 the consumer manipulations (including dm^2 scales in the reach scale manipulation)	Sample location ID by 4-5 columns: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ see section 2, Table 5 for biome abbreviations) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014) 5. Within reach location: TOP (= upstream/start of the reach), BOT (= downstream/end of the reach); only applicable for certain variables
Synoptic (=3)	Data collected at 10-100s m^2, sites spread out across the steam network thus collectively > 100s m^2	Sample location ID by 3-4 columns: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ see section 2, Table 5 for biome abbreviations) 2. Site type: S = Synoptic 3. Site number: 1-20 4. Within reach location: TOP (= upstream/start of the reach), BOT (= downstream/end of the reach); 2014 only for certain variables

Table 14. Detailed description of all tables in the database (see table 12 for abbreviations of topic details). Site descriptor variables are summarized under the heading Scale as data were collected at three different scales (1=Patch, 2=Reach, 3=Synoptic) that also reflect different details of site designations and are thus included in the database table designation (final number) (see Table 13). See section 2, Table 5, for details on biome abbreviations. Each table also has a comments column in text format. SF6pulse, physical and stoichiometric data via web link.

Table name	Main variable	Scale (columns for site description)	Variables (units)	Primary key	Submission file	Data collection/ analysis methods
Calibrate Baro	Barometric pressure	NA	Timestamp (yyyy-mm-dd hh:mm:ss) One column per sensor plus a column for NEON standard (=calibrated)	Timestamp	NA	Section 3.4.1.
Calibrate Light	Light	NA	Date (yyyy-mm-dd) Time (hh:mm:ss) One column per sensor plus two columns for standards (PAR_NEON; PAR_Dodds)	Date + Time	NA	Section 3.4.1.
Calibrate Temp	Temperature	NA	Timestamp (yyyy-mm-dd hh:mm:ss) One column per sensor plus a column for NEON standard (=calibrated)	Timestamp	NA	Section 3.4.1.
Calibrate BaroSite	Barometric pressure	1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ)	Timestamp (yyyy-mm-dd hh:mm:ss) One column per sensor	Biome + Timestamp	AAA999999_CalibrateBaroSite	Section 3.4.1.
Calibrate LightSite	Light	1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ)	Date (yyyy-mm-dd) Time (hh:mm:ss) One column per sensor	Biome + Date + Time	AAA999999_CalibrateLightSite	Section 3.4.1.
Calibrate TempSite	Temperature	1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ)	Timestamp (yyyy-mm-dd hh:mm:ss) One column per sensor	Biome + Timestamp	AAA999999_CalibrateTempSite	Section 3.4.1.
BOMS11	Benthic organic matter	Patch: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: P = Patch 5. Patch number: 1-8 (2013 only) 6. Patch treatment: IN (consumers reduced), OUT (consumers ambient)	BOM type (CBOM or FBOM) Date collected (yyyy-mm-dd) Dry mass (mg m^{-2}) AFDM (mg m^{-2}) Ashed mass (mg m^{-2})	Scale + BOM type + Date	AAA999999_BOM_reach_patch	Sections 3.8.2., 3.8.4, 4.3., 4.6.

BOMS12	Benthic organic matter	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014) 5. Patch number: 1-8 (2013), 1-10 (2014)	BOM type (CBOM or FBOM) Date collected (yyyy-mm-dd) Dry mass (mg m^{-2}) AFDM (mg m^{-2}) Ashed mass (mg m^{-2})	Scale + BOM type + Date	AAA999999_BOM_reach_patch	Sections 3.6, 3.8.2., 3.8.4, 4.3., 4.6.
BOMS13	Benthic organic matter	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20 4. Transect: 1-10	BOM type (CBOM or FBOM) Date collected (yyyy-mm-dd) Dry mass (mg m^{-2}) AFDM (mg m^{-2}) Ashed mass (mg m^{-2})	Scale + BOM type + Date	AAA999999_BOM_synoptic	Sections 3.6, 4.3.
BOMSYN	Benthic organic matter	Method comparison (basket to synoptic): 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient) 5. Reference patch: 1-10	BOM type (CBOM or FBOM) Date collected (yyyy-mm-dd) Dry mass (mg m^{-2}) AFDM (mg m^{-2}) Ashed mass (mg m^{-2})	Scale + BOM type + Date	AAA999999_BOM_reach_synstyle	Sections 3.6, 4.3.

