Temp-Pref Tracker

Version 2.5

User Manual

Generated February 20, 2026

Webcam-based thermal preference tracking
with real-time DAQ hardware integration

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1. Overview

Temp-Pref Tracker is a Windows desktop application for real-time, webcam-based object tracking designed for thermal preference research. It uses an infrared / low light sensitive USB camera for contrast-based contour detection to follow a subject (i.e., a fish) inside a two-choice thermal arena and records timestamped X-Y coordinates at one-second intervals.

When paired with a Measurement Computing (MCC) USB DAQ device, the software can also monitor temperature via analog input channels, control heating and cooling hardware via digital outputs, and trigger a mixing pump based on the subject's position.

When specimen mass is provided, the software can also calculate an optional internal core / body temperature estimate from the calibrated chamber temperature surrounding the tracked specimen.

Key capabilities:

- Real-time contrast-based object tracking
- Automatic brightness-based tracking calibration with user adjustable sensitivity sliders
- Adjustable X-threshold line that splits the arena into left/right zones
- Mask drawing tools to isolate the region of interest
- DAQ hardware: dual-channel / chamber temperature monitoring with 2-point calibration
- Digital output control for heating, cooling, and mixing pump trigger
    - with safety limits, rate limits, and temperature-difference maintenance
- System Control trials with configurable heat/cool cycling
- Optional internal core / body temperature estimation using specimen mass and chamber temperature
- Data export to timestamped CVS files
- Settings persistence via JSON files
- Trial (7-day) and licensed activation modes

2. System Requirements & Installation

Hardware Requirements

- Windows 10 or Windows 11
- USB webcam with infrared sensitivity or low light sensitivity (to enable nighttime tracking)
- MCC USB-1208LS or compatible DAQ device with InstaCal drivers installed
- Heating and cooling hardware connected to DAQ analog/digital outputs
- IR background illumination of the tracking area to allow tracking at night

Installing Temp-Pref Tracker

Temp-Pref Tracker is distributed as a standalone Windows installer. No separate Python installation or additional software is required.

1. Obtain the installer file: TempPrefTrackerv2Installer.zip from www.FishResilience.com / Softwares
2. Unpack the zip file and double-click the installer to launch the setup wizard
3. Follow the on-screen prompts:
    - Review the information page and click Next
    - Choose the installation folder (default: Program Files\Temp-Pref Tracker)
    - Optionally create a Desktop shortcut (recommended)
4. Click Install to complete the installation
5. Optionally launch Temp-Pref Tracker immediately when the installer finishes

Running the Application

After installation, launch Temp-Pref Tracker using any of these methods:

- Double-click the "Temp-Pref Tracker" shortcut on your Desktop
- Open it from the Start Menu under "Temp-Pref Tracker"
- Run TempPrefTracker.exe directly from the installation folder

DAQ Hardware Setup (Optional)

To use the USB-1208LS DAQ device for temperature monitoring and hardware control, you must install the MCC InstaCal and drivers from Digilent/MCC before connecting the device. Make sure the device is installed as a 4-channel differential. The DAQ Controller within Temp-Pref Tracker will automatically detect the device once the drivers are installed and the hardware is plugged in.

Note: If no DAQ hardware is connected, all tracking and camera features work normally. Temperature fields will display "---" until a DAQ Controller is connected.

3. Trial Version vs Licensed Version

Trial (Demo) Version

When launched without a valid license, the software runs in 7-day demo mode:

- An orange banner appears at the top: "DEMO - 7 DAY TRIAL (time left: X days)"
- An "Add License" button is shown in the header bar
- The "6. Save Trial Data" button is disabled - you cannot export trial CSV files
- All other features (camera, tracking, calibration, DAQ control, System Control) are fully functional

Licensed Version

With a valid license activated:

- The demo banner and "Add License" button are hidden
- The "6. Save Trial Data" button is enabled for full data export
- The license is tied to your machine and cannot be relocated to another machine

