STUDY PROTOCOL

Title: Establishing Personalized Heat-Strain Thresholds for Agricultural Workers Through Integrated Environmental and Physiological Monitoring

Background

Exposure to extreme heat is a pressing occupational health problem globally, and is becoming a growing issue for workers who work outdoors (1). Agricultural workers constitute one of the most susceptible occupational groups as they perform physically demanding tasks under direct exposure to environmental heat and usually are limited and/or not guaranteed to have access to shade, cooling infrastructure or rest periods integrated into their work schedule. Furthermore, in tropical and subtropical low- to middle-income settings, where a larger proportion of the workforce is engaged in outdoor or heat-exposed work and occupational heat-protection systems are not well established (2, 3). Anthropogenic climate change is causing the frequency, duration and intensity of extreme heat events to increase significantly, thereby worsening its health effects on workers exposed to heat (4). As a result, occupational heat stress is not only increasingly viewed as a problem of environmental exposure but also as an important public health, labor productivity, and occupational safety challenge (5, 6).

There are several negative physiological consequences associated with extreme heat exposure. Dehydration, heat exhaustion and syncope, electrolyte imbalance and heat stroke are acute outcomes (7). Frequent exposure to hot temperatures can lead to persistent health impacts like chronic kidney disease and cardiovascular stress (8, 9). Recent evidence on agricultural and other outdoor worker populations suggests that repeated heat exposure combined with heavy physical workload, followed by dehydration and inadequate recovery may play a role in kidney injury and chronic kidney disease of unclear or non-traditional etiology (10, 11). Beyond the direct health impacts, occupational heat stress may decrease work capacity due to increased fatigue, reduced work pace, deterrent from working for long durations without taking rest breaks, and less ability to maintain physically demanding activities (5). Long-term exposure to such high temperature may increase the risk of fatigue, dizziness, lack of concentration, and physical exhaustion which in turn may cause workplace mistakes or place workers at a higher risk of falls, injuries and other safety incidents. Conversely, a systematic review and meta-analysis reported associations between exposure to occupational heat strain, objectively measured decreases in work capacity and increased risk of adverse health outcomes, emphasizing the value of integrated multi-exposure as well as physiological monitoring systems for workplace assessment of workers exposed to environmental heat (5).

Heat exposure among agricultural workers may not end when the workday is completed. Nighttime indoor temperatures may also influence the extent to which workers are able to recover physiologically from daytime heat strain. This is particularly important in Bangladesh, where many agricultural workers may return from outdoor fieldwork to dwellings that remain hot during the evening and night, with limited access to mechanical cooling. Housing materials, ventilation, shade, and indoor heat-mitigation options could therefore contribute substantially to nighttime heat exposure, which subsequently affects sleep, hydration recovery, and thermoregulatory recovery after daytime work (12-14).

Existing global occupational guidelines for heat exposure typically use environmentally-based indices such as Wet Bulb Globe Temperature (WBGT) or Universal Thermal Climate Index (UTCI). These indices combine meteorological parameters, such as air temperature, humidity, wind speed and solar or radiant heat, to estimate the environmental heat stress. They are also beneficial because they represent standardized and pragmatic metrics of heat exposure, can be directly computed from routinely available meteorological data, and generally emphasize workplace heat-risk assessment and heat-warning systems. But these environmental indices do not directly reflect the physiological responses of individuals subject to heat stress (15). Environmental-based measures do not take into account individual susceptibility to heat, such as the metabolic load created by physical work involving high-energy expenditure, hydration status, clothing, and acclimatization or behavioral responses to their work (3). Physiological strain may differ greatly, even among individuals that have been exposed to identical environmental conditions. This variation is influenced by hydration status, metabolic workload, acclimatization, physical fitness, body composition and behavioral adaptations such as changes to fluid intake and rest patterns. Thus, environmental heat indices alone may not completely reflect risk of heat strain in the individual worker (16). This limitation is especially relevant for agricultural workers, since work intensity, posture, type of work and access to drinking water or rest can vary prominently between workers and within the working day (17).

Physiological indicators of heat strain and dehydration include direct measurements, such as core body temperature and plasma osmolarity; indirect measures, such as serum electrolytes; or urine hydration markers (15). Core body temperature is important because an increase in internal temperature also represents the relationship between heat exposure from environment, metabolism, and evapo-heat loss through sweat, skin blood flow, and behavioural change (5). Wearable and ingestible monitoring is can be deployed in real-world occupational settings, enabling continuous measurement of physiological responses (18). In recent years, the use of wearable sensors, heart-rate monitors, accelerometers and ingestible temperature capsules to measure physical workload, heat strain and core-temperature responses have gained acceptance in field-based occupational studies (17-20). These technologies, in combination with concurrent environmental measurements and biological markers of hydration, can provide a more comprehensive assessment of heat-related risk when compared to single measures alone (19).

Metabolic heat production, which occurs during physical activity, is another source of heat outside of environmental heat. Agricultural work is usually a physically demanding form of labour involving activities such as digging, harvesting, carrying loads and bending that generate a considerable amount of internal heat as consequence of muscular metabolism (21). If metabolic heat production coincides with significant environmental heat exposure, the adaptive capacity of the overall thermoregulatory response can be overwhelmed, which potentially amplifies baseline core body temperature and a negative hydration status (22). External and internal heat load may initiate a vicious circle of sweating, fluid loss, cardiovascular strain, less efficient skin blood-flow and heat dissipation due to elevated core temperature when no optimal fluid intake or rest is undertaken (6, 17).

Although metabolic workload is known to play an important role in physiological responses to extreme heat in occupational settings, few field studies have concurrently assessed environmental heat exposure, physical activity, thermoregulatory responses, and physiological biomarkers in agricultural workers. Even fewer studies have examined heat exposure across both occupational and residential environments, including the role of indoor nighttime temperatures and housing conditions in shaping opportunities for physiological recovery after work. Most available heat-stress assessments rely primarily on environmental heat indices or selected physiological measurements and therefore do not adequately capture worker-specific thresholds at which combined environmental heat exposure, metabolic workload, hydration status, and post-work recovery conditions lead to measurable dehydration or thermoregulatory strain (3, 12, 14, 19, 23).

In response to this gap, this study aims to integrate location-specific continuous environmental monitoring with wearable activity tracking, wireless core temperature measurement and biological markers of hydration. The study will also assess housing characteristics data to investigate how dwelling conditions might shape nighttime indoor heat exposure and post-work recovery. One of the key strengths of this study is its integration of monitoring heat exposure across the daily context in which Bangladeshi agricultural workers experience and recover from potential heat strain. This study will create a more comprehensive assessment of heat vulnerability among Bangladeshi agricultural workers by measuring both outdoor occupational heat and indoor residential heat, physical and behavioral responses to heat, hydration biomarkers, and housing characteristics. By identifying these environmental and physiological thresholds, this study will translate integrated heat-stress measurements into tractable indicators that can guide adequate heat monitoring and protection for agricultural workers.

Research Question and Aim

Research Question

How can thermoregulatory and physiological measurements be used to develop individualized heat-stress thresholds of agricultural workers during periods of extreme heat?

Research Aim

To develop individualized heat-stress thresholds for Bangladeshi agricultural workers by integrating outdoor occupational heat exposure, indoor residential heat exposure, physical activity, housing characteristics, thermoregulatory responses, and physiological markers of dehydration during routine agricultural work.

Research Objectives

Objective 1: To examine how thermoregulatory and physiological responses change based on varying levels of outdoor occupational heat exposure, indoor residential heat exposure, physical activity intensity and housing characteristics among Bangladeshi agricultural workers.

Objective 2: To assess individualized thresholds for heat-stress signals of dehydration risk and thermoregulatory strain in Bangladeshi agricultural workers performing routine field work.

