Investigations with NEON: Variability of macroinvertebrates versus urban and rural temperature dynamics in streams (INVURTS).

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If you have any questions, comments, additional resources, strategies you used for implementation, lessons learned, or suggestions to improve our protocols, please email us at **invurts@gmail.com**

Background

Inspired by a previous EREN project that investigated the effect of riparian forest cover on stream temperature (Simmons et al. 2015), we are investigating the interactions of temporal variability of stream temperature and aquatic macroinvertebrate communities. Large-scale climate differences (biomes) and more local land-use changes (urban, suburban, and agricultural development) affect water temperature regimes. To assess potential trends and interactions at a broad spatial scale, we plan to combine data from sites in the NEON network and sites at participating institutions. See a map of our current sites and the NEON network here.

Thermal regimes encompass the magnitude, frequency, duration, timing, and rate of change (variability) of stream temperatures. However, assessments of temperature change are frequently restricted to assessing the change in mean temperatures. Compared with studies of the biological effects of changing mean temperatures, relatively little field research has investigated how stream macroinvertebrates respond to the level of variability in habitat temperatures (Simmons et al. 2015, Steel et al. 2017). Increased variability, i.e. rate of change at scales from one day to one year, is expected to increase organismal stress, change development rates and alter intra- and inter-species interactions (Vasseur et al. 2014, Steel et al. 2017), particularly if changes are occurring more rapidly.

To date, there are a few field studies with evidence for differences in temperature variability affecting stream communities. For example, species composition of macroinvertebrates and overall food-chain lengths are negatively affected by increasing temperature variability in alpine streams (Hette-Tronquart et al. 2013). Based on temperate-stream field and lab studies, Vannote and Sweeney (1980) outlined several predictions for how changing temperature regime could negatively affect macroinvertebrate species composition and population abundance. However, Stanley and Short (1988) did intensive field research on mayfly species in four Texas streams and concluded that the abundance and biomass responses of a species to more variable temperature depended on the species (i.e. native range and life history). Shah et al. (2017) compared tropical and temperate locations and found that thermal tolerances of EPT taxa were aligned with the thermal variabilities of their native habitats. With climate change and land use changes not only increasing mean temperatures but also causing more extremes and variability within and among years and seasons, understanding these impacts is ever more pressing.

Recruiting participants to represent a diverse geographic region, the INVURTS project investigates questions about macroinvertebrate responses to changing temperature regimes, specifically temperature variability. Assemblages are expected to interact with not only the magnitude and scale of the disturbance, but also local resilience (e.g. species richness). Any changes in select species may also depend on their larger context (e.g. biogeography, species-specific constraints).

Therefore, the INVURTS project is organized to test three very general hypotheses. Firstly and secondly, we plan to use community diversity data collected from sites representing a broad range of climates and urban-rural land-use impacts. Thirdly, we have also included an analysis of population data for testing ideas about how temperature constrains the range and success of individual species:

Hypotheses/Objectives:

- **Hypothesis 1 (Intermediate Disturbance Hypothesis)**: More extreme (high or low) daily/seasonal/annual temperature variability will decrease biodiversity of stream macroinvertebrates due to species' limits, therefore, highest biodiversity is expected at sites with predictable, intermediate levels of variability.
- **Hypothesis 2 (Urban Stream Syndrome)**: Stream macroinvertebrate biodiversities in urban streams are more similar to each other across NEON Domains than to other sites within a given NEON Domain, because as urban streams become more homogenized in their physico-chemical characteristics (Walsh et al., 2005), including temperature regime, they become more similar in their community composition.
- **Hypothesis 3 (Thermal Equilibrium Hypothesis)**: An optimum equilibrium between temperature & growth exists; therefore, populations of a species at suboptimal sites are expected to exhibit low population density and smaller individuals versus more optimal sites.

Summary of Field Methods:

To test these hypotheses, participants will:

- **Identify one or more sample locations.**
- **Install a temperature logger** or have publicly available stream temperature data within 1 km of your location. We ask for one year (or more!) of continuous temperature data. We prefer temperature data collection starts ~3 months before stream sampling.
- **Capture macroinvertebrate samples** and/or **take environmental DNA samples** at least one season per year of temperature data.