Activating a License

1. Click the "Add License" button in the demo banner
2. Enter the license key provided to you
3. The application will validate the key and activate your license
4. Restart the application to confirm activation

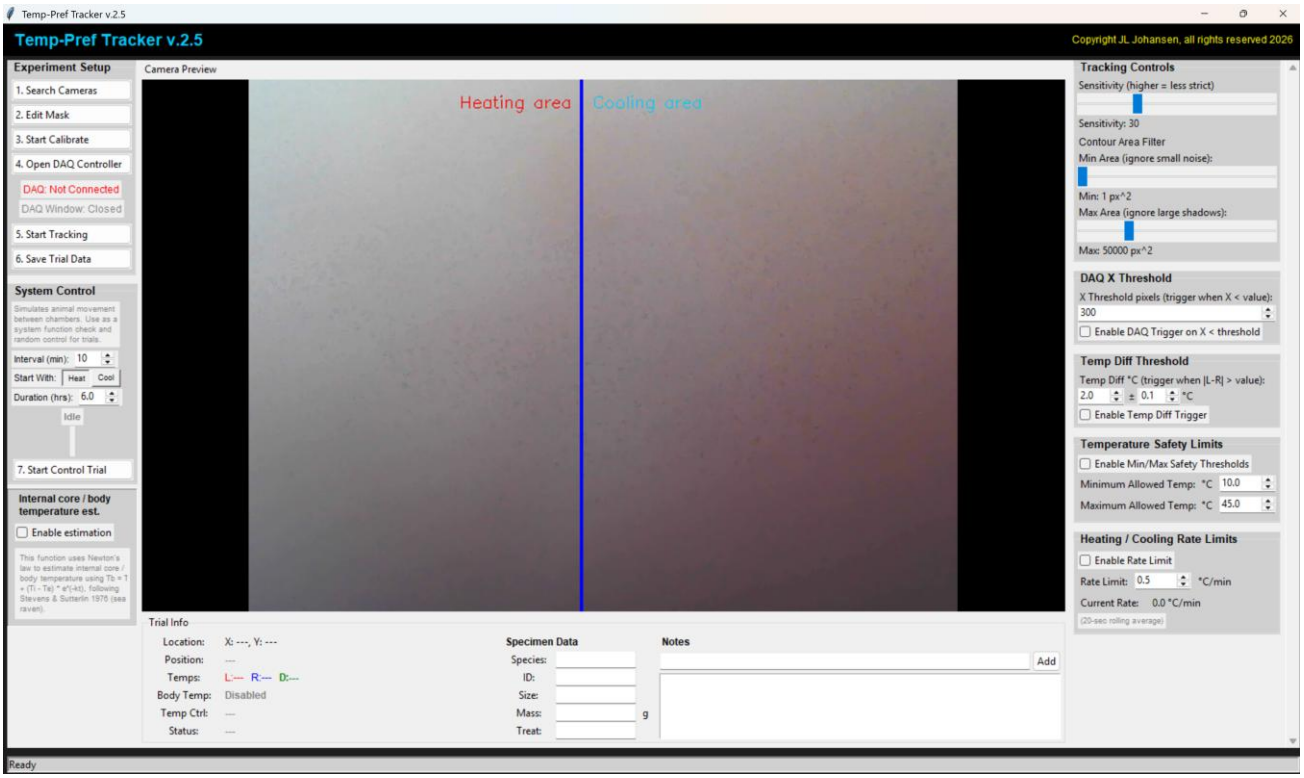
To acquire a license, contact:

Jacob L Johansen - jacob.l.johansen@gmail.com

Note, we aim to keep license fees to an absolute minimum, just enough to keep the software updated and website active. A student license lasts 3 years and cost US$50. A lab license doesn’t expire and cost US$100.

4. Application Layout

The application window is divided into several regions:



Click the title "Temp-Pref Tracker v2.5" in the header bar to view the changelog.

The main window is organized into a left control panel and a right video display area.

