

# Wetland Macroinvertebrate Sampling & MacroIBI App Guide

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*A complete workflow for field sampling, lab sorting, identification, and IBI calculation.*

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## 1. Overview

This guide explains how to collect, sort, identify, and score wetland macroinvertebrates using the MPCA protocol and the MacroIBI R/Shiny application. No prior R experience is required, this document walks you through installation and use of the app step-by-step.

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## 2. Equipment Checklist

### Required Field Equipment

- Two D-frame dip nets (500 µm mesh)
- Hardware cloth + frame
- Plastic bin for catching material
- 200 µm sieve
- Squirt bottles (1 water, 1 alcohol)
- Forceps (one per person)
- Wetland Invertebrate Visit Form
- GPS unit
- Pencils, clipboards
- 100% reagent alcohol
- Chest waders
- Plastic jars for storage
- Permanent marker

### Optional Field Equipment

- Maps for navigation
- Scissors for labels

- Camera to capture site conditions
- Cooler or crate for transporting samples

## Required Lab Equipment

- Stereoscopes (2x-4x magnification)
  - Petri dishes
  - Identification guides
  - Squirt bottles (water or alcohol)
  - Dissection tools
  - Computer with R and the **macroibi** package
- 

## 3. Collecting Macroinvertebrate Samples

### When to Sample

- Sample **June–early July** while larvae are mature but wetlands retain water.
- Sampling too late risks dried wetlands or transient species.
- If multiple samples are planned, keep all events within this timeframe.

### Where to Sample

Prioritize habitat zones in this order:

1. Emergent vegetation (richest fauna)
2. Floating-leaf vegetation
3. Submerged vegetation
4. Shallow open water

Record the zone(s) used.

### Sampling Procedure

#### 1. Identify the sampling area.

Select the highest-priority habitat available and work within a 10-15 m radius.

#### 2. Collect two efforts per sample.

For each effort, perform **3-5 strong sweeps** through vegetation and water.

*Avoid scraping mud; restart the effort if so.*

#### 3. Sort for 10 minutes.

Dump both efforts onto hardware cloth above the bin.

Start a 10-minute timer and begin sorting (see next section).

#### 4. Repeat for the second sample.

Stay in the same zone but choose new micro-locations.

Samples may be combined or kept separate for processing; both ultimately contribute to one IBI evaluation.

**Tip:** With larger crews, some people can begin the second sample while others finish the 10-minute sort; this saves significant time.

## 4. Sorting Samples in the Field

### During the 10-Minute Sorting Window

1. Rinse vegetation so organisms fall into the pans.
2. Pick organisms with forceps into the water-filled pans.
3. After 10 minutes, return vegetation to the wetland; all organisms in the bin become the sample.

### After Vegetation Removal

1. Pour pans through the 200 µm sieve; flush snails/leeches into the sieve.
2. Back-flush the sieve **with alcohol only** into sample jars.
  - Aim for ~80% final concentration.
  - Split jars if >1/3 full.
3. Label jars (site ID, date, sample #, jar #, crew initials).
4. Store in hazardous-materials room; check periodically for evaporation.

## 5. Lab Identification & Enumeration

### 1. Software Setup

Ensure R + MacroIBI are installed (see next section).

### 2. Prepare Workspace

Set up stereoscopes, petri dishes, tools, guides, and your sample.

### 3. Identify & Count Everything

- Every individual in the sample must be identified and counted.
- Family-level ID is required; genus ideal; species when possible.
- One person should handle data entry; others relay counts.
- Pre-sort into visually similar piles to speed identification.
- Samples may be rinsed with water to reduce irritation during the process, BUT;
- If samples must remain preserved after ID, do not use any water.

### 4. Enter Data Periodically

Relay taxa and counts to the data handler for entry into the app.

- Combined samples may all be entered into "Dipnet 1."
- See Section 7 for full app workflow.