Instructor overview of Field Methods

********Blue underlined text* **indicates link to more information.*****

Alternatively, you can *use this link to access the entire folder with all documents/template spreadsheets etc. If you want to edit any files for your own purposes, you will need to download a copy. Comments are enabled - help us improve our materials during the project phase with needed clarifications, details etc!*

Find a study location and identify your Fall, Spring, Summer sampling windows:

To participate in this project, you should have access to a wadeable stream that you can generate/access temperature data for and sample in at least one of the sampling windows (Fall,

Spring, Summer) by collecting data on the macroinvertebrate community composition using traditional methods such as a kicknet or dip net and/or collecting and filtering water to extract environmental DNA.

Identify an already installed temperature logger or install your own:

Our hypothesis focuses on the effect of temperature variability. This means that we need hourly temperature data for an extended time period before/after you sample the biological community for at least one year of data per site. If you can access a location near (within 1km) of a temperature logger with publicly available data that is great. If not, you should install a temperature logger as soon as possible. While most loggers will last for years, we would ask that you download and submit your temperature data quarterly[1]. As we send out updates quarterly-ish, we will include reminders to submit data.

Complete a habitat assessment:

The watershed and stream characteristics can be important determinants for thermal regimes and the level of temperature variability. You will be asked to complete a widely implemented habitat assessment survey to gather data on your stream for each sample.

Collect data on macroinvertebrate community composition

Option 1: Sample macroinvertebrates in the field, identify them in the lab, and archive them for future further identification.

We have adapted macroinvertebrate sampling protocols to create an easy-to-follow overview of different sampling techniques you can use appropriate to the characteristics of your stream site to generate a representative sample of the aquatic macroinvertebrate community. There are three sampling windows, you are not expected to sample in all three[2] but ideally you are able to sample in at least one!

Faculty are not expected to be experts at identifying their own macroinvertebrates to participate in this project. Identifying to order is not super hard. Identifying to family can be done using keys such as macroinvertebrates.org and provides some good experiences to students.

You will be asked to organize and archive your specimens in Ethanol for potential future identification to a lower taxonomic level[3] and to submit the provided spreadsheet template with data recorded to the taxonomic level (Family whenever possible, Order when unsure) that you are comfortable with.

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[1] Depending on your stream and how accessible it is to you, it is advisable to download your data even more regularly than that (e.g. every few weeks). Temperature loggers have been known to be washed away or removed by curious passersby and we would hate to lose your data.

[2] See option 2 for a less labor intensive way of generating biological data on a more regular basis.

[3]If you have some taxonomic expertise and would be willing to help with this aspect of the project, please get in touch with us!

Option 2: Take a water sample and filter it for environmental DNA

Environmental DNA can be used to generate a complementary data set to characterize biological communities next to traditional techniques. We will supply you with a sampling kit that you can use to filter water samples (3 field replicates + 1 field/lab blank per sampling window) using a set up as simple as a vacuum flask, Buchner filter, and (hand) vacuum pump as well as preserve the filters and mail them back. We will then extract DNA and metabarcode samples and perform some bioinformatics "magic" to identify macroinverts present in the sample.

Remember to record your data and submit it

For this project, you will be gathering a range of different types of data sets. We have templates for spreadsheets to help with data recording. For each data set you will be asked to submit files in a specific format with specific naming conventions that are designed to make it easier for us (and you) to wrangle all the individual data sets into a single set across individual sites for each data set and combine data sets in a straightforward way for downstream analysis. Eventually, we will be staging raw data files and code on how to wrangle them into a complete data set for analysis on **github.** Check back for updates as our project makes progress!

Data Analysis, Presentation, and Publication

Periodically, we like to present our data at conferences and workshops. All participants are given the option to be included as authors. ***Regularly submitting data as you gather it, helps us improve the project and better understand how to analyze our data to answer our research questions***. If you are interested in analyzing a subset of INVURTS data to present, or have a research question you want to pursue, feel free to reach out!

Authorship and other details regarding the preparation of papers from this project will follow the **EREN Authorship Guidelines** [Note: These were revised July 2024.]

Course implementation

Student skills & learning outcomes

Activities for this project are broad in questions/skills they address and can be implemented in a wide-range of courses for entry-level science and non-majors courses to upper-level courses in ecology and environmental science, as well as independent research projects completed by individual students.

Required skills

- No biological background is needed to participate in this activity and you will be able to tailor how much background information students need compared to what new knowledge they build during this unit based on your class level. Helpful background info could include basic invertebrate taxonomy and functional feeding groups.