Left panel (top to bottom):

- Camera selector
- Masking Tool
- Tracking Calibration
- DAQ Controller
- Start / Stop Tracking
- Data Recording
- Control Trial
- Internal core / body temperature calculation

Middle panel:

- Live camera preview with real-time tracking overlay (cross-hair, center dot, threshold line)
- Trial Info at the bottom with tracking state and actions / notes

Right panel:

- Tracking Controls
- DAQ X threshold
- Temp Diff Threshold
- Temperature Safety Limits
- Heating / Cooling Rate Limits

5. Getting Started - First-Time Trial Walkthrough

Follow these numbered steps in order. The buttons in the Experiment Setup panel are numbered 1-7 to guide you through the workflow.

Step 1: Search for Cameras

1. Click "1. Search Cameras"
2. The application scans for connected USB cameras
3. A dialog appears listing available cameras - select one
4. The live camera feed appears in the Camera Preview area
5. Adjust your camera to just capture the area of interest

Step 2: Draw a Mask

1. Click "2. Edit Mask"
2. The mask editing toolbar appears above the camera preview
3. Use the drawing tools to define the region where the organism can move:
   - Draw - paint the mask area (non-shaded = tracked region)
   - Erase - remove parts of the mask
   - Clear - reset the entire mask
   - Drag the areas and adjust the brush size slider as needed
4. Click "Save Mask" when finished (allowing you to load a previously saved mask)
5. Only the unmasked area will be used for tracking

Tip: Draw the mask to cover only the arena/chamber area, excluding edges, labels, or other visual clutter.

Step 3: Calibrate

1. Place the organism in the arena so it is visible on camera (or wait until it is visible on its own)
2. Click "3. Start Calibrate"
3. The camera feed freezes and a crosshair appears
4. Click directly on the organism - a 10x10 pixel region is sampled to determine the object's brightness
5. Click “Capture Object”. The software automatically optimizes the Sensitivity, Min Area, and Max Area sliders.
6. A green tracking indicator (circle + crosshair) should appear on the organism, as well as a yellow outline of the tracked organism.
7. If detection looks good, click "Done" to accept. If not, click the organism again to re-sample or use the sliders to adjust sensitivity. Lower values detect only high-contrast objects; higher values detect subtler differences. Fine-tune while watching the preview.

Note: Calibration captures the average brightness of the object and tests different sensitivity values to find the combination that produces exactly one clean contour matching the organism's size.

Step 4: Open the DAQ Controller

1. Click "4. Open DAQ Controller"
2. A separate DAQ Controller window opens

The DAQ Controller provides hardware device management, temperature monitoring with calibration, digital output control, and settings persistence. The window uses a two-column layout with device controls on the left and calibration on the right.

The calibrated left/right chamber temperatures from the DAQ Controller are also used by the optional internal core / body temperature estimation feature in the main tracker window.

4.1 Device Discovery

The top-left section handles connecting to your USB DAQ hardware.

Control	Description
Discover Devices	Scans board numbers 0-7 for connected MCC DAQ devices via InstaCal. Logs all found devices.
Board #	Spinbox (0-7) to select which board number to connect to. Auto-set when a device is discovered.
Connect	Connects to the selected board. Configures the digital port for output. Logs wiring instructions for the temperature sensors.
Disconnect	Turns off all outputs and releases the board.
Flash LED	Sends a brief test signal to the board to confirm connectivity (the board LED flashes).

If no DAQ hardware is connected or InstaCal drivers are not installed, discovery will find no devices. Tracking and camera features in the main application still work normally without a DAQ connection.

4.2 Temperature Monitoring

Displays real-time temperature readings from the left and right analog input channels:

- Left / Right - Large temperature displays showing the current calibrated temperature in degrees C.
- Diff - The absolute difference between left and right probes. Changes color based on magnitude.

Digital Output (Mixing Pump)

Below the temperature displays, the digital output row controls the mixing pump trigger:

Control	Description
Port	Spinbox to select the digital port (A, B, or C).
Bit	Spinbox (0-7) to select the bit within that port.
Update	Applies the port/bit selection to the hardware.
Test	Sends a 10-second HIGH pulse to the selected port/bit (i.e. 5V), then returns to LOW (0V). Useful forverifying wiring.
Output Status	Shows current state: HIGH (green) or LOW (red).