## 6. Installing the MacroIBI App

This section walks you through installing everything you need to run MacroIBI. **No prior experience with R or programming is required.** Follow each step in order.

## What You're Installing

You need two free programs:

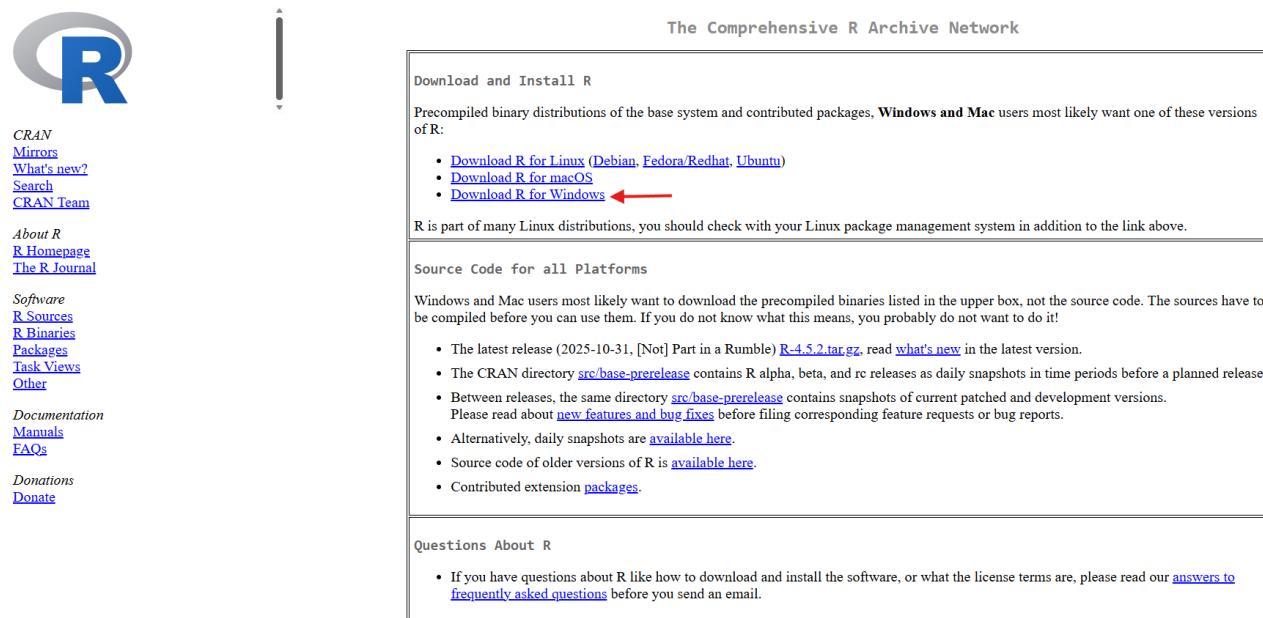
Program	What It Does
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<b>R</b>	The engine that powers the app. You won't interact with it directly.
<b>RStudio</b>	The program you'll actually use. It provides a user-friendly interface for running R commands.

Think of R as the engine and RStudio as the dashboard of a car — you need both, but you only interact with the dashboard.

## Step 1: Install R

1. Go to <https://cran.r-project.org>
2. Click the download link for your operating system:
  - **Windows:** Click "Download R for Windows" → "base" → "Download R-4.x.x for Windows"
  - **Mac:** Click "Download R for macOS" → Choose the version for your Mac (Apple Silicon or Intel)
3. Open the downloaded file and run the installer
4. **Accept all default options** — just keep clicking "Next" or "Continue"



The screenshot shows the CRAN website's 'Download and Install R' section. On the left, there's a large blue 'R' logo and a sidebar with links like 'CRAN MIRRORS', 'About R', 'Software', 'Documentation', and 'Donations'. The main content area has three sections: 'Download and Install R', 'Source Code for all Platforms', and 'Questions About R'. In the 'Download and Install R' section, there's a list of download links for Linux, macOS, and Windows. The 'Download R for Windows' link is highlighted with a red arrow. Below this, a note says 'R is part of many Linux distributions, you should check with your Linux package management system in addition to the link above.' The 'Source Code for all Platforms' section explains how to get source code for Windows and Mac users. The 'Questions About R' section provides links for answers to frequently asked questions.

