CHAPTER 6

Reproduction and growth in rodents

Introduction

Breeding is the main reason why populations increase in size. This is especially true of many rodent species that are capable of rapid population growth, especially when conditions are favourable. Rapid population growth is generally due to a combination of two factors—namely, a **high reproductive potential** and a **short period of maturation** to sexual maturity.

The reproductive potential of a species can be thought of as the possible number of offspring that a typical female can produce during her life. This is affected by four main factors:

- length of the gestation period (i.e. the period between conception and delivery)
- litter size (i.e. number of offspring per delivery)
- length of time between delivery and the next conception
- the reproductive life of females (i.e. the period of time from the first litter to the last litter or until death).

Rodents typically have short gestation periods, with high litter sizes and an ability to fall pregnant again within a few days of delivery. These factors alone would ensure a high reproductive potential. However, many rodents also attain sexual maturity at very early ages, due mainly to rapid growth during the first few weeks of life. This latter factor is particularly important in allowing a population to respond to relatively short-term increases in the availability of food. The particularly short period to sexual maturity of many murid rodents is without doubt one of the main reasons why so many of the major agricultural pests belong to this one family of mammals.

In this chapter, we provide information on the reproductive anatomy of rodents, the changes that occur both during and after pregnancy, and the process of growth and maturation of the young. We also discuss some key reproductive parameters that will assist you to make sense of your observations of reproductive activity within a population of rodents.

Basic reproductive anatomy

The external features of the reproductive system were described in Chapter 4. Here we will concentrate on features of internal anatomy. These are usually examined by making a careful incision along the midline of the belly, starting from just below the ribcage and running down to just above the genital papilla. Care must be taken not to cut into the intestine or any embryos that may be present in the

abdominal cavity. To this end, it is best to make the incision with a pair of sharp scissors rather than with a scalpel blade.

Male reproductive tract

The male reproductive tract (Figure 6.1) consists of the paired testes, epididymes and ducti deferens, accessory sex glands and the centrally located penis. Elements of the **urinary tract** are also labelled on Figure 6.1—notably the paired kidneys and ureters, and the centrally located bladder.

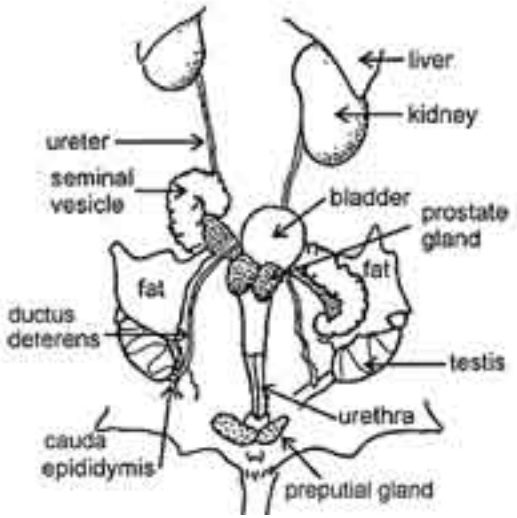

Figure 6.1 Male reproductive and urinary tracts of a typical murid rodent (redrawn by M. van Wensveen, after Cook 1965).

The **testes** produce sperm and also synthesise and release male sex hormones. In a juvenile rat, they are located high in the abdomen, just behind the kidneys. With maturation, the testes enlarge in size and move backwards—first into a position at the base of the tail, and finally into the scrotum. Sperm are produced in the testes, then move into and through the **epididymes**, where they mature and are stored. Contractions associated with sexual stimulation move the sperm out of the epididymes and through a tubular transport tract beginning with the **ducti deferens**. Secretions are added by various **accessory sex glands** (e.g. prostate glands and seminal vesicle) to produce an ejaculate that exits the body along the urethra, a canal supported by the penis.

In many rodents, large **preputial glands** are present on either side of the penis. These release strong-smelling fluids into the urine that are important in various kinds of behaviour, including scent marking.

Female reproductive tract

The female reproductive tract (Figure 6.2) consists of the paired **ovaries**, the **Y-shaped uterus** within its elongate **uterine horns** and basal stem, and the centrally located **vagina**. The urinary tract consists of the same elements as in a male. In many rodents, females also possess large **clitoral glands** on either side of the genital papilla, with ducts opening into the genital papilla.

Juvenile female rodents have an **imperforate** vagina, which is to say that it is sealed over by a thin layer of skin called the **hymen**. The hymen appears as a small, shiny patch of skin just behind the genital papilla. Internally, the uterine horns are narrow and thin-walled, and have an inconspicuous blood supply. The ovaries and fallopian tubes are also small.

With the onset of sexual maturity, the ovaries enlarge and start to secrete female sex hormones. The effects of these hormones are felt throughout the body:

- the ovaries begin to produce mature eggs
- the uterine horns elongate and become thicker, and develop a more conspicuous blood supply

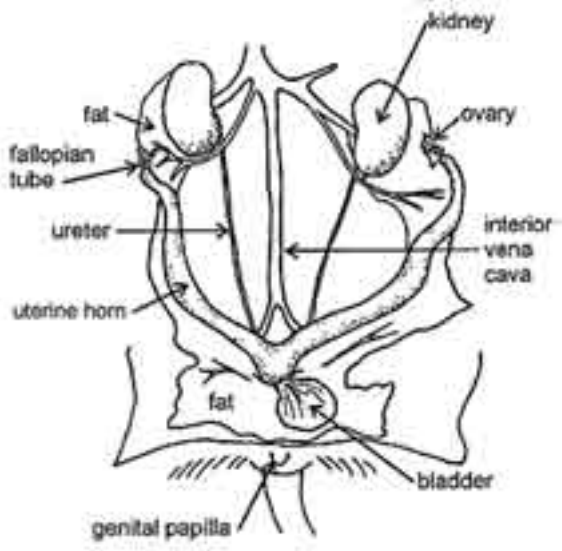

Figure 6.2 Female reproductive and urinary tracts of a typical murid rodent (redrawn by M. van Wensveen, after Cook 1965).

- the hymen is resorbed, resulting in an open or **perforate vagina**
- the teats and associated mammary tissue enlarge.

Pregnancy and embryonic development

Mature eggs are released from the ovaries into the **fallopian tubes** (see Figure 6.2) every four days on average in the pest species of *Rattus*, every five days in *Mus domesticus* and every 3–8 days in species of *Bandicota* (see Chapter 11 for information on particular species). If conception occurs, the fertilised eggs remain in the fallopian tubes for up to three days before they move into the adjacent uterine horn. Here, the fertilised eggs, now at the blastula stage of development, move into small pockets in the wall of the uterus where they will undergo implantation and embryonic development. A critical part of this process is the formation of a **placenta** between each embryo and the wall of the uterus, starting around day 7. The placenta establishes a blood supply (via the **umbilical cord**) between the female and each developing embryo, and provides the embryo with nutrition for rapid growth.

The **gestation period** (the period from conception to delivery) varies greatly between different groups of rodents. However, among the major groups of pest rodents, the gestation period is usually very short, falling between 19–25 days.

If conception occurs while a female is still lactating from a previous litter, the blastulae may not implant immediately after entering the uterus. Instead, they may remain in a period of suspended development, until triggered by some hormonal cue to resume the process of implantation. In laboratory rats (*Rattus norvegicus*), implantation is commonly delayed by a period of 6–9 days (i.e. occurs on day 8–11 after mating), but in some 10% of cases this is extended to a delay of 12–19 days. The length of this delay is related to the strength of the sucking stimulus and thus to the previous litter size. The average gestation period for lactating laboratory rats is 26 days, compared with 22 days for non-lactating females. In laboratory strains of the house mouse (*Mus musculus domesticus*), the delay in implantation is typically shorter, with only 5% extending implantation beyond day 7. The average gestation period for lactating mice is 24 days, compared with 20 days for non-lactating females.

The gestation period is divided into three time intervals called **trimesters**—each trimester making up approximately one-third of the total period. Here we illustrate the major stages of embryonic development for a rat with a total gestation period of 20–22 days, such as occurs in *Rattus rattus*.

Trimester 1

In the early stages of trimester 1 (up to days 5–6), the only evidence of pregnancy will be an obvious increase in blood supply to the uterine horns. At this stage, there is no obvious swelling of the uterine walls, hence it will not be possible to count the number of embryos. After 5–6 days, the embryos begin to interact directly with the tissues of the uterine wall, which responds by forming a series of distinct bulges, one for each embryo (Figure 6.3). These are easy to count, even though they measure less than 5 mm in diameter.

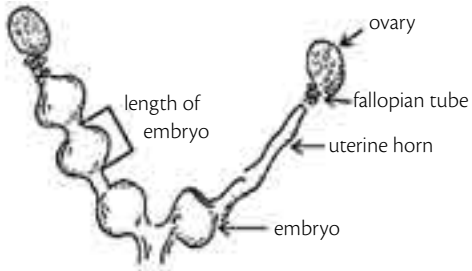

Figure 6.3 Condition of the uterus at late trimester 1 of embryonic development in a rat (after Theiler 1972). The recommended method of measuring the uterine swelling is also shown.

Trimester 2

This is a period of rapid internal development of the embryo, marked by the beginnings of the nervous, circulatory and alimentary systems, and of the skeletal column. Limbs initially appear as 'buds'

without distinct toes (Figure 6.4). The placenta also develops rapidly during this period to provide the nutrition needed for embryonic growth.

Trimester 3

In this trimester, the body grows rapidly in preparation for birth. Fingers and toes form and then separate, details of the ears, eyes and skin emerge, and elements of the circulatory system become visible through the pale skin (Figure 6.5).

Within a single species, a good relative measure of the stage of development is a simple linear measurement of a uterine swelling. We prefer to measure this parallel to the long axis of the uterine horn, as shown in Figure 6.3. Although this information is not yet available for many species, it can be gathered during the early part of a study for each of the captured species.

Embryos can fail in their development at any stage. If this occurs during trimesters 1–2 or early in trimester 3, the embryo will be resorbed by the uterus. A resorbing embryo will gradually decrease in size, while the remaining live embryos get larger. For this reason, it is sometimes possible to tell them apart. However, embryos that fail during the first trimester may be impossible to detect other than by microscopic examination of the ovary. Embryos that die during the last few days of pregnancy will be delivered as stillbirths.

Delivery of the young is triggered by further hormonal activity on the part of the ovaries. This causes the uterus to contract, leading to expulsion of the young, and to detachment of the placentae from the wall of the uterus. As each placenta pulls away, it leaves behind an open wound in the uterus, one for each embryo. The scar tissue that forms over each wound is called a **placental scar**. These scars are visible through the uterine wall (Figure 6.6).

Immediately after delivery, the placental scars are large and reddish brown, still with an obvious blood

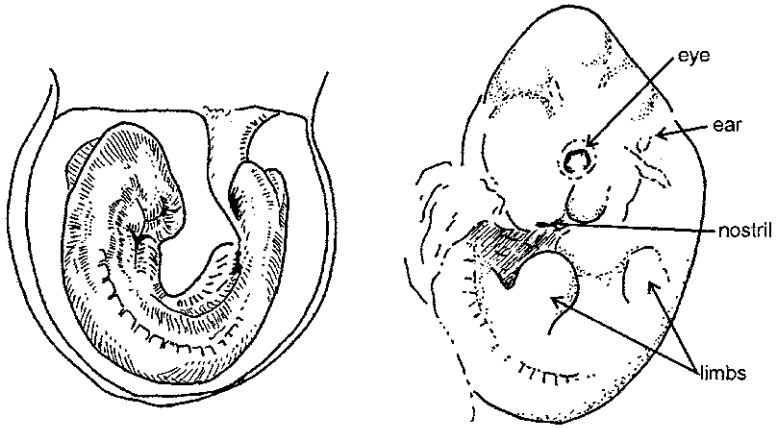

Figure 6.4 Early (left) and late (right) trimester 2 of embryonic development in rats (after Theiler 1972).

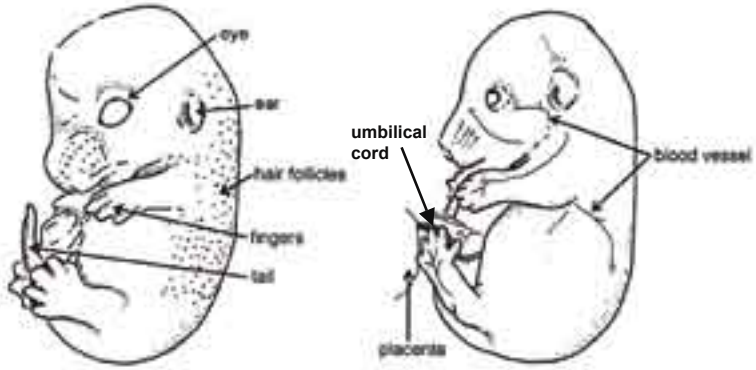

Figure 6.5 Early (left) and mid (right) trimester 3 of embryonic development in rats (after Theiler 1972).

supply. As the uterine horns thicken and narrow, the scars become smaller and take on a yellowish colour, and they lose their blood supply. Over time, they first become darker, and then smaller and less distinct. In the laboratory rat, placental scars generally remain visible throughout the adult life of a female.

By carefully examining the wall of the uterus, it is often possible to distinguish various sets of scars, based on differences in the size and intensity of the scars. However, where a female has experienced three or more pregnancies, it may not be possible to distinguish among scars of the earliest sets.

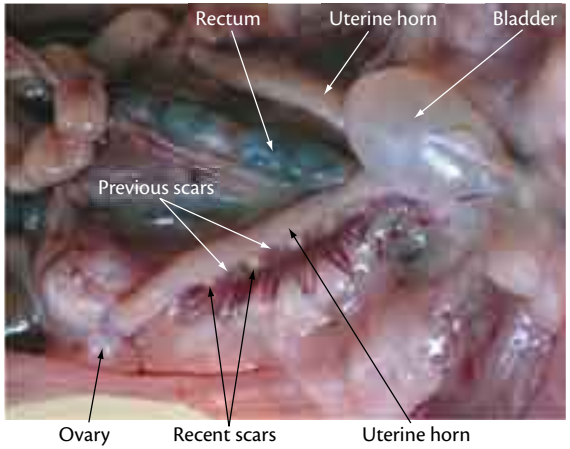

Figure 6.6 Dissection of a female rat showing a uterine horn with two sets of placental scars. The rectum (lower gut) has been pulled aside to expose the reproductive tract. The rich blood supply to the uterine horn is also clearly visible.

Growth and maturation after birth

The sequence and timing of maturation is very similar in all of the major pest species of rodents (Table 6.1). Newborn pups weigh just over 1 g in *Mus* species, and from 3–6 g in the pest species of *Rattus* and *Bandicota*. Newborns of all species are hairless except for small vibrissae on the snout. The eyes and ear canals are closed, and the external ear (pinna) is flattened against the head (Figure 6.7).

By the end of day 3, the pinna is usually erect. Fine dorsal hairs are visible to the naked eye by day 3–5 and teats are first seen on females as small, pigmented spots during the same period. At around day 7–9, the incisors start to erupt, the belly becomes covered with fur, and the pups begin to stand and walk. By day 13–14, the external ear canal is open and the pinna has become thinner and starts to enlarge. The eyes may open from day 11–12 in *Rattus* and *Mus*, but not until after day 14 in *Bandicota* species (Figure 6.8).

Pups of all species begin to take solid food brought into the nest by adults from the end of the second week. However, weaning is generally not completed until the end of week 3 or 4. After weaning, young rats and mice are effectively independent from the mother, although in some species they may continue to inhabit the same burrow complex for some time.

Table 6.1 Summary of key events and parameters in the development and growth of the young of some of the major rodent pest species. All values are in days, except for 'Weight at birth' which is in grams. A question mark (?) indicates that data are not available. Value prefixed with a 'c.' are approximate mean values.

Key event or parameter	*Rattus rattus*	*Rattus norvegicus*	*Rattus exulans*	*Mus musculus*	*Bandicota bengalensis*	*Bandicota indica*
Gestation period	20–22	20–25	19–21	19–20	21–25	21–25
Weight at birth (g)	3.0–6.4	4.3–6.2	2.8–3.1	1.2	3.5–5.0	?
Pinna of ear unfolds	2–5	2–3	2–5	2–3	?	?
Dorsal hairs are visible	3–5	3–5	3–5	2–3	?	?
Incisors erupt	7–12	9–13	7–11	5–7	?	?
Ear canal opens/pinna thins and elongates	10–14	c.12	11–14	c.13	?	?
Eyes open	11–15	13–17	12–16	12–14	14–18	18–22
Weaning occurs	23–28	21–28	21–28	c.24	c.25	c.28

Figure 6.7 Two- or three-day-old pups of *Rattus rattus*. No fur is present and the pinna has not yet unfolded. Each pup is about 50 mm in length.

Figure 6.8 A pup of *Bandicota bengalensis*, probably around day 13–14. The ears have started to thin and enlarge, but the eyes remain closed.

Attainment of sexual maturity

The length of time to sexual maturity varies between species. Although the onset of breeding is presumably limited by maturation of the reproductive organs, it is also influenced by many environmental factors, including the nutritional state of the young animals, aspects of social behaviour linked to population density and habitat structure, and possibly also crop maturation.

In wild populations of *Rattus norvegicus*, ovulation (oestrous) cycles may start at any time from 40 days of age but with a mean age of around 100 days. The vagina opens at any time over the same period, with a mean age of 72 days in a laboratory-reared population. In laboratory-reared males, testes descend into the scrotal sac during the period 15–51 days of age. Occasional mature sperm are present in the epididymes from around 50 days, but at much higher densities from 70 days onwards.

In *Rattus argentiventer*, females show an open or perforate vagina at a mean age of 33 days, and are pregnant at a mean age of 49 days. Males show descent of the testes from 26 days, with scrotal testes in more than 90% of individuals by day 40. Mature sperm are first observed in the epididymes from 70–90 days.

In laboratory mice (*Mus musculus domesticus*), vaginal perforation occurs between 28–49 days, with a mean of 35 days. Ovulation commences soon thereafter, but the first mating is often delayed by one or two weeks. Male mice become sexually mature slightly later than females.

Life span and menopause

Even when reared under optimum conditions, most rodents have a maximum life span of only 2–3 years. Females of *Rattus norvegicus* and *Mus musculus domesticus* stop ovulating (i.e. enter **menopause**) altogether between 12–18 months of age, although average litter size declines well before this age. Under natural conditions, very few individuals are likely to survive to such advanced ages.

Assessing reproductive activity from external characteristics

In capture–mark–release studies, we have to rely on external signs to assess reproductive activity.