Research Design and Methods

Study Design

A prospective observational cohort design will be used to measure naturally occurring environmental heat exposure, physical activity, thermoregulatory responses and physiological markers of dehydration during regular agricultural activities. A prospective design will allow measurement of exposure and outcomes during the same work shift, improve temporal alignment between UTCI, metabolic loading, core body temperature and biological markers, and reduce reliance on retrospective self-report. It is appropriate to use observational designs when the purpose is to study exposure-outcome relationships without experimental manipulation, and transparent reporting of such designs has been supported by the STROBE statement (24). Approaches such as these have been successfully employed in field-based occupational studies to evaluate environmental heat exposure, dehydration status, urine specific gravity and core temperature among agricultural and outdoor workers during summer months (6, 17).

The study will be conducted from July to December 2026 in Rajshahi and Jessore districts of Bangladesh. This time frame is also projected to encompass different levels of heat conditions, ranging from extremely hot workdays to relatively cooler working conditions. This will enable comparison of thermoregulatory and physiological responses across high-, moderate- and low-levels of environmental heat exposure.

Observation Structure

The main unit of observation is the worker-day, which we define as one completed work shift in agriculture performed by one participant under supervision. The study will enroll 200 individual farmers and collect the same number of worker-day observations, where each farmer contributes one observation

Each farmer will be observed for approximately 6 hours, corresponding to a routine agricultural work shift. The observation period will consist of continuous outdoor monitoring of the environment, core body temperature, wearable activity monitor assessment, hourly structured observation, fluid-intake recording, and sample collection.

The study will focus on a wide range of naturally occurring heating conditions. Irrespective of whether the UTCI value exceeds a specified threshold, all worker-day observations will be stored. We will define heat exposure as relatively cool (<32°C), intermediate (32°C and <36°C) and high (≥36°C) (25, 26). This would enable the full exposure-response relationship to be assessed, including possible thresholds for heat strain, without discarding observations that fall within the intermediate UTCI range.

These 200 farmers will also be involved in the household-based indoor temperature monitoring component. An indoor temperature and relative humidity sensor will be placed once in the regular sleeping or resting area of each participant at the Day 1 household visit and retrieved on Day 3. The sensor will collect both post-work and nighttime residential heat exposure corresponding to the participant's single worker-day observation. Self-reported participant questionnaires will assess housing characteristics, nighttime thermal comfort, sleep conditions, food and fluid intake and perceived recovery. Inside the household, there will be no direct observation of participants.

Table 1: Planned Observation Structure for Prospective Field Monitoring of Agricultural Workers

Parameter	Specification
Total participants	200 farmers
Total worker-day observations	200
Observations per participant	One work-shift observation
Work-shift duration	Approximately 6 hours
UTCI exposure range	All naturally occurring UTCI values will be included
Descriptive heat-exposure categories	Comparatively cooler: UTCI <32°C; intermediate: UTCI 32°C to <36°C; high: UTCI ≥36°C

Study Setting and Population

Study Setting

The study will be conducted out in high exposure settings typical of rural agricultural communities in Bangladesh where workers routinely perform field-based agricultural work. Such tasks can be planting, harvesting, irrigation, soil preparation, crop maintenance, weeding, carrying loads, and other forms of manual agricultural labour. The natural sites selected for the open-field trials will be representative of actual field-based farming scenarios, where workers are exposed to direct solar radiation and high ambient temperature, humidity or wind speeds.

Environmental monitoring equipment (e.g. indoor climate measurements) will be fixed at approximately 10 metres from the participant's routine work area. This location will be utilized to record the microclimatic characteristics encountered by an agricultural worker when performing normal agricultural tasks. Sampling shall be avoided in non-representative micro-environments (e.g. deep shade, very enclosed areas where air is trapped and/or areas immediately adjacent to buildings) unless such conditions replicate the actual exposure pattern of workers.

Study Sites

The study will be implemented in some selected agricultural areas of Rajshahi and Jashore districts. Rajshahi will represent a high heat-exposed agricultural environment in north-western Bangladesh and Jashore a heat-exposed agricultural environment in south-western Bangladesh (27).

From each district, one upazila will be selected, total two upazilas. Then two unions will be chosen from each identified upazila, making it four in total. The final selection would be made in consultation with the Department of Agricultural Extension, based on agricultural intensity, cropping calendar, expected heat exposure, field suitability & accessibility.

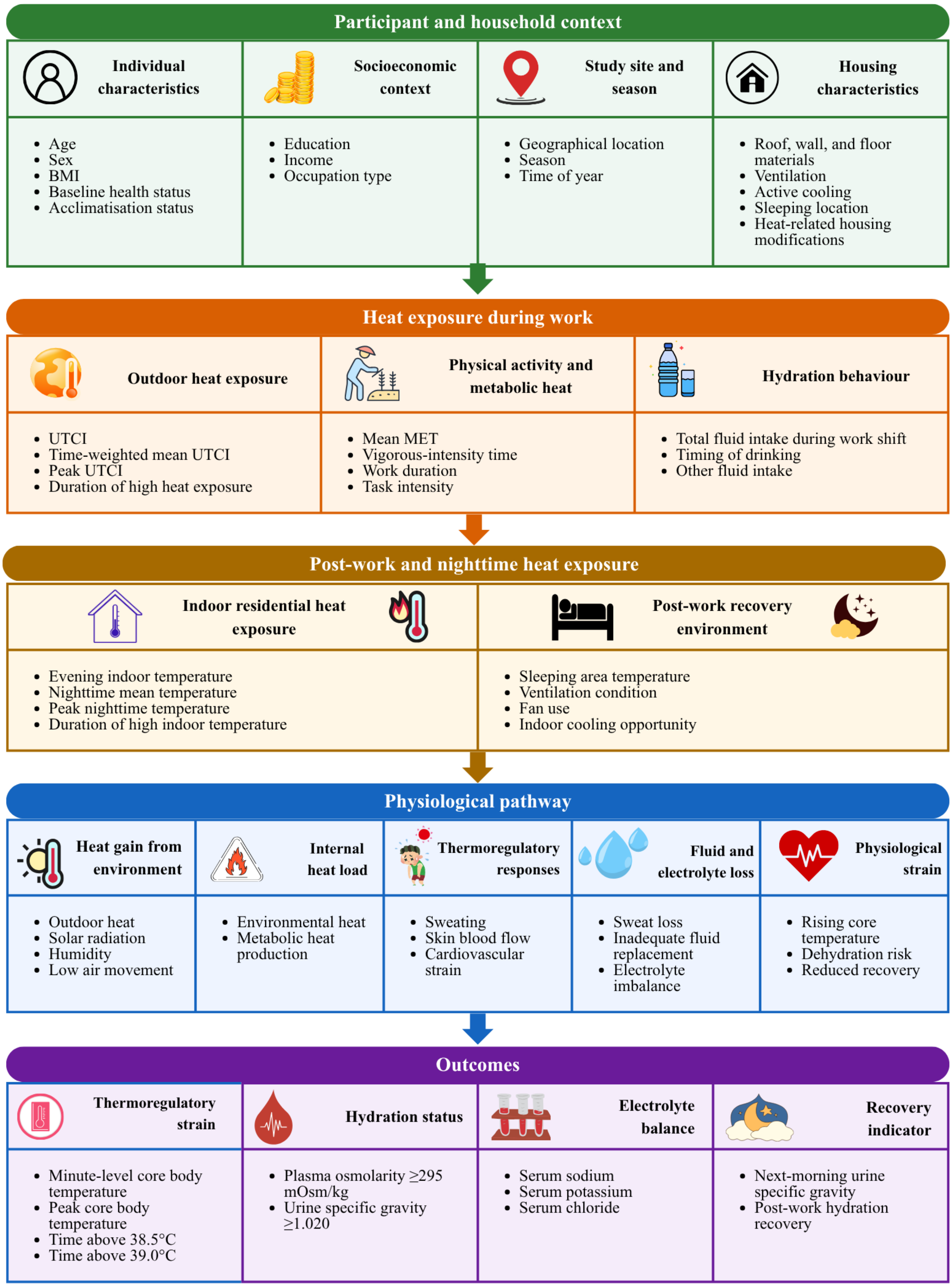
The study will include 200 farmers, about half from each district, 50 farmer each chosen union and from selected upazila. About 20% of participants will be women. Recruitment will also target farmers from the full eligible age range of 18-60 years. They may also follow participants age 18-29, 30-44, and 45-60 years across groups in order to capture thresholds with wide age representation.