✓ **Success:** The installer finishes without errors. You don't need to open R directly.

## Step 2: Install RStudio

1. Go to <https://posit.co/download/rstudio-desktop/>
2. Scroll down to "All Installers" and click the download for your operating system
3. Open the downloaded file and run the installer
4. **Accept all default options**



creators of RStudio, built for the next generation of data science workflows with R and Python.

## 1: Install R

RStudio requires R 3.6.0+. Choose a version of R that matches your computer's operating system.

*R is not a Posit product. By clicking on the link below to download and install R, you are leaving the Posit website. Posit disclaims any obligations and all liability with respect to R and the R website.*

[DOWNLOAD AND INSTALL R](#)

## 2: Install RStudio

[DOWNLOAD RSTUDIO DESKTOP FOR WINDOWS](#)



Size: 314.17 MB | [SHA-256: 525403C0](#) | Version: 2026.01.0+392 | Released: 2026-01-06

✓ **Success:** RStudio appears in your applications/programs.

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### Step 3: Install Rtools (Windows Only — Required)

**Mac users:** Skip to Step 4.

**This step is required on Windows.** Rtools allows R to install packages that need compilation.

1. Go to <https://cran.r-project.org/bin/windows/Rtools/>
2. Download "Rtools44" (or the version matching your R version)
3. Run the installer
4. **Accept all default options**

[RTools: Toolchains for building R and R packages from source on Windows](#)

Choose your version of Rtools:

[RTools 4.5](#)  
[RTools 4.4](#)  
[RTools 4.3](#)  
[RTools 4.2](#)  
[RTools 4.0](#)  
[old versions of RTools](#)



for R versions from 4.5.0 (R-prerelease and R-devel)  
 for R versions 4.4.x (R-release)  
 for R versions 4.3.x (R-oldrelease)  
 for R versions 4.2.x  
 for R from version 4.0.0 to 4.1.3  
 for R versions prior to 4.0.0

