

Appendix 5: Sampling protocols for studying ecological engineering for rice pest suppression in irrigated tropical rice (as adapted at Ho Chi Minh City Workshop)

Overview

As in all rigorous research, the methods to be used are determined by the hypothesis to be tested. Accordingly it is worth making this hypothesis explicit:

H⁰: Implementing ecological engineering improves biological control of pests by enhancing biodiversity compared with farmer's practices using insecticides (control).

The following sections that set our sampling protocols are organized according to our collection of data to test this key hypothesis. It is important that data collection is careful and that the agreed protocols are followed because of the following reasons: Our experimental design is one, multi-site (Thailand, Vietnam, China (Guilin), China (Jinhua)) experiment with each site having a single replicate of each of the two treatments: ecological engineering versus *farmer's practices using insecticides (control)*. Accordingly, if any site uses different methods or fails to collect data for any given aspect the whole experiment is compromised. Note that although it is critical to collect data from the ecological engineering and the control areas on all sites in a consistent manner the integrity of the design does not require us to use identical ecological engineering methods on all sites. There is no problem with sites using different flower species, sowing dates, varieties etc. Ecological engineering is about implementing management practices that are appropriate for a given locality and acceptable to farmer practice.

So remember: okay to implement locally applicable ecological engineering practices but MUST use the agreed sampling methods.

Some sites may wish to test supplementary hypotheses. This is encouraged provided that they do not compromise the testing of our key hypothesis. What does this mean? It would be a bad idea to have replicated plots of sprayed and unsprayed plots/fields in the ecological engineering and insecticide spraying (control) areas. Doing this would lead to increased numbers of natural enemies in the insecticide spraying (control) area and decreased numbers in the ecological engineering area. Accordingly, the insecticide spraying (control) area on each of our four sites should be managed according to conventional farmer practice whereby insecticide use is the mainstay of pest management.

It would be okay to test supplementary hypotheses that do not compromise the key hypothesis. Examples of these are:

- Sow small, replicated plots of different flower species in one part of the eco-engineering area and sample these to see which attracts the most natural enemies.
- Laboratory bioassays to measure effects of different flower species on parasitoid fitness (see Ricehoppers site for detail on this)
- Mark natural enemies in flowers with rubidium chloride and follow the movement of these into rice.
- Analyze gut contents of predators to identify prey species consumed (see Ricehoppers site for detail on this).

We plan to seek additional funding to develop molecular (DNA ‘barcoding’) identification methods for natural enemies captured in this project so you will notice later sections specify use of 100% ethanol for specimen storage. This will maximize DNA quality and is especially important for samples collected by sweep net and for parasitoids reared from egg trap plants because these specimens can be placed immediately into preservative without any degradation.

Sampling methods for key hypothesis

H^o: Implementing ecological engineering improves biological control of pests by enhancing biodiversity compared with farmer’s practices using insecticides (control).

Testing this hypothesis will require staff at each of the four sites to collect data from their ecological engineering area and the *farmer’s practices using insecticides (control)* area in following manner.

Sampling in the two areas should be with the same equipment, using the same staff and as far as possible on the same day and approximately the same time of the day so that any differences between the two experimental treatments is attributable to the treatments and not an artifact of sampling.

The sampling methods outlined below should be used in the *approximate centre of rice fields (i.e. at least 10m from the field margin) and near the rice bunds (i.e. about 1m from the bunds) and from 10 fields in the ecological engineering and 10 fields in the farmer practice with insecticide spray (control) areas*. Sampling by different methods should not be done on the same point, which was sampled before using another method (refer to sampling lay out). If different varieties have been sown in each of these areas (or there is some other difference such as fertilizer use/ sowing date/ irrigation etc) then fields should be matched as far as possible so that there is a ‘like for like’ field in the ecological engineering and farmer practice with insecticide spray (control) areas.