In males, the only indicator of sexual maturity is the condition of the testes and scotal sac. Although the process of testicular enlargement and descent is a gradual one, we find it useful to distinguish three conditions of the testes:

- non-descended (scrotal sac undeveloped)
- partially descended (scrotal sac visible but not to the full extent, generally lacking a distinct epididymal pouch)
- fully descended (scrotal sac developed to the full extent, generally with a distinct epididymal pouch).

As noted in Chapter 4, the testes in species of *Bandicota* and *Nesokia* do not attain such a large size relative to body size as they do in the species of *Mus* and *Rattus*, and they would probably be scored as partially descended, even in fully adult individuals.

Males are generally not the primary focus for breeding studies. However, in a reproductive study you may wish to check for the presence of sperm in a urine sample. This is usually done by placing a urine droplet on a microscope slide and examining it at approximately 20–40 times magnification for evidence of motile sperm.

In females, the external signs of sexual maturity include an open vagina and enlargement of the teats. Signs of sexual activity are less obvious. The best indication is the presence of a yellowish vaginal plug that forms from vaginal secretions and ejaculate, and persists for 1–2 days after mating.

Pregnancy is usually evident by day 13, at which time the abdomen should be visibly enlarged. The teats also become more prominent during the final week or so of pregnancy. With some experience, it is possible to confirm whether or not a live animal is pregnant by using a technique termed **palpation**. To do this, run your thumb and first finger down each side of the lower abdomen, applying gentle pressure over the area covering the uterus and intestines. Embryos will feel smooth and round. In contrast, faeces will feel harder and more discrete.

It is generally impossible to detect a first trimester pregnancy by palpation, and it requires experience to accurately detect the second trimester embryos. By the time embryos have entered the third trimester they are much more obvious. Because an animal should not be recorded as pregnant unless the researcher is positive that this is the case, the palpation method will usually result in a serious underestimate of the pregnancy rate. Also be aware that palpation can lead to prenatal losses if it is not done gently.

A female that is currently nursing a litter of pups will have at least some enlarged teats and active mammary glands. Teats that are producing milk will be swollen at the base and often lack fur around their base; active status can be confirmed by gently squeezing the base of the teat until a droplet of milk is released. If the number of young is less than the number of available teats, some of the teats and associated glands may be inactive. For this reason, you may need to check more than one teat to confirm active lactation.

At the end of a period of breeding activity, the teats of an adult female rodent become smaller and the fur will grow back around the bases. However, the teats remain larger and more raised than those of a sexually immature individual.

We recommend that teats be scored as one of three categories (Figure 6.9):

- low and indistinct (fur at base)
- raised but not lactating (fur at base)
- raised and lactating (no fur at base).

Figure 6.9 Classes of teat on female rats. Top, teats are low and indistinct in a juvenile rat; middle, teats are raised but have fur around the base in a non-lactating adult; bottom, teats are raised and lack fur around the base in a lactating adult rat.

Assessing reproductive activity from internal characteristics

It is possible to obtain much more information on breeding activity where rodents are being sacrificed as a routine part of a population study.

In male rats, the condition of the testes should be examined to confirm whether or not the animal has reached sexual maturity. This is particularly important for those species (e.g. *Bandicota* and *Nesokia* spp.) where the scrotal sacs never become very prominent. A fully mature male in which sperm is being actively produced will have large testes, each with a prominent blood supply, and enlarged epididymes with highly convoluted, sperm-filled tubules. During periods when sperm is not being produced, the testes and epididymes reduce somewhat in size, the epididymal tubules become harder to see, and the blood supply to the testis becomes less obvious.

If greater certainty is needed, you can check for the presence of mature sperm in the **cauda epididymis**, the bulb-shaped part of the epididymis that projects posterior to the testis (Figure 6.1). This is done by carefully removing this part of the epididymis with sharp scissors and smearing the cut surface across a microscope slide. The sperm can be made more visible by application of a general stain such as gentian violet.

In female rats, the full picture of reproductive activity can only be obtained by examining both the ovaries and the uterus. However, accurate interpretation of the ovary generally requires histological examination, hence we will concentrate here on features of the uterine horns that can be observed by eye or with a dissecting microscope.

The uterine horns will generally fall into one of the following categories:

- very thin and short, with a poorly developed blood supply (Figure 6.10a). This condition is typical of juveniles; the vagina is either imperforate or very recently opened
- slightly thicker and more elongated, with a more obvious blood supply but without embryos or placental scars (Figure 6.10b). This condition is typical of an individual that is entering its first breeding season. The vagina should be checked for presence of a vaginal plug—this will indicate that mating has occurred
- thicker and with embryos present in one or both uterine horns (Figure 6.10c). Early-stage embryos take the form of small swellings. As the embryo and placenta develop, the uterine horns become wider and thinner-walled. If no placental scars are visible, the animal is probably in its first pregnancy. However, scars can be difficult to see when the pregnancy is in the third trimester and the wall of the uterus is very stretched (Figure 6.10d)
- elongate and wide, with very thin walls, but without embryos (Figure 6.10e). This condition is typical of the period immediately after delivery of the young. The placental scars appear as large discolourations of the uterine wall
- elongate and thick walled, with obvious placental scars but no visible embryos (Figure 6.10f). The uterine horns compact and thicken within a few days of delivery, ready to receive a new batch of fertilised eggs.

As mentioned earlier, recent placental scars are large and either reddish-brown or yellowish in colour. These become smaller and darker with time, but they probably remain visible through life. A female may have numerous sets of scars, and it should be possible to distinguish two or more sets based on their size and colour. However, once three or more sets are present, it may become difficult to distinguish between the earlier sets.

A count of the total number of scars is a useful measure of the reproductive output of a female. However, for two reasons, the total number of scars cannot be used as an exact count of the number of young produced during the life of the animal. Firstly, resorbed embryos also leave scars in the uterine horns. Inclusion of resorbed embryos would lead to an *overestimate* of the number of live young. Secondly, where there have been two or more separate pregnancies, it is possible that some of the later placentae have formed over the top of previous scars. In this case, a count of scars will *underestimate* the total number of young.

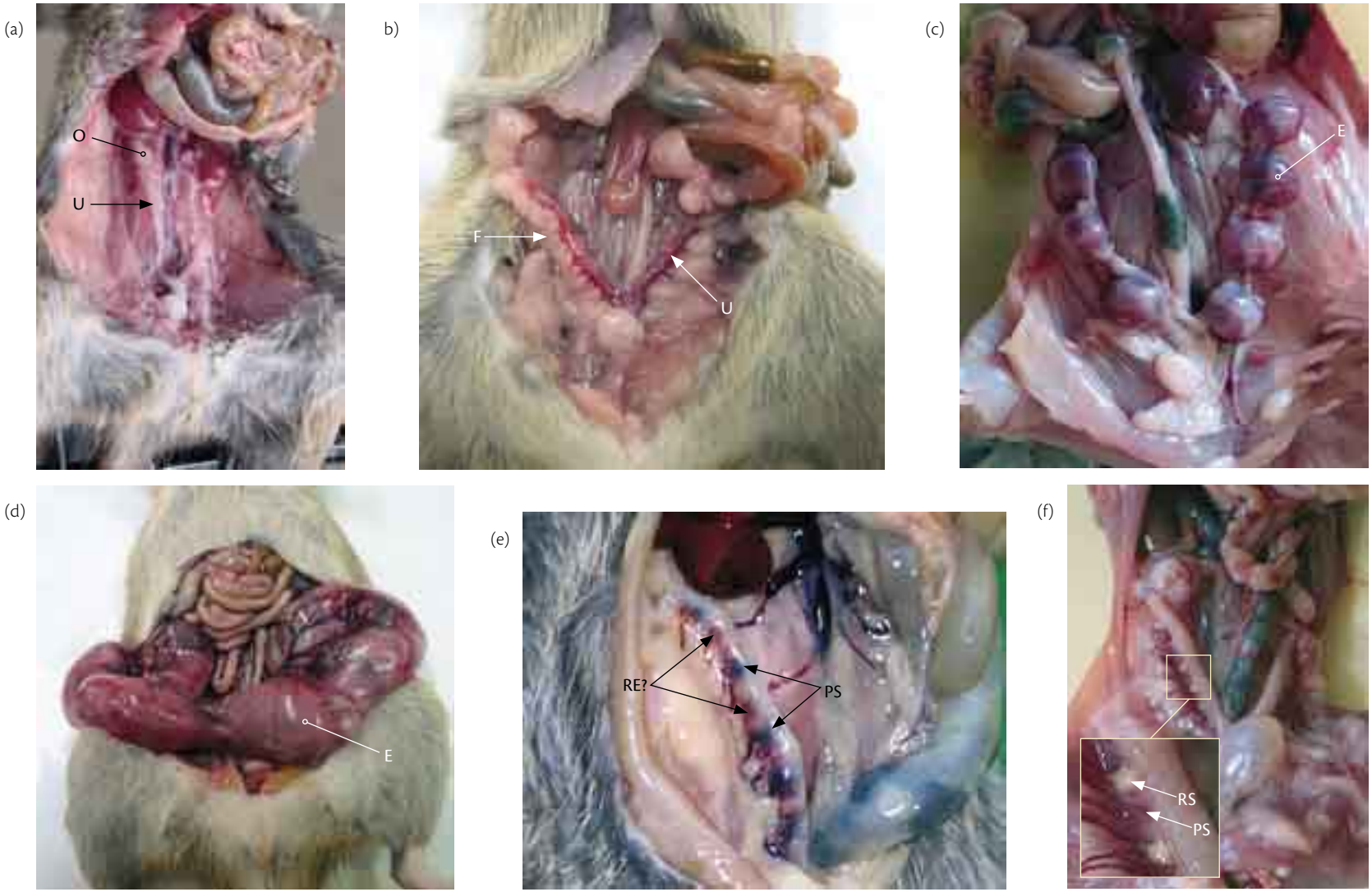

Figure 6.10 Comparison of the female reproductive tract: (a) juvenile—showing the ovary (O), and the uterine horn (U) which is very thin with an inconspicuous blood supply; (b) subadult entering its first breeding cycle—uterine horn is thicker, with conspicuous blood supply. Note extensive fat deposits (F) along uterine horns; (c) adult in second trimester of pregnancy with seven healthy embryos (E); (d) adult in advanced third trimester of pregnancy—the embryos (E) are clearly visible through the highly stretched uterine wall; (e) adult that has recently given birth. Within a few days, the large, dark placental scars (PS) will reduce in size and become paler. The smaller discolorations, each of which has a separate blood supply, probably represent resorbing embryos (RE?); (f) adult showing two sets of placental scars—the larger, yellowish scars (RS) are from a recent litter, while the smaller, darker scars (PS) are from a previous litter.

Key reproductive parameters

For population studies, it is important to determine the following key reproductive parameters.

Commencement and cessation of the breeding season

There is some debate over whether it is female or male reproductive activity or behaviour that really controls the timing of breeding activity. However, in either case, it is the occurrence of pregnancy in females that defines the **effective breeding season** and for this reason, we will maintain our focus on female reproductive condition.

Not all rodent species show a discrete breeding season. However, most of the pest rodents seem to stop breeding during periods of extended fallow, when food is scarce or of low quality.

The breeding season of a population can be said to start with the first successful mating after a period of non-breeding. Where the gestation period of a species is known, the date of conception of a pregnant female can be estimated by observing the trimester of development and counting back the likely number of days since conception. The breeding season is finished when the last litter of pups is weaned. This can be estimated directly by digging up numerous burrows and observing the growth stage of the litters. Alternatively, it can be counted forward from the last captures of pregnant females (provided the average time to weaning is known). The last captures of newly weaned young could also provide the same information.

Percentage of adult females in breeding condition

Not all adult females will necessarily breed continuously through any particular season. The intensity of effective breeding activity can be estimated in two ways:

- calculating the proportion of adult females that are pregnant during a given trapping period (by palpation or from presence of embryos in the uterus by necropsy)
- calculating the proportion of adult females that are lactating during a given trapping period.

During the breeding period, the proportions of females that are pregnant versus lactating will probably shift, but the changes should be complementary. A useful index of overall breeding activity is the proportion of adult females that are pregnant and/or lactating.

Because the earliest stages of pregnancy are difficult to detect, either by palpation or by necropsy, the pregnancy rate is always underestimated by a significant factor. With necropsy data, pregnancy may go undetected for the first 5–6 days, representing 25% or so of the total gestation period. For estimates based on palpation, the proportion of undetected pregnancies may be much higher. However, in both cases, the exact proportion will depend on the age distribution of the pregnancies, which can be expected to shift through the breeding season. Thus, during the early part of a breeding season, a very high proportion of pregnancies may go undetected. Later, as the number of new pregnancies falls away, a much higher proportion of pregnancies would be detected by either method.

As noted before, any females that live beyond 12–18 months of age probably stop ovulating. Under natural conditions, very few individuals are likely to survive this long, hence the inclusion of post-oestrus females is unlikely to cause any significant bias in estimates of pregnancy rate.

Percentage of adult females that produce multiple litters within one season

Females that have produced multiple litters within one season will be simultaneously pregnant and lactating. You would also expect to see recent scars on the uterus, especially if conception has occurred immediately after birth. However, these scars may be difficult to see if the second pregnancy is in the second or third trimester and the uterine wall is very

stretched. Removing the embryos from the uterine horns will generally make it easier to count the previous scars.

Average litter size

The average number of young per delivery is an important determinant of the potential rate of population increase. This is usually estimated from the number of embryos present in pregnant females or from counts of recent scars in recently post-natal females. Because some mortality occurs at all stages of pregnancy including birth, these counts are likely to slightly overestimate actual litter sizes. Captive breeding of rodents allows a greater degree of control over litter sizes. However, captive-born litters may be either be smaller or larger than those produced under wild conditions, depending on how well the particular species responds to the artificial diet and living conditions.

Within any one species of rodent, litter size is usually positively correlated to body weight (i.e. larger individuals have more young). In addition, there is often a difference between the number of pups in the first and subsequent litters—this may be partly due to continued growth of the female. Litter size usually peaks around the third or fourth litter, and then falls after that.

Pre-weaning mortality rate

Although litter size is very high in many species of rodents, there is sometimes also a high rate of pre-weaning mortality. This occurs as a result of starvation, predation by animals such as snakes and carnivorous invertebrates, and infanticide both by the mother and by other members of the same species. Because these events generally take place below ground, they are very difficult to observe or even estimate. Perhaps the best way of estimating these parameters is to excavate a sample of burrows at various times through the breeding season. The number and size of surviving pups can then be compared with the number of recent scars as a measure of original litter size.

Recording reproductive data

In Appendix 2, we have provided an example data sheet for recording breeding information. We recommend that you record the capture or sampling day as a **Julian date**, by which is meant the number of the day from day 1 through to day 365 (366 in a leap year). Dates entered in this way are easier to manipulate in computer applications and mathematical models than dates entered in the traditional calendar format (e.g. 2–11–2001). Tables for calculating the Julian date for both normal and leap years are given in Appendix 3.

Further reading

Baker, D.E.J. 1979. Reproduction and breeding. In: Baker, H.J., Lindsey, J.R. and Weisbroth, S.H., ed., The laboratory rat: volume I, biology and diseases. New York, Academic Press, 153–168.

Cook, M.J. 1965. The anatomy of the laboratory mouse. London, Academic Press, 143 p.

Cowan, P.E. 1981. Early growth and development of roof rats, *Rattus rattus* L. Mammalia, 45, 239–250.

Davis, D.E. 1948. The placental scar as a measure of fertility in rats. Journal of Wildlife Management, 12, 162–166.

Davis, D.E. 1953. The characteristics of rat populations. The Quarterly Review of Biology, 28, 373–401.

Greene, E.C. 1968. The anatomy of the rat. New York, Hafner Publishing.

Harrison, J.L. 1952. Reproduction in rats of the subgenus *Rattus*. Proceedings of the Zoological Society of London, 121, 673–694.

Lam, Y.M. 1983. Reproduction in the ricefield rat *Rattus argentiventer*. Malayan Nature Journal, 36, 249–282.

Pelikán, J. 1981. Patterns of reproduction in the house mouse. Symposium of the Zoological Society of London, 47, 205–229.

Singleton, G.R., Krebs, C.J., Davis, S., Chambers, L.K. and Brown, P.R. 2001. Reproductive changes in fluctuating house mouse populations in southeastern Australia. Proceedings of the Royal Society of London, Series B, 268, 1741–1748.

Theiler, K. 1972. The house mouse. Development and normal stages from fertilization to 4 weeks of age. Berlin, Springer-Verlag.

Tristiani, H. and Murakami, O. 1998. Reproduction and survival of the ricefield rat *Rattus argentiventer* on rice plant diet. Belgian Journal of Zoology, 128, 167–175.

Walton, D.W., Brooks, J.E., Tun, UM.M. and Naing, U. Hla 1978. Observations on reproductive activity among female *Bandicota bengalensis* in Rangoon. Acta Theriologica, 23, 489–501.

CHAPTER 7

Studies of movement

Introduction

Movements of rodents and other animals are studied for many different reasons. One common reason is to understand the way in which individual animals use their local environment. Where are nests or burrows situated in relation to feeding areas? How far does an animal move in one night or over longer periods such as a week or month? Do males and females have different patterns of movement? What pattern of movement do juveniles follow when they become independent? How do movements of one animal affect the movements of others in the same area? Information of this kind is essential to building a complete picture of any species' biology, and is also valuable when looking for ways in which a pest species might be controlled through habitat manipulation or specific management actions.

Another reason to study movements is to understand the contribution of immigration and emigration to changes in local population density or community composition. Indeed, without knowing something about the seasonal and longer-term pattern of movements, it is often difficult to know whether local changes in population density are due to increased breeding or survival or to changes in the pattern of habitat use by members of a more stable population.

These examples emphasise the fact that studies of movement can be used both to frame and to test hypotheses. Gathering information on how members of a particular species move around in their environment is one part of putting together a basic biological picture for the species. When combined with information on population densities and breeding activity, this knowledge can be used to develop specific hypotheses about how the species functions in time and space. Testing these hypotheses often requires additional studies of movement, but this time the observations must be made within the context of a carefully designed and replicated study that will provide data of appropriate quality and quantity.

Some basic concepts

Animals move around in the environment for many different reasons and at differing levels of regularity. Daily patterns of movement are generally motivated by the need to locate food and water, to avoid predators and to maintain social interactions. Less regular movements might be undertaken to protect resources or to find a mate to reproduce. In some species, occasional, larger-scale movements interrupt the regular pattern. These occasional

movements often result in the construction of a new nest or burrow and the establishment of new feeding areas. Such events might be triggered by the depletion of local food supplies, by some disturbance in the previous location, by social conflict following the arrival of competitors in the area, or by an environmental change (e.g. rising watertable) that makes a previous locality unsuitable for continued use.