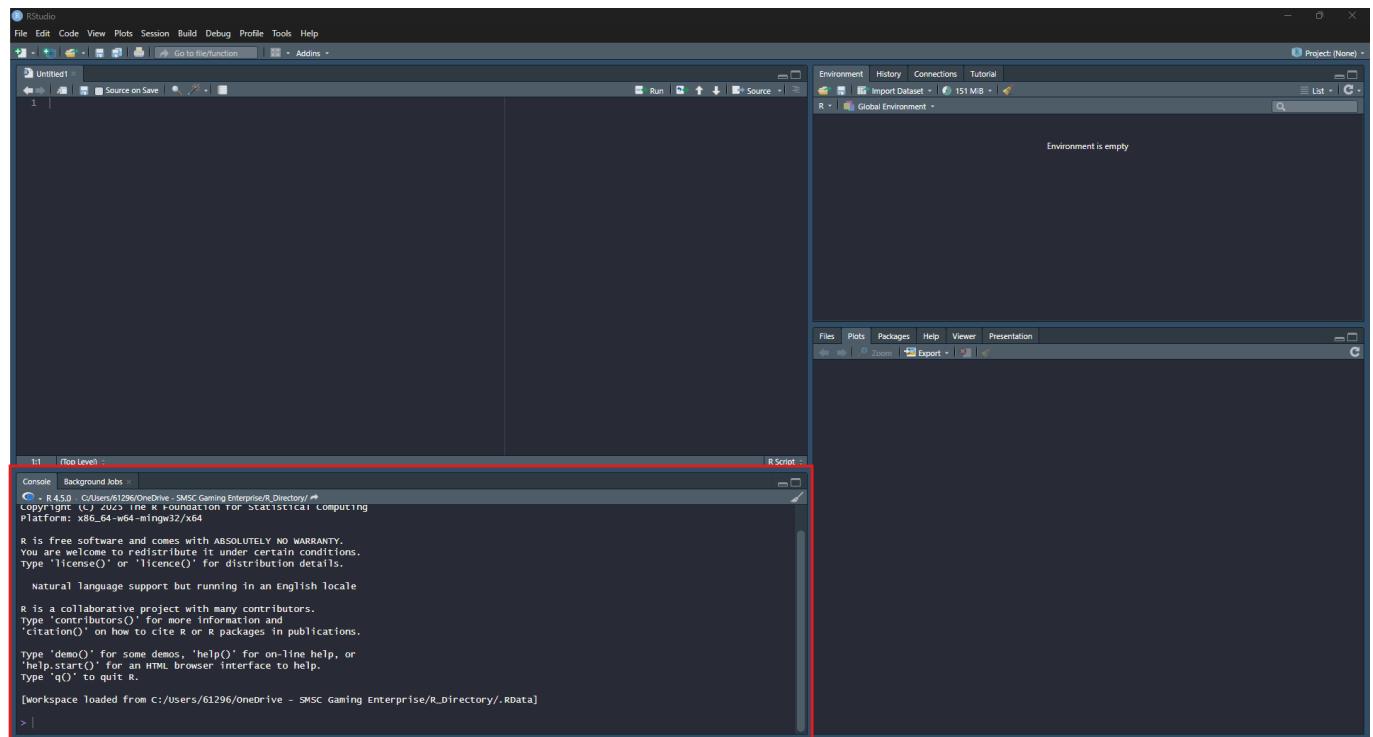
✓ **Success:** The installer completes. You won't see Rtools anywhere — it works behind the scenes.

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### Step 4: Open RStudio

- **Windows:** Click the Start menu and search for "RStudio", then click to open
- **Mac:** Open Finder → Applications → RStudio

When RStudio opens, you'll see a window divided into panels. Look for the **Console** — it's the panel with a > symbol where you can type commands. This is usually in the bottom-left area.



## Step 5: Install the MacroIBI Package

Now you'll type commands in the Console. Here's how:

1. **Click in the Console** (next to the > symbol)
2. **Type or copy-paste** the command shown below
3. **Press Enter** to run it
4. **Wait** for the > to reappear before typing the next command

### First command — Install the "remotes" helper package:

```
install.packages("remotes")
```

You'll see text scrolling as R downloads and installs the package. **This is normal.**