For 10 fields in the ecological engineering and 10 fields in farmer practice with insecticide spray (control) take sample at the seedling, tillering, booting and milking stages of rice growth (i.e. samples at each of the four major growth stages) (refer to the general rice growth stages illustrated below) using the following methods:

- Yellow sticky trap (three traps mounted on wooden stakes to be just above the crop canopy with 5 meters distance between traps installed at the center of the rice field and near the rice bund, collected after 24 hr). There will be one hundred twenty samples for each sampling period.
- Yellow pan trap (three traps mounted on stand at approximately the same height of vegetation or just below the canopy level with 5 meters distance between traps at the center of the rice field and near the rice bund, left in place for 24 hr). There will be one hundred twenty samples for each sampling period.
- Blower-vac sampler (single sample from an undisturbed part of the center of the field and near the rice bund). There will be forty samples for each sampling period.

- Sweep net (30 sweeps whilst walking slowly through an undisturbed part of the center of the field at approximately 0.5 m/sec and 30 sweeps on the rice hills at least 1m from the rice bunds). There will be forty samples for each sampling period.

All the sampling methods should be used at the center part of the rice fields as well as close to (about 1 m) the rice bunds.

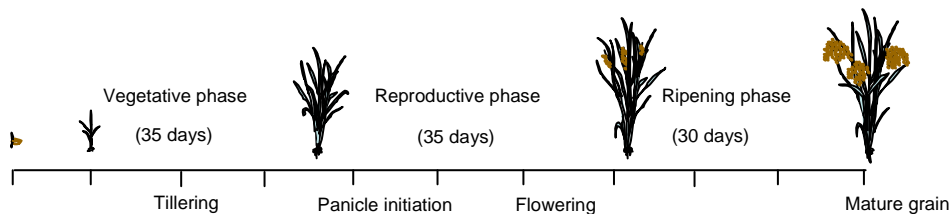
In addition to sampling on different growth stages of the rice crop, take sample of parasitoids during peak abundance of planthoppers (this will be determined based on the population of hoppers from blow-vac and sweep net samplings) using bait trap method.

- Bait traps for egg parasitism (one bait plant with BPH eggs on the center and one near (1 m) from the rice bund for each field) will be carried out in 5 fields in the ecological engineering and 5 fields in farmer practice with insecticide spray (control) at peak time of each generation of rice planthoppers. The trappings will be done 2-3 times for each site. There will be twenty samples for each sampling day.

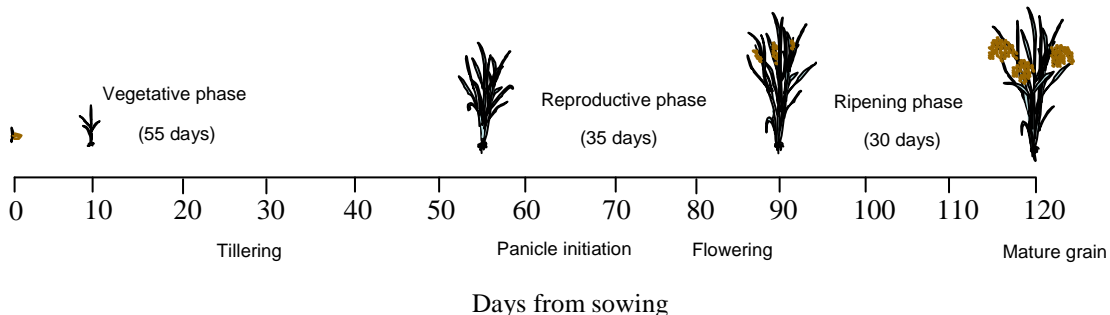
Additional method is the use of sticky plate to count the number of hoppers per unit area. This will be done once every week for the 10 ecological engineering fields and 10 fields in farmer practice with insecticide spray (control).

Further detail on each of these methods is set out later in this document.