The farmer lists will be collected from the respective Upazila Agriculture Offices of the Department of Agricultural Extension which will assist eligibility screening and recruitment process for participants.

Study Population

Adult agricultural workers aged 18-60 years who are currently engaged in outdoor agricultural field work will be included as study participants. Both male and female workers will be eligible for participation. Agricultural workers are the target population, as they experience chronic outdoor environmental heat during the day and engage in strenuous physical work generating significant metabolic heat.

Conceptual framework



Study Activities

Each participant undergoes a structured three-day monitoring cycle. Day 1 is a household visit conducted the evening before the observation day. Day 2 is the full workplace observation day. Day 3 is a follow-up household visit conducted the morning after Day 2.

Table 1: Overview of three-day study visit structure

Visit Day	Location	Primary Activities
Day 1 (Evening)	Participant's Household	Eligibility assessment; consent; baseline survey (demographics, work history, heat KAP, housing characteristics); indoor sensor installation; handover of first-morning urine collection pot
Day 2 (Work Shift)	Agricultural Field Site	Environmental monitoring setup; collection of first-morning urine on arrival; acute screening and enrolment; pre-shift blood sample; T-pill activation and ingestion; anthropometry; Actigraph placement; graded water bottle and urine pot issue; 6-hour work observation with hourly surveys and observation checklists; post-shift blood, urine, and weight; handover of overnight urine pot and water bottle
Day 3 (Morning)	Participant's Household	Sleep quality and thermal comfort survey; overnight urine collection; water bottle retrieval; indoor sensor retrieval

Day 1 Activities: Eligibility screening, informed consent, baseline survey, and indoor sensor installation

Day 1 activities take place at the participant's household, conducted by a trained Field Research Assistant (FRA) in the evening typically between 17:00 and 20:00 when the farmer is expected to be at home after returning from the field. The Day 1 visit must be completed before the Day 2 workplace observation. No measurement or sample collection takes place on Day 1; this visit is dedicated entirely to screening, consent, survey administration, sensor installation, and preparation for Day 2.

1.1 Framers listing and community mobilization

Before any Day 1 household visits begin at a new study site, field supervisors will prepare a listing of potentially eligible agricultural workers. Field supervisors will prepare the farmer listing using two or more of the following sources, depending on what is locally available at each site:

- Agricultural extension office records listing registered smallholder farmers in the area.
- Union parishad or local government ward records that document household composition and livelihood activities.

- Direct community mapping by field staff during a site familiarisation visit, walking through the farming area and documenting households where adult agricultural workers reside.

The farmer listing will record the following information for each identified household: household head's name, names of eligible-aged adult members engaged in farming, estimated number of working days per week in the field, household address or location description, and preferred contact time for a household visit. Each listed farmer will be assigned a preliminary Listing Number.

1.2 Eligibility assessment at household

On arrival at the household, the FRA will introduce themselves, explain the general purpose of the study in simple, non-technical language, and ask to meet the listed farmer. Eligibility assessment is completed before any mention of consent. The FRA will work through the eligibility checklist on KoboToolbox form HTS-Screen-01. Farmers who clearly do not meet eligibility criteria-for example, those known to have had major gastrointestinal surgery, those with severe cardiovascular disease, or those who are currently pregnant will be excluded from the visit list and documented as screen failures with the reason recorded.

1.2.1 Inclusion Criteria Assessment

The FRA will confirm that the farmer meets all of the following inclusion criteria before proceeding:

- Adult male or female aged 18 to 60 years (confirmed by asking the farmer their age or by review of a national identity document where available).
- Currently engaged in outdoor agricultural field work for at least three days per week during the study period.
- Physically capable of performing routine field tasks such as planting, harvesting, weeding, or carrying loads.
- Willing to undergo all study procedures: physiological monitoring with wearable devices, swallowing an ingestible temperature capsule (T-pill), providing blood and urine samples, and being observed during a full work shift.
- Able and willing to provide written informed consent.

1.2.2 Exclusion Criteria Assessment

The FRA will screen for each of the following exclusion criteria using direct questioning, review of any available health documents, and where necessary, consultation with a family member or community health worker:

- Known or suspected current gastrointestinal obstruction, or a history of gastrointestinal surgery (e.g. bowel resection, intestinal bypass, colostomy) that would make ingestion of the capsule unsafe.
- Physician-diagnosed severe cardiovascular disease, including unstable angina, recent myocardial infarction (within 6 months), or severe heart failure.
- Current pregnancy or breastfeeding.
- Presence of implanted electronic devices, such as a cardiac pacemaker or insulin pump, that may be subject to electromagnetic interference from the capsule receiver.
- Current use of diuretics, anticholinergic medications, or other medications known to substantially impair thermoregulation or sweat response.

- Acute febrile illness on the day of the visit axillary temperature will not be measured on Day 1, but the farmer will be asked whether they are currently experiencing fever, diarrhoea, or vomiting. A formal temperature check takes place on Day 2.

1.3 Informed consent

Informed written consent is obtained only after the farmer has been confirmed eligible. Consent covers all three study days and all procedures, including the household visits (Day 1 and Day 3), indoor sensor installation, and the full Day 2 workplace observation protocol (physiological monitoring, blood and urine sampling, and observation).

1.4 Participant Enrolment and ID Assignment

Immediately after the signed consent form is witnessed and countersigned, the FRA will enrol the farmer into the study. A unique Participant ID will be assigned in the format: HTS-[Site Code]-[3-digit sequential number] (for example, HTS-RAJ-001 for the first participant enrolled at the Rajshahi site). The Participant ID is entered into KoboToolbox form HTS-D1-Q, and a pre-printed ID label is affixed to all study materials prepared for that participant: the Day 1 survey form, the indoor sensor, and the urine pot.

The FRA will record the participant's Participant ID in the study master roster, which is maintained by the field supervisor and updated after each Day 1 visit. The master roster is stored on a password-protected field tablet and backed up to the secure cloud server each evening.

1.5 Baseline Survey Administration

Immediately following enrolment, the FRA will administer the Day 1 baseline survey using KoboToolbox form HTS-D1-Q. This is an interviewer-administered questionnaire; the FRA reads each question aloud and records the farmer's response. The survey is estimated to take 25 to 35 minutes. It covers four thematic modules.

1.5.1 Module A: Sociodemographic Characteristics

This module collects basic identifying and socioeconomic characteristics that will be used as covariates in the statistical analysis and for sub-group comparisons. The FRA will ask about:

- Age in completed years.
- Sex.
- Marital status.
- Highest level of formal education completed.
- Approximate monthly household income (using response bands, not exact figures, to reduce respondent discomfort).
- Number of household members currently living in the home.

1.5.2 Module B: Occupational History and Work Characteristics

This module characterises the farmer's typical work patterns and exposure conditions. These data inform the interpretation of Day 2 physiological findings and help contextualise the participant's level of acclimatisation. The FRA will ask about:

- Total years engaged in agricultural field work.
- Average number of days per week spent working in the field during the current season.

- Typical work shift start and end times.
- Main types of agricultural tasks performed (planting, harvesting, weeding, carrying loads, irrigation, soil preparation, crop maintenance, or other — tick all that apply).
- Whether the farmer regularly takes rest breaks during the work shift, and if so, the typical duration and number of breaks.
- Whether shade is typically available at the worksite, and if so, how often the farmer uses it.
- Whether drinking water is typically available at the worksite, and the usual source of water.
- Experience of any prior heat-related illness in the past 12 months (heat cramps, heat exhaustion, fainting, vomiting, or heat stroke), and whether medical care was sought.