The area used by an individual animal in the course of its regular pattern of activities is sometimes referred to as its **territory**. However, this term has connotations relating to the defence of resources and we prefer instead to use the neutral term **home range** to refer to the area used on a day-to-day basis. A home range might be a territory if it is defended. A territory is always, at the same time, a home range. Other useful concepts are **range span**—the largest distance across a home range; and **range overlap**—the proportion of the home range that is used by more than one animal of the same species.

Movements between habitats are sometimes stimulated by changes in the availability of food resources or shelter. Such changes are particularly dramatic in agricultural landscapes, where the harvest of mature crops or tillage of fallow fields can represent a crisis for the rodent community. However, patterns of movement may also reflect differences in the rate of reproduction and population growth between habitats. Ecologists sometimes distinguish between source habitats and sink habitats. A **source** habitat is one where breeding takes place at sufficiently high rate to sustain the population, whilst also supporting a net emigration of animals away from the habitat. A **sink** habitat is one where little or no breeding takes place, and where the population is replenished primarily through immigration. Source habitats thus supply sink habitats with animals.

Techniques for studying movement

A variety of field methods are available to study patterns of movement. Most of these methods are time-consuming and some require the use of expensive equipment. As with any other component of an ecological study, movement studies should be guided by one or more specific questions or hypotheses. These will help you to identify the most appropriate methods and to design a study with adequate sample sizes and, if necessary, with appropriate replication (see Chapter 2).

Capture–mark–release trapping

Capture–mark–release studies, as described in Chapter 5, often provide some information about the local movements of rodents. However, unless large numbers of traps are set across sufficiently large areas, the likelihood of obtaining any significant information about movements within and between habitats is slight.

The technique of marking groups of individuals with a common ear-punch is worth considering if you suspect that there are periods of mass movement of animals between habitats. However, this will only be practical if you are able to capture a sufficiently high proportion of the total population in each of the habitats.

Spool-and-line methods

Spool-and-line methods have been used since the 1920s to study movements of mammals. The method involves attaching a spool of fine thread to a captured animal. The loose end of the thread is attached to a fixed object at the point of release such that the thread spools out or unwinds as the animal moves away. Commercially available spools are enclosed in shrink-wrap, leaving an open end where the thread comes out. For rodents, the spool is fixed to the back of the animal with a non-toxic, fast-drying glue (Figure 7.1).

Where the animal is trapped and released close to its burrow or nest, it will often not emerge again until the following night. Provided that the animal does not dislodge the spool in the meantime, the thread will then track its movements through one or more subsequent activity periods. The number of periods

Figure 7.1 Gluing a tracking spool to the back of a rat. In this case, the spool is glued to the fur. For longer-term tracking, over several days, it would be advisable to glue the spool to the skin after first shaving a patch of fur.

represented will depend on the distance travelled by the animal relative to the length of thread in the spool. In general, the weight of the spool should not exceed 5–10% of the body weight of the animal. For a rat-sized rodent weighing 100–150 g, this usually means a thread length around 100–300 m long. An exhausted spool will usually fall off after a few days. If the spool is attached during the animal's active period, the first 20–30 m of line should be disregarded since the released animal may not show natural behaviour during the 'escape'.

The spool-and-line method can be used to answer many basic ecological questions. It can be used to locate the nests or burrow sites of a cryptic species, to confirm that a particular species is responsible for damage observed within an area of crops, or to determine the general mode of habitat use (e.g. use of trees). It is also sometimes used to quantify the pattern of habitat use, based on the proportion of the line that passes through different habitats. However, this type of information is not always easy to interpret because the distance travelled through each habitat may not simply equate to time spent in the habitats or reflect their relative importance to the animal.

Spool-and-line tracking of a large sample of animals within a population will allow you to calculate values that we refer to as **average nightly range** and **average nightly range span**. Unless you are working with a highly sedentary species, these values will almost certainly be smaller than average home range and range span values estimated for the same population.

Spool-and-line methods are simple to use and relatively cheap. The main limitation of the technique is that each animal is usually tracked for only one or a few nights. Repeated capture and spooling of the same individual is not recommended, as this is likely to impact on its behaviour. The method is most appropriate in areas with moderately dense ground cover, providing numerous points for attachment of the thread and minimal chance of disturbance by large animals. Under open conditions, there is much greater potential for disturbance of the thread by wind and livestock.

Radio-tracking

The development of small radio-transmitters caused a revolution in the study of animal movements. Other methods are either effective only for very short periods, as in the spool-and-line method, or they are effective only if a marked animal returns to a certain location, as in capture–mark–release trapping and the use of passive integrated transponder (PIT) tags (described below). In contrast, a radio-collared animal can be followed to its exact location, provided that it stays within the range of a receiver. This is an invaluable advantage, especially for the study of highly mobile species. However, radio-tracking equipment is expensive (each collar costs >US$50) and the radio-tracking process is labour-intensive and sometimes very difficult in rugged or densely vegetated habitat.

Radio-tracking is the most versatile of the methods described here. It can be used at a very simple, descriptive level to locate nest of burrows of highly secretive species or to follow and observe highly mobile species that might otherwise be very difficult to locate. More intensive tracking of individuals can provide information on home-range size, on patterns of habitat use (including the timing of activity) and on social behaviour (contact with other members of the same species). Finally, if tracking is continued for sufficiently long periods, you might also obtain useful information about patterns of dispersal and survival.

Radio-tracking methods also can be used in the context of more structured experiments. This will often involve selecting contrasting pairs of sites that differ according to some key attribute. For example, to test the hypothesis that rodents will travel further from a refuge habitat to attack crops at the ripening stage than at maximum tillering, you would need to investigate rodent movements at sites that differ only in the crop stages. The contrasting pair would need to be replicated, giving a minimum of four sites in total. Another kind of study might involve tracking different sub-populations within a single locality. This approach could be used to test the hypothesis that male rodents have larger home ranges than female rodents within a common habitat. Again, for a real test of either hypothesis, replication is needed, with tracking of both males and females in at least two different sites.

Practical considerations sometimes limit the number of sites and/or animals that can be tracked within a single time period. For this reason, the design of many radio-tracking activities is a compromise between methodological and practical issues.

Equipment

Transmitters

Transmitters emit a radio signal which is detected using an antenna and receiver (see below). The usual signal band is 150–151 MHz but this may vary from country to country. For use on rodents, radio-transmitters have an external antenna and are fitted to plastic collars (Figure 7.2).

Transmitters differ in size and weight, mainly determined by the size and durability of the battery and whether or not an amplification system (second stage) is incorporated. Larger transmitters, suitable for use on a rat-sized animal, should last for 2–3 months and emit a strong signal that can be located many hundreds of metres away. Small transmitters, suitable for mouse-sized animals, will last for only 2–4 weeks and emit a weaker signal that may not carry much beyond 150 m. Some commercially available transmitters can be turned off using a small magnetic switch.

Figure 7.2 A radio-transmitter fitted to a plastic collar. This transmitter and collar set is suitable for attachment to a rat-sized rodent such as *Rattus argentiventer* or *Bandicota bengalensis*.

It is a good idea to attach a small piece of highly reflective tape to the base of the antenna on each transmitter. This is more easily detected by torch light than the animal's eye shine, and will help to minimise any disturbance of the animal. Even a brief glimpse of the reflective tape also will remove any doubt that an animal seen scampering away is the one wearing the radio-collar.

Antenna and receiver

The most commonly used antenna is a three-element, folding 'Yagi' (Figure 7.3). However, you can also make a simple but effective antenna from about 3–5 m of coaxial cable fixed to a wooden or plastic pole. The antenna is connected to a receiver unit that can be tuned to the individual signals emitted

Figure 7.3 Radio-tracking with a three-element, folding 'Yagi' antenna.

by each of the transmitters in use. Receivers are expensive pieces of equipment and great care should be taken to keep them clean and dry. Ideally, you should have at least one backup receiver with you in case of equipment failure.

Field procedure

A radio-tracking study involves the following key steps:

- selection of sites
- capture, collaring and release of animals
- tracking and marking of radio-locations
- mapping of habitat and radio-locations
- knowing when to stop
- recovery of radio-collars
- data analysis.

Ideally, all animals should be collared and tracked simultaneously across all sites, so that weather conditions etc. are standardised within the samples. However, in many cases, it may not be possible logistically to radio-track at all sites simultaneously, as this would require multiple sets of tracking equipment and a large number of people. Additionally, in many cases, the animals are captured, collared and released over a number of successive days and this means that the radio-tracking effort, even at one site, is often staggered in time.

Where simultaneous radio-tracking is not practical, you should make sure that your sites or sub-populations are interspersed in time. For example, if you need to radio-track at two treatment and two control sites, these should be alternated (i.e. treatment 1 → control 1 → treatment 2 → control 2). This will allow you to analyse the data in two ways: by site type (treatment versus control) and by tracking period (either early versus late, or using sampling order in a rank correlation analysis).

Selecting study sites

Apart from the general issues relating to experimental design, there are some important practical considerations when selecting a site for radio-tracking:

- avoid sites with overhead power lines, which can interfere with the signal
- think about general site access (including wet-weather access) and site security (possible theft of traps, posts and harassment of field workers)
- if possible, avoid working close to houses or other buildings—radio-tracking will be done late at night as well as during the day and disturbance of nearby residents should be minimised
- be aware of the location of large channels, creeks or rivers that may need to be crossed during the night
- if possible, select sites with elevated channel banks, dunes or other high points, which will improve detection of signal (if these are not present and the site is completely flat, you may need to consider using ladders to help to locate any animals that have moved away)
- if possible, select sites where there is some prior information about the rodent population.

Catching animals and fixing radio-collars

In all experimental studies, we make the assumption that the procedure does not significantly alter the natural behaviour of the animal. In radio-tracking studies, it is important that the initial capture and handling of the animal does not cause excessive stress or disruption to its usual activity pattern. For this reason, we strongly discourage the use of any capture method that involves major disturbance of nest sites, such as excavation of burrows. We also recommend that all animals are collared and released as soon as possible after the time of capture.

With these limitations, most radio-tracking studies will probably need to begin with a period of intensive trapping, either using single-capture traps or linear trap–barrier systems (see Chapter 3). However, in some cases, it may be possible to capture animals by driving them into nets or by flushing them from daytime retreats in wood- or straw-piles or the thatched roofs of houses. When using the flushing method, it is important to erect a plastic fence or net around the habitat to minimise the chance of escape or injury to the animals.

As mentioned above, the transmitter should not exceed 5–10% of the animal's body weight. Where the study involves two or more species of different adult size, or adults and juveniles of the one species, it may be necessary to have at least two different-size transmitters at hand. Before fitting the collar, record basic information about the captured animal—the species, sex, age—and take some basic measurements (at least the animal's weight). Each collar will have a unique frequency and associated channel number. This number can be used to identify all of the data associated with that particular rat (e.g. rat no. 24: *Rattus rattus*, male, weight 48 g; capture location, time and date).

The job of fitting a radio-collar is best done by two people—one to hold the animal steady while the other fits and adjusts the collar (Figure 7.4). For a rat-sized animal, this is best done with the animal partially enclosed within a cloth bag. First, adjust the collar's tie until it will slide easily over the animal's head. Gradually tighten the collar until it will no longer slide back over the ears, but not so much that it will restrict breathing. It should be possible to rotate a correctly fitted collar around the animal's neck, but without leaving any space for the animal to insert a fore-limb between its neck and the collar.

Once the collar is fitted, place the animal back inside a bag, bucket or trap and observe its behaviour over a period of a few minutes. If the animal is moving freely and the collar appears to be firm, restrain the animal again and cut away the excess cable tie. If it is too tight and the animal is having difficulty breathing, cut the collar off and try again after the animal has had a rest. It is important to collar an animal quickly and efficiently so that the animal does not become too stressed, as this may affect their movements once released. The animal should then be released close to the point of capture. The release point should be marked with a piece of flagging tape labelled with the rat number and the date.

Figure 7.4 Fitting a radio-collar to a *Berylmys berdmorei* (left); and a *Mus musculus* with radio-collar attached (right).

Radio-tracking and marking radio-locations

Although radio-tracking can be done by one person alone, for safety reasons we recommend that each team consists of two people. This is particularly important for night work or tracking in rugged terrain.

Begin radio-tracking a day or two after initial capture and release. This should give the animal time to get over any capture stress and to become used to carrying the radio-collar. Initially, tracking will be slow as you become familiar with the local terrain and the usual location of each animal. Most rodent species are nocturnal and will be spending the daytime inside one or more burrows or nests. It is probably best to begin a radio-tracking session with what is called a **daytime fix**. Using the original capture location as a guide or starting point, tune the receiver to the specific frequency or channel of the particular radio-collar. Holding the antenna vertically, and with the gain (volume) up full, perform a slow sweep of the surrounding area. Use the fine-tuning on the receiver to obtain the best sound—a clear pulse, sounding like 'choc'. You will probably

hear a range of high to low pitched pulses, but the middle frequency is usually the clearest. Point the antenna to where the pulse is strongest, then turn the volume down until the signal loses the low and high pitch pulses. Repeat the sweep and the adjustments of tuning and volume until you are confident about the direction of the signal. If there is no signal, you may have to search around or move on to the next animal and try again later.

Once you have identified a general direction, make a mental note of the bearing and then move off at an angle of approximately 30° from that bearing. Listen for the signal at regular intervals, always performing a general sweep to make sure that you have the direction correctly fixed. If you get contradictory signals, begin the whole process again (signals are sometimes bounced around and your original fix may have been an echo).

In areas with dense ground cover of crops or weeds, it is unlikely that you will actually see the collared animal, at least not without causing an unacceptable level of disturbance. In such situations, most radio-locations will be obtained through the general method known as **triangulation**. This is illustrated in Figure 7.5, using the example of a rat that is sheltering in the centre of a rice paddy.

Unless a collared animal has been seen, the only way to be absolutely certain of its location is to perform a complete circle around the source of the signal. During the daytime, there is little risk of disturbing the animal, so you can afford to make increasingly smaller circles until you have found the exact position. When tracking in areas with tree cover or buildings, be aware of the possibility that the signal may be emanating from a nest located above your head. This may result in confusing signals unless the antenna is pointed directly at the nest site.

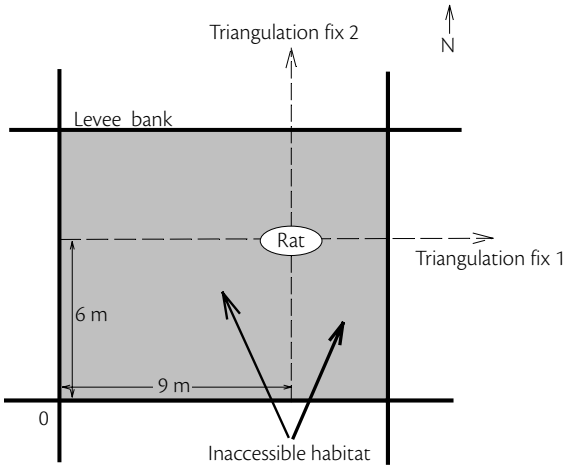

Figure 7.5 Fixing the approximate location of an animal through the process of triangulation. From a starting point (0; bottom left hand corner), pace or measure the distance in the north–south direction to where the signal is strongest and perpendicular to the levee bank (6 m). Do the same in the east–west direction (9 m). The point of intersection of the two fixes is the approximate location of the animal. Where levee banks do not run north–south or east–west, you may need to use a compass to determine the correct angle of each triangulation measurement.

The radio-location should be examined closely, but in a way that will not flush out a resting animal. In many cases, you will probably find an active burrow entrance or a nest. Occasionally, this first fix will lead you to a loose radio-collar that an animal has managed to dislodge. Other possibilities, such as tracking the collar to a large snake (with rat and collar inside), should also be considered.

Each radio-location can either be recorded directly according to a coordinate system (see below) or it can be marked with flagging tape for later recording. If the latter option is chosen, the tape must be clearly labelled with the animal's number and the date and time of the fix. We strongly recommend that you also make some general notes about the location. This will help you to relocate the tape and will also be valuable if the tape is lost or disturbed. Make sure that the tape is clearly visible from all angles.

For **night fixes**, it is important that your own movements do not disturb or influence the animal's behaviour or movement pattern. Hence, it is even more important that you use the triangulation method for all tracking. Do not be tempted simply to move in the direction of the initial fix, as you may find that you are actually driving the animal ahead of you.

Because most rodents are nocturnal in their feeding and general movement patterns, we usually try to obtain a number of fixes spread through the night.

Keep in mind that tracking at night is usually much slower than during the day. This is partly due to the greater difficulties of moving around in the dark, but also because the animals may have moved tens or even hundreds of metres away from the location of the initial daytime fix.

Many species are more active during the early part of the night and then again in the hours before dawn, but this may not be true of all species or even all individuals within one species. One approach is to randomise the time at which fixes are taken through the night for each animal. However, there are also practical limitations to consider (such as the need to sleep!). Another important consideration is to leave sufficient time between fixes for the same animal. Generally, in small rodents there should be about two hours between taking successive fixes.

The process for recording night fixes is the same as that described above for the initial daytime fix. However, we strongly recommend the use of labelled flagging tape rather than on-the-spot calculations of positions, mainly because it is much more difficult at night to maintain correct orientation within the landscape. When marking a fix, it is usually best not to risk disturbing the animal by approaching the exact radio-location, but instead to attach the flagging tape where you completed the fix and mark the tape with a direction (use a compass, if possible) and approximate distance (e.g. rat no. 58; 25 m at 210°N of here; 2130 h; 31/7/2003). Additional notes should be taken on each fix, including whether or not the animal was seen or heard, and if so, what it was doing (e.g. climbing in low shrub, running along low bund).

Mapping habitat and radio-locations

Drawing a good map of the study site is an important part of any radio-tracking study. The map should be drawn at a scale that is appropriate to the questions being asked and to the expected scale of movements of the study animals (usually 1 mm = 1 m). The map should also include a link to the coordinate system that you are using to record radio-locations.

A typical map of an agricultural landscape will contain:

- major channels, secondary channels, main levees and fence lines
- boundaries of the major habitat types (e.g. rice paddy, barley crop, vegetable crop, sugarcane, forest remnants, fallow)
- buildings, houses or edge of village
- location of marker posts or other reference points used for recording radio-locations
- other significant features (e.g. haystacks, trees used by rodents).

For each major crop type, you should make detailed notes of the growth stage (e.g. for rice: transplanting, milky stage, ripening, harvesting, stubble).