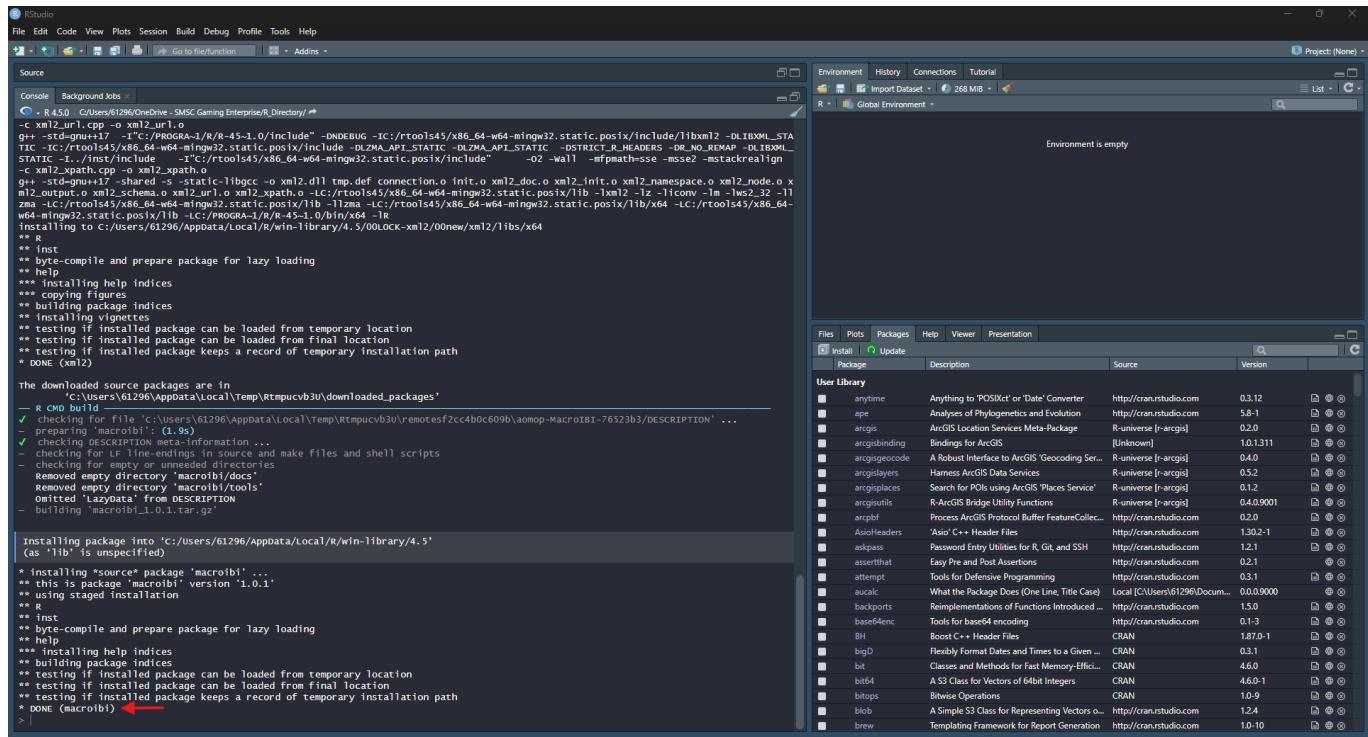
✓ **Success:** You see a message containing **package 'remotes' successfully unpacked** and the > prompt returns.

### Second command — Install MacroIBI:

```
remotes::install_github("aomop/MacroIBI")
```

This downloads MacroIBI from the internet. It may take 1-2 minutes. You'll see lots of text — **this is normal**.

✓ **Success:** The console says \* DONE (macroibi) and the > prompt returns.



```

R 4.5.0 -- Copyright (C) 2022 The R Foundation for Statistical Computing
  www.R-project.org
  License: GPL (>= 2)
  Platform: x86_64-w64-mingw32/x64_64-bit

  R is free software and comes with ABSOLUTELY NO WARRANTY.
  You are welcome to redistribute it under certain conditions.
  Type 'license()' or 'licence()' for distribution details.

  Natural language support but running in an English locale
  R is a language and environment for statistical computing and graphics
  and is based on the S language and environment which was developed at
  the Department of Statistics, University of Vienna, Austria, in the 1990's.
  The R development team is located at the R Core Team
  R Core Team (2022). R: A Language and Environment for Statistical Computing.
  Vienna, Austria: R Foundation for Statistical Computing.
  ISBN 3-900051-07-0
  https://www.R-project.org

  R is distributed under the terms of the GNU General Public License (GPL),
  version 2 or later. See the file COPYING in the R source code distribution
  for more information.

  Recommended citation:
  R Core Team (2022). R: A Language and Environment for Statistical Computing.
  Vienna, Austria: R Foundation for Statistical Computing.
  ISBN 3-900051-07-0.
  https://www.R-project.org

  Type 'citation("R")' or 'citation("rstats")' for citing R or its documentation.

  Attaching package: 'macroibi'

  The downloaded source packages are in
  'C:/Users/61296/AppData/Local/RtmpRtmptcvb3U/downloaded_packages'
  ✓ R CMD check
  ✓ checking for file 'C:/Users/61296/AppData/Local/RtmpRtmptcvb3U/remotesf2cc4b0c609b/`aomop-MacroIBI-76523b3/DESCRIPTION' ...
  ✓ preparing 'macroibi': (1.9s)
  ✓ checking DESCRIPTION meta-information ...
  - checking for LF line-endings in source and make files and shell scripts
  - checking for empty or unneeded directories
  - removing unused dependencies
  - removing unused executables and objects
  Removed 1 unused directory: 'macroibi/tools'
  Omitted 'LazyData' from DESCRIPTION
  - building 'macroibi_1.0.1.tar.gz'

  installing package into 'C:/Users/61296/AppData/Local/R/win-library/4.5'
  (as 'lib' is unspecified)

  * installing *source* package 'macroibi' ...
  ** this package 'macroibi' version '1.0.1'
  ** using staged installation
  ** R
  ** inst
  ** byte
  ** byte-compile and prepare package for lazy loading
  ** help
  *** installing help indices
  *** building package indices
  *** testing if installed package can be loaded from temporary location
  *** testing if installed package can be loaded from final location
  *** testing if installed package keeps a record of temporary installation path
  * DONE (macroibi) ←
  > |