CHEM12	Water chemistry	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014) 5. Within reach location: TOP (= upstream/start of reach), BOT (= downstream/end of reach); NA (2014)	Date (yyyy-mm-dd) Ammonium (µg N L^{-1}) Detection limit (NH4-N) Nitrate (µg N L^{-1}) Detection limit (NO3-N) Soluble Reactive Phosphorus (µg P L^{-1}) Detection limit (SRP) Total Dissolved Nitrogen (µg N L^{-1}) Detection limit (TDN) Total Dissolved Phosphorus (µg P L^{-1}) Detection limit (TDP) Dissolved Organic Carbon (µg C L^{-1}) Detection limit (DOC) Particulate Phosphorus (µg P L^{-1}) Detection limit (PP) Particulate Nitrogen (µg N L^{-1}) Detection limit (PN) Particulate Carbon (µg C L^{-1}) Detection limit (PC)	Scale + Date	AAA999999 _waterchem _reach	Sections 3.3., 4.2.
CHEM13	Water chemistry	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20	Date (yyyy-mm-dd) Ammonium (µg N L^{-1}) Detection limit (NH4-N) Nitrate (µg N L^{-1}) Detection limit (NO3-N) Soluble Reactive Phosphorus (µg P L^{-1}) Detection limit (SRP) Total Dissolved Nitrogen (µg N L^{-1}) Detection limit (TDN) Total Dissolved Phosphorus (µg P L^{-1}) Detection limit (TDP) Dissolved Organic Carbon (µg C L^{-1}) Detection limit (DOC) Particulate Phosphorus (µg P L^{-1}) Detection limit (PP) Particulate Nitrogen (µg N L^{-1}) Detection limit (PN) Particulate Carbon (µg C L^{-1}) Detection limit (PC)	Scale + Date	AAA999999 _waterchem _synoptic	Sections 3.3., 4.2.

CONS21	Invertebrates	Patch: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: P = Patch 5. Patch number: 1-8 (2013 only) 6. Patch treatment (i.e., ExclosureType): IN (consumers reduced), OUT (consumers ambient)	Date (yyyy-mm-dd) SpeciesID Length (mm) Count	Scale + Date + SpeciesID + Length	AAA999999_Inverts_reach_patch	Section 3.8.4, 4.6.
CONS22	Invertebrates	Patch: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014) 5. Patch number: 1-8 (2013), 1-10 (2014)	Date (yyyy-mm-dd) SpeciesID Length (mm) Count	Scale + Date + SpeciesID + Length	AAA999999_Inverts_reach_patch	Section 3.8.4, 4.6.
CONS32	Macro-consumers	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced)	Date (yyyy-mm-dd) Pass (text, unique to Biome) Species (ID, see Species Table) Length (mm) Count (if no value = 1)	none	AAA999999_consumer_reach	Section 3.2.
CONS33	Macro-consumers	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20	Date (yyyy-mm-dd) Pass (text, unique to Biome) Species (ID, see Species Table) Length (mm) Count (if no value = 1)	none	AAA999999_consumer_synoptic	Section 3.2.