In a relatively flat, open landscape, you should begin by staking out a grid with regularly spaced wooden or bamboo posts (20 m spacing for rats, 10 m spacing for mice) using a compass to orient the lines. Initially, the grid should be centred on the area where the majority of mice or rats have been trapped. However, it can be progressively extended to include all of the areas used by the radio-collared animals. The grid will serve a dual purpose. It can be used to draw an accurate map of the site and it can also provide a set of reference points for recording radio-locations. For simplicity of future analysis, it is a good idea to orientate the grid to run north–south and east–west. To map a site using this method, you will need tape measures, marker posts (e.g. garden stakes, bamboo posts), flagging tape and marking pens, a compass, ruler and large sheets of graph paper.

In more complex habitat or situations where the radio-collared animals are dispersed over much larger areas, it is often not practical to use the grid method for mapping. In such cases, you should start by producing a larger-scale schematic map that shows the distribution of major habitats and landscape features. This can be measured out with a long tape and compass, or by pacing along compass bearings. In areas where rodent activity is concentrated, you can then produce more detailed maps, either by establishing a local grid or by a tape and compass survey. You should link these detailed maps back to specific features on the large-scale schematic map so that a composite diagram can be produced. If

possible, fix the coordinates of the large-scale map using a global positioning system (GPS).

You should always try to complete drafts of all maps in the field to make sure that all necessary detail has been recorded before you leave the field site.

Knowing when to stop radio-tracking

How do you know when you have enough data on each animal? The answer to this question depends to a large extent on your initial questions. If your goal is simply to learn as much as possible about the natural history of the animals you are following, then the answer is that you should keep tracking until you fail to make any new observations or until the equipment or field resources give out. However, most radio-tracking studies have more specific goals. For example, you may be interested in how rodents respond to an environmental change, such as a cropping cycle or flooding event. In such cases, the duration of a study may be determined by the environmental schedule. Alternatively, you may be interested in estimating certain parameters of spatial behaviour within a static environment, such as home range, range span and range overlap, as introduced earlier.

The statistical methods used to estimate parameters of spatial use allow you to calculate an appropriate number of fixes. As with most statistical methods, progressively larger samples result in smaller proportional error values and tighter confidence intervals. In general, home-range estimates based on fewer than 15 fixes often have proportionally large errors. Increasing the sample size to 30 fixes will substantially reduce the error; however, going from 30 to 50 or even 100 fixes does not really improve the degree of certainty much for all the extra effort. As a general rule of thumb, 20–30 fixes per individual will give a good estimation of home range (and of range overlap when multiple individuals are tracked at one site). Some people prefer to include only night fixes (i.e. those taken during periods of activity) in this total. One good reason for this is that the daytime fixes are often repeats of the same location, i.e. a nest or burrow site, and this violates the statistical assumption that the fixes are independent representations of the home range. Night fixes that are taken too close together in time are also of suspect value for the same reason. Range-span values are less closely related to sample size due to the fact that the value is sensitive to a single, large excursion by the animal in any direction.

Where a particular radio-collared animal has not moved over a period of two or more days, you should consider the possibility that it has either died at that location or that the collar has been dislodged. In either case, it is probably best to investigate the radio-location carefully and retrieve the carcass and/or the collar. If this occurs early during a study period, it may be necessary to fix the collar to a new animal and recommence tracking.

Recovering radio-collars

Radio-collars should be recovered at the end of the radio-tracking study. Hence, you should not allow the battery to run down completely, otherwise you may not be able to find it. Radio-transmitters are expensive and it is also considered unethical to leave animals collared for longer periods than necessary. For most transmitters, the battery can be replaced or recharged to restore them to full function.

Collared animals can be recaptured in traps set close to their nest or burrow, or they can be flushed directly from their daytime retreat, using a plastic fence or netting to encircle the animal. Where an animal is tracked back to a burrow system, this can be fumigated or excavated to retrieve the animal and the collar. Close examination of nests and burrows will also tell you whether the animal was living singly or communally and whether a radio-tracked female was rearing pups. This information may allow you to interpret otherwise unexplained variation in the pattern of movements between individuals or groups of animals (e.g. between pregnant or nursing versus non-breeding females). If recaptured animals are sacrificed, even more information can be obtained by examining their reproductive condition and history and even their disease status (e.g. parasite load may influence behaviour).

Data analysis

Several computer packages are available for analyses of radio-tracking data. One of the more widely used programs is RangesV, which offers a large range of methods to analyse spatial and temporal patterns of habitat use. The more user-friendly Ranges 6 has just become available on the Internet. In both versions, radio-location data can be imported from spreadsheet or database computer files (e.g. Excel, Access) and the results and graphs can be exported to other applications. Less comprehensive packages for the analyses of radio-telemetry data can be obtained for free from various websites (see Further reading).

Bait markers

Bait markers (or 'biomarkers') work on the general principle that a food item containing an identifiable marker is provided at a known location and point in time. Some time later, animals are caught and analysed individually for the presence of the marker. Depending on the type of bait marker used, evidence of food uptake can be found in faeces or 'scats' (e.g. wool threads, plastic beads), in external tissues such as claws and hair (e.g. rhodamine B, DuPont oil blue A) or in internal tissues such as blood, bones and teeth, and the intestinal tract (e.g. radioactive markers, rhodamine B, tetracycline).

Bait markers are often used to study feeding behaviour. For example, a bait marker can be used to find out which species consume a particular food item, or which species eat from a particular location, such as a grain store. Bait markers can also be used to study movement patterns, typically by posing the question: Where do the animals come from that eat the bait? Finally, bait markers can be used to study aspects of social behaviour, such as intraspecific competition for food or access to particular habitats.

The preparation and application of bait containing a biomarker is usually inexpensive and does not require much labour, even when it is used on a large scale. However, the analysis of samples may require special and expensive equipment and it is usually time-consuming. We will illustrate this class of methods with information on one particular biomarker, rhodamine B.

Rhodamine B

The non-toxic xanthene dye rhodamine B (RB) has been used as a bait marker in several studies involving small mammals. The substance is palatable to rodents and it can be detected under ultraviolet (UV) light in many tissues, including whiskers and blood. In house mice, uptake in bait of 3 mg RB results in the detectable presence after 12 hours of RB in both internal and external tissues (intestines, blood, whiskers) and in excretions (urine, faeces). It remains visible under normal light for up to four days in urine and the digestive tract, and in faeces for up to two days. RB is detectable in blood serum for up to 84 hours using a fluorometer and in whiskers for up to 7 weeks after ingestion. Sampling whiskers or blood has the added advantage that the same individual can be sampled repeatedly. RB is detectable for similar periods in rats and other small mammals.

Bait preparation and delivery

RB can be mixed with grain kernels to produce a 'home-made' bait, or it can be offered as commercially produced, dry extruded pellets (Figure 7.6). Whatever bait is used, care must be taken to ensure that the mixing process gives an even concentration of RB throughout the bait. A concentration of 0.5% RB is palatable to rodents.

Figure 7.6 Rodent bait pellets containing 0.5% rhodamine B under ambient light (left) and under ultraviolet light (right).

RB particles will stick to skin, laboratory benches and equipment, staining everything that comes in contact with it. It is important to have designated RB mixing areas and equipment to avoid contamination of other equipment and materials. Rubber gloves, a lab coat and a face mask should be worn when mixing bait.

Bait can be broadcast or distributed in bait stations. The advantage of using bait stations is that the bait can be provided for a known time period only and at specific locations.

Sampling and detection

Depending on the research questions, animals may be sampled in the general vicinity of a bait station (e.g. for a comparison of bait uptake between sexes or between young and old individuals) or at various distances away from the bait station (e.g. to estimate the foraging range of animals).

For tissues other than vibrissae (whiskers), sampling for RB will need to be carried out within a few days of bait provision. If vibrissae are used, several weeks can elapse between bait distribution and sampling.

Vibrissae —for a particular sampling episode, pluck with tweezers at least two vibrissae (one from either side of the nose) from a restrained live animal. Examine them for the presence of RB-coloured bands under a UV spotlight. Alternatively, examine using a fluorescence microscope at 10× magnification. An animal is scored as RB-positive if at least one vibrissa shows orange fluorescence in the hair bulb or a band of orange fluorescence partway along the shaft (Figure 7.7).

Blood—draw from the suborbital sinus or caudal vein if the animal will be released, or from cardiac puncture if it will be euthanased. Centrifuge a 100 µL sample at 10,000 rpm for 3 min. Remove the serum and freeze at –20°C until it can be analysed with a fluorometer. After thawing, dilute two 20 µL subsamples of blood serum each with 80 µL double-distilled water. Scan the subsamples with a fluorometer and record the photons generated by RB fluorescence as counts per 0.2 s. An animal is considered RB-positive if the fluorometer reading is higher than the average value +3 standard errors of the reading obtained from a series of control samples from mice that have not eaten any RB-bait.

Other tissues—to screen intestine or other tissues for the presence of RB, first necropsy the animal. Freeze the tissues at –18°C until analysis. Inspect the samples for pink colouration under normal light or under a UV spotlight. Comparison with RB-free control animals is necessary to guarantee accurate results.

Figure 7.7 Rat vibrissa (whisker) examined under a fluorescence microscope (ultraviolet light) showing a fluorescent rhodamine B band.

PIT tags

Passive integrated transponder (PIT) tags can be used to monitor small-scale spatial and temporal activity of rodents. A PIT tag is a microchip encapsulated in a glass tube (5 mm long) (Figure 7.8). The tube is implanted under the skin of an animal. These tags are routinely used by veterinarians to individually identify domestic animals.

Studies using PIT tags are usually aimed at small-scale movement patterns (e.g. time of movement in and out of burrows), foraging behaviour (Which animals visit particular feeding places?) or social behaviour (e.g. Which animals share the same burrow?).

PIT tags have no internal power supply but they become energised when they come in close proximity to an electromagnetic field generated by the antenna or a reading device. The reading device retrieves the identification number stored in the chip and records this information along with the date and time that the reading occurred. This information can be downloaded from the reading device and provides a detailed record of which animals have passed by the antenna and at exactly what times.

The advantage of PIT tags is that the activity of free-ranging animals can be observed without external attachments to the animal (spool, radio-transmitter). Disadvantages include the short detection range of

the readers (approximately 50 mm, depending on tag orientation to the antenna) and the high cost of the PIT reader system (US$3 per PIT tag, US$500 for a hand-held reader, >US$3000 for automated reading systems).

The basic equipment for PIT tag studies is the PIT tags, a device to inject a PIT tag under the skin of an animal, a reading device and a computer. Reading devices may be hand-held, where each animal is scanned manually, or automated, with the antenna connected to a data logging system.

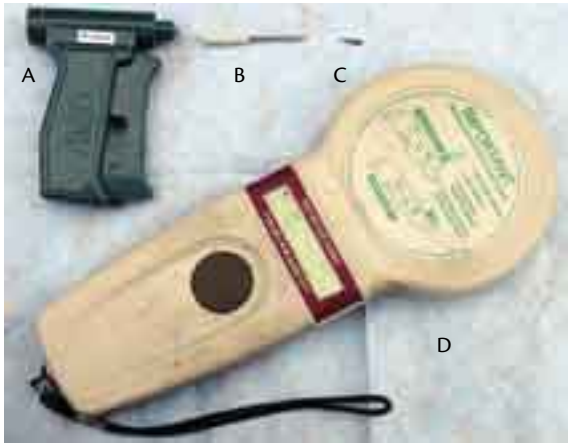

Figure 7.8 A passive integrated transponder (PIT) injector (A) with needle (B) and PIT tag (C), and a hand-held reading device (D).

Further reading

Spool-and-line methods

Boonstra, R. and Craine, I.T.M. 1986. Natal nest location and small mammal tracking with a spool and line technique. Canadian Journal of Zoology, 64, 1034–1036.

Hawkins, C.E. and McDonald, D.W. 1992. A spool-and-line method for investigating the movements of badgers, *Meles meles*. Mammalia, 56, 322–325.

Radio- tracking

Anatrack Ltd 2003. Ranges 6. Wareham, Dorset, UK, Anatrack Ltd. On the Internet: <http://www.anatrack.com/>.

Andreassen, H.P., Ims, R.A., Stenseth, N.C. and Yoccoz, N.G. 1993. Investigating space use by means of radiotelemetry and other methods: a methodological guide. In: Stenseth, N.C. and Ims, R.A., ed., The biology of lemmings. London, Academic Press, 589–618.

Ecology Software Server 2003. Home range. Illinois Natural History Survey. On the Internet: <http://nhsbig.inhs.uiuc.edu/wes/home_range.html>. Provides descriptions and links to available software.

Gallerani Lawson, E.J. and Rodgers, A.R. 1997. Differences in home-range size computed in commonly used software programs. Wildlife Society Bulletin, 25, 721–729.

Harris, S., Cresswell, W.J., Forde, P.G., Trewhella, W.J., Woollard, T. and Wray, S. 1990. Home-range analysis using radio-tracking data—a review of the problems and techniques particularly as applied to the study of mammals. Mammal Review, 20, 97–123.

Kenward, R.E. and Hodder, K.H. 1996. Ranges V. An analysis system for biological location data. Wareham, UK, Institute of Terrestrial Ecology, Furzebrook Research Station, 66 p.

Samuel, M.D. and Kenow, K.P. 1992. Evaluating habitat selection with radio-telemetry triangulation error. Journal of Wildlife Management, 56, 725–734.

Seaman, D.E., Griffith, B. and Powell, R.A. 1998. KERNELHR: a program for estimating animal home ranges. Wildlife Society Bulletin, 26, 95–100.

Seaman, D.E., Millspaugh, J.J., Kernohan, B.J., Brundige, G.C., Raedele, K.J. and Gitzen, R.A. 1999. Effects of sample size on kernel home range estimates. Journal of Wildlife Management, 63, 739–747.

Swihart, R.K. and Slade, N.A. 1985a. Influence of sampling interval on estimates of home-range size. Journal of Wildlife Management, 49, 1019–1025.

Swihart, R.K. and Slade, N.A. 1985b. Testing for independence of observations in animal movements. Ecology, 66, 1176–1184.

Worton, B.J. 1987. A review of models of home range for animal movement. Ecological Modelling, 38, 277–298.

Worton, B.J. 1989. Kernel methods for estimating the utilization distribution in home-range studies. Ecology, 70, 164–168.

Bait markers

Evans, J. and Griffith, R.E. 1973. A fluorescent tracer and marker for animal studies. Journal of Wildlife Management, 37, 73–81.

Fisher, P. 1999. Review of using rhodamine B as a marker for wildlife studies. Wildlife Society Bulletin, 27, 318–329.

Jacob, J., Jones, D.A. and Singleton, G.R. 2002. Retention of the bait marker rhodamine B in wild house mice. Wildlife Research, 29, 159–165.

Papillon, Y., Buffiere, L. and Butet, A. 2002. Rhodamine B as a collective marker for studying movements of small mammals. Acta Theriologica, 47, 491-497.

Taylor, M.K. and Lee, J.L. 1994. Tetracycline as a biomarker for polar bears. Wildlife Society Bulletin, 22, 83–89.

PIT tags

Biotelemetry 2003. PIT tags. On the Internet: <http://www.biotelem.org/pit.htm>. Descriptions and information on suppliers.

Dell'Omo, G., Shore, R.F. and Lipp, H.P. 1998. An automated system, based on microchips, for monitoring individual activity in wild small mammals. Journal of Experimental Zoology, 280, 97–99.

Hutching, M.R. and Harris, S. 1996. An active transponder system for remotely monitoring animals activity at specific sites. Journal of Zoology, 240, 798–804.

CHAPTER 8

Techniques for disease studies

Introduction

Diseases probably play an important role in regulating natural populations of many vertebrate species. Human biology provides some of the best examples of how diseases can limit the ability of a species to occupy what, in all other respects, is a suitable environment. For example, before effective medical treatment was developed against trypanosomiasis (sleeping sickness), large areas of West Africa were largely unpopulated due to the high prevalence of this debilitating and fatal disease.

Knowledge about wildlife diseases is often most detailed for what are called **zoonotic diseases** or **zoonoses**. These are diseases that can be transmitted between animal hosts and humans. Rodents carry many zoonotic diseases, such as the plague, arenaviruses and hantaviruses, rat typhus, lungworm infection and leptospirosis. Several of these diseases have played a major role in shaping the course of human history, and some of them continue to cause suffering and hardship in many parts of the world. In addition, new rodent-borne zoonoses are identified on a regular basis. For example, between 1995 and 1999, more than 25 new hantaviruses and arenaviruses were identified in rodents.

Despite the obvious clinical and economic importance of rodent-borne zoonoses, their basic biology is, in general, poorly understood. With few exceptions, little is known about which species of rodents are the major reservoir of each disease, how long the infective life stages of each pathogen (e.g. bacteria, viruses, spirochaetes or helminths) persist in domestic and rural environments, how these diseases are transmitted in wild rodent populations and then to humans, how prevalent these diseases are in both the rodent and human populations, and the basic human epidemiology of these diseases (i.e. incidence of infection, morbidity rates, transmission rates, age and sex-related effects, and effects of socioeconomic status).

The impact of rodent diseases on human livelihoods, in both urban and agricultural communities, also is poorly documented. However, the available evidence suggests that the impact on human health is increasing in developing countries. This trend is probably linked to increased:

- movements of people between rural and urban areas
- movement of people between countries
- human population density, which amplifies the ability of a disease to spread through populations
- clearance of natural habitats, which leads to a higher incidence of rodent–human contact.

Despite these trends, little research is being done on the epidemiology of rodent diseases in either Asia or the Pacific region. The situation is similar in Europe and Africa.

In the Asian context, our most detailed knowledge about a rodent-borne disease relates to leptospirosis. This disease is reported from Indonesia, Vietnam, Australia and the Pacific Islands. Although generally not fatal, leptospirosis is nonetheless having a major impact on rural communities in many developing countries. Surveys conducted in north-eastern Thailand showed a marked increase in the number of diagnosed cases of leptospirosis from 1995 to 2000, with a maximum of 14,608 cases and 365 deaths reported in hospitals in the year 2000. Since 2000, the number of reported cases in north-eastern Thailand has declined, despite increased public awareness and improved hospital testing. This hints at some natural cycle, either in the general environmental prevalence of leptospirosis, or in rodent populations specifically.

Most deaths from leptospirosis involve rice farmers who are regularly exposed to infection as they work their fields. The early symptoms are influenza-like and can easily be mistaken for malaria and dengue fever. Often the disease is neglected in the rural areas until serious clinical damage has occurred. This is unfortunate because the disease, if diagnosed early, can be treated effectively using antibiotics. By improving farmers' knowledge and practices for rat management, the prevalence and impact of various zoonoses, particularly leptospirosis, could be greatly reduced.