4.3 Temperature Control (Heating / Cooling)

Configures the digital output channels used for heating and cooling hardware. Each row has the same controls:

Control	Description
Port	Spinbox to select the digital port for the output.
Bit	Spinbox to select the bit within that port.
Update	Applies the port/bit configuration to the hardware.
Test	Sends a 10-second HIGH pulse to confirm the output is wired correctly, then returns to LOW.

Separate rows are provided for the Heating output and the Cooling output, each with their own port/bit/test controls. Output Status indicators show the live state of both outputs (HIGH or LOW).

Chamber Assignment

Below the heating/cooling rows, the chamber assignment section defines which physical chamber is heated and which is cooled:

- LEFT = Heat, RIGHT = Cool (default) - or vice versa.
- Swap button - Reverses the assignment (LEFT becomes Cool, RIGHT becomes Heat). The labels update to reflect the current assignment.

This assignment determines how the main tracker position-based control works: when the organism is on the LEFT side and LEFT = Heat, the system heats the left chamber and cools the right.

4.4 2-Point Temperature Calibration

The right column contains the calibration system that converts raw voltage readings from the DAQ analog inputs into accurate temperature values in degrees C.

Live Voltage Display

- Left / Right voltage - Shows the current raw voltage reading from each analog input channel, updated in real time.
- Averaging window - Spinbox to set how many seconds of voltage readings to average (smooths out noise). Useful for getting a stable reading when setting calibration points.

Analog Input Channel Selection

- Left AI Channel - Spinbox to select which analog input channel (0-7) reads the left temperature probe.
- Right AI Channel - Spinbox to select the analog input channel for the right probe.

LEFT Channel Calibration

Two-point calibration for the left temperature probe:

4.4.1 Point 1: Place the probe at a known temperature. Enter that temperature in the degrees C field and click Save Pt1. The current averaged voltage is captured and paired with your entered temperature. The status label shows the saved voltage and temperature.

4.4.2 Point 2: Move the probe to a different known temperature. Enter the temperature and click Save Pt2. Once both points are saved, a linear calibration (slope and offset) is automatically calculated.

4.4.3 The Calibration status label shows either Not complete or the calculated equation (e.g., T = 10.05 x V + 0.32).

RIGHT Channel Calibration

Identical process for the right probe - two points with separate Save Pt1 / Save Pt2 buttons, and automatic slope/offset calculation.

Clearing Calibration

- Clear Left Calib - Resets the left channel calibration to defaults (slope = 1.0, offset = 0.0).
- Clear Right Calib - Resets the right channel calibration.

How calibration works:

The two known (voltage, temperature) pairs define a linear relationship: T = slope x V + offset. All subsequent voltage readings are converted to temperature using this equation. Calibration is saved with your settings file and persists between sessions.

> **TIP**
> For best accuracy, choose two calibration points that span the range of temperatures you expect in your experiment (e.g., one cold point and one warm point).

4.5 Settings File

The DAQ Controller automatically saves all settings to a JSON file whenever you change a port, bit, or channel value.

Control	Description
Save Settings As...	Opens a file dialog to save the current settings to a new JSON file.
Load Settings...	Opens a file dialog to load settings from a previously saved JSON file.
Current	Displays the name of the currently active settings file (default: daq_settings.json).

What is saved:
Board number, digital output port/bit, heating port/bit, cooling port/bit, left/right AI channels, averaging window, calibration data (all four points plus calculated coefficients), and chamber assignment.

4.6 Event Log

The bottom of the DAQ Controller window contains a scrollable event log that records all significant events:

- Device discovery results
- Connection and disconnection events
- Port configuration changes
- Calibration point captures and calculations
- Temperature read errors
- Output state changes

The log is useful for diagnosing hardware issues and confirming that commands are reaching the DAQ device.