METB11	Oxygen	Patch: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Patch number: 1-8 (2013), 1-10 (2014) 5. Patch treatment: IN (consumers reduced), OUT (consumers ambient); NA (2014)	Timestamp (yyyy-mm-dd hh:mm:ss) Rate (ER = dark; NEP = light treatment) Dissolved oxygen (mg/L) Temperature (° C) Barometric pressure (kPa) Unit ID Folder (file name given during logging)	Scale + Timestamp	AAA999999_DO_patch	Section 3.8.1.
MET11_Blk	Oxygen	Calibration run without substrata: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Replicate 4. Patch Treatment	Timestamp (yyyy-mm-dd hh:mm:ss) Rate (ER = dark; NEP = light treatment) Dissolved oxygen (mg/L) Temperature (° C) Barometric pressure (kPa) Unit ID Folder (file name given during logging)	none	AAA999999_DO_patch_blk	Section 3.8.1.
METB11L	Oxygen	Test for linearity in oxygen changes: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Replicate	Timestamp (yyyy-mm-dd hh:mm:ss) Rate (ER = dark treatment; NEP = light treatment, 1L, 2L, 3L, 4L, 5L = Layers of mesh) Dissolved oxygen (mg/L) Temperature (° C) Barometric pressure (kPa) Unit ID Folder (file name given during logging)	Scale + Timestamp	AAA999999_DO_patch_linearitylight	Section 3.8.1.
METB12	Oxygen	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014) 5. Within reach location: TOP (= upstream/start of reach), BOT (= downstream/end of reach)	Timestamp (yyyy-mm-dd hh:mm:ss) Dissolved oxygen (mg/L) Temperature (° C) Barometric pressure (kPa) Unit ID	Scale + Timestamp	AAA999999_DO_reach	Section 3.4.2.

METB12C	Oxygen	Calibration run before/after deployment (reach): 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014) 5. Within reach location: TOP (= upstream/start of reach), BOT (= downstream/end of reach)	Timestamp (yyyy-mm-dd hh:mm:ss) Dissolved oxygen (mg/L) Temperature (° C) Barometric pressure (kPa) Unit ID Folder (file name given during logging; CAL)	Scale + Timestamp	AAA999999_DO_reach_cal	Section 3.4.2.
METB13	Oxygen	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20 4. Within reach location: TOP (= upstream/start of reach), BOT (= downstream/end of reach); 2014 only	Timestamp (yyyy-mm-dd hh:mm:ss) Dissolved oxygen (mg/L) Temperature (° C) Barometric pressure (kPa) Unit ID Folder (file name given during logging)	Scale + Timestamp	AAA999999_DO_synoptic; AAA999999_DO_synoptic(year2)	Section 3.4.2.
METB13C	Oxygen	Calibration run before/after deployment (synoptic): 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20 4. Within reach location: TOP (= upstream/start of reach), BOT (= downstream/end of reach); 2014 only	Timestamp (yyyy-mm-dd hh:mm:ss) Dissolved oxygen (mg/L) Temperature (° C) Barometric pressure (kPa) Unit ID Folder (file name given during logging; CAL)	Scale + Timestamp + UnitID	AAA999999_DO_synoptic_cal; AAA999999_DO_synoptic_cal(year2)	Section 3.4.2.
METB21	Light	Patch: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Patch number: 1-8 (2013), 1-10 (2014) 5. Patch treatment: IN (consumers reduced), OUT (consumers ambient); NA (2014)	Timestamp (yyyy-mm-dd hh:mm:ss) Lux (unitless) PAR (µE /cm2) Rate (ER = dark treatment; NEP = light treatment) Unit ID Calibration Date (yyyy-mm-dd) R2 (of lux to PAR conversion) Slope (of lux to PAR conversion)	Scale + Timestamp	AAA999999_Light_patch	Section 3.8.1.