100-day variety



120-day variety



General rice growth stages

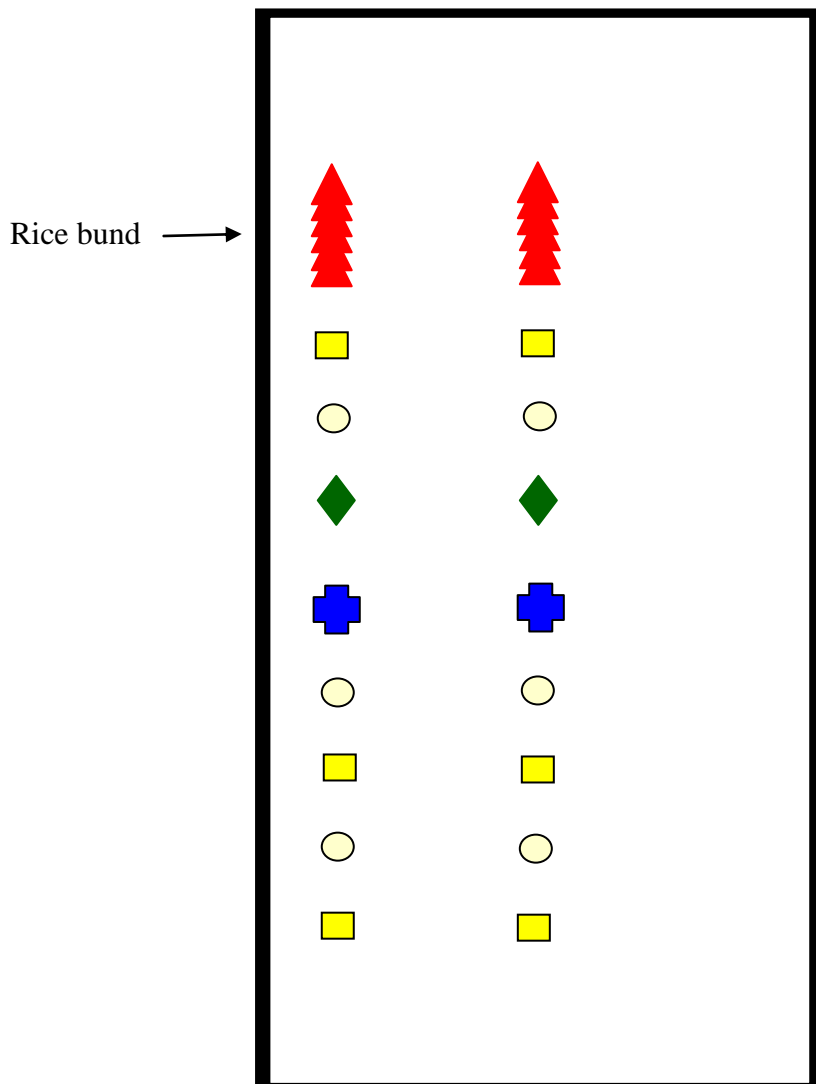
Sampling schedule:

1. Direct seeded rice


Seedling stage: 2-3 weeks after seeding
Tillering stage: 5-6 weeks after seeding
Booting stage: 8-9 weeks after seeding
Milking stage: 10-12 weeks after seeding

2. Transplanted rice

Seedling stage: about 2 weeks after transplanting
Tillering: 4-5 weeks after transplanting
Booting: 8-9 weeks after transplanting
Milking: 11-14 weeks after transplanting





Sampling lay out of different methods in one field

 Sweep net (which is about 15 m long covering 30 sweeps)

 Yellow sticky trap

 Yellow pan trap

 Egg bait trap

 Blow-vac machine

Taxonomy and identification

Arthropod biodiversity may be studied through sampling, counting and identifying the specimens. Wherever identification to *named* species is not possible, individuals should be identified to ‘morphospecies’ (otherwise known as recognizable taxonomic unit). This means that specimens are sorted into categories in which all individuals are identical. For example ‘Ichneumonid #1 or Coccinellid # 3). Bulk, unsorted samples are best stored in 70-100% alcohol allow follow up identification. (Scope for DNA barcoding is being investigated?). All the predator specimens should be sorted as early as possible and preserved in 100 % ethanol for further testing. Scrupulous attention needs to be paid to labeling such bulk samples and individual specimens (e.g. date collected, site, exact position or plot number if from within an experiment, collector’s name are the minimum).