```

If you see red text mentioning "Rtools": Go back to Step 3 and install Rtools, then try this command again.

## Step 6: Launch the App

Type these two commands (press Enter after each):

```
library(macroibi)
run_macroibi()
```

✓ **Success:** A web browser window opens showing the "Wetland IBI Dashboard". You're ready to use the app!

## Quick Verification (Optional)

To confirm everything works, try the demo mode:

```
library(macroibi)
run_macroibi(demo_mode = TRUE)
```

Click "OK" on the demo message, then click **Load Autosave** on the left sidebar. If you see demo files listed, everything is working correctly.

## Closing the App

When you're done:

- **Close the browser tab**, OR
- **In RStudio**, click the red **Stop** button (●) in the Console area, OR
- **Press the Escape key** while clicked in the Console

## Don't Panic: Understanding Console Messages

When running R commands, you'll see various messages in the Console. Here's what they mean:

What You See	What It Means	Action Needed
<b>Black text scrolling by</b>	Normal progress messages	None — just wait
<b>Blue text</b>	Informational notes	None — just informational

What You See	What It Means	Action Needed
<b>Red text with "Warning"</b>	A heads-up, but not an error	Usually none — warnings often don't prevent success
<b>Red text with "Error"</b>	Something went wrong	See troubleshooting below
<b>Key point:</b> Red text doesn't always mean failure! Warnings are common and usually harmless. Only "Error" messages indicate a real problem.		

## Troubleshooting

### "Error: package 'remotes' is not available"

Run `install.packages("remotes")` first, then try again.

### Red text mentioning "Rtools" or "compilation" (Windows)

Install Rtools from Step 3, restart RStudio, and try again.

### "Cannot open URL" or network errors

Check your internet connection. If you're on a work network, try from home or contact IT about firewall restrictions.

### PDF/image export not working

The PDF export feature requires additional software. In the Console, run:

```
webshot2::install_phantomjs()
```

### App won't start / "could not find function"

Make sure you ran `library(macroibi)` first. You need to run this command every time you open RStudio.

### "Object not found" errors while using the app

Try closing and relaunching the app. If the problem persists, clear your data and re-enter it.

### Still stuck?

Contact Sam Swanson at [sam.swanson@shakopeedakota.org](mailto:sam.swanson@shakopeedakota.org) — happy to help!

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## Quick Reference Card

Copy this and keep it handy:

MacroIBI QUICK REFERENCE

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TO START THE APP (every time):

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1. Open RStudio
2. In the Console, type:

```
library(macroibi)
run_macroibi()
```

TO CLOSE THE APP:

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- Close the browser tab, OR
- Press Escape in the RStudio Console

AUTOSAVE LOCATION:

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Windows: C:\Users\[You]\AppData\Local\R\macroibi\data\  
Mac: ~/Library/Application Support/macroibi/data/

NEED HELP?