Many rodent-borne diseases can infect a variety of other hosts, including livestock and companion animals (cats and dogs). In some cases, these diseases also affect the health of livestock, leading to weight loss, reduced fertility or even death. Examples of diseases that can affect both rodents and livestock include leptospirosis (in pigs and cattle), erysipelas and trichinella (in pigs), tapeworm and other helminths (probably in all livestock). For communities who live in close proximity to their livestock, such as many of the Hill Tribe peoples of Southeast Asia, the cycle of transmission between rodents, livestock and people may be even more complex, and the levels of risk perhaps higher again.

The potential use of diseases or parasites as biological control agents for rodent management has been explored in several countries, including Australia and Malaysia. Biological control can act either on the animal's reproductive system (i.e. by reducing fertility) or on the fitness or mortality rate of infected adults. To be acceptable, biological control must be specific to the pest species. Before options for biological control can be explored for any target rodent species, we need to know, at a minimum, which disease agents are present in the natural rodent populations, the prevalence of infection (proportion of animals infected) for each disease, the processes of transmission, and the impact of each disease at the individual and population levels.

This chapter describes the sampling techniques used for population surveys of helminths, viruses and bacteria. It is not a comprehensive guide to disease sampling but should provide a useful introduction to the subject and associated techniques. Anyone wishing to work in this area is encouraged to contact local health agencies to discuss the most pressing health issues and appropriate sampling procedures.

Helminths

The major groups of helminths

The three most common groups of helminths are nematodes, cestodes and trematodes. Those recorded in Southeast Asia and the Pacific region are listed in Box 8.1.

Nematodes

Nematodes are also called roundworms. They are non-segmented, with an elongated, round body. The body wall is cuticular and there are no cilia (hairs). Sexes are usually separate and the larvae resemble the adults. They have a simple internal structure, with a distinct mouth, straight intestine terminating in an anus, and a simple nervous system (Figure 8.1).

Nematodes commonly occur in the stomach, small intestine, caecum, large intestine, liver, lungs and body cavity of rodents. They occur less frequently in the heart, kidney, eye, mouth, tongue, oesophagus and muscle tissue.

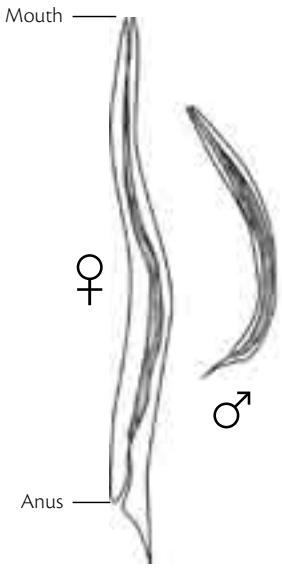

Figure 8.1 A typical nematode body form.

Trematodes

Trematodes are also called flatworms (or flukes) because they have a flattened body. The body wall is thicker than that of a nematode. There are generally two suckers—one located on the ventral surface which is used for attachment, and one surrounding the mouth (Figure 8.2). They also have a simple internal structure, with a distinct pharynx and a blind, forked intestine, but no anus.

Trematodes are most often found in the gut, liver, bile duct, gall bladder, lungs, pancreatic duct, ureter and bladder of the host.

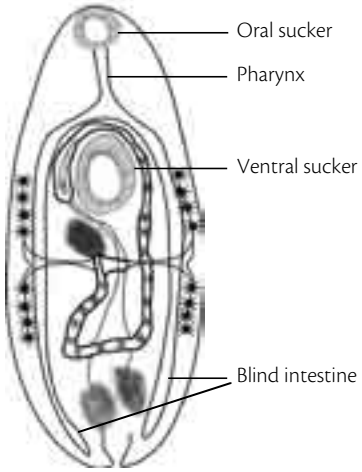

Figure 8.2 A typical trematode body form.

Cestodes

Cestodes are also known as tapeworms. They have segmented bodies and a tough outer surface (Figure 8.3). There are two main external body parts: the scolex, which has hooks and suckers used for attachment (this is the equivalent of a head and is not segmented); and the proglottids or segments, each of which carries one or two reproductive systems. Cestodes lack an alimentary canal.

Adult cestodes are found in the gut and bile ducts that enter the gut. Larval cestodes occur in organs such as the lungs and liver.

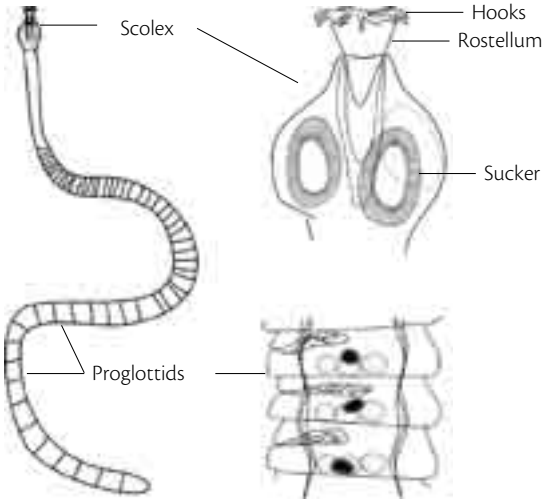

Figure 8.3 A typical cestode body form with details of the scolex and representative proglottids.

Where and how to look for helminths

Laboratory procedures

Rats are easiest to necropsy for parasites when they are freshly dead. If this is not possible, rats can be frozen and the necropsy conducted at a later date, after thawing.

Essential equipment includes good-quality forceps and scissors, glass Petri dishes, a stereomicroscope and light source, gloves and rubbish bags. Glass containers, suitable labels (jewellers' tags are good), pencils and formalin (see Chapter 4) will be needed for labelling and preserving specimens.

Where possible, work on a clean laboratory surface and use clean glassware. Wear disposable gloves and place the used gloves, other used disposable items, and the necropsied rat body in a strong bag at the end of the session. Where possible, incinerate the bag. All used equipment and benches should be thoroughly cleaned with detergent and water after the session and sterilised with 70% ethanol, if available.

Organ examination

For helminth examinations, we recommend the following procedures:

Skin (with fur)**:** after skinning the animal, place the skin in saline solution (0.8% NaCl) and stretch it out. Parasites will be drawn out into the saline.

Tongue: remove from mouth and flatten between two Petri dishes. Examine under a stereomicroscope with not less than 12× magnification.

Oesophagus: as for tongue.

Lung: remove a sample of lung and flatten between two Petri dishes. Examine under a stereomicroscope with not less than 25× magnification.

Liver: examine the surface visually first—some nematodes (e.g. *Calodium*; formerly *Capillaria*) create distinctive white tracks along the surface of the liver. If necessary, cut the sample into smaller pieces and flatten each piece between two Petri dishes and examine under 12× magnification.

Stomach: open the stomach and tease out the stomach contents onto a Petri dish. Examine both the stomach lining and the contents under not less than 12× magnification.

Duodenum and small intestine: extract the tissue from the body and 'unwind'. Spread out in a Petri dish so there is no overlap. Flatten with another Petri dish and examine under not less than 12× magnification.

Caecum and large intestine: as for duodenum and small intestine.

Box 8.1 Previously recorded helminths

Helminth diseases that are a potential risk to humans or livestock in Southeast Asia and the Pacific region are listed below.

***Paragonimus* spp.:** these trematodes are widespread throughout East and Southeast Asia and have a large number of mammalian hosts, including rodents which can act as reservoirs.

***Hymenolepis* spp.:** these cestodes infect humans throughout southern Asia. The exact role of rodents in transmission is unclear.

***Railletina* spp.:** rodents are the primary host for these cestodes. Infection occurs by ingestion of food contaminated with the intermediate host (arthropods including beetles and house flies).

***Schistosoma japonicum*:** infection by this trematode is one of the most serious health problems in the developing world. Humans are the primary host but many wild and domestic animals also act as reservoirs.

***Angiostrongylus cantonensis*:** the adult form of this nematode lives in rodent lung tissue. Infection of humans occurs by ingestion of the intermediate or paratenic hosts—generally a gastropod (snail or slug) or freshwater prawns or terrestrial crabs. The parasite is carried by many rodent species throughout Southeast Asia. Infection in humans is of concern because the larval nematodes migrate to the spinal cord and brain; this condition can be fatal in young children.

Calodium hepaticum (formerly *Capillaria hepatica*)**:** an extremely common nematode infection of rodents. Humans may be infected, but infections are rarely fatal.

***Trichinella spiralis*:** nematodes that infect rodents through the ingestion of infected pig meat. Similarly, the infection is passed on to humans by ingestion of infected meat.

Measures of helminth infection

There are two main measures of helminth infection:

Prevalence of infection: this is simply a measure of the percentage of animals infected with a particular parasite. For example, if 25 rice-field rats (*Rattus argentiventer*) from a sample of 75 rats had their livers infected with the nematode *Molinacuaria indonesiensis*, then the prevalence would be 33%.

Intensity of infection: this requires the number of individual helminths of a particular species to be counted per animal. The mean intensity is a population measure that refers to the mean level of infection per rodent. This mean is calculated from only those animals that are infected. So, working from the previous example, we would conduct counts of the number of parasites in each of the 25 animals infected with *M. indonesiensis* and then estimate the mean level of infection in these 25 animals only, omitting the animals that had no parasites.

Preserving specimens of helminths

Unless you are very familiar with their taxonomy, parasites can be difficult to identify. If you are unsure about a specimen, it may be best to preserve the parasite and seek assistance from a specialist.

Carefully extract the specimen from the organ or body part. Try to keep the body in one piece. If this is not possible, then preserve all the pieces, as they may be needed to determine species and sex. Different parasites must be preserved in different ways:

Nematodes: preserve the specimen in hot 2–5% formalin (approximately 80°C).

Trematodes: preserve the specimen in hot 2–5% formalin. If there are two specimens, preserve one in cold 2–5% formalin and one in hot 2–5% formalin.

Cestodes: remove the tissue sample containing the parasite and place in a Petri dish of water. If the parasite is in the gut, open the gut to let the water bring the parasite out. Do not scrape the parasite out as this might break the parasite body or head and damage or lose hooks that may be present. Once the parasite is free of the tissue and relaxed in the water for 10–15 minutes, preserve in hot 2–5% formalin.

If possible, use small glass containers with screw-on lids that fit securely. For each specimen, record on a small piece of card (with pencil) the species of the host animal, the location and habitat of the host, the date, the collector's name, the tissue from which the sample was collected, and what you know about the parasite. Put this card into the solution with the parasite.

If you send the specimen to an expert in another country, make sure to follow all regulations for the import/export of biological material.

WARNING: do not inhale fumes from hot formalin. This fixative is a strong irritant and the fumes can damage your eyes or respiratory tract, and may cause cancer with prolonged exposure. Formalin should be heated in a well-ventilated area.

Viruses and microbial diseases

Viruses and microbial organisms of various kinds can infect many different types of tissues within the body. Those recorded in Southeast Asia and the Pacific region are listed in Box 8.2. Methods used in the isolation of particular pathogens are often quite specific and require particular culture media and environmental conditions. For this reason, most epidemiological studies begin with a serological survey based on blood samples.

When an animal is invaded by a potential pathogen such as a virus or bacterium, the white blood cells react to proteins on the surface of the pathogen and form antibodies that are specific to its molecular structure. These antibodies are found in the blood serum and specific tests can be performed to identify particular antibodies. Note, however, that most serological tests do not tell us whether the animal is currently infected with a replicating virus or bacterium, only that the animal has been exposed to the pathogenic agent sometime during its life.

We will concentrate here on methods used to collect, preserve and analyse blood samples.

Box 8.2 Previously recorded viral and microbial diseases

This section concentrates on rodent-borne zoonotic diseases — they present a potential risk to humans or livestock in Southeast Asia and the Pacific region.

Hantaan virus (haemorrhagic fever): there is a group of hantaviruses that has been detected in urban populations of rodents in many parts of the world. The virus is passed from host to host via infected saliva, urine and faeces. Some strains have little effect on humans; others cause major illnesses with a wide variety of symptoms.

Tick typhus (*Rickettsia conori*): the principal reservoir for this disease is the dog, but rodents are also important reservoirs. The disease in humans results from a bite from an infected tick. The tick particularly involved in transmission is found throughout Asia.

Scrub typhus (*Orientia tsutsugamushi*): a variety of rodents throughout Asia are the principal reservoir for this disease, which is transmitted by larval trombiculid mites called 'chiggers'. Mortality rates in humans are low if treatment is sought early.

Murine typhus (*Rickettsia typhi*): reported throughout Southeast Asia, this disease is spread by flea bites or contact with infected faeces or crushed fleas. The disease causes a wide range of symptoms in humans, but the mortality rate is low.

Queensland tick typhus or spotted fever (*Rickettsia australis*): occurs down the eastern coast of Australia and is carried by ixodid ticks. Natural reservoirs of the pathogenic organism appear to be marsupial mice, bandicoots, possums, rats and mice. The disease causes a wide range of symptoms in humans, but the mortality rate is low.

Leptospirosis: caused by a variety of spirochaetes, leptospirosis is one of the most prevalent zoonotic diseases carried by rodents in rice fields. Almost all rodent species in Southeast Asia can act as hosts. Human infection occurs when an open wound comes into contact with water, moist soil or vegetation contaminated by rat urine. The mortality rate is low for most strains. The symptoms are similar to influenza and last from several days to three weeks. Symptoms of leptospirosis can be confused with those of malaria and dengue fever, and many cases are probably misdiagnosed. People working in rodent-infested plantations or fields are most at risk.

Rat bite fever (*Spirillum minor*): caused by a spirochaete, this disease is transmitted by rodent bites and is found throughout the world. Incubation takes several weeks and symptoms usually appear after the wound has healed.

Plague (*Yersinia pestis*): a bacterial disease that can be treated with antibiotics if diagnosed early. The cycle of this disease is mammal to flea to mammal, with rodents as the primary host. Whilst advances in medical science make it unlikely that plague will erupt again in global pandemic, as it did on various occasions through history, it still presents a serious health problem in many parts of the world. The last major epidemic of plague in Asia and Australia occurred in the first decade of the 20th century.

Salmonellosis: *Salmonella* bacteria infect humans worldwide, usually through ingestion of water or food contaminated by faeces of an infected animal but also through eating incorrectly prepared foods. There are many strains with variable severity of impact.

Toxoplasmosis: caused by a coccidian *Toxoplasma gondii*, for which the domestic cat is the primary host. Many other mammals, including rats and mice, may act as intermediate hosts.

Collecting and processing blood samples

It is best to take blood samples from freshly caught rodents. Never take blood samples for viral testing from animals that have been housed together for more than three days. Transfer of infection by close contact may lead to a virus being present in all animals to be sampled, giving false prevalence results.

The following procedure can be followed for the collection of sera for viral testing.

- Anaesthetise animals one at a time using carbon dioxide (as described in Chapter 3), until they are unconscious but not dead. If bottled carbon dioxide is not available, then carbon monoxide can be used via exhaust fumes from a petrol-fuelled car (diesel fumes are not effective). However, we strongly recommend the use of carbon dioxide.
- Open the chest cavity and draw up to 3 mL of blood directly from the heart using a needle and syringe. A 5 mL syringe and 21 gauge needle is an efficient combination. If possible, angle the needle up into the ventricle of the heart, along the line of the body. Try to avoid air bubbles as these may lead to lysis of the sample (broken blood cells). New equipment must be used for each animal. After collecting the blood, use cervical dislocation to ensure that the animal is dead.
- Remove the needle from the syringe and transfer the blood to small plastic tubes with lids (2.5 mL Eppendorf tubes are ideal). These tubes should be clearly labelled with a number or code that identifies the individual rodent. Note that rapidly forcing blood through a needle will result in lysis of the sample, hence the importance of removing the needle.
- Put the sheath back on the used needle, and store the needle and syringe in a solid container. At the end of the sampling session, the container should be incinerated, if possible.
- Leave samples for approximately 1 hour at room temperature (<30°C) or until a blood clot has formed in the tube.
- Score the sample by separating the clot from the walls of the tube using a clean needle or pipette for each sample. Alternatively, you can use a probing instrument if it is dipped in alcohol and sterilised with a flame between samples.
- If you have a centrifuge, leave the samples for 1 hour and then spin them to increase the yield of sera. Make sure that the lids are secured. Ideally, spin the samples for 5 minutes at 2500–3000 rpm. If you do not have a centrifuge, store samples overnight in a refrigerator.
- Remove the sera (clear liquid) into tubes with a pipette. Use a new pipette for each sample. When possible, separate the serum into at least two tubes—this provides a backup in case something happens to the first sample and will also allow you to do other tests at a later date.
- Label the tubes clearly with the rodent's identification number, the date, and number of samples. A black permanent marker pen is recommended. Clear labelling of samples is essential. Samples that have illegible or smeared labels are usually worthless.
- Once all of the sera have been collected, store the tubes immediately in an upright position in a freezer. Ideally, storage should be at or below −50°C. However, sera can be stored for up to a month at −18°C, the temperature of a basic household freezer.

Lyophilising (freeze-drying) samples

Samples that have been prepared by lyopholisation or freeze-drying can be transported to a testing laboratory without having to remain frozen. This is a major advantage if samples have to be transported a long distance, or if the local transport systems are unreliable.

If you have access to a lyophiliser or freeze-dryer, carefully follow the directions of the manufacturer of the equipment. If samples to be lyophilised are frozen, thaw at approximately 4°C, either on ice or in a refrigerator. If the samples are to be lyophilised the next day, thaw them in a refrigerator overnight. It is vital that someone is in attendance while the samples are being lyophilised. If there is a blackout or if the samples are not fully lyophilised by the end of the day, remove them from the lyophiliser, recap

them and refreeze immediately. When you are ready to begin again, thaw the samples and then begin the process again.

When the samples are fully lyophilised, recap them well. If possible, cover the lids with parafilm or thick tape. The lyophilised samples can be stored in a clearly labelled plastic bag and placed within an airtight container until they are ready to be sent for testing.

Sampling design for rodent disease studies

Sampling for rodent diseases is unfortunately often dictated by logistics and money. Samples are taken when and where opportunity permits, and statistical analyses are designed around the available data.

Two strategies for sampling are described in this section: one determines a sample size in advance and the other does not. There are limitations to each, but they can be used as a guide when embarking on sampling for disease.

A general rule, applicable to both sampling strategies, is that you should try to obtain a cross-section of the population, as there may be an age or sex bias in any disease prevalence. It may also be possible to maximise the use of animals by taking blood or tissue samples from animals that have been killed for some other purpose (e.g. for taxonomic or breeding studies).

Optimal sample size for detecting a disease (predetermined sample size)

To determine the sample size required to investigate whether a population is infected or not with a particular pathogen, the following equation can be used:

$$n = \frac{[1-(1-CL)^{1/d}][N-(d-1)]}{2}$$

where n = the required sample size
N = the total population size
d = the number of diseased animals in the population
CL = the confidence level as a fraction.