- For identification purposes, some familiarity with the concept of a dichotomous key is helpful, but all students who participate in macroinvertebrate specimen identification will learn to use a dichotomous key and vocabulary/taxonomy of invertebrates.
- For data analysis, students should have background knowledge of basic algebra and categorization of discrete vs. continuous variables and have a fundamental knowledge of how to interpret standard plots (scatterplot, histogram, barplot).

Acquired skills (will vary depending on implemented research/teaching modules)

- Variety of field methods to sample aquatic macroinvertebrates.
- Using a dichotomous key to identify aquatic macroinvertebrates.
- Analyze and visualize stream temperature variability using R or Excel.
- Analyze macroinvertebrate data sets (diversity stats) using R or Excel.
- Determine watershed characteristics using **StreamStats**, **Model My Watershed**, and/or other GIS tools.

Student activities

Instructors can choose options from our "menu" to fit their course format/level and desired learning outcomes. Given the broad scope of methods implemented for this project classroom implementation can range from one or two lab periods in a course to having research students working on data collection over the semester. These modules can form a semester-long project (CURE-style) that goes through habitat assessment (GIS), to field sampling, identification in the lab, development of local hypotheses, evaluation etc.

- One class period for preparing students for field work, for example stream sampling methods, habitat assessment practice, organization of groups, what to wear, etc. Doing these things will allow for lab time to be used more efficiently (i.e. more time for sampling)
- (optional) one class period introducing macroinvertebrates, functional feeding groups, practice identifying to Order using examples, brainstorming questions about who lives where, draft testable hypotheses, etc - see **local hypothesis testing starter kit**)
- 1 lab period for field sampling and habitat assessment (depending time to reach site, 1-2 hrs + transport time).
- 1 - 2 lab periods for macroinvertebrate identification.
- Additional lab period(s) to analyze habitat, macroinvertebrate, and/or temperature data sets (especially if students are developing local hypotheses).
- Additional lab periods for teaching modules on habitat/watershed characterization (GIS), impact of thermal regimes on habitat availability, eDNA/bioinformatics, invertebrate diversity/feeding groups etc.

Options for accessibility: Field work is frequently a memorable experience for students as they are pushed out of their comfort zone. However, for some students safely accessing and participating can be difficult depending on your sample site. Increasingly, for some students this might be the first time they are experiencing the "wild outdoors." We recommend making sure

these students are able to participate by assigning them specific roles such as data manager, lead on sample ID from the safety stream bank, or habitat/watershed characterization.

Planned and available teaching modules

We are still in the process of developing most of the teaching modules that build on the basic questions and procedures of the project. These will be made available via these links as they are completed - if you are interested in helping us develop one of these modules, or have developed another one you are interested in sharing please let us know. If you have students doing directed/independent research feel free to share their posters/theses with us to add to our research project idea repository.

Here are some modules we're developing, drafts are available in our teaching materials library. We're happy to share our experiences and hear from you!

- **Temperature variability module**: Temperature is one of the most important factors determining habitat availability. For cold-water fishes such as brook trout, more variable temperatures can be especially stressful - can you identify streams that should be prioritized for conservation as potential cold-water refugia?
- **Watershed and riparian properties (StreamStats)**: Understanding the influence of watershed and riparian properties on stream ecosystems is critical for effective conservation and management - how are the macroinvertebrate communities in your stream different/similar to streams with similar/different watersheds? Are there patterns related to land use and shared biomes?
- **Macroinvertebrates (functional feeding groups)**: Species richness and diversity are not the only way we can measure the diversity of a community. For example, aquatic macroinvertebrates are also classified by their function group including shredders, grazers, filter feeders, and predators each of which contributes to nutrient cycling and energy transfer within the ecosystem - what can you learn about the ecological functioning of your stream from the macroinvertebrates present in your ecosystem?
- **Idea starter kit to test local hypothesis** (macroinvertebrate communities): Aquatic macroinvertebrates are important bioindicators of stream health and fill a wide range of ecosystem roles. Similarly, streams are heterogeneous ecosystems with lots of microhabitats. What questions/hypotheses can you formulate about the aquatic macroinvertebrates/stream ecosystems and how can you use the data collected by you and your classmates to test your ideas?
- **Introduction to metabarcoding for community characterization** [*TBD*]: With rapidly increasing availability of sequencing and computational power metabarcoding has become an increasingly powerful tool to characterize and monitor biological communities by amplifying and sequencing specific genetic markers (frequently ribosomal RNA genes) in a sample that contains DNA from a variety of organisms - can you master the fundamental steps of processing millions of individual reads

(sequences) to identify unique sequences present in the data set and match those to a reference database to determine which species are present in your data set? Do your genetic results match traditional data sets based on morphological IDs?