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Contact: sam.swanson@shakopeedakota.org

## 7. Using the MacroIBI App

Start the dashboard:

```
library(macroibi)
run_macroibi()
```

Enter the **sample title** and **date**, then click **Let's go!**

You can also continue without metadata if you plan to upload data or reload an autosave.

### Step 1 — Enable Autosave

Before entering data, find the **Autosave Settings** on the left side of the Data tab and **Enable Auto-Save**

Autosave will:

- periodically save your progress
- store autosaves per user
- allow recovery after crashes
- provide history for reports and comparisons

You can reload autosaves using **Load Autosave**.

**Important:** Data Summary and Full Report features rely on having autosaves available.

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## Step 2 — Enter Taxa and Counts

Still on the data tab, use **Select Taxon** to search and add taxa.

The app assigns each taxon to the correct group automatically.

Each row displays:

- Taxon name
- Dipnet 1 Count
- Dipnet 2 Count
- Sum Count (auto-calculated)

Each group footer updates live:

- Total Taxa
- Percent of Total Sample
- Total Individuals

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## Step 3 — Review Metrics

Switch to **Results** to see real-time IBI calculations.

Displayed metrics include:

- Total Individuals
- EOT richness
- Snail richness
- All-taxa richness
- Corixid ratio
- Abundance of EOT
- **Overall IBI score (0–50)**

Hover over “*How are these calculated?*” for metric formulas.

### Metric definitions and scoring (offline reference)

- **Total Individuals** — Sum of all individuals entered across both dipnets and all taxonomic groups.
- **EOT richness** — Number of unique taxa in Dragonflies, Mayflies, Damselflies, and Caddisflies (EOT Orders).
- **Snail richness** — Number of unique taxa in Gastropoda.
- **All-taxa richness** — Sum of unique taxa across all groups entered in the app.
- **Corixid ratio** — ( $\text{Corixidae individuals} / (\text{all true bugs} + \text{beetles})$ ); higher ratios indicate potential nutrient loading, so scoring decreases as the ratio rises.
- **Abundance of EOT** — ( $\text{Total EOT individuals} / \text{Total individuals}$ ).

- **IBI Score (0–50)** — Sum of five adjusted metric scores (each 0–10). Scores are scaled between anchor percentiles observed in reference data:
    - *Decrease with disturbance*: EOT richness (1–12 taxa), snail richness (1–10 taxa), all-taxa richness (10–40 taxa), abundance of EOT (0–0.16). Values below the minimum score 0; values at/above the upper anchor score 10.
    - *Increase with disturbance*: Corixid ratio uses anchors 0 (best) to 1.0 (worst) with a 5th–95th percentile band of 0–0.82 guiding the scale; values above the maximum anchor score 0.
    - The final IBI score is the sum of the capped component scores (maximum 50).
- 

## Step 4 — Export Final Outputs

Available downloads:

1. **Raw Data CSV** For archiving or re-uploading into the app. Use only CSVs exported from MacroIBI without external edits; uploading unrelated or manually prepared files can break metric calculations.
2. **Results CSV** A basic CSV file with the final calculated scores
3. **Table Image (PNG)**  
A formatted metric table with titles and dates.
4. **Data Summary (PDF)**  
A concise one-page snapshot of metrics.
5. **Full Report (PDF)**  
Comprehensive report including:
  - current metrics
  - comparisons to other autosaved sessions

*Reports require autosave history to generate comparisons.*

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## 8. Additional Features

### Taxonomic Hierarchy Viewer

Use **Show/Hide Taxonomic Hierarchy** to view a taxonomic tree of selected taxa; this helps verify correct grouping.

### Reloading Old Work

Upload any previously exported **Raw Data CSV** to restore tables exactly as saved.

### Clearing Data

**Clear All Data** resets everything.

***This cannot be undone if the data isn't saved.***

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