Step 5: Start Tracking

1. Click "5. Start Tracking"
2. The application begins tracking the organism's position in real time
3. The Trial Info panel updates with Location, Position, and Temperatures, once per second
4. Click the button again ("Stop Tracking") to stop

Step 6: Save Trial Data

1. Fill in the Specimen Data fields (Species, Specimen ID, Size, Treatment)
2. Add any Notes using the "Add" button in the Trial Info panel
3. Click "6. Save Trial Data"
4. A file dialog appears - choose a location and filename for the CSV file
5. The application begins recording trial data to the CSV every second
6. If you are using internal core / body temperature estimation, enter the specimen Mass in the Specimen Data panel and enable estimation before saving so the CSV can record the estimate and mass metadata.
7. When finished, click the button again ("Stop Saving") to close the file

Important: This button is disabled in the demo/trial version. A license is required to save trial data.

Step 7: Start a System Control Trial (optional)

See Section 6.8 - System Control for details on automated heat/cool cycling experiments.

6 Feature Reference

6.1 Experiment Setup (left side of the window)

The left-side panel contains the primary workflow buttons:

Button	Function
1. Search Cameras	Scans for and connects to USB webcams
2. Edit Mask	Opens mask drawing tools to define tracking region
3. Start Calibrate	Freezes frame and begins brightness calibration
4. Open DAQ Controller	Opens DAQ Controller window for temperature monitoring
5. Start Tracking	Begins/stops real-time object tracking
6. Save Trial Data	Begins/stops recording trial data to CSV (licensed only)

Between buttons 4 and 5, status labels show DAQ connection status and current left/right probe temperatures.

6.2 Trial Info Panel (bottom/middle below the camera feed)

Located below the camera preview. Shows real-time experiment data.

Live Data (Left Column)

- Body Temp - Displays the running internal core / body temperature estimate when estimation is enabled.

Field	Description
Location	Which chamber the organism is in: LEFT, RIGHT, or LOST
Position	Current X, Y coordinates of the tracked object
Temp Left	Left probe temperature (C) from DAQ
Temp Right	Right probe temperature (C) from DAQ
Temp Diff	Absolute difference between left and right probes
Temp Ctrl	Current temperature control state
Status	Tracking status and setting change notifications

Specimen Data (Middle Column)

- Mass - Specimen mass in grams. Required for the optional internal core / body temperature estimation feature.

Field	Description
Species	Free-text entry for species name
Specimen ID	Unique identifier for the specimen
Size	Specimen size (any format)
Treatment	Experimental treatment/condition label

Notes (Right Area)

- A text display area showing experiment notes
- Click "Add" to add a timestamped note
- Notes are included in the saved trial CSV under the "=== NOTES ===" section

6.1 Tracking Controls (Right side of the widow)

Sensitivity Slider (1-100)

- Controls the brightness threshold for object detection
- Lower values = stricter matching (closer to calibrated brightness)
- Higher values = more permissive (may pick up noise)
- Set automatically during calibration; fine-tune manually if needed

Contour Area Filter

- Min Area slider - minimum contour size (pixels). Filters out small noise.
- Max Area slider - maximum contour size. Filters out large background regions.
- Both are auto-set during calibration

6.2 DAQ X Threshold

Controls which side of the arena is considered "left" vs "right":

- X Threshold spinbox (0-640): The vertical dividing line shows current division. Objects with X < threshold are "LEFT"; X >=threshold are "RIGHT".
- Enable checkbox: When enabled, the DAQ outputs are triggered based on organism position.