METB21L	Light	Test for linearity in oxygen changes: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Replicate	Timestamp (yyyy-mm-dd hh:mm:ss) Lux (unitless) PAR (µE /cm2) Rate (ER = dark treatment; NEP = light treatment, 1L, 2L, 3L, 4L, 5L = Layers of mesh) Unit ID Calibration Date (yyyy-mm-dd) R2 (of lux to PAR conversion) Slope (of lux to PAR conversion)	Scale + Timestamp	AAA999999_Light_patch_linearitylight	Section 3.8.1.
METB22	Light	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014) 5. Within reach location: TOP (= upstream/start of reach), BOT (= downstream/end of reach)	Timestamp (yyyy-mm-dd hh:mm:ss) Lux (unitless) PAR (µE /cm2) Unit ID Calibration Date (yyyy-mm-dd) R2 (of lux to PAR conversion) Slope (of lux to PAR conversion)	Scale + Timestamp	AAA999999_Light_reach	Section 3.4.2.
METB23	Light	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20 4. Within reach location: TOP (= upstream/start of reach), BOT (= downstream/end of reach); 2014 only	Timestamp (yyyy-mm-dd hh:mm:ss) Lux (unitless) PAR (µE /cm2) Unit ID Calibration Date (yyyy-mm-dd) R2 (of lux to PAR conversion) Slope (of lux to PAR conversion)	Scale + Timestamp	AAA999999_Light_synoptic AAA999999_Light_synoptic(year2)	Section 3.4.2.
METB32	Travel time	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014)	Date (yyyy-mm-dd) Method (text; pulse or plateau) Travel time (min m^{-1})	Scale + Date + Method	AAA999999_traveltime_reach	Sections 3.4.3., 4.1.

METB33	Travel time	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20	Date (yyyy-mm-dd) Method (text; pulse or plateau) Travel time (min m^{-1})	Scale + Date + Method	AAA999999_traveltime_synoptic	Sections 3.4.3., 4.1.
METB42	Discharge	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014)	Timestamp (yyyy-mm-dd hh:mm:ss) Specific conductance (Sp; µS cm^{-1}) NaCl concentration (mg L^{-1}) Slope (Sp-NaCl conversion) Intercept (Sp-NaCl conversion) Relationship type (Sp-NaCl) NaCl added (g) Bromide conductance (mV) Br^- concentration (mg L^{-1}) Slope (mV-NaBr conversion) Intercept (mV-NaBr conversion) Relationship type (mV-NaCl) NaBr added (g)	Scale + Timestamp	AAA999999_SlugDischarge_reach	Sections 3.1.6., 3.4.3.
METB43	Discharge	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20	Timestamp (yyyy-mm-dd hh:mm:ss) Specific conductance (Sp; µS cm^{-1}) NaCl concentration (mg L^{-1}) Slope (Sp-NaCl conversion) Intercept (Sp-NaCl conversion) Relationship type (Sp-NaCl) NaCl added (g) Bromide conductance (mV) Br^- concentration (mg L^{-1}) Slope (mV-NaBr conversion) Intercept (mV-NaBr conversion) Relationship type (mV-NaCl) NaBr added (g)	Scale + Timestamp	AAA999999_SlugDischarge_synoptic	Sections 3.1.6., 3.4.3.

METB52	Aeration	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014)	Date (dd-MMM-YYY) Distance from injection site (m) Cl^- concentration (mg L^{-1}) Cl^- stock solution (g L^{-1}) Br^- concentration (mg L^{-1}) Br^- stock solution (mg L^{-1}) Rhodamine (fluorescence) Rhodamine stock solution (fluorescence) SF6 (peak area) SF6 stock solution (ml L^{-1}) Pump rate (ml min^{-1}) Temperature (°C)	Scale + Date + Distance	AAA999999_SF6plateau_reach	Sections 3.4.3., 4.1.
METB53	Aeration	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20	Date (dd-MMM-YYY) Distance from injection site (m) Cl^- concentration (mg L^{-1}) Cl^- stock solution (g L^{-1}) Br^- concentration (mg L^{-1}) Br^- stock solution (mg L^{-1}) Rhodamine (fluorescence) Rhodamine stock solution (fluorescence) SF6 (peak area) SF6 stock solution (ml L^{-1}) Pump rate (ml min^{-1}) Temperature (°C)	Scale + Date + Distance	AAA999999_SF6plateau_synoptic	Sections 3.4.3., 4.1.
NUTU11	Nutrient uptake	Patch: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Patch number: 1-8 (2013), 1-10 (2014) 5. Patch treatment: IN (consumers reduced), OUT (consumers ambient); NA (2014)	Date (yyyy-mm-dd) Sampling time (hh:mm) Chamber volume Ammonium (µg N L^{-1})	Scale + Date + Sampling time	AAA999999_ammuntake_patch	Sections 3.8.1, 4.2.