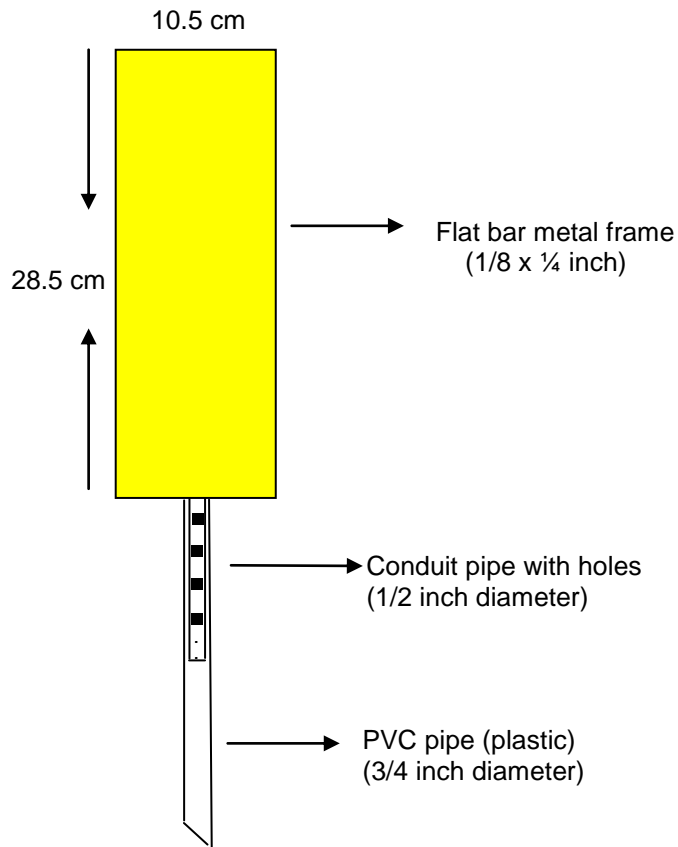
Several sampling techniques are to be used in the overall IRRI/ADB project.

Yellow sticky trap

Yellow sticky trap is designed to attract a variety of insects. The yellow color of the trap attracts the insects and the sticky coating captures them. Its dimension is 28.5 cm x 10.5 cm. A flat bar metal frame holds the yellow sticky strap and its base is attached to a conduit pipe, which is then inserted into a rounded PVC pipe. Both pipes are with holes for easy adjusting during the different growth stages of the rice plant.

Sampling by yellow sticky trap

1. Install the yellow sticky trap just above the rice canopy for 24 hours.
2. Retrieve the trap after 24 hours.
3. Cover the sticky trap with a transparent plastic sheet after retrieval.
4. Slice or cut the trap longitudinally into 2-3 parts for easy handling under the microscope.
5. Identify the natural enemies that stucked on the traps.
6. Keep the yellow sticky trap refrigerated if identification is to be delayed. Do the sampling on seedling, tillering, booting, and milking stages of the rice crop.
7. The standardized sticky trap will be provided by Dr Lv.
8. Do the sampling on seedling, tillering, booting, and milking stages of the rice crop.



Yellow pan trap

Many small day-active insects are attracted to the color yellow. Yellow pan traps collect insects that are attracted to the color. They are inexpensive and simple means of passively sampling insects in an area. This trapping method uses small pans filled with a mixture of water and liquid detergent. The pans are then placed on the ground in conspicuous places in the morning. When flying insects land on the surface of the water they rapidly sink and drown. At the end of the day or after 1-2 days, the water is strained through a fine sieve and the specimens are collected.