1.5.3 Module C: Heat-Related Knowledge, Attitudes, and Practices

This module assesses what the farmer knows about heat-related health risks, their attitudes towards heat protection, and the practices they currently follow during hot weather. Findings will be used to characterise the sample and to contextualise heat-protective behaviour observed on Day 2. The FRA will ask about:

- How the farmer would describe the heat experienced at work during the hottest months, using a five-level verbal scale from 'not hot' to 'extremely hot'.
- Whether the farmer knows the warning signs that indicate they should stop work because of heat, and if so, what those signs are (recorded verbatim).
- The farmer's belief about whether drinking sufficient water during work prevents heat illness (five-point Likert scale from 'strongly agree' to 'strongly disagree').
- Perceived barriers to resting or drinking enough water during hot work
- Whether the farmer has ever received training or information about working safely in the heat, and if so, from whom.
- Specific practices the farmer currently uses to cope with heat: resting in shade, drinking more water, changing work timing, wearing specific clothing, or other strategies.

1.5.4 Module D: Housing Characteristics

Housing characteristics capture the residential thermal environment that the farmer returns to after the work shift. These data are important for understanding post-work heat recovery and will be linked to the indoor temperature sensor data collected over the 48-hour monitoring period. The FRA will ask about and, where possible, visually confirm:

- Primary roof material (corrugated iron or tin, concrete, thatch, asbestos, or other).
- Primary wall material (brick, concrete, bamboo or jute, mud, or other).
- Primary floor material (concrete, mud, tile, or other).
- Number of windows in the sleeping room.
- Whether cross-ventilation is present in the sleeping room (openings on more than one wall).
- Whether the household has electricity supply.
- Types of cooling devices available in the household
- Where the farmer usually sleeps at night
- Whether the farmer usually uses a mosquito bed net when sleeping.

- Presence of trees or shade structures near the dwelling that provide daytime shade.

1.6 Indoor Temperature and Humidity Sensor Installation

Following completion of the baseline survey, the FRA will install a hygrochon iButton temperature and relative humidity sensor in the participant's household. This sensor will record the residential thermal environment continuously from Day 1 evening through to Day 3 morning, capturing the conditions the farmer experiences during post-work recovery, sleep, and the overnight period between Day 2 and Day 3. The data from the sensor will be used to characterise indoor heat exposure as a potential modifier of overnight physiological recovery.

The FRA will retrieve the pre-labelled sensor assigned to this participant from the field equipment bag. Before installation, the FRA will confirm: (a) the sensor serial number matches the Participant ID label, (b) the sensor has been successfully initialised with the correct date, time, and recording interval (5 minutes) using the iButton software on the field tablet, and (c) the sensor battery charge is sufficient for the expected 48-hour monitoring period.

1.7 First-Morning Urine Collection Pot Handover

As the final step of the Day 1 household visit, the FRA will hand over a single labelled urine collection pot to the participant. This pot will be used by the farmer to collect their first-morning void urine on Day 2 — the first urine passed upon waking, before eating, drinking, or beginning any activity. This sample reflects the participant's baseline hydration status before the Day 2 work shift begins, providing a critical pre-work comparator for the end-of-shift and next-morning samples.

1.7.1 Preparation of the Urine Pot

Before the Day 1 household visit, the field supervisor will have prepared and labelled the urine collection pot for each enrolled participant. The label affixed to the pot will include: Participant ID, study site, date of collection (Day 2 date), the words 'FIRST MORNING URINE- BEFORE EATING OR DRINKING and a clear instruction that the entire void should be collected (not a partial sample). The pot will be a clean, sterile 60 mL container with a secure lid.

1.7.2 Instructions to the Participant

The FRA will provide clear verbal instructions to the participant, supported by a written instruction card attached to the pot in Bangla. The instructions are:

- Tomorrow morning (Day 2), when you wake up, the very first urine you pass should go into this pot- before eating anything, before drinking any water, and before starting any activity.
- Please collect as much of the first urine as possible into the pot. Fill it and close the lid tightly.
- Keep the pot in a cool, shaded location until the field team arrives at the field in the morning. Do not leave it in the sun.
- Bring the pot with you when you come to work in the field tomorrow. The field team will collect it from you at the worksite in the morning.
- If you forget or are unable to bring the pot, please tell the field team when you arrive. This is important information and should not be hidden.

The FRA will confirm that the participant has understood the instructions by asking them to repeat back the key steps in their own words. The FRA will record in HTS-D1-Q that the pot was handed over, that instructions were given, and whether the participant demonstrated understanding.

1.8 Day 1 Closing and Preparation for Day 2

Before leaving the household, the FRA will complete the following closing steps:

- Confirm the participant's worksite location and the expected time they will arrive at the field on Day 2. Record this in HTS-D1-Q.
- Confirm the participant's mobile phone number (if available) for Day 2 coordination.
- Remind the participant that the field team will arrive at the worksite before them and will be ready when they arrive.
- Submit the completed HTS-D1-Q form in KoboToolbox before leaving the household. Do not save it as a draft.
- Inform the field supervisor of the enrolment, the participant's worksite location, and the expected shift start time so that Day 2 logistics can be coordinated.

The agrometeorological weather forecast will be checked prior to all the data-collection and field observation schemes will be planned accordingly. On the day prior to data collection, the field team will check forecasts and will check again the next morning before proceeding on with field observations.

Rainfall conditions will be assessed using the following agrometeorological rainfall categories (28):

Rainfall category	Rainfall amount
Light rain	1-10 mm/day
Moderate rain	11-22 mm/day
Moderately heavy rain	23-43 mm/day
Heavy rain	44-88 mm/day
Very heavy rain	>88 mm/day

- If light rain is forecast or if a light rain occurs during the fieldwork, data collection would be paused (during rainfall) and resumed after the end of the rain, if participant is safe, equipment protected and ordinary agricultural activity continues. Recording of all interruptions, changes in working activity or execution away from the planned observation.
- If moderate rain or heavier rainfall is expected during the day, field observation will be postponed to another day.
- In the event of moderate or heavier rain beginning unexpectedly during a field-based survey, observation will be ceased, all equipment secured, and the session recorded as uncompleted/rescheduled.

- Field observation will be delayed or suspended when thunderstorm, lightning, waterlogging of field site, unsuitable (unsafe) field conditions or any condition that may pose a risk to participants or field staff.

Day 2 Activities (workplace observation day)

Day 2 will be the primary data collection day. All physiological monitoring, biological sampling, and continuous environmental measurement will take place on this day. The full observation period will last for six hours.

Table 2: Overview of Day 2 activities

Timing	Phase	Key Activities
Before farmers arrive	Field Site Setup	Environmental monitoring unit assembled; equipment checked and calibrated; area surveyed
On farmer arrival	Arrival Procedures	First-morning urine collected; urine specific gravity measured; acute screening; enrolment confirmation; pre-shift blood sample
After blood sample	Device Deployment	T-pill activated and swallowed; anthropometry (height, weight); Actigraph fitted to waist; graded water bottle and graded urine pot issued; farmer begins usual work
Hours 1-6	Work Observation	Hourly HRI symptoms and thermal sensation survey; FRA observation checklist completed each hour; continuous core temperature, actigraphy, and environmental data collection
After 6 hours	Post-Shift	Post-shift blood and urine samples; post-shift weight; handover of overnight urine pot and water bottle with instructions

2.1 Field Site Setup - Before Farmers Arrive

The field team will arrive at the agricultural worksite at least 60 minutes before the farmers are expected to start work. This provides sufficient time to set up the environmental monitoring unit, check all equipment, and be ready to receive participants on their arrival.