6.3 Temp Diff Threshold

Maintains a target temperature difference between the two chambers:

- Threshold spinbox: The desired temperature difference (C) between probes
- +/- Hysteresis spinbox (0.05-1.00): Tolerance band around the target
- Enable checkbox: Activates the temperature difference control

How it works:

The system has three independent physical outputs:

1. Heating (left chamber heater)
2. Cooling (right chamber cooler)
3. Mixing pump (circulates water between chambers to equalize temperatures)

When the organism's position is known, the primary heating/cooling follows the organism. The diff trigger then controls two secondary outputs:

- Diff too high (above target + hysteresis): Mixing pump ON, opposite output OFF
- Diff too low (below target - hysteresis): Mixing pump OFF, opposite output ON
- Within tolerance band: Both OFF (maintaining current state)

When position is lost, the system uses mixing and heating/cooling to maintain the last known difference target.

6.4 Temperature Safety Limits

Prevents temperatures from exceeding safe bounds:

- Enable checkbox: Activates safety monitoring
- Min Temperature (-20 to 50 C): If either probe drops below, cooling is suspended
- Max Temperature (-20 to 60 C): If either probe rises above, heating is suspended

When triggered, the offending output is immediately disabled, a safety event is logged, and the status label updates.

6.7 Heating / Cooling Rate Limits

Prevents temperatures from changing too quickly:

- Enable checkbox: Activates rate monitoring
- Rate Limit (0.02-2.00 C/min): Maximum allowed rate of change
- Current Rate display: Shows the calculated rate in real time

How it works:

- Rate is calculated using a 20-second rolling average of mean temperature ((left + right) / 2)
- If positive rate exceeds the limit, heating is blocked
- If negative rate exceeds the limit, cooling is blocked
- A minimum 2-second interval between toggles prevents rapid switching

6.8 Internal Core / Body Temperature Estimation

Provides an optional estimate of internal core / body temperature in the main tracker window.

- Enable estimation checkbox: Located in the "Internal core / body temperature est." panel on the left side of the main window.
- Mass field: Enter specimen Mass in grams in the Specimen Data panel before enabling estimation.
- Body Temp display: The Trial Info panel shows the current estimate in degrees C.

How it works:

- The estimate uses Newton's law of cooling / warming: Tb = Te + (Ti - Te) * e^(-kt).
- The mass-dependent constant is calculated as k = 3.32 * mass^-0.536.
- Te is taken from the chamber currently occupied by the tracked specimen, or from the active heating/cooling phase during a System Control trial.
- When enabled, the estimate initializes to the current chamber temperature and updates continuously while valid position and temperature data are available.
- Constants are derived Stevens & Sutterlin 1976 using sea ravens as the model fish

6.9 System Control

Provides automated heating/cooling cycling for control trials. This allows you to test the “temperature preference” of an organism moving between the two chambers at random / the “referred temperature” of your system

Settings:

Setting	Description
Heat / Cool	Toggle buttons to select mode for each phase
Interval	Duration of each heat/cool cycle (seconds)
Duration	Total runtime (minutes), or 0 for unlimited
Start Mode	Which phase to begin with: "Heat" or "Cool"

Button: "7. Start Control Trial" (left side of the window)

1. Click to begin the automated control trial
2. The system alternates between heating and cooling phases
3. Status labels show Status, Phase (with countdown), and Time elapsed
4. Click again ("Stop Control Trial") to end

Note: During a control trial, the system overrides manual heating/cooling. Safety limits and rate limits still apply. Temp diff maintenance works alongside control cycling.

7. Data Output Formats

TPref-XY.txt - Real-Time Tracking Log

Written continuously during tracking (overwritten each session):

```
# Timestamp(s), X, Y
2025-01-15 14:30:01, 245, 189
2025-01-15 14:30:02, 247, 190
2025-01-15 14:30:03, -, -
```

- One line per second; "-, -" indicates position lost
- File is overwritten when tracking starts

Trial CSV - Full Experiment Record

Saved via "6. Save Trial Data" (licensed version only). Contains four sections:

Section 1: Trial Information

Includes specimen metadata entered in the Trial Info panel, including Mass when it has been provided.

```
=== TRIAL INFORMATION ===
Trial Type: Experimental
Trial Start: 2025-01-15 14:30:00
Species: Zebrafish
Specimen ID: ZF-001
Size: 3.2cm
Treatment: Control
```