Sampling arthropods by yellow pan trap

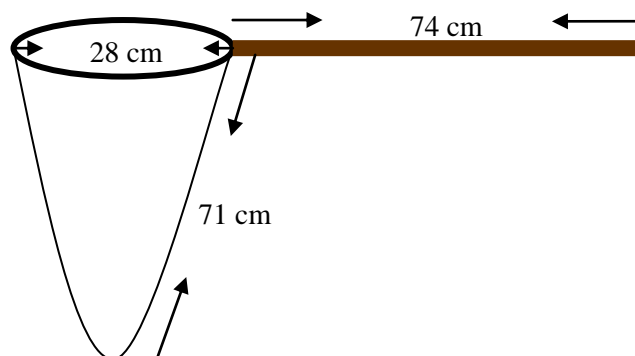
1. Use 500 ml circular plastic 'take away food container' (with 17 cm diameter and 5 cm depth). Deeper bowls experience less evaporation in hot climates.
2. Cut one hole near top of bowl and cover with mesh. In excessive rain this allows water to flow out of the bowl without losing any samples.
3. Paint with two top coats of yellow UV paint (e.g. RJ London acrylic spray paint).
4. Place bowls at approximately the same height of vegetation (50-100 cm) or just below the canopy level using a wire frame.
5. Add a mixture of 400 ml water and 1.2 g of sodium benzoate preservative and one drop of liquid detergent (washing-up liquid).
6. Cover each bowl with a coarse wire mesh to prevent scavenging of insects by birds.
7. Leave out for 24 hrs at a time.
8. Use an aquarium net or fine sieve to collect the insects and place in 100 % ethanol.



Yellow pan trap at vegetative stage of the rice crop.

Insect sweep net

The use of sweep net is a simple and inexpensive way to monitor the presence of a variety of arthropods in the ecosystem. If sampling effort is consistent (e.g. 30 sweeps whilst walking slowly through vegetation) samples can also be used to infer relative abundance of arthropods within a vegetation type. The sweep net is a funnel-shaped net, which is made-up of a nylon or similar synthetic fabric. It is important that the net is mounted on a rigid metal ring rather than wire. This allows the net to be swept through dense vegetation, dislodging arthropods. The net's ring is attached to a long wood or metal handle. A standard sweep net has a diameter of 28 cm with a length of 71 cm long. The stick handle is about 74 cm long.



A typical sweep net

How to use a sweep net

1. Hold the sweep net near the end of the handle with the hoop end nearest to the ground in front of you.
2. Swing the net from side to side in a full 180° arc or forming a semicircle. Keep the circular frame of the open end of the net perpendicular to the ground and pointing to the direction of the swing.
3. Sweep one stroke per step as you casually walk through the field or down the row. Do not swing the net up and down.
4. In short vegetation, swing the net as deeply as possible.
5. In taller vegetation, sweep only deeply enough to keep upper edge of the sweep net opening even with the top of the plants.
6. The net should not go more than 25 cm below the top of the plants during sampling.

Sampling arthropods by a sweep net

1. Sampling must be done when all the morning dew has evaporated. Avoid sampling in raining and wet weather.
2. Do thirty sweeps on the center of the field and another thirty sweeps on the rice hills next to the bunds or about 1 m from the bunds.
3. Swing the net as hard as possible after the last sweep. This will allow the insects to be deposited at the funnel end of the net.
4. Close the net by gripping the mid section by the palm.
5. Invert the net and put the collected insects in plastic bags and label with tags.
6. Transfer the collected insects in labeled zip loc or plastic bag with 100% ethanol.
7. Bring the zip loc to the laboratory and transfer into labeled vials maintaining the 100% ethanol. Record the time from sampling to transferring into labeled vials.
8. Identify all the insects based on guilds.
9. Do the sampling on seedling, tillering, booting, and milking stages of the rice crop.



Each passage of the net is considered one sweep.



Hold firmly the end of the net after the sweep.



Transfer all collected insects.