2.1.1 Environmental Monitoring Unit Setup

The portable environmental monitoring unit will be assembled and positioned at the field site to record outdoor heat exposure throughout the 6-hour observation period. The unit measures the environmental variables required to calculate the Universal Thermal Climate Index (UTCI):

air temperature, relative humidity, wind speed, and mean radiant temperature. The portable outdoor environmental monitoring equipment will be positioned within 10 metres from participant's main working area. SOP 5.4 in Appendix D specifies how to perform equipment set up, calibration checks, recording frequency and outlining procedures for the calculation of UTCI.

2.1.2 Equipment and Consumables Check

Before farmers arrive, the FRA will conduct a systematic check of all equipment and consumables needed for the day. The field supervisor will co-sign the equipment checklist (HTS-Equip-01) to confirm readiness. The following items must be confirmed ready:

- T-pill capsules (e-Celsius performance): check batch number and expiry date; confirm the e-Celsius Activator is charged and functional; confirm the receiver has been initialised for each participant and battery is full.
- Actigraph devices: confirm that each device has been initialised with the correct Participant ID, date, time, and 30-second epoch; battery at 100%; clip attachment in good condition.
- Graded water bottles (2 L, clear, with measurement markings): one per participant, clean and empty, labelled with Participant ID.
- Graded urine collection pots (2L, clear, with measurement markings): one per participant for during-shift urine collection, labelled with Participant ID.
- End-of-shift urine pots (labelled separately) and overnight urine pots (labelled for Day 3 morning collection): prepared and labelled for each participant.
- Blood sample collection materials: 21-gauge butterfly needles, 5 mL EDTA tubes (purple cap), 5 mL plain serum tubes (red or gold cap), tourniquet, antiseptic swabs, sterile gauze, adhesive bandages, sharps container, biohazard bags.
- Portable urine refractometer: confirmed clean, calibrated with distilled water (should read SG 1.000), lens cleaned.
- Cooler box with sufficient ice packs: pre-cooled to 2-8°C and confirmed with a thermometer.
- Portable weighing scale: set on a level, firm surface; zeroed; last calibration date within the week.
- Portable stadiometer: assembled and vertical; last calibration check confirmed.
- KoboToolbox field tablet: charged; all Day 2 forms (HTS-D2-Q, HTS-Obs-01) loaded and accessible offline.

2.2 Farmer Arrival and First-Morning Urine Collection

As each farmer arrives at the worksite, the FRA will greet them and immediately begin the arrival sequence. The first-morning urine sample must be collected and processed before any other Day 2 procedure begins.

2.2.1 Collection of the First-Morning Urine Sample

1. On greeting the farmer, the FRA will first ask whether they collected the urine in the pot provided on Day 1 evening and whether they have brought it with them. If yes, the FRA will receive the pot from the farmer.
2. If the farmer did not bring the pot, or was unable to collect the sample (forgot to void into the pot, lost the pot), the FRA will document this as a missing first morning urine

sample in HTS-D2-Q and will handover another pot to collect pre-shift urine sample and will continue next procedures.

2.2.2 Urine Specific Gravity Measurement

1. The FRA will open the urine pot and use a disposable transfer pipette to place two to three drops of urine on the prism of the portable urine refractometer.
3. The FRA will close the daylight plate, hold the refractometer towards a light source, and read the urine specific gravity (USG) value on the scale through the eyepiece. The reading is taken at the boundary line between the clear and shaded areas on the scale.
4. The USG reading will be recorded immediately in KoboToolbox form HTS-D2-Q, along with the time of reading and the sample type (first-morning urine from home, or first morning urine collected at worksite).
5. The refractometer prism will be rinsed with distilled water and dried with a lint-free cloth after each reading.
6. The remaining urine sample will be transferred to the labelled pre-shift urine collection tube, sealed, and placed immediately in the cooler box at 2-8°C for laboratory transport.

2.3 Acute Condition Screening and Enrolment for Day 2 Observation

Before any physiological device is deployed or any invasive procedure is performed, the FRA will conduct a brief acute health screening to check whether the farmer is in a condition suitable for safe participation in the observation on this specific day.

2.3.1 Acute Screening Procedures

1. The FRA will measure the farmer's axillary temperature using a digital axillary thermometer. The measurement will be taken before any physical exertion. Record the temperature in HTS-D2-Q.
7. The FRA will ask the farmer the following questions: (a) Do you have fever or feel feverish today? (b) Do you currently have diarrhoea or vomiting? (c) Do you have any unusual pain or physical complaint that is preventing you from working today? (d) Have you taken any new medication since yesterday evening?
8. The FRA will visually observe the farmer for signs of acute illness: pallor, excessive sweating at rest, confusion or disorientation, laboured breathing, or obvious physical incapacitation.

2.3.2 Day 2 Enrolment Decision

If the axillary temperature is above 37.5°C, or if the farmer reports diarrhoea, vomiting, or any condition that could confound the physiological measurements or increase the risk of heat-related illness during the observation, the farmer will not be enrolled for Day 2 on that day. The FRA will:

- Explain to the farmer, kindly, that they cannot participate in today's observation because of their current health condition.
- Advise the farmer to rest and seek medical care if symptoms worsen.
- Document the reason for deferral in HTS-D2-Q under the acute screening section.
- Notify the field supervisor and schedule an alternative Day 2 visit date if the farmer remains enrolled and wishes to continue after recovery.

If the farmer passes the acute screening (axillary temperature $\leq 37.5°C$ and no acute conditions identified), the FRA will confirm their enrolment for Day 2 observation and proceed immediately to the pre-shift blood sample collection.

2.4 Pre-Shift Blood Sample Collection

Immediately after enrolment is confirmed, a pre-shift venous blood sample will be collected from the farmer by the trained phlebotomist or nurse who is part of the field team. SOP 5.6 in Appendix D will describe the sample collection, labelling, storage, and maintenance of cold chain during transport. Blood samples will be collected for HbA1c, creatinine, cystatin-C and osmolarity.

2.5 T-Pill (Ingestible Core Temperature Capsule) Activation and Ingestion

The e-Celsius Performance ingestible telemetry capsule (referred to throughout as the T-pill) will be used to measure the farmer's gastrointestinal core body temperature continuously throughout the 6-hour observation period and beyond. The capsule will be ingested 40 minutes before the work shift begins to allow the gastrointestinal temperature reading to stabilise and reach equilibrium with true core temperature. Administration, recording and safety monitoring of capsules will follow SOP 5.3 Appendix D.

2.6 Anthropometric Measurements

Height and body weight will be measured after T-pill ingestion and before work begins. Measurements will be repeated post-shift.

BMI will be calculated automatically by KoboToolbox using the formula: BMI = weight (kg) ÷ height² (m²). Body surface area (BSA) will be calculated offline using the Du Bois formula: BSA (m²) = 0.007184 × height (cm)^0.725 × weight (kg)^0.425.

2.7 Actigraph Placement on the Waist

Physical activity during the work shift will be monitored using a triaxial ActiGraph accelerometer device. Participants will wear a triaxial accelerometer on the waist during the entire duration of an agricultural work shift. SOP 5.5 in Appendix D will be followed for device initialization, placement, battery check and time synchronization.

2.8 Graded Water Bottle and Graded Urine Collection Pot Issue

Immediately before the farmer begins their usual work activities, the FRA will issue each farmer two items: a graded water bottle and a graded urine collection pot. These two items will be used throughout the 6-hour observation period to quantify the farmer's total fluid intake and total urine output, respectively.

2.8.1 Water intake monitoring

1. Issue the pre-labelled 1.5 L clear graduated water bottle to the farmer. The bottle will be filled with clean drinking water to the brim and the exact initial volume (2000 mL) will be recorded in HTS-D2-Q before handing it to the farmer.
2. Instruct the farmer clearly and firmly: 'Please drink only from this bottle throughout today's work period. If you need more water, please tell us and we will refill the bottle and record the refill. Please do not drink from any other source, including rivers, irrigation channels, other people's containers, or water brought by family members. This is very important for our measurements.'