Table 8.1 is given as an example.

As can be seen from the table, for detection of disease at very low prevalence, the sample size needed is very high. At high disease prevalence, the necessary sample size for detection is low, even when the population is large.

Sequential sampling (no fixed sample size)

Even where the optimal sample size can be estimated, it is often not possible to meet the required numbers, either because of inadequate field time (especially with detailed parasitology) or because of budget limitations (especially when testing for more than one virus).

A method that can minimise the sample size (and therefore save time and money) is sequential

Table 8.1 Calculations of the sample sizes (numbers in cells) required to accurately determine (at 90% confidence limits) the prevalence of a disease (%*d*) within populations of variable size (*N*).

	N								
%*d*	10	20	50	100	150	200	500	1000	5000
1%	10	20	50	90	117	136	184	205	224
5%	10	18	30	36	39	40	43	44	45
10%	9	13	18	20	20	21	21	22	22
20%	6	8	9	10	10	10	10	10	10
50%	3	3	3	3	3	3	3	3	3
75%	2	2	2	2	2	2	2	2	2

sampling. This type of sampling does not rely on choosing a sample size in advance. Instead, observations are made one at a time, and after each observation a decision is made either to stop sampling or to continue sampling. An example is given in Figure 8.4 (with 90% confidence limits).

This method is useful for determining the prevalence of a parasite, but could not be used to determine the prevalence of a bacterium or virus unless facilities were available for immediate testing.

Prevalence is defined as the percentage of animals infected by a particular disease agent. If the prevalence is low or high, there is little variance in estimates, therefore sample sizes of around 30 animals are typically adequate. If the prevalence is between 30% and 70%, then sample sizes will generally need to be around 60 to 70 animals.

The sample size required to provide a 90% or 95% confidence interval of the prevalence can be calculated from published tables. These tables and other useful tools for quantitative epidemiological studies are also available on the Internet (see Further reading).

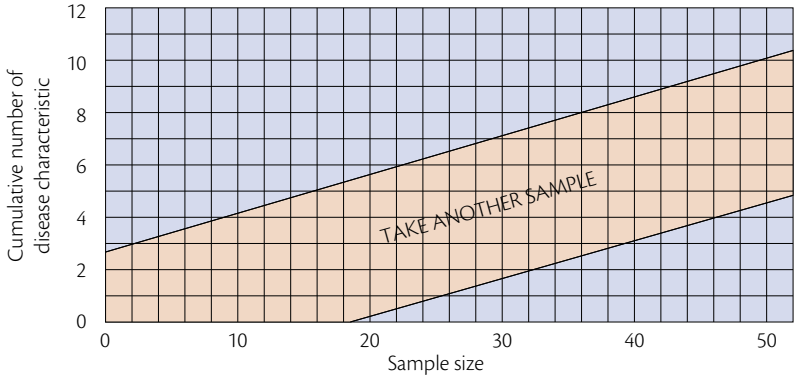

Figure 8.4 An example of sequential sampling to determine the prevalence of a particular disease (for example, a particular parasite). Animals are examined one by one for the presence of the parasite. Animals continue to be examined until the result is either over the top line or below the bottom line of the graph. If the result falls in the top section, we are 90% confident that the prevalence is greater than 20%. If the result is in the bottom section, we are 90% confident that the prevalence is less than 10%. If the result falls between the two lines, the prevalence is uncertain and another sample is taken. A detailed description of sequential sampling can be found in Krebs (1999) *Ecological Methodology*, *Chapter 9*.

Further reading

Begon, M. 2003. Disease: health effects on humans, population effects on rodents. In: Singleton, G.R., Hinds, L.A., Krebs, C.J. and Spratt, D.M., ed., Rats, mice and people: rodent biology and management. ACIAR Monograph No. 96. Canberra, Australian Centre for International Agricultural Research, 13–19.

De Blas, N., Ortega, C., Frankena, K., Noordhuizen, J. and Trusfield, M. 2000. Win Episcope, version 2.0. Also available via the Internet: <http://www.clive.ed.ac.uk/winepiscope/>. Contains quantitative tools for disease studies, including tables for estimating the sample sizes required for detection of disease in populations at various confidence intervals. It also describes diagnostic tests and contains course notes on analytical observational studies of diseases of animals.

Gratz, N.G. 1994. Rodents as carriers of diseases. In: Buckle, A.P. and Smith, R.H., ed., Rodent pests and their control. Wallingford, UK, CAB International, 85–108.

Krebs, C.J. 1999. Ecological methodology. Menlo Park, California, Benjamin Cummins, 620 p.

Mills, J.M. 1999. The role of rodents in emerging human disease: examples from the hantaviruses and the arenaviruses. In: Singleton, G.R., Hinds, L.A., Leirs, H. and Zhang, Z., ed., Ecologically-based management of rodent pests. ACIAR Monograph No. 59. Canberra, Australian Centre for International Agricultural Research, 134–160.

Nitatpattana, N., Henrich, T., Palabodeewat, S., Tangkanakul, W., Poonsuksombat, D., Chauvancy, G., Barbazan, P., Yoksan, S and Gonzalez, J.-P. 2002. Hantaan virus antibody prevalence in rodent populations of several provinces of northeastern Thailand. Tropical Medicine and International Health, 7, 1–6.

Prociv, P., Spratt, D.M. and Carlisle, M.S. 2000. Neuro-angiostrongyliasis: unresolved issues. International Journal for Parasitology, 30, 1295–1303.

Singleton, G., Farroway, L., Chambers, L., Lawson, M., Smith, A. and Hinds, L. 2002. Ecological basis for fertility control of house mice using immunocontraceptive vaccines. Reproduction Supplement, 60, 31–39.

Spratt, D.M. 1990. The role of helminths in the biological control of mammals. International Journal for Parasitology, 20, 543–550.

Tangkanakul, W., Tharmaphornpil. P., Plikaytis, B.D., Bragg, S., Poonsuksombat, D., Choomkasien, P., Kingnate, D. and Ashford, D.A. 2000. Risk factors associated with leptospirosis in northeastern Thailand, 1998. American Journal of Tropical Medicine and Hygiene, 63, 204–208.

CHAPTER 9

Assessing crop damage and yield losses

Introduction

The ultimate goal of most rodent management activities is to reduce the impact of rodents on crop production. To measure the impact of our actions, we need simple and effective methods for assessing the level and consequences of damage inflicted by rodents.

Rodents can attack crops at any stage during production and storage. It is convenient to break the resulting impact into two components, namely:

- **preharvest** impact, caused by rodents to growing crops, through to harvest
- **postharvest** impact, caused by rodents during any period of storage.

When talking about rodent impacts on crops, it is essential to distinguish between crop damage and crop loss. Rodent **crop damage** is the actual physical harm inflicted by rodents on crops or produce. It can occur at any stage during the production and storage of crops, and includes the excavation and consumption of newly sown seed, the cutting and removal of tillers and attached panicles in cereal crops, and the gnawing of tubers or fruits. In stored crops, damage includes both direct consumption and contamination with urine or faeces.

Crop loss caused by rodents is measured at the point of harvest for preharvest impacts, or at the point of consumption or sale for postharvest. These losses are the cumulative result of damage that occurs during crop growth and storage, respectively. The relationship between damage and loss is very complex, especially in the case of preharvest impacts, and it often not possible to directly equate the two figures.

In most rodent management projects, our ultimate goal is to reduce crop losses caused by rodents. The most direct way of measuring the success of any rodent management system is therefore to measure the yield at harvest and at the point of sale or consumption, and to compare these values either with the situation before rodent control measures were adopted or at similar sites where no measures were taken. Good estimates of yield are also needed to calculate the potential economic benefit of any rodent control method. This involves calculating the value of any extra crop produced, either in energetic or cash terms, and then weighing this benefit against the cost of the rodent management actions, including both materials and labour. In a final benefit to cost analysis, we might also consider other factors or side benefits, such as potential improvements to human or livestock health.

Although our primary focus is generally on crop losses caused by rodents, there are various situations in which it is necessary or advisable to measure rodent damage to crops. Firstly, measurements of damage are often necessary during the problem definition phase of a new project (see Chapter 1), where we need to get a quick quantitative estimate of rodent impacts across a range of crop types. By combining damage estimates with farmer knowledge of past and present crop losses, we can quickly build a good general picture of rodent impacts in a new area. A second reason why we might want to study rodent damage in addition to yield loss is to understand the relationship between the timing and intensity of crop damage, and any changes in rodent abundance, breeding activity and movement patterns. For example, we might wish to know whether rodent damage is more or less intense at particular stages of crop growth, and whether these periods are connected to the onset of breeding activity or to periods of dispersal. A third reason is that crop losses are caused not only by rodents but by other pests and diseases as well, hence some measurement of damage together with yield loss is necessary to attribute the losses to each of these factors. Finally, as we will explain in the final section of this chapter, the relationship between rodent abundance and crop damage in any given cropping system is of great theoretical and practical interest, as it is this relationship that will allow us to set targets for rodent control.

In this chapter, we describe some techniques that can be used to estimate both the level of rodent damage and crop loss in field crops, and the level of damage and loss to stored foods. You may need to modify or adapt these techniques to work in particular crop types or field conditions.

Methods for estimating damage

With experience, it is usually easy enough to distinguish damage caused by rodents from that caused by insects or other pests. However, quantification of rodent damage is complicated by two issues. The first is the complex relationship between the timing of the rodent damage and its impact on final crop yields. The second is the fact that rodent damage is often unevenly distributed within the agricultural landscape.

Timing of damage

Damage can occur at any time during the growth of crops through to the time of harvest. The impact of this damage on final crop yields will depend on both the severity and timing of the damage, and on the ability of the particular type of crop to compensate for any damage by putting on extra growth following damage.

In cereal crops, **growth compensation** has two components—tiller regrowth and panicle filling. Any tiller that is cut through by a rodent is likely to regrow. If this occurs before the maximum-tillering stage, the tiller may go through normal panicle initiation. These tillers may be shorter than undamaged ones but they often produce a normal-sized panicle. A tiller that is cut after the plant has entered the panicle-initiation stage generally will not be able to produce a new panicle. However, the plant may compensate for this loss by diverting its resources into the remaining panicles. This can lead to panicles with larger or more numerous grains. Once a cereal plant enters the panicle-ripening stage, it is unable to compensate for any subsequent rodent damage. Crop damage that occurs during the ripening phase will have the most immediate impact on crop yield. However, we should not underestimate the potential impact of damage at earlier stages. The point at which growth compensation will cease to be effective against rodent damage needs to be investigated for each crop type.

It is important to emphasise from the outset that assessment of crop damage at one point of time may not provide a good estimate of yield loss. For example, in rice crops, estimates of damage are usually taken in the week before harvest. This will only detect fresh damage and will not reflect the cumulative damage from the maximum-tillering stage through until harvest. The few estimates available for rice crops indicate that estimates of

damage taken in the week or so before harvest would need to be multiplied by four or five times to estimate yield loss. However, it should be noted that these estimates pertain to lowland irrigated rice crops and to damage mainly caused by *Rattus argentiventer*. Many more studies are needed of this important relationship.

Spatial distribution of damage

The distribution of rodent damage is often uneven within a single field or among a group of fields (Figure 9.1). In many cases, areas of particularly heavy damage are adjacent to local features that provide refuge or breeding habitats for rodents, such as large bunds or channel banks. However, in several parts of Southeast Asia, the highest rat damage is often found in the middle of rice fields rather than around the edges, producing the so-called 'stadium effect' (Figure 9.2). This unusual pattern presumably reflects some aspects of the feeding behaviour of the major pest species.

Damage assessment is simplest where the damage is randomly distributed in a field and more complex when it is very uneven or patchy in distribution (Figure 9.3). In the following sections, we will describe methods that are suitable for estimating damage that is randomly distributed within a field (Figure 9.3a) or distributed in a structured manner (Figure 9.3b–e).

Researchers have compared different sampling designs for plant disease and insects and found that sample size was more important than sampling pattern when the disease distribution was random, while the sampling pattern was more important when disease distribution was aggregated or patchy.

Figure 9.1 Localised patches of heavy rat damage to rice in Myanmar. This damage was most likely caused by *Bandicota bengalensis*.

Figure 9.2 'Stadium effect' of rodent damage to rice in Indonesia (indicated by arrow).

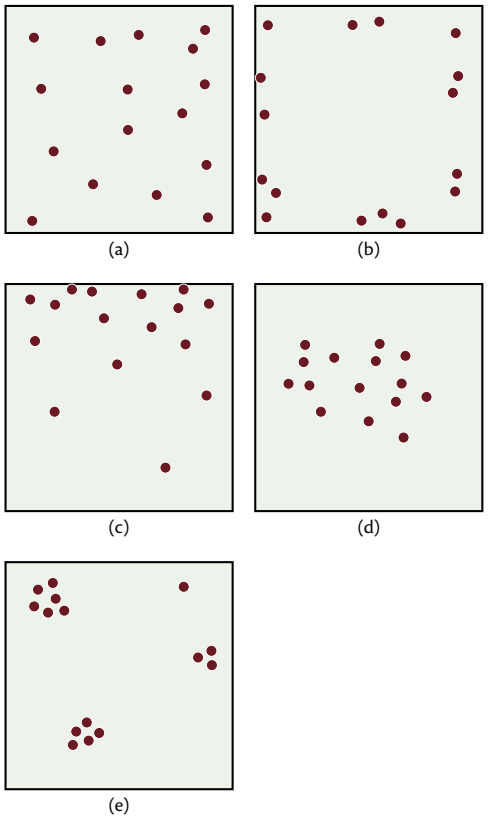

Figure 9.3 Five different patterns of damage within a field: (a) random damage; (b) highly structured damage (close to margins of field); (c) highly structured damage (decreasing away from upper margin of field); (d) highly structured damage (in centre of field — 'stadium effect'); (e) highly structured (clumped) damage.

Estimating damage at sowing/transplanting

Rodents often damage crop plants immediately after sowing or transplanting, or during the first week or two of plant growth. The seeds or germinating plants provide a high-quality food resource for rodents. Typically, this damage results in the complete removal of individual plants. Provided that the level of damage is not too severe or where damage is evenly spaced, the remaining plants may be able to compensate for this damage by putting on extra growth. However, in some cases, the early crop damage is sufficiently heavy or in large enough patches to cause significant losses in crop production.

Early crop damage is generally easy to detect but difficult to quantify. We will describe separate methods that can be used for crops that are sown and those that are transplanted.

Sown crops

Many crops are direct seeded, either by broadcasting by hand, mechanised sowing, or dibble-stick methods. Rodents often enter the fields to dig up and consume the newly sown seed, or to feed on the early shoots after germination (Figure 9.4).

In order to estimate the level of damage to seeds or new shoots, we need information on (1) the quantity of seed that was spread; and (2) the number of seeds or seedlings that were removed or damaged. Obtaining this information can be difficult.

One way to estimate the proportion of seed lost to rodents is to compare the number of plants that germinate per unit area with the quantity of seed that was sown across the same area. Farmers generally know the seeding density and it is easy to count emergent plants using a quadrat sampling method. However, this technique will overestimate the level of damage if some of the sown seed failed to germinate. (Our experience with wheat seed in Australia is that only 60–80% of sown seeds will germinate.)

Figure 9.4 Examples of rodent damage to newly planted crops. Top: *Rattus argentiventer* footprints in a recently sown rice crop where seeds are germinating. Bottom: a newly sown wheat field in Australia, showing evidence of digging for seed by *Mus musculus*.

An alternative way of measuring the extent of rodent damage to early crop stages is to compare the number of emerging plants in areas that have been damaged by rodents with areas that have been protected from rodent damage. The usual method is to set up exclusion plots (see below). Three or more exclusion plots are required to achieve adequate replication, and the unprotected crop should be sampled with quadrats of the same size as the plots. The distribution of these quadrats should adequately reflect the pattern of damage—either randomly placed if the damage appears to be randomly distributed, or arranged as a stratified random sample if the damage appears to be patterned in some way (see below).

Data from exclusion plots are used to determine damage according to the following formula:

$$\text{Damage rate } (\%) = \left(1 - \frac{\text{Number of plants in unprotected area}}{\text{Number of plants in protected area}} \right) \times 100$$

Transplanted crops

Rodent damage in a seedbed is probably best estimated by using the exclusion plot method.

For transplanted crops, the number of seedlings per hill and the density of hills are generally known to a fair degree of precision. Where such information is available, reasonable estimates of rodent damage can be obtained by using a quadrat sampling method. An exclusion plot method would probably yield more reliable estimates, but would involve considerably greater labour input and cost.

Exclusion plots

Exclusion plots are representative areas of crop that are protected against rodent damage by a rodent-proof fence or barrier. The reduction in crop yield caused by rodent damage is calculated by comparing the yield of the protected crop with unprotected areas in the surrounding field.

The main consideration when designing an exclusion plot is that the barrier will effectively stop all rodent pests from climbing, burrowing or gnawing their way into the enclosure. The choice of fencing material and dimensions of the barrier will need to take into account both the size of the particular pest species and its climbing and digging capabilities (see Box 9.1).

Box 9.1 Design of exclusion plots

Two examples will serve to illustrate some of these design principles. In Australian wheat crops, small plastic fences were built to protect small areas against damage by house mice, *Mus domesticus*. Each exclusion plot measured 2 × 2 m, from which the central area of 1 m^2 was harvested to determine yield. The plastic fence was 200 µm thick and 0.6 m high, with the bottom 10 cm dug into the ground. The fence was supported by metal fencing pickets and held taut against wind by wire strung through a fold along the top of the plastic and fixed to the top of the pickets. The plots were erected as soon as possible after sowing (i.e. the afternoon after the farmer had sown the crop). Exclusion plots were set at varying distances from the edge of the crop (e.g. 10, 20, 50 m), with two plots set at each distance to achieve replication. Counts of plants at emergence of the crop were conducted from the central 1 × 1 m area of the plot and related to the abundance of mice at different sites.

In deepwater rice crops in Bangladesh, researchers set exclusion plots that were 5 × 5 m (Figure 9.5). These were constructed of wire netting fixed to poles at the corners and sides, and with a 30 cm strip of galvanised metal sheet at the top to prevent rats from getting a foothold. The base of the fence was buried 10 cm in to the ground. Fences were made 195 cm high, which was 35–45 cm higher than the maximum flood depths of previous seasons. During peak floods, the fence was further extended using plastic sheeting. The full exclusion plots were harvested in this particular study to compare yields.