Section 2: Active Settings

Records whether Internal Temp Estimation was enabled. When enabled and a mass value is available, the header also records the specimen Mass in grams.

```
=== ACTIVE SETTINGS ===
DAQ X Threshold: 320 (Enabled)
Temp Diff Threshold: 2.0 +/-0.10 C (Trigger Enabled)
Temperature Safety Limits: Enabled (Min: 10.0C, Max: 40.0C)
Heating/Cooling Rate Limit: Enabled (0.50 C/min)
```

Section 3: Notes

```
=== NOTES ===
[14:30:15] Specimen placed in left chamber
[14:35:22] Sensitivity changed from 45 to 50
```

Includes manually added notes and automatically logged setting changes.

Section 4: Tracking Data Columns

Tracking rows include Specimen Temp (C), which reports the chamber temperature at the organism's current location, and Internal Temp Estimate (C), which reports the optional running estimate or LOST when position is lost.

Column	Description
Time Since Start	Elapsed time (HH:MM:SS)
Date	Date (YYYY-MM-DD)
Time of Day	Clock time (HH:MM:SS)

X	Object X coordinate, or "-" if lost

Y	Object Y coordinate, or "-" if lost
Location	LEFT, RIGHT, LOST, LEFT-SIM, or RIGHT-SIM
Chamber Type	HEATING, COOLING, MAINTAINING, CONTROL, or UNKNOWN
Temp Left (C)	Left probe temperature, or "---"
Temp Right (C)	Right probe temperature, or "---"
Temp Diff (C)	Absolute difference, or "---"
Specimen Temp	Temperature at specimen location, or LOST / "---"
Specimen internal temp	Estimated internal temperature of the specimen using Newtons law functions
Safety Event	Safety event description (blank if none)

Note: If the CSV file is open in another program (e.g., Excel), data is buffered in memory and written once the file becomes available.

8. Changelog

Version 2.5

- Added optional internal core / body temperature estimation based on calibrated chamber temperature and specimen mass
- Added specimen Mass input and Body Temp display support for the internal core / body temperature estimation workflow
- Trial CSV export now records internal temperature estimation status, specimen mass, and the Internal Temp Estimate (C) data column when enabled

Version 2.0

- Temperature safety limits (min/max bounds)
- Heating/cooling rate limits with 20-second rolling average
- System Control trials with automated heat/cool cycling
- Temperature difference threshold with hysteresis control
- 3-output control: heating, cooling, and mixing pump
- Setting change logging to trial data notes
- Trial data CSV export with full metadata headers

Version 1.0

- Basic webcam object tracking
- Mask drawing tools
- Brightness calibration
- DAQ X threshold triggering
- Coordinate logging to TPref-XY.txt

9. Troubleshooting

Problem	Solution
No cameras found	Ensure USB camera is plugged in and recognized by Windows. Try a different USB port.
Tracking is erratic	Re-run calibration. Adjust Sensitivity and Contour Area filters. Ensure mask covers only the arena.
Temperatures show "---"	Open DAQ Controller and verify device is connected. Check that InstaCal drivers are installed and temperature probes are attached.
DAQ not found	Install InstaCal drivers from MCC/Digilent. Ensure USB-1208LS is plugged in.
"Save Trial Data" disabled	You are in demo mode. Activate a license to enable data saving.
CSV file locked warning	Close any program with the CSV open (e.g., Excel), or wait for auto-flush.
Application won't start	Try reinstalling using TempPrefTrackerv2Installer.exe. Ensure Windows 10/11.
Tracking lost frequently	Improve lighting. Ensure organism contrasts with background. Re-calibrate.

10. Contact & Licensing

For license keys, technical support, or feature requests:

- Open the License Activation dialog to view and copy your Machine ID.
- Send your Machine ID to jacob.l.johansen@gmail.com as well as your license type request (student/full)
- A payment option will be provided
- and license key tied to your machine will be provided.

This software is provided as-is for research purposes. The license is non-transferable and locked to the hardware on which it is activated.