3. Inform the farmer that they are free to drink as much or as little as they wish- there are no restrictions on how much they drink. The instruction is only about the source of water. The farmer's natural drinking behaviour must not be interfered with.
4. Tell the farmer they will take the water bottle home after the work shift ends and continue drinking from it until the following morning. The bottle and any remaining water will be collected by the field team on Day 3 or when they next meet the farmer.

2.8.2 Urine output monitoring

1. Issue the pre-labelled graduated urine collection pot (300-500 mL capacity) to the farmer. This pot is distinct from the post-shift urine sample pot and from the overnight pot that will be given at the end of the shift.
2. Instruct the farmer: 'Each time you need to urinate during work today, please urinate directly into this pot. After you have passed urine, one of our team members will record the volume, and then you can empty the pot privately. Please do not urinate elsewhere during the work period if you can avoid it. If you do need to urinate and cannot reach the pot in time, please tell us so we can record it as an unrecorded void.'
3. Explain that the team will come to each farmer at least once an hour and will check the pot at that time. Farmers are encouraged to tell the team immediately after urinating so that the volume can be recorded promptly.
4. Record the issuance time and the initial pot volume (0 mL) in HTS-D2-Q.

2.9 Six-Hour Work Observation Period

On Day 2, participants will be observed for ~6 hours during a routine agricultural work shift using a structured observation checklist. Hourly observation rounds will document work task, posture, exposure to sun, shade, rest breaks, fluid intake, core temperature, and the prompt for any safety concerns. The frequency of observations will be increased if any symptoms appear or the core temperature reaches predefined alert levels. Farmers will participate in short survey (5 minutes) for thermal sensation and perceived exertion, HRI symptoms. The farmer continues to rest or resume work immediately after the survey.

2.10 Post-Shift Procedures

At the end of 6 hours of observation from the work start time, the FRA will inform each farmer that the monitoring period has ended and that a final set of measurements will now be taken. Post-shift procedures must be completed promptly and in sequence. The biological samples collected at this stage reflect the physiological impact of the full 6-hour exposure to environmental heat and physical work, and any delay in collection or sample processing will compromise the validity of the measurements.

2.10.1 Post-Shift Blood and Urine Sample Collection

9. Record the exact work shift end time in HTS-D2-Q.
10. Ask the farmer to sit and rest quietly for 3 to 5 minutes before venepuncture, as they have been physically active.
11. Collect the post-shift venous blood sample following the same procedure as the pre-shift collection (Section 2.4). Draw blood into one EDTA tube (5 mL) and one plain serum tube (5 mL). Label both tubes 'Post-shift blood' with Participant ID, date, and time. Place immediately in the cooler box at 2-8°C. Record in HTS-D2-Q.
12. Collect the post-shift urine sample: provide the farmer with the pre-labelled post-shift urine collection tube or pot. Ask the farmer to void directly into the container. The FRA

will measure the urine specific gravity immediately using the portable refractometer (following the same procedure as Section 2.2.2) and record the USG and urine colour (using the standardised urine colour chart) in HTS-D2-Q. Transfer 30 mL of the urine into the labelled sample tube, seal, and place in the cooler box.

13. Record the total volume of urine collected in the post-shift sample, as well as the time of collection.

2.10.2 Post-Shift Body Weight Measurement

2. Immediately after sample collection, measure the farmer's body weight using the same procedure as the pre-shift measurement (Section 2.6.2). Use the same scale, in the same position, with the farmer wearing the same clothing. Record the post-shift weight in HTS-D2-Q with the exact time, labelled as 'Post-shift weight'.

14. Calculate the absolute weight change (pre-shift weight minus post-shift weight, in kg) and the percentage body weight change. Record both in HTS-D2-Q.

2.10.3 Overnight Urine Pot and Water Bottle Handover

As the final step of Day 2, the FRA will hand over two items to the farmer: the overnight urine collection pot (for Day 3 first-morning void) and the study water bottle (to continue tracking fluid intake overnight).

1. Hand the farmer the pre-labelled overnight urine collection pot. It is labelled 'Day 3 First Morning Urine - Before Eating or Drinking. Instruct the farmer to collect their first urine void on Day 3 morning into this pot before eating, drinking, or starting any activity, following exactly the same instructions as provided on Day 1 evening.
2. Hand the farmer the same study water bottle (the graduated 1.5 L bottle that was issued before the shift). Refill it to its total capacity if necessary and record the refill volume. Instruct the farmer: 'Please continue drinking from this bottle tonight and tomorrow morning. When our team visits you tomorrow (or when you bring it to the field), we will measure how much water you have drunk from this bottle since the end of your work today. This helps us understand how much you drink to recover from the heat.'
3. Confirm the farmer understands both instructions. Ask them to repeat back the key points. Record in HTS-D2-Q that both the overnight urine pot and the water bottle have been issued, with instructions given and understood.
4. Remind the farmer that a field team member will visit their household on Day 3 morning to collect the urine pot, measure the remaining water in the bottle, and ask a few brief questions about their sleep and recovery.

Day 3: Next-Morning Recovery Assessment

The next morning after each monitored work shift, field staff will collect next-morning biological samples to evaluate post-work and overnight recovery. Urine samples will be collected for creatinine, IGFBP7 and NGAL. Blood will be collected for osmolarity, as well as creatinine and cystatin-C.

A recovery survey will also be conducted on Day 3. In this survey, participants will be asked about sleep-related questions, food consumed during the nighttime, fluid intake at nighttime, thermal comfort in sleep and perceived recovery and fatigue, exertion or body-strain scale and any heat related symptoms after return home from work.

On Day 3, after recording approximately 48 hours of monitoring the indoor temperature and relative humidity sensor will be collected. Field staff will examine the device, download the data, record any problems with the device or deviations from protocol and match indoor sensor data with Day 1, Day 2 and Day 3 records of a given participant using a unique Participant ID.

Biological Sample Storage and Analysis

Biological samples will be collected during three timepoints: pre-shift and post-shift on Day 2, and next morning on Day 3. They will be analysed for hydration status, renal function, kidney stress or injury biomarkers and recovery following post-work.

Time point	Urine markers	Blood markers
Pre-shift	USG, albumin, creatinine, IGFBP7, TIMP-2, NGAL	HbA1c, creatinine, cystatin-C, osmolarity
Post-shift	USG, creatinine, IGFBP7, TIMP-2, NGAL	Creatinine, cystatin-C, osmolarity
Next morning	Creatinine, IGFBP7, NGAL	Creatinine, cystatin-C, osmolarity

All of these biological samples will be labelled with the values Participant ID, date, timepoint and sample type. Samples will be stored and transported using cold-chain conditions as described in SOP 5.6 as provided in Appendix D. The final sample volume, tube type to be used, processing procedure, storage temperature, and laboratory analysis plan will be determined by working with the laboratory team and documented in SOP 5.6 prior to data collection starting.

Data Collection

Data collection will be structured across three study days (day 1: household-based eligibility screening, baseline survey, household assessment and indoor sensor installation; day 2: field-based physiological monitoring and hourly observation; day 3: one next-morning recovery assessment).

Day 1 participant survey and Day 2 structured observation checklist will be distinct. The Day 1 participant survey will consist of eligibility screening, demographic and socioeconomic characteristics, usual farming profile, self-reported health status, heat-related knowledge, attitude and practice, household characteristics, water access, and usual water-intake behaviour.

Structured observations on Day 2 will only be performed during the shift where agricultural work is conducted. This would include time-varying field information of current task, posture, direct sunlight exposure, shade availability, number and duration of rest breaks, water intake, other fluid intake, thermal sensation score, perceived exertion, current core temperature reading, and safety action observed. The observation rounds will be performed on an hourly basis, and more frequently if certain symptoms or core temperature warning signs arise.

The Day 3 recovery survey will include sleep-related questions, nighttime food consumption, nighttime fluid intake, thermal comfort, perceived recovery, fatigue or exertion scale, and heat-related symptoms after work.