Invert the net and transfer the insects in labeled zip loc or plastic bag.



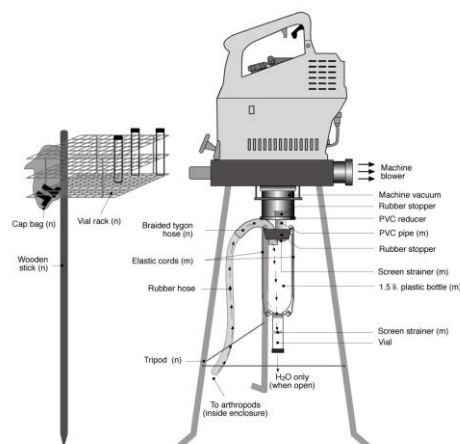
Close the zip loc.

last

The efficiency of a sweep net may vary depending on many factors. Different weather conditions, wind speed, air temperature, and intensity of solar radiation may affect the number of insects in the area while sweeping. Different habitats, especially the height of the plants, time of day, reflecting different cycles of behavior of the species, and different styles of sweeping are also factors to be considered.

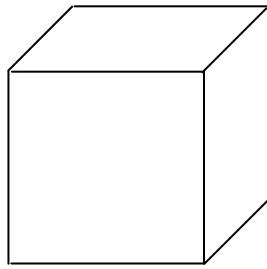
Blower-vac machine

Blower-vac machine may be used for more quantitative studies of insects in rice. It is operated by a gasoline-powered motor. The machine sucks the insects from rice plants by vacuum pressure. This machine is similar to that described by Arida and Heong (1992). However, instead of a plastic bucket, it will use a plastic bin.

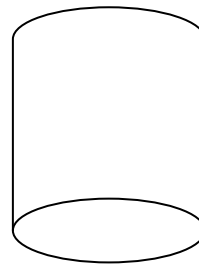


A modified blower-vac apparatus for sampling arthropods. Arrows indicate the flow of air, water and arthropods through the apparatus. Symbols: (n) new or (m) modified part from

the original blower-vac apparatus.



A



B

(A) A square and (B) a circular sampling enclosure, which can enclose (A) 4 hills or 0.25 m² for transplanted rice and (B) 0.1 m² for direct seeded rice, as shown in the above picture, should be prepared for sampling.

Sampling of arthropods by blower-vac machine

1. To sample using the blower-vac, drop the plastic bin enclosure over the rice plants.
2. Suck the arthropods from the nylon net sleeve, the air column, the plant surfaces and finally the water surface. The suction time will depend until all the insects are collected (suction time will later be prolonged as rice crop matures).
3. Place the collected insects in labeled vials with 70% ethanol.
4. Sort and identify all the insects based on guilds as early as possible and all the predator specimens should be kept in vials with 100% ethanol and store at -18 to 20°C.
5. Do the sampling on seedling, tillering, booting, and milking stages of the rice crop in the morning. Avoid sampling during the afternoon.



A blower-vac machine in action in the field.

Bait traps for egg parasitization

Egg trap is special trap used for investigating natural enemies related to egg stage.

1. Use about 30-day-old rice plants susceptible to BPH.
2. Thin the rice plants to 5 tillers each pot.
3. Introduce five gravid female adults to the rice plants for oviposition in the morning.
4. Remove the adults after 24 hours.
5. Bring the plants with newly laid eggs in the morning to the rice field and expose for 72 hours.
6. Retrieve the rice plants with eggs after 72 hours and bring to the greenhouse or laboratory and cover with a mylar cage for another 3 days.
7. Use a black cloth to cover the cage, but leave a hole with glass tube and light at top to attract parasitoids after their emergence.
8. Check daily the glass tube for parasitoids and count the number of planthopper nymphs that emerge. Identify the species and take records.
9. Calculate parasitism rate for each pot, as well as the mean parasitism rate for each area.
10. Do the bait traps at the peak time for each planthopper generation.