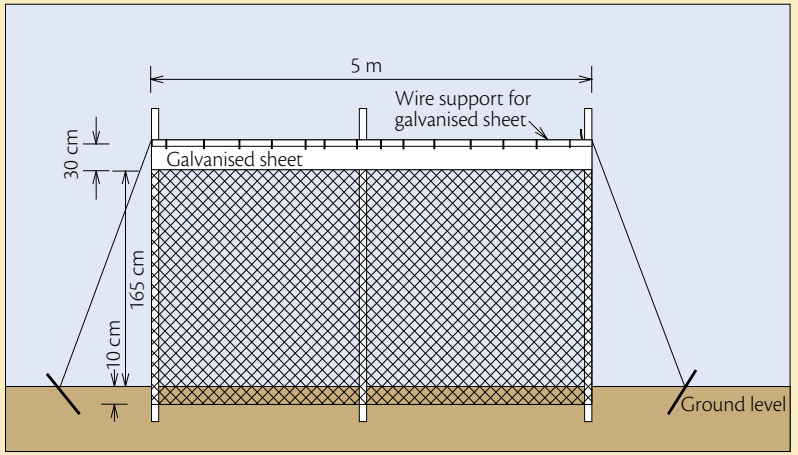

Figure 9.5 Wire exclusion plot used to protect deepwater rice from rats in Bangladesh (adapted from Islam et al. 1993).

Another important consideration is that the fence of an exclusion plot does not influence crop growth within the plot. This is probably of greatest concern where a plastic fencing material is used, because this may lead to changes in local air movements and light and humidity levels. One commonly used method for overcoming this problem is to calculate crop yields from the central portion of the exclusion plot, excluding any areas that grow close to the fence, so the larger the plots the better. Barriers constructed from open mesh wire are probably better in this regard, although they may not be effective against some rodents that have excellent climbing ability. All types of fences present the possibility that they may provide perching sites for birds, thereby increasing bird damage to the exclusion plot.

Estimating damage at later stages of cereal crops

Larger rodents usually gnaw through a panicle-bearing tiller near its base, leaving behind a neatly cut surface with a characteristic 45° angle (Figure 9.6). They then feed on the panicle where it falls or drag the tiller away to a safe place, such as a burrow. Very small rodent species, such as species of *Mus*, will climb the tillers and either snip away the panicle or else feed on individual grains without removing the panicle. Different methods are required to assess damage of each type.

We can estimate the proportion of damage to cereal plants at any particular stage of crop growth by examining a sample of individual plants. For each plant, we can record the number of tillers that are uncut, recently cut, previously cut and regrowing, or previously cut and not regrowing. The sum of these will give the total number of tillers for the individual plant. This is a laborious process when repeated on a large scale, hence you will need to decide how many times you can afford to repeat the process.

If damage assessment can be done only once, then we recommend that you do it as close as possible to harvest time. For the reasons set out above, this will provide a *minimum* estimate of yield loss.

If damage assessment can be carried out more than once, we recommend that it is done at the booting (panicle initiation) stage and again just before harvest. The choice of booting stage reflects widespread reports and field observations that rodent damage is particularly intense at this stage.

Figure 9.6 Rat damage to rice tillers (circled). Note the 45° angle of the cut through the tillers.

Different methods are needed to quantify direct panicle damage caused by mice or other small climbing rodents. For panicles at the ripening stage, the usual method is to estimate the proportion of grains that have been removed or damaged. For earlier stages of growth (e.g. tillers at the booting stage), you should count the number of damaged versus undamaged tillers.

Random and stratified random sampling

Random sampling methods are appropriate where the damage appears to be genuinely random in its distribution, even where the underlying landscape shows discrete structured variation. Selecting sampling points or quadrats in such a situation is simply a matter of deciding on a plot size (e.g. 1 m^2 quadrats) and sampling density (e.g. 5% of the total area), and then generating random numbers (from tables or by using a hand calculator) to identify the sampling points.

Stratified random sampling methods are appropriate where damage does not appear to be random, regardless of whether the underlying variation is discrete or continuous (Figure 9.7). An example of discrete variation is where rodent distributions are influenced by the presence of two or more clearly defined soil types or habitats (Figure 9.7a). An example of continuous variation is the distance of the

crop from an irrigation canal. Structured variation of this kind is illustrated in Figure 9.7b. Our recommendation is to always use stratified sampling.

The sampling procedure for stratified random sampling begins with the definition of the **strata**—the layers of variation within the landscape. If these are discrete, you will first need to calculate the relative proportion of each stratum within the total study area. The number of sampling points or quadrats placed within each stratum is then scaled to reflect these relative proportions. This scale may be a direct proportional one, or it may be biased towards greater sampling of the more common units or greater sampling of the less common units, depending on the primary purpose of the sampling. Within each stratum, sampling points or quadrats should be chosen randomly, using the process described above.

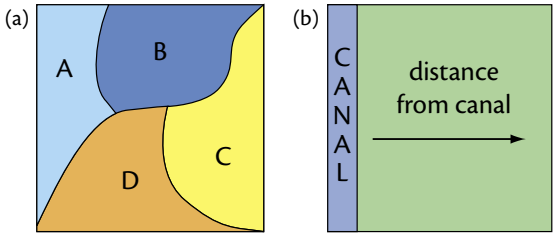

Figure 9.7 Two kinds of underlying structure that may exist within a landscape. Examples of **discrete** or categorical variation (a) would be areas planted with different crop types or with underlying soil types. **Continuous** variation (b) is observed along a gradient away from a key factor—in this case, the distance from refuge habitat along a major canal.

For sampling of continuous variation, you will first need to decide upon the key factor that encapsulates the variation. In the example introduced above, this would be the distance from the irrigation canal. The next step in this example would be to decide whether the gradient of variation is likely to be a linear function of distance, or some more complex function. An appropriate sampling density at any point along the gradient is then decided on the same grounds as those already mentioned for the sampling for discrete strata. Once again, sampling points or quadrats should be chosen randomly at each position along the gradient.

In a situation where it is not obvious whether rodent damage is spread randomly or unevenly within a field, we recommend that stratified random sampling is used. The results of this sampling will always be equal to or better than the precision that you can get from random sampling with no stratification.

A worked example of stratified random sampling of rodent damage in a rice field is given in Box 9.2.

Where the rodent damage in a field is obviously very patchy (e.g. Figure 9.3e), neither of the fully random nor stratified random sampling methods is likely to give a reliable estimate unless the sampling density is extremely high. In such a case, you may need to consider using a different approach based on the principle of adaptive sampling (for details, see Krebs 1999, Ecological Methodology, Chapter 8). However, all of these methods are more complicated to apply in the field and we do not recommend their use unless you think the stratified random sampling method is giving very inaccurate damage estimates.

Estimating damage to vegetable and upland crops

Methods used to assess damage in vegetables and other upland crops need to embody the same principles of randomisation and adequate sampling intensities as those used for cereal crops. However, two factors combine to make the process of quantifying rodent damage to these crops more straightforward. The first is that the damage generally affects the fruits, pods, cobs or tubers (see Figure 9.10), and rarely has any significant effect on vegetative growth. Because a damaged tuber or fruit is generally not considered edible or saleable, simple counts of the numbers of damaged versus undamaged fruits or tubers are generally adequate to quantify the extent of damage. However, this approach fails to take into account any potential compensation in the size of remaining undamaged fruit or tubers following damage.

Any method for scoring damage may need some adjustment for particular kinds of vegetable and upland crops. For example, damage to maize crops is usually counted as the number of cobs on fallen tillers and gnawed cobs on standing tillers. Damage

Box 9.2 A protocol for stratified random sampling of rodent damage in rice crops

We have used the method described here to quantify rodent damage to transplanted rice crops in Indonesia and Vietnam. In both of these studies, the level of rat damage was usually highest in the middle of the crop and lowest around the edges.

The method is an example of a stratified random sampling approach based on a continuous gradient—distance from the edge of the field. Our example is based on sampling of a rectangular field with dimensions 500 m × 300 m.

To begin, establish a baseline along the long axis of the field. Set four transects perpendicular to the baseline, running in from the edge of the crop (Figure 9.8), and spaced at 20 m intervals. If possible, try to keep transects 1 and 4 at least 50 m away from other roads, major channels or villages, as these may produce atypical levels of damage. To fully sample the variation within the field, we would define and sample the five strata that represent five equal-width zones from the edge to the centre of the field (Figure 9.8).

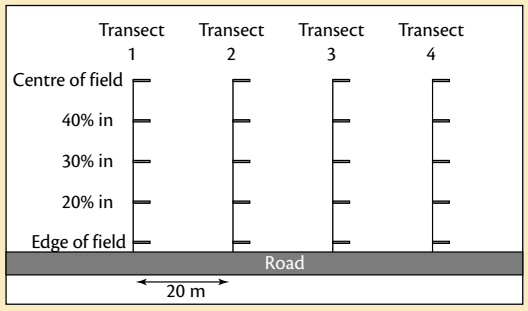

Figure 9.8 Layout of transects to measure damage by rodents in a rice field.

At each point, assess 10 plants along a line perpendicular to the transect. Score every fifth plant, as shown in Figure 9.9.

For each plant, count the numbers of:

- tillers with recent damage by rodents
- undamaged tillers bearing mature panicles
- undamaged tillers that either lack or bear immature panicles (perhaps indicating earlier damage by rodents).

Record the information on a standard damage assessment data sheet (an example is provided in Appendix 4).

Sampling of 10 plants at each sampling point provides an estimate of the proportion of tillers damaged within each stratum. The four transects are replicates, so for each of the five strata we have examined 40 plants for damage. The total number of counted plants is 200 for the entire field. Each plant examined will have one or more tillers. Given these data, the estimated proportion of rodent damage for the entire field is given by the equation:

$$\hat{p}_{ST} = \frac{\sum N_h \hat{p}_h}{N} \quad (1)$$

where:

$\hat{p}_{ST}$ = Stratified mean proportion damaged by rodents
N_h = Size of stratum h (in number of sample units)
$\hat{p}_h$ = Estimated proportion damaged for stratum h
N = Total field size (in number of sample units)

The standard error of this stratified mean proportion is:

$$SE(\hat{p}_{ST}) = \frac{1}{N}\sqrt{\sum\left[\frac{N_h^2 (N_h - n_h)\hat{p}_h \hat{q}_h}{(N_h - 1)(n_h - 1)}\right]} \quad (2)$$

where:

$SE(\hat{p}_{ST})$ = Standard error of the stratified mean proportion
$\hat{q}_h = 1 - \hat{p}_h$
n_h = Sample size in stratum h (= 4 in this case)

and all other terms are as defined above.

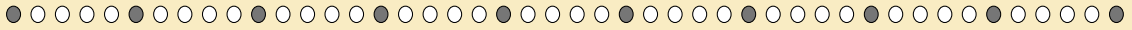

Figure 9.9 Measure damage on every fifth plant.

Box 9.2 (cont'd)

Confidence limits for the stratified mean proportion are obtained using the *t*-distribution:

$$\hat{p}_{ST} \pm t_{\alpha}(\text{standard error of } \hat{p}_{ST}) \qquad (3)$$

The additional information needed to calculate the stratified mean proportion of damage is the size of the five strata and the total size of the field, and the key point is that these 'sizes' must be in 'sample size units'. If the five strata occupy equal areas within the sampling area, the size of each stratum is 20% of the total sampling area, so only one parameter must be estimated. If you know the size of the sampling area in m^2, and you know the average area sampled to obtain 10 plants, you can get the total size of the field in sampling units by division. Note that these estimates can be approximations, and the resulting damage estimate is robust.

To estimate damage in a field, the area represented by each stratum must be calculated, taking into account the geometry of the field. Note that the outer strata (i.e. those close to the edge) will cover larger areas than those towards the centre of the field.

assessment for cassava and other root crops may need to use another measure based on counts of underground tubers, while damage to groundnut could be based either on counts of damaged pods or on counts of lost or damaged nuts.

Damage in vegetables and other upland crops is generally assessed shortly before harvest. The simplest method involves the use of *transect counts*, as illustrated in Box 9.3. Other useful methods include *quadrat sampling* and *variable area transects*. References to these alternative methods are included in Further reading.

Figure 9.10 Examples of rodent damage to maize (top) and ripening tomato (bottom).

Estimating preharvest yield loss

There are two established methods for estimating yield loss.

The first method is to convert damage estimates into yield losses. As already mentioned, this relationship is complicated by two factors:

- the possibility that damage has occurred throughout the growing period, with a cumulative effect on yield at harvest
- the phenomenon of growth compensation by plants following damage.

One way of learning about the relationship between damage and loss is by simulating rodent damage to crops. Experiments have been conducted in which rice plants were cut experimentally at different intensities and at different stages over the growth period of the crop. As expected, the results showed that damage inflicted at later growth stages caused a proportionally greater reduction in yield at harvest than damage at earlier stages. Compensatory growth was observed in all treatment plots and the yield was fully compensated if damage occurred early. Studies of this type can be conducted relatively easily, but sufficient people are required to implement the treatments.

Box 9.3 Transect method for damage assessment in vegetables and other upland crops

This method is easiest to use in well-organised fields with crops planted in straight rows. However, the same method can be adapted for less structured fields.

Divide the area of crop into four equal-sized areas and assign one transect to each area (Figure 9.11). Begin each transect away from the edge of the plot. Assess rodent damage on every second plant until you obtain estimates for 10 plants on each transect. For each plant, count the number of tillers, pods or cobs damaged by rodents and the number of undamaged tillers, pods or cobs. Record all the information on a standard damage assessment data sheet (see Appendix 4), taking care to record the crop type. The data can be analysed in the same manner as shown in equation 1 in Box 9.2.

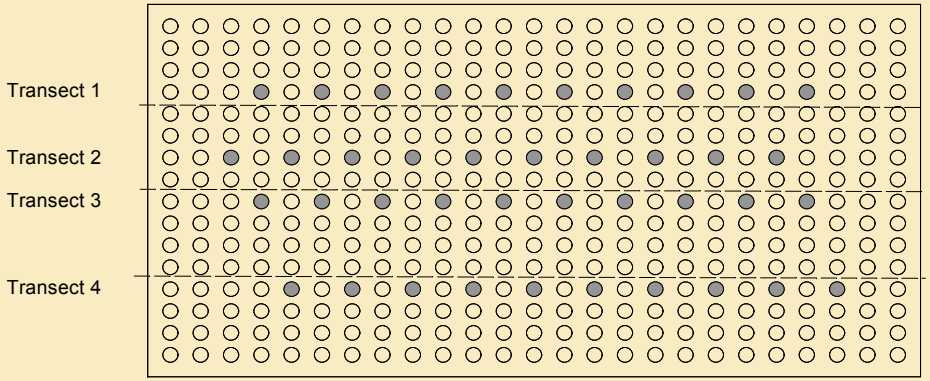

Figure 9.11 An example of the layout of the four transects to sample for damage in small vegetable plots.

The second method is constructing exclusion plots (see Box 9.1). This provides a more direct way of calculating yield loss but care is needed to ensure good experimental design, including sufficient replication.

Estimating postharvest damage and loss

Postharvest damage to stored vegetables or fruits is usually obvious from the signs of gnawing. In contrast, damage to stored cereal grain is not so easily observed and often must be inferred from general signs of rodent activity in and around the storage containers, such as the presence of faeces, hairs or urine smears. Contamination of stored grain reduces its value and the presence of rodent saliva or urine also poses a risk for the transmission of diseases.

Postharvest losses are rarely taken into account in the calculation of rodent impacts. This situation reflects two deeply held beliefs. The first is that total postharvest loss is often difficult to estimate with any degree of reliability. The second is that rodent damage to stored grain is difficult to distinguish from damage caused by other pests. Although there is some element of truth behind both of these beliefs, there are also experimental approaches that may help overcome the difficulties.

The reliability of estimates of postharvest loss to stored grain is really a function of the level of record keeping of ingoing and outgoing produce. In a situation where produce is stored for a period before sale, the calculation of total loss is generally straightforward, assuming that the quantity of harvested crop and the quantity sold are both reliably documented. Contamination of produce during its time in storage may also reduce its final sale value. Although the total financial loss may be easy to calculate in such cases, it may be difficult to attribute this loss to any one particular pest.

Much greater difficulty will be encountered in situations where stored grain or other crop produce is used either exclusively or primarily for household consumption. Under these circumstances, the crop produce is generally held for long periods and used in small amounts each day. Records of usage are rarely, if ever, kept, hence it is often very difficult to calculate exactly how much of the stored crop has been used by the household and how much has been consumed by rodents and other pests.

One method that is currently being trialled in several parts of the world is to monitor grain loss from a container placed within the larger storage area. This method is described in some detail in Box 9.4. Limitations of the method include the fact that it is only sensitive to losses from the open surface of the store, and would not record losses from penetration of the store container from below, and the possibility that feeding from the container either occurs at higher or lower intensity than that from the general surface of the store. We have tried to estimate the extent of any feeding bias by measuring the level of contamination by hairs and faeces of both the container and the surface of the general store area. The results obtained thus far from these studies appear promising.

The relationship between rodent abundance and rodent damage

Although it is probably true in general terms that more rodents will produce more damage, this relationship may not be a simple linear one. Many aspects of rodent ecology and behaviour are **density-dependent**, which is to say that they change in response to changes in population density. A simple example is a shift in diet from one preferred food item to a broader range of foodstuffs as population pressure starts to limit access to the various food resources. Another example might be a decrease in the breeding rate among adult females as population densities rise, perhaps due to competition for nesting sites or to increased social tensions. These complex ecological and behavioural interactions may lead to variable levels of crop damage at different population densities.

There are two reasons why it is important to understand this relationship. The first is that we might be able to predict the likelihood of serious crop damage based on some information on rodent abundance. For example, if we know that critical levels of crop damage are only likely to occur if population density exceeds a certain threshold level (e.g. >20 individuals per hectare), we may wish to monitor rodent abundance during the early part of a season and then use our knowledge of potential population growth rates to forecast the likelihood of serious damage. Typically, this information would be fed into a decision analysis that also included the cost of any rodent control actions and the potential losses associated with not taking those actions.

The second reason for wanting to know about the relationship between rodent abundance and damage is to set appropriate management goals (Figure 9.12). To illustrate this process, let us assume that our management goal is to keep rodent damage below a certain specified level, such as below 5% (measured in a standard way). Our first step would be to consult the relationship between rodent abundance and damage to estimate the corresponding population density. We would then ask what management actions would be required to keep the rodent population density at or below this level. If the cost of these actions was unacceptably high, we might then revise our original goal to find a point where the benefit to cost ratio is acceptable (e.g. keep damage below 10%).

Box 9.4 Estimating postharvest loss from a grain store

The method described here is suitable for monitoring loss from open storage units of the kind used widely across South and Southeast Asia. These units are generally made from woven bamboo, sometimes sealed with mud or animal dung. The storage units are often raised on stilts or placed on a low platform but they are sometimes placed directly on the ground.