Biological sample forms will record pre-shift, post-shift, and next-morning blood and urine sample collection, sample time, sample type, labelling, storage, cold-chain maintenance, and transport. Device-monitoring forms will record indoor sensor installation and retrieval, capsule administration, receiver function, accelerometer placement, outdoor environmental monitoring, data download, and equipment problems.

All data collected for field survey and observation will only be using KoboToolbox encrypted password-protected electronic forms based on the developed study questionnaire. The forms will consist of mandatory fields, range checks, skip logic, consistency check and standardized response codes. Questionnaire data, biological samples, device data, and observation records will be linked using a unique Participant ID. Core temperature receivers, accelerometers and environmental monitoring devices will collect sensor data with continuous sampling intervals and safely download the recorded data daily to password-protect field tablets within 24 hours post-collection. The Daily Field Workflow Checklist in Appendix A will guide the timing and sequence of field activities. All files will be labelled using standardized file names containing site ID, participant ID, date, and data type.

Data Safety Monitoring Plan (DSMP)

Data Quality Assurance

Data will be ensured through quality at the field, laboratory and data-management levels. Training of field staff will include study procedures, ethics and participant safety, KoboToolbox, device setup and use for biological sample collection and handling, data flow management and data security.

Implement range checks, skip logic, required fields and consistency check in KoboToolbox forms to minimize data-entry errors. Field supervisors will review data completeness on a daily basis and data managers will perform regular quality control against field notes, sensor files and sample transport logs.

The time on each device will be synchronized prior to every observation day, allowing for temporally linked environmental, core-temperature, accelerometry, observation fluid-intake and biological-sample data. Before lock of the database; missing data, unlikely values, missing sensors, non-wear windows and deviations from cold-chain and protocol shall be flagged and resolved. Appendices C and D will prescribe field procedures, along with equipment calibration.

Participant Confidentiality

All study forms, biological samples, sensor files, analytic datasets, reports and publications will identify participants only by study identification number. The linkage file that links participant names or identifying information to study identification numbers will be kept on an encrypted drive to which only authorized study personnel have access.

Electronic data will be stored in password-protected files and access to study personnel on the cloud for backup purposes. Biological samples will only be labelled with participant ID, date, time and type of sample. The results of the study will be presented in aggregate form and no participant individual could ever be identified from any report, presentation, thesis or journal publication.

Safety Monitoring

Heat-Related Illness Response Protocol

Participant safety will be actively monitored during each 6 hours of work shift. Data collection will occur through structured observation rounds by field staff every hour, and monitoring frequency will be increased in the event that participants report symptoms or if core temperature reaches predefined alert levels. Symptoms of concern are includes, dizziness, light-headedness, headache, nausea and/or vomiting confusion syncope extreme fatigue and altered mental status.

Trigger	Immediate Action	Responsible Personnel
Core temp 38.5-38.9°C	Alert supervisor; increase observation frequency to every 15 min; offer cool water	Field Research Assistant
Core temp 39.0-39.4°C	Cease work immediately; move to shade; cool with wet cloth; oral rehydration; contact medical officer	Field Supervisor + Medical Officer
Core temp ≥ 39.5°C or confusion / vomiting	Treat as emergency; call emergency services; begin active cooling (cool water immersion if possible); do not leave participant	All available staff
Symptomatic dehydration (dizziness, syncope)	Cease work; supine position; oral rehydration; contact medical officer	Field Research Assistant + Supervisor

Data Safety Monitoring

A Data Safety Monitoring Committee made up of two independent clinical researchers and a biostatistician will review safety data following every 50 worker-day observations. The committee will review adverse events including, but not limited to, protocol deviations, heat-related illness events, withdrawals of participants from study and serious safety issues. If safety concerns arise, the committee will be empowered to recommend protocol modification, temporary suspension or cessation of data collection.

Sample Size

A total of 200 agricultural workers (200 worker-day observations) will be recruited for this study. Each farmer will be observed for a regular agricultural work period of around 6 hours.

The full scope of UTCI values will be covered in the study. This will help to characterise the exposure–response relationship between UTCI and core body temperature and explore potential heat-strain thresholds.

Sample Allocation

District	Selected upazilas	Selected unions	Farmers per union	Total farmers
Rajshahi	1	2	50	100
Jashore	1	2	50	100
Total	**2**	**4**	—	**200**

This study will comprise about 20% of the overall sample being female farmers. The study will intend to recruit around 160 male and 40 female farmers. Selected union will recruit about 40 male and 10 female farmers. Where upazila recruitment of female farmers is lower, the implementation might re-distribute farmer recruitments across other upazilas to maintaining 20% female participation.

Outcome Measures

Primary Outcome

The primary outcome of this study is core body temperature, measured continuously and non-invasively throughout the six-hour work shift using the e-Celsius Performance ingestible telemetry capsule.

Primary Outcome Variable	Operational Definition and Units
Time-weighted mean core temperature (T_mean)	Arithmetic mean of all valid 1-minute T_gi readings during the 6-hour shift (°C).
Peak core temperature (T_peak)	Maximum T_gi recorded at any single 1-minute reading during the 6-hour shift (°C).
Time above 38.5°C (T>38.5)	Total duration in minutes during the 6-hour shift at which T_gi was recorded at or above 38.5°C. Reflects the dose of high thermoregulatory strain.

Secondary Outcomes

Hydration markers	Pre-shift, post-shift Plasma osmolarity
	Pre-shift, post-shift and next morning Urine specific gravity (USG)
	Post-shift body weight change
Renal strain markers	Serum creatinine
	Serum cystatin-C
	Urine albumin

	Urine creatinine
	Urine NGAL (Neutrophil Gelatinase-Associated Lipocalin)
	Urine IGFBP7 (Insulin-like Growth Factor Binding Protein 7)
	Urine TIMP-2 (Tissue Inhibitor of Metalloproteinase 2)
Metabolic heat production	Estimated metabolic heat production (M)
Subjective and symptomatic outcome	Borg CR-10 perceived exertion (RPE)
	Fatigue score
	Thirst score
	Thermal sensation
	Thermal comfort
	Heat-related illness symptoms
	Overnight sleep quality

Statistical Analysis

The primary analytical aim of this study is to characterise the relationship between environmental heat exposure (UTCI) and core body temperature across the UTCI distribution observed in Rajshahi and Jashore districts, including two selected upazilas and four selected unions, and to identify the UTCI level at which core temperature begins to rise in a clinically-the thermoregulatory strain threshold.

Descriptive analysis will be conducted to summarize participant characteristics, housing characteristics, outdoor and indoor heat exposure, physical activity, fluid intake, core body temperature, serum electrolytes, and dehydration markers. Continuous variables will be summarized using mean and standard deviation or median and interquartile range, depending on distribution. Categorical variables will be summarized using frequency and percentage.

The primary analysis will use restricted cubic spline regression or a generalized additive model to allow for a potentially non-linear relationship between time-weighted mean UTCI during the shift and time-weighted mean core temperature (T_mean) across worker-day observations. A second analysis model will examine the minute-by-minute core temperature record as a function of concurrent and lagged UTCI using a generalized additive mixed model with a random intercept for each participant and first-order autoregressive correlation structure (to account for correlation between consecutive measurements). As each participant will contribute an observation for one worker-day, measurements are minute-level but nested within participants. This model will only include measurements taken during the six-hour agricultural work shift, and not those taken while at home.

The association between time-weighted mean UTCI and (a) post-shift plasma osmolarity and (b) post-shift USG will be examined using multivariable linear regression or generalized additive models with plasma osmolarity or USG as the outcome and pre-shift plasma osmolarity or pre-shift USG included as a covariate to adjust for baseline hydration status.

Binary dehydration outcomes (plasma osmolarity $\geq$ 295 mOsm/kg; USG $\geq$ 1.020) will be analysed using mixed-effects logistic regression with the same covariate structure and participant random intercept. Odds ratios and 95% confidence intervals will be reported.