Rice plants with eggs ready for field exposition



A wooden stick is pegged on the bait trap in the field



The rice plant is enclosed with a mylar cage after field exposition.

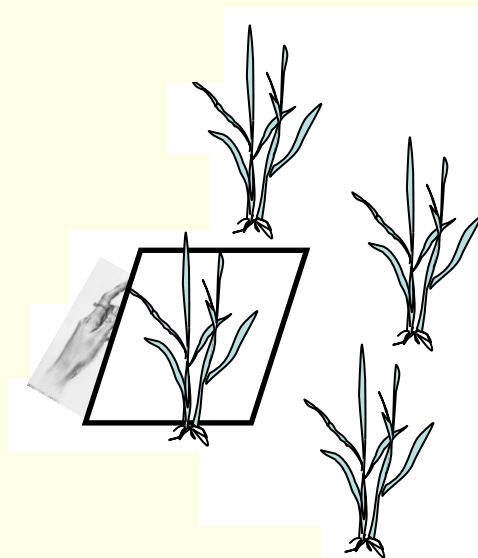
A black cloth covers the mylar cage with a glass tube on top for parasitoids.

Counting of hoppers/m²

White enamel plates coated with sticky substance can be used to count the number of hoppers per hill.

Sampling of hoppers by hill

1. Use a white enamel plate with 30 x 45 cm dimension.
2. Spread kerosene (or petroleum jelly) or anything sticky on the plate. (Leave a small space uncoated for easy handling).
3. Position the plate close enough to the base of the rice hills or plants carefully to avoid agitating the planthoppers and tap the hills several times with the hand to collect the hopper.
4. For transplanted fields, tap 2 hills at a time and sample 5 times for each field. For direct seeded fields, use 0.05 m² for each sampling and sample 5 times for each field.
5. Bring the plate to the laboratory and count the number of hoppers based on age (young nymphs from 1st – 3rd instars and mature nymphs from 4th – 5th instars) and forms (short-winged or brachypterous and long-winged or macropterous) that stick to the board.
6. Do the sampling at 7 day intervals.



Sampling of hoppers using an enamel plate.

Evaluating predation quantitatively using triplex RT-PCR

The triplex RT-PCR could be used to evaluate species-specific predation by all the predators qualitatively and compare relative predation quantitatively for particular predator species among sites. All the samples taken by the sampling methods mentioned above could be used for evaluation by the RT-PCR method if the samples could be kept in 100% ethanol.

1. Samples (mainly predators) taken by ***Blower-vac machine and Insect sweep net*** (samples taken each time should be put into a nylon stocking with a label (field number and sampling date), then keep in bottle with 70% ethyl alcohol in fields as soon as possible (the longer the samples are kept without ethyl alcohol, the greater the predation will be and DNA quality will also decline).
2. All the samples should be sorted as early as possible (within a week) and all the predators are transferred into labeled vials with 100% ethanol by species or groups after sorting or identification.
3. The vials with predators should be kept in freezer at minus 18-20°C.
4. Testing. All the samples will be collected before November next year and will be tested by ZJU.

Identification of arthropod samples from all sampling techniques

1. All the samples should be sorted out first as early as possible (within 1-2 weeks after sampling) as first step for further identification.
2. All the predator specimens should be kept in labeled vials with 100% ethanol and should be stored in minus 18-20°C (for PCR analysis later).
3. Sort, count and identify the collected arthropods to species level (if possible).
4. Group the sampled arthropods based on guilds (predators/omnivores and parasitoids/parasites) described by Moran and Southwood (1982).

Data analysis

1. The raw data will be entered into Excel file using a standardized data sheet (refer to attachment).
2. Analysis will follow.

References:

- Arida GS, Heong KL. 1992. Blower-Vac: a new suction apparatus for sampling rice arthropods. *International Rice Research Newsletter* 17(6):30-31.
- Moran VC, Southwood TRE. 1982. The guild composition of arthropod communities in trees. *J. Animal Ecol.* 51:289-306.