Sold? use this form if you are interested in participating: Tell us about yourself, your research questions, your class, and we'll reach out to you.

Materials and Cost List

Cost to participate will depend on which pieces of equipment/reagents are already on hand. Here is an overview of what materials could be part of your set-up so you can assess what you already have on hand and what you would need to acquire, details on set ups and alternatives are found in each of the specific protocols.

Item	Use	Cost range/Potential source
Onset® HOBO® TidbiT MX2203 Temperature Data Logger or HOBO Water Temperature Pro v2 Data Logger similar temperature logger	In situ temperature recording (instrument)	$140 - 150, e.g. Forestry Suppliers
Temperature logger encasing, e.g. HOBO hard case/boat	In situ temperature recording (protection)	$50 - $100 or a lot cheaper if building your own.
Rebar, rubberized steel cables, earth anchor, e.g. Duckbil	In situ temperature recording (optional anchoring depending on conditions)	$20-50
Clipboards	Habitat assessment	$10-20
Meter sticks, meter tapes	Habitat assessment	$10-35
Thermometers	Habitat assessment	
Turbidity Tubes, Sediment Sticks (optional)	Habitat assessment	$20 (soil sticks) - $120 (turbidity tube)
Kick net, Serber sampler, Hess sampler (600 um mesh)	Macroinvertebrate sampling	~$150 e.g., https://www.carolina.com/environmental-science-field-collection/net-kick/652165.pr

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Triangle or D-frame net (600 um mesh)	Macroinvertebrate sampling	~$150-250, e.g., https://www.carolina.com/environmental-science-field-collection/net-d-frame-aquatic/651297.pr
Sieve bucket or sieve (600 um mesh or #30)	Macroinvertebrate sampling	~$25-125, e.g., https://www.forestry-suppliers.com/p/77255/50011/watermark-wash-bucket-littoral-samples
White wash tubs or picking trays	Macroinvertebrate sampling	$5-30
Squirt bottles	Macroinvertebrate sampling	
Forceps of varying sizes	Macroinvertebrate sampling	
95% Ethanol	Macroinvertebrate sampling	
Sample containers	Macroinvertebrate sampling	
Forceps, needle tools, finger bowls, etc.	Macroinvertebrate ID	
Dissecting microscopes	Macroinvertebrate ID	
Sample jars, specimen storage containers, ethanol	Macroinvertebrate ID and archive	
Polypropylene Nalgene bottles or glass bottles	eDNA samples	E.g. **Nalgene (6 pack)** or **glass bottles** **(case of 10)**
See also PPCO (polypropylene co-polymer) Nalgene bottles (autoclavable)	eDNA samples	e.g.**Nalgene 6-pack**
Buchner Filter (47mm)	eDNA samples	$20-60 e.g. **option filter only** or **option for complete set up** ($60)
Vacuum Flask	eDNA samples	$20-30 e.g. **glass flask** or **option for complete set up** ($60)
Vacuum pump & tubing	eDNA samples	$30-50 for **hand pump** or

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		option for complete set up ($60)

Additional helpful resources

This is a preliminary short-list of helpful resources to familiarize students with concepts and techniques. Detailed protocols and teaching modules contain additional resources. If you have resources that have been helpful to your student that you have developed and are willing to share or are otherwise publicly available please let us know.

- sampling macroinvertebrates (Ohio U, 6') -- Video demonstrates kick nets and D-nets
- Ohio's Scenic Rivers (ODNR, 2022, 3')
- OSR monitoring program (ODNR, 1')
- Riffles, pools and runs (U Windsor)
- Macroinvertebrate ID (Stroud Water Research Center)
- Macroinvertebrates.org also comes as an App: PocketMacros
- Filtering water for eDNA – demonstrates set up using Buchner filter

References

All of these and more can be found in our Zotero library

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