NUTU12	Nutrient uptake	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014)	Date (yyyy-mm-dd) Sampling time (hh:mm) Sample number (1-50) Ammonium (µg N L^{-1}) Chloride tracer (mg Cl^{-} L^{-1}) Bromide tracer (mg Br^{-} L^{-1})	none	AAA999999_ammuntake_reach	Sections 3.5., 4.2.
NUTU13	Nutrient uptake	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20	Date (yyyy-mm-dd) Sampling time (hh:mm) Sample number (1-50) Ammonium (µg N L^{-1}) Chloride tracer (mg Cl^{-} L^{-1}) Bromide tracer (mg Br^{-} L^{-1})	none	AAA999999_ammuntake_synoptic	Sections 3.5., 4.2.
PBIO11	Producer biomass	Patch: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: P = Patch 5. Patch number: 1-8 (2013) 6. Patch treatment: IN (consumers reduced), OUT (consumers ambient)	Date collected (yyyy-mm-dd) Substrate area (cm^2) Chlorophyll *a* (mg m^{-2})	Scale + Date	AAA999999_Chla_reach_patch	Sections 3.8.2., 4.4.
PBIO12	Producer biomass	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014) 5. Patch number: 1-8 (2013), 1-10 (2014)	Date collected (yyyy-mm-dd) Substrate area (cm^2) Chlorophyll *a* (mg m^{-2})	Scale + Date	AAA999999_Chla_reach_patch	Sections 3.8.2., 4.4.
PBIO13	Producer biomass	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20 4. Transect (unique to Biome)	Date collected (yyyy-mm-dd) Substrate area (cm^2) Chlorophyll *a* (mg m^{-2})	Scale + Date	AAA999999_Chla_synoptic	Sections 3.6., 4.4.

PBIOSYN	Producer biomass	Method comparison (basket to synoptic): 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient) 5. Reference patch: 1-10	Date collected (yyyy-mm-dd) Substrate area (cm^2) Chlorophyll *a* ($mg\ m^{-2}$)	Scale + Date	AAA999999_Chla_reach_synstyle	Sections 3.6., 3.8.2., 4.4.
SITE12	Site characteristics	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014)	Longitude (degree decimal) Latitude (degree decimal) Coordinate system Reach length (m) Slope (degree) Number of transects Spacing of transects (m)	Scale	AAA999999_sitecharacteristics_reach	Sections 3.1.1., 3.1.7.
SITE12	Site characteristics	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20	Longitude (degree decimal) Latitude (degree decimal) Coordinate system Reach length (m) Slope (degree) Number of transects Spacing of transects (m)	Scale + reach length	AAA999999_sitecharacteristics_synoptic	Sections 3.1.1., 3.1.7.
SITE22	Depth, Substrate	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014)	Date (yyyy-mm-dd) Transect (m) Substrate (mm) Depth (cm)	none	AAA999999_depthsubstrate_reach	Sections 3.1.2., 3.1.3.
SITE23	Depth, Substrate	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20	Date (yyyy-mm-dd) Transect (m) Substrate (mm) Depth (cm)	none	AAA999999_depthsubstrate_synoptic	Sections 3.1.2., 3.1.3.