Renal strain markers (serum cystatin-C, urine creatinine, urine NGAL, urine IGFBP7, and urine TIMP-2) will be analysed as a nested secondary outcome group, characterising the subclinical renal response to occupational heat exposure and dehydration across three time-points: pre-shift (Day 2 morning), post-shift (Day 2 end-of-shift), and overnight recovery (Day 3 morning). For each renal marker, a linear mixed-effects model will be fitted with the post-shift value (or the post-shift minus pre-shift change) as the outcome, time-weighted mean UTCI as the primary exposure, and the pre-shift value of the same marker as a covariate measuring within-shift change in each renal biomarker attributable to heat exposure, adjusted for baseline status. Fixed-effect covariates match the primary model: MET, total fluid intake, age, sex, BMI, and study site. Participant will be included as a random intercept.

Ethics

This will be a nested study within the parent study entitled: "Evaluating a Multisectoral Heat-Health Intervention Model to Reduce Heat-Related Illness Among Agricultural Workers in Rural Bangladesh: A Pre-Post Implementation Study." The design of the main study has been approved by the Research Review Committee (RRC) and the Ethical Review Committee (ERC) of icddr,b under protocol reference PR-25026.

The current protocol which includes, updated study procedures, questionnaires, biological sample collection and analysis plan, physiological and environmental monitoring procedures and all relevant standard operating procedures will be submitted to the RRC and ERC as an amendment to protocol PR-25026 prior to participant recruitment and collection of data.

Participants will be asked to provide their written informed consent before enrolment, and prior to the undertaking of any study-related procedures. Participation is voluntary, and participants can withdraw at any time without penalty or loss of services or other benefits to which they are entitled.

Unique study identification numbers will be used to identify all study data, biological samples, and devices generated records. Data that identifies a participant will be stored separately in password-protected and encrypted files. Only authorized study personnel will have access. Biological specimens will be collected, processed, stored, transported and analysed per the approved amendment, applicable standard operating procedures by icddr,b, and relevant institutional ethical and biosafety regulations.

References

1. Gubernot DM, Anderson GB, Hunting KL. The epidemiology of occupational heat exposure in the United States: a review of the literature and assessment of research needs in a changing climate. International Journal of Biometeorology. 2014;58(8):1779-88.
2. Kjellstrom T, Holmer I, Lemke B. Workplace heat stress, health and productivity-an increasing challenge for low and middle-income countries during climate change. Global health action. 2009;2(1):2047.
3. Spector JT, Sheffield PE. Re-evaluating occupational heat stress in a changing climate. Annals of occupational hygiene. 2014;58(8):936-42.
4. El Khayat M, Halwani DA, Hneiny L, Alameddine I, Haidar MA, Habib RR. Impacts of Climate Change and Heat Stress on Farmworkers' Health: A Scoping Review. Frontiers in Public Health. 2022;Volume 10 - 2022.
5. Flouris AD, Dinas PC, Ioannou LG, Nybo L, Havenith G, Kenny GP, et al. Workers' health and productivity under occupational heat strain: a systematic review and meta-analysis. The Lancet Planetary Health. 2018;2(12):e521-e31.
6. Ioannou LG, Mantzios K, Tsoutsoubi L, Nintou E, Vliora M, Gkiata P, et al. Occupational heat stress: multi-country observations and interventions. International journal of environmental research and public health. 2021;18(12):6303.
7. Amoadu M, Ansah EW, Sarfo JO, Hormenu T. Impact of climate change and heat stress on workers' health and productivity: A scoping review. The Journal of Climate Change and Health. 2023;12:100249.
8. Correa-Rotter R, Wesseling C, Johnson RJ. CKD of Unknown Origin in Central America: The Case for a Mesoamerican Nephropathy. American Journal of Kidney Diseases. 2014;63(3):506-20.
9. Herath C, Jayasumana C, De Silva PMCS, De Silva PHC, Siribaddana S, De Broe ME. Kidney Diseases in Agricultural Communities: A Case Against Heat-Stress Nephropathy. Kidney International Reports. 2018;3(2):271-80.
10. Jayasekara K, Kulasooriya P, Wijayasiri K, Rajapakse E, Dulshika D, Bandara P, et al. Relevance of heat stress and dehydration to chronic kidney disease (CKDu) in Sri Lanka. Preventive medicine reports. 2019;15:100928.
11. Moyce S, Mitchell D, Armitage T, Tancredi D, Joseph J, Schenker M. Heat strain, volume depletion and kidney function in California agricultural workers. Occupational and environmental medicine. 2017;74(6):402-9.
12. McGirr A, Chowdhury S, Rabbi MF, Hasan MM, Sagar MS, Ibnat N, et al. High indoor temperatures increase reporting of acute symptoms: finding mitigating solutions for the climate-vulnerable of Bangladesh. BMC Public Health. 2025;25(1):2121.
13. Minor K, Bjerre-Nielsen A, Jonasdottir SS, Lehmann S, Obradovich N. Rising temperatures erode human sleep globally. One Earth. 2022;5(5):534-49.
14. Krisher L, Villarreal Hernandez K, Li Y, Butler-Dawson J, Jaramillo D, Yoder HA, et al. Heat exposure, heat strain, and off-work recovery of Guatemalan sugarcane workers. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2025;328(6):R703-R17.
15. Gao C, Kuklane K, Östergren P-O, Kjellstrom T. Occupational heat stress assessment and protective strategies in the context of climate change. International Journal of Biometeorology. 2018;62(3):359-71.

16. Mac VV, Elon L, Smith DJ, Tovar-Aguilar A, Economos E, Flocks J, et al. A modified physiological strain index for workplace-based assessment of heat strain experienced by agricultural workers. American Journal of Industrial Medicine. 2021;64(4):258-65.
17. Wagoner RS, López-Gálvez NI, de Zapien JG, Griffin SC, Canales RA, Beamer PI. An occupational heat stress and hydration assessment of agricultural workers in North Mexico. International journal of environmental research and public health. 2020;17(6):2102.
18. Notley SR, Flouris AD, Kenny GP. On the use of wearable physiological monitors to assess heat strain during occupational heat stress. Applied Physiology, Nutrition, and Metabolism. 2018;43(9):869-81.
19. Cannady R, Warner C, Yoder A, Miller J, Crosby K, Elswick D, et al. The implications of real-time and wearable technology use for occupational heat stress: A scoping review. Safety Science. 2024;177:106600.
20. Dolson CM, Harlow ER, Phelan DM, Gabbett TJ, Gaal B, McMellen C, et al. Wearable sensor technology to predict core body temperature: a systematic review. Sensors. 2022;22(19):7639.
21. Nath A, Sahu S. Predicting Heat-Induced Physiological Strain and Dehydration Among Male Agricultural Workers in Eastern India: Implications of Climate Change. Journal of Occupational and Environmental Medicine. 2026;68(1):e69-e76.
22. Kenny GP, Jay O. Thermometry, Calorimetry, and Mean Body Temperature during Heat Stress. Comprehensive Physiology. 2013;3(4):1689-719.
23. Kovats RS, Hajat S. Heat stress and public health: a critical review. Annual review of public health. 2008;29(2008):41-55.
24. Vandenbroucke JP, Von Elm E, Altman DG, Gøtzsche PC, Mulrow CD, Pocock SJ, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. International journal of surgery. 2014;12(12):1500-24.
25. Bröde P, Fiala D, Błażejczyk K, Holmér I, Jendritzky G, Kampmann B, et al. Deriving the operational procedure for the Universal Thermal Climate Index (UTCI). International journal of biometeorology. 2012;56(3):481-94.
26. Blazejczyk K, Epstein Y, Jendritzky G, Staiger H, Tinz B. Comparison of UTCI to selected thermal indices. International journal of biometeorology. 2012;56(3):515-35.
27. Karmakar S. Patterns of climate change and its impacts in northwestern Bangladesh. J Eng Sci. 2019;10:33-48.
28. Agro-Meteorological Division BMD. Agrometeorological Weather Forecast for the period: 10-05-2026 to 16-05-2026. 2026.