SITE32	Canopy cover	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014)	Date (yyyy-mm-dd) Transect (m) CanopyN (points open 0-96) CanopyE (points open 0-96) Canopy S (points open 0-96) CanopyW (points open 0-96)	Scale + Date + Transect	AAA999999_canopy_reach	Section 3.1.5.
SITE33	Canopy cover	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20	Date (yyyy-mm-dd) Transect (m) CanopyN (points open 0-96) CanopyE (points open 0-96) Canopy S (points open 0-96) CanopyW (points open 0-96)	Scale + Date + Transect	AAA999999_canopy_synoptic	Section 3.1.5.
SITE42	Discharge	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014)	Date (yyyy-mm-dd) Discharge (Q; $m^3 s^{-1}$)	Scale + Date	AAA999999_discharge_reach	Sections 3.1.6., 3.4.3.
SITE43	Discharge	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20	Date (yyyy-mm-dd) Discharge (Q; $m^3 s^{-1}$)	Scale + Date	AAA999999_discharge_synoptic	Sections 3.1.6., 3.4.3.
SITE52	Width	Reach: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: E = Experimental 3. Site number: 1-6 4. Reach type: C = Control (consumers ambient), T = Treatment (consumers reduced); C or NA (2014)	Date (yyyy-mm-dd) Width (m)	none	AAA999999_widths_reach	Sections 3.1.2.

SITE53	Width	Synoptic: 1. Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) 2. Site type: S = Synoptic 3. Site number: 1-20	Date (yyyy-mm-dd) Width (m)	none	AAA999999_widths_synoptic	Sections 3.1.2.
SlopeR2		Lux to PAR conversion	Unit ID (Probe#) Slope R^2	none	NA	Section 3.4.1.
Species	Macro-consumer species	NA	SpeciesID Biome (AND, ARC, AUS, CPC, CWT, KNZ, LUQ) Group (Fish, Amphibian, Crustacea) Family Genus Species Common name Trophic guild (Omnivore, Algivore, Insectivore, Carnivore) Position (Benthic, Water column) Intercept Slope Citation (for length to weight conversion)	Species ID	AAA999999_Macroconsumer_species	Section 3.2.

InvertsSpecies	Invertebrate species	NA	SpeciesID Taxon Family Sub-falmily Genus Species Life stage (A=Adult, L=Larva, N=Nymph, P=Pupa, T=Terrestrial, LT=Terrestrial Larva, AT=Terrestrial Adult) FFG (CF=Collector-Filterer, CG=Collector-Gatherer, Ch?, FF=Filter-Feeder, Ominvore, P=Predator, Pa=Parasite, Ph=Piercer-Herbivor, Sc=Scraper, Sh=Shredder) a b Type (i.e., Power, Exp, Constant) Biomass type (mg) (DM=drymass (mg), DM µg = drymass (µg), AFDM=ash-free drymass) Source Biomass conversion (citation)	Species ID	AAA999999_Inverts_species	Section 4.6.

6.3.3. Raw data entry qa/qc

If possible, data were assigned a primary key to avoid duplicate lines (e.g., not possible for macroconsumers as each individual consumer was measured and noted separately). Additionally, to avoid issues of site identification assignments sensor data were checked for changes in site description related to changes in timestamps. All variables were given an allowed range to avoid unreasonable values due to typos. Certain data were corrected after initial data analyses to correct potential entry errors. Missing values were entered as -9999.

6.4. Backup procedures

All notebooks were scanned and the scans uploaded to the ***scanned notebooks*** folder in the "data" folder of the "SCALER all" Dropbox. All data files to be incorporated into the database (i.e., all files listed in Table 11) were placed in the ***new data*** folder in the "data" folder of the "SCALER all" Dropbox. Raw data were then incorporated into the database by the data manager and files moved to the "processed data" folder. The Dropbox folder was backed up by the data manager. All data were also backed up on individual or lab-based external hard drives.

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8. Glossary

Biome – The five (+2) different areas participating in the SCALER project are referred to as biomes to separate and avoid confusion with site (see below).
Habitat – A type of habitat (e.g., riffle, run,, pool), but often also used to describe the patch (~1 m^2) scale manipulation of consumers or location of basket placement.
Reach – One of the three reaches in the consumer manipulation areas (i.e., control, patch/habitat, treatment).
Site – Study area within a biome, either one of the 20 synoptic ones, or one of the 6 consumer manipulation areas.