A wide close-weave basket of known diameter and weight is partially filled with a standard quantity of unmilled paddy rice (e.g. 5 kg). This is placed on the surface of the rice within the storage container. The store owner is asked not to take rice from or add rice to the basket. The basket with its contained paddy is weighed at regular intervals to chart the rate of loss of grain (Figure 9.12). When the quantity of remaining paddy falls below a certain level (e.g. below 1 kg), the basket is refilled to its original weight. The moisture content of the rice in the basket and near the surface of the grain store is recorded each time, using a standard field gauge. These values allow the weight of the basket rice to be adjusted as necessary to match that of the general stored rice.

The rate of consumption of paddy from the basket is calculated as a loss rate per unit surface area (e.g. if 0.5 kg of rice is removed over an 8 week period from a basket with a surface area of 0.5 m^2, the loss rate is then 0.125 kg/m^2/week). This value can be multiplied by the surface area of the grain store to calculate an overall rate of loss from that store.

This method relies on several critical assumptions. The first is that the rate of consumption from the basket is equivalent to that from the surface of the wider grain storage. This may not be the case if the rodents either feed preferentially from the basket or else avoid the basket. A method to control for any bias in feeding location is discussed below. The second is that no grain is lost from damage to the base or sides of the grain store. This may be difficult to monitor where the store is placed directly on the ground. The third is that no loss occurs as a result of animals scuffing rice out of the basket. This is more difficult to control, other than to make the basket quite deep, at the risk of reducing access by some species of rodents.

Figure 9.12 Postharvest loss experiment in Bangladesh.

If feeding in the basket is a truly random sample of behaviour within the store, we could expect the same level of contamination in each, and the same level of damage to remaining grains. To measure contamination, we take a standard container (e.g. a cup) from each of the basket and the surface of the surrounding grain store and count faeces and hairs in both samples. We then take a subsample of 100 paddy grains and count the number of unfilled grains, the number showing rodent tooth marks and the number showing insect damage (typically visible as bore holes). If we do find a difference in the level of contamination or damage, we would then need to consider whether the level of contamination is proportional to the amount of feeding activity. A good way to start would be to ask whether the level of contamination and damage are correlated across a range of replicated samples.

The same approach might be used in a crisis management situation where high levels of rodent damage are occurring. In such contexts, we would consult the relationship between rodent abundance and damage to find out what proportion of the existing population would need to be culled to bring the population density back to acceptable levels of associated damage. This information would help us determine appropriate methods and to estimate the cost of the necessary actions.

To determine the relationship between rodent abundance and damage for any particular combination of rodent species and cropping system, you will need data from across over a wide range of rodent population densities and levels of damage. As explained in Chapter 5 and in this chapter, each of these measurements is fraught with complexity, related to factors such as growth compensation and availability of alternative food in the case of rodent damage, and trappability and the mobile and highly dynamic nature of the rodent population itself in the case of rodent abundance. To accommodate this variability, it will be necessary to obtain numerous data points, which in turn implies considerable field effort. However, as indicated above, the heuristic value of the relationship between rodent abundance and damage is sufficiently great that the effort will be richly rewarded.

In **Model 1** (Figure 9.13, left), damage is directly proportional to the abundance of rodents, up to a point where 100% of the crop is damaged. Below this threshold, a reduction of rodent abundance by a given percentage will result in a reduction of damage by the same percentage. Above the threshold, the reduction of damage will be less than the reduction of rodent abundance.

In **Model 2** (Figure 9.13, centre), the amount of extra damage decreases as rodent abundance increases (this might occur if it becomes progressively more difficult for rodents to find undamaged plants). Under this model, the proportional reduction in damage will always be lower than the reduction in rodent abundance, but particularly so in the upper part of the curve.

In **Model 3** (Figure 9.13, right), rodent damage increases more rapidly above a certain threshold in rodent abundance (this might occur if rodents switch to eating and damaging the crop only above a certain population density). In this situation, any decrease in rodent abundance will result in a proportionally higher decrease in damage, especially if rodent abundance moves from above to below the threshold value.

There are likely to be other types of abundance–damage relationships. By knowing the shape of the curve in any particular situation, it should be possible to develop targets for control. A critical value for developing targets is the threshold of damage that farmers are willing to accept.

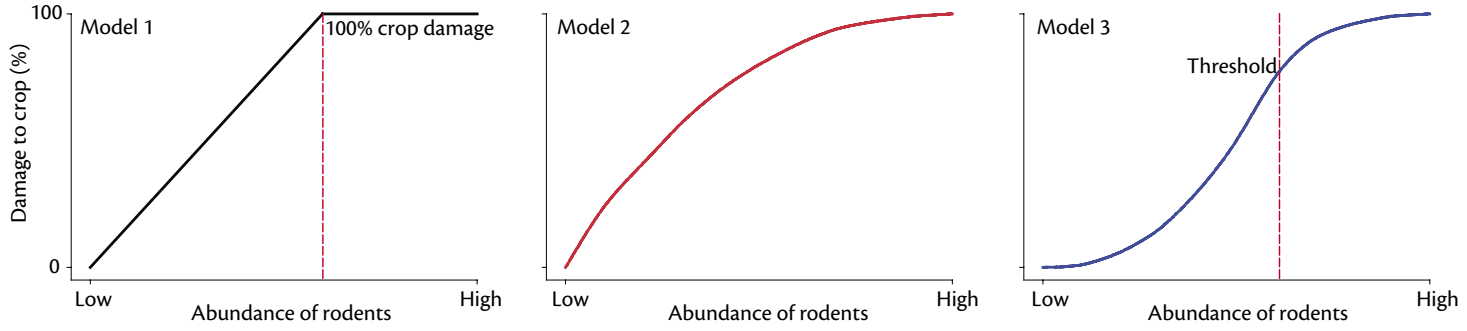

Figure 9.13 Conceptual models of the relationship between rodent abundance and rodent damage to crops.

Further reading

Benigno, E.A. 1980. Rat damage distribution in rice and corn fields. Philippine Agriculturalist, 63, 239–250.

Brown, P.R. and Singleton, G.R. 2001. Relationship between mouse abundance and damage to crops. Proceedings of the 12th Australasian Vertebrate Pest Conference, Melbourne. Melbourne, Department of Natural Resources and Environment, 273–277.

Brown, P.R., Singleton, G.R., Tann, C.R. and Mock, I. 2003. Increasing sowing depth to reduce mouse damage to winter crops. Crop Protection, 22, 653–660.

Buckle, A.P. 1994. Damage assessment and damage surveys. In: Buckle, A.P. and Smith, R.H., ed., Rodent pests and their control. Wallingford, Oxon, UK, CAB International, 219–248.

Buckle, A.P., Yong, Y.C. and Rowe, F.P. 1979. Yield response of the rice variety Improved Mashuri to simulated rat damage. Malaysian Agriculture Journal, 52, 135–144.

Engeman, R.M. and Sterner, R.T. 2002. A comparison of potential labor-saving sampling methods for assessing large mammal damage in corn. Crop Protection, 21, 101–105.

FAO (Food and Agriculture Organization of the United Nations) 1980. Assessment and collection of data on post-harvest foodgrain losses. Rome, FAO Statistics Division, 71 p.

Hone, J. 1994. Analysis of vertebrate pest control. Cambridge, Cambridge University Press.

Islam, Z., Morton, R.G. and Jupp, B.P. 1993. The effects of rat damage on deepwater rice yields in Bangladesh. International Journal of Pest Management, 39, 250–254.

Krebs, C.J. 1999. Ecological methodology. Menlo Park, California, Benjamin Cummins, 620 p.

Mulungu, L.S., Makundi, R.H. and Leirs, H. 2003. Robustness of techniques for estimating rat damage and yield loss in maize fields. In: Singleton, G.R., Hinds, L., Krebs, C.J. and Spratt, D.M., ed., Rats, mice and people: rodent biology and management. ACIAR Monograph No. 96. Canberra, Australian Centre for International Agricultural Research, 224–228.

Poché, R.M., Haque, M.E., Main, M.Y., Sultana, P. and Karim, M.A. 1981. Rice yield reduction by simulated rat damage in Bangladesh. Tropical Pest Management, 27, 242–246.

Rennison, B.D. 1979. Monitoring rat damage in West Malaysia: a consultant's report and recommendations for the Malaysia/UK Rice Rat Control Project. Rodent Pests Department, Ministry of Agriculture, Fisheries and Food, UK, 29 p.

Shepard, B.M. and Ferrer, E.R. 1990. Sampling insects and disease in rice. In: Crop loss assessment in rice. International workshop on crop loss assessment to improve pest management in rice and rice-based cropping systems in South and Southeast Asia, 11–17 October 1987. Manila, Philippines, International Rice Research Institute, 107–130.

Walker, P.T. 1990. Determining pest–loss relationships and quantifying loss. In: {eds?}Crop loss assessment in rice. Manila, Philippines, International Rice Research Institute, 151–160.

CHAPTER 10

The 'human factor' in rodent management studies

Introduction

In Chapter 1, we suggested that the viability of any rodent management option should be judged against each of three criteria:

- ecological sustainability
- cultural acceptability
- socioeconomic sustainability.

So far in this volume, we have focused on methods that will allow you to gather information relevant to the first and last (in part) of these criteria. In this chapter, we introduce some methods that should allow you to explore the cultural and socioeconomic context of rodent management. Our treatment of these methods is much less comprehensive than for the biological and agricultural methods. This is partly because we do not have specific expertise in these fields. However, it is also because the consideration of cultural and socioeconomic factors is a relatively new development in the area of agricultural research in general, and even more so in the field of rodent management. We hope that publications listed under Further reading will provide interested readers with a pathway into relevant literature.

A conceptual framework

We can learn much about cultural and social issues by listening to what people say and by observing what they do. In the context of rodent management projects, where the research phase is often of quite short duration, the challenge is to organise and interpret these observations in ways that help us to understand why people would choose to take certain actions and not others.

A useful conceptual framework is available from previous studies of farmers' beliefs and associated decision-making behaviour in relation to insect pest management. These studies in turn draw upon a much larger body of theoretical literature related to **decision-making** as a process or system.

Two simple flow models help to illustrate how decision-making theory can help make sense of human behaviour. The first is an example of what is termed a 'belief model'. It illustrates the notion that people's behaviour is influenced by their perceptions of risks and benefits associated with particular pests and management actions. Each of the four major components of this model (Figure 10.1) can be quantified to some extent, either by calculating the monetary value of potential benefits or losses, or by ranking the importance of various influences on a subjective scale (i.e. as more or less important).

The second model illustrates a broader 'theory of reasoned action' (Figure 10.2). This model emphasises the social context of human behaviour by indicating that a person's behaviour is often a compromise between what they would like to do, based on their personal preferences, and what they feel they 'should' do, based on the beliefs, attitudes and values of other family members, neighbours and the wider society. This compromise is mediated by the strength of each individual's motivation to comply with the societal pressures or 'norms'.

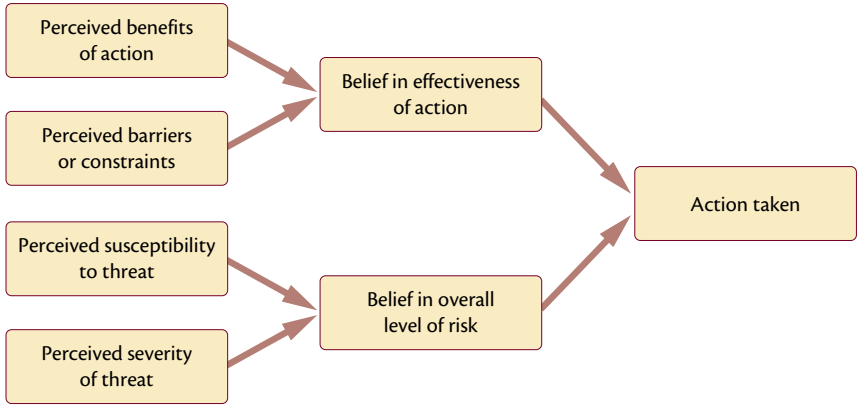

Figure 10.1 Belief model designed for studying behaviour in regard to pest management (modified from Heong and Escalada 1999, Fig. 1).

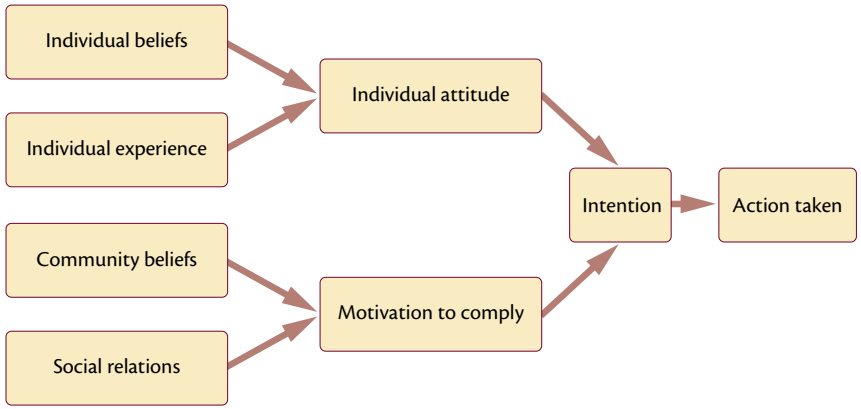

Figure 10.2 Behavioural model based on the theory of reasoned action (modified from Heong and Escalada 1999, Fig. 2).

Some basic tools and methods

Many of the tools and methods that we recommend for exploring the socioeconomic and cultural issues associated with rodent management have a long history of use in the field of participatory research (see Box 10.1). A good general introduction to participatory methods is found in another recent ACIAR monograph (see Further reading: Horne and Stur 2003).

An important aspect of participatory methods is that they allow community members to contribute both to the recognition of problems and to the development of solutions. This creates a sense of ownership and understanding that builds their confidence and capacity for learning. The participatory methods also may help build a close relationship among the team members, such that improved communication can take place in an atmosphere of mutual trust and respect.

Community resource maps

These are a good way to begin in a new project area. You will need large pieces of paper and pens or crayons. Invite a small, representative group of local community members (a 'focus group') to draw a map of the important physical features and resources used by their community. This would normally include infrastructure, such as buildings, roads and canals, and the location of major cropping areas. It should

also include features that are particularly important to rodent ecology, such as food storage areas and any areas that people regard as significant breeding habitat. A number of different people should be asked to contribute to the map in order to achieve a balanced representation of local resources (Figure 10.3). The process of compiling the map itself may help you understand how local resources are structured and accessed by different groups within the community and how different people perceive the nature of their rodent problems. The completed map also can be used as a reference point for subsequent activities such as construction of the seasonal calendar and in problem diagnosis.

Box 10.1 Participatory approaches to research

Participatory approaches to agricultural research and development (R&D) arose in the 1980s as it became clear that the adoption of various new technologies by farming communities—especially in traditional smallholder farming systems—was not always as rapid or as high as expected by those who developed them. Researchers started to question whether the traditional R&D approach, where scientists develop new crops or associated methods on research farms and then 'release' them into the wider world, was really the most effective way to help the rural poor. Might it not be better to first consult with farmers about their problems and priorities, and perhaps even to explore the appropriateness of possible solutions before investing time and effort into their development?

From these early steps, a whole new area of research methodology has developed within which we can distinguish various contrasting approaches, such as farmer participatory research (FPR), action research (AR), adaptive management (AM), and even active adaptive management (AAM). These methods share a common emphasis on interaction between the developers and the potential users of proposed new technology or practices, but they differ in two main respects. The first is the nature and extent of the interaction among the various stakeholders (researchers, extension staff, users), which ranges from a process of consultation through to a true partnership or collaboration. The second is the nature of the research process itself, which follows fairly traditional lines (i.e. hypothesis formulation and testing) under FPR, but leans towards the immediate implementation and progressive readjustment of management actions under AR, AM and AAM. However, it is generally agreed by those working in the area that there is no one 'right' way to do participatory research and that the choice of method should depend on both the goals and objectives of the project and the particular socio-cultural context.

Figure 10.3 Participants conferring on their community resource (village) map during a focus-group session in Cambodia.

Seasonal calendars

A seasonal calendar is a simple graphical representation of the important environmental, agricultural and social events that take place during the course of a typical year. The same group of community members that produces the village resource map often produces the seasonal calendar. The same basic materials are required.

A good way to start is to ask when the new 'year' is thought to begin—this may be the planting time of a particular crop, or it may some astrological event such as Lunar New Year. Using this as a starting point, draw a matrix with months along the top. Then invite the focus group to identify the major crop types and write these down the margin. For each of the crop types, the growing phases and

associated activities should be recorded month-by-month across the page. For example, in a lowland rice crop production area, the major growth phases are tillering, panicle initiation, ripening etc., and the key activities would typically include seed-bed construction, land preparation, transplanting, weeding, harvest, and threshing. At this stage, it may be useful to ask participants to indicate the timing and severity of rodent and other pest damage in relation to each crop. Because these problems are often more severe in some places than others, it may be useful to relate these observations back to the community resource map (Figure 10.4). At the same time, you might ask general questions about the methods that people are currently using to manage rodents in the various habitats.

Figure 10.4 Cambodian focus-group participants relating their seasonal calendar back to their community resource map.

Major environmental events such as the start of the wet season and likely periods of flooding or water shortage should also be recorded on the calendar. Finally, the calendar should record other key activities that might require significant investment of labour or cash (e.g. fishing, craft activities) or periods of involvement in social activities such a festivals or community work.

Historical calendars

A historical calendar attempts to document some of the major events or changes that have affected a community's livelihood in the recent past. A first draft is often produced in the context of a focus-group meeting, but the calendar can be revisited many times on the basis of new information from as many different individuals as possible. Individual interviews should be sought with the oldest men and women in the community.

A good way to begin is to ask about the visible infrastructure. When did the community come to occupy its current location? When was the school built? By referring to the community resource map, you could ask when certain resources were developed (e.g. when a canal was built, when a particular cropping area was established). A next step could be to ask about major environmental events such as major floods, serious droughts, or particularly extensive forest fires. In many upland areas of Southeast Asia, people will often identify major rodent outbreaks as a kind of historical disaster, but at least in the first instance, you should not prompt such observations but rather allow them to emerge. It is natural for people to emphasise what the particular researcher wishes to hear, hence it is important to avoid leading questions.

In many areas, major political events and associated displacement or movement of people may also have played a major role in shaping the present cultural landscape. You may need to explore these factors in a sensitive manner and perhaps through individual interviews rather than in a group context.

Once the general history of the community is established, you might then inquire about some of the more subtle changes that may have affected people's livelihoods. How has their access to markets changed over the years? Have they been placing more emphasis on certain crops at the expense of others? Have they changed their residential pattern or style of housing or storage of foods? What kinds of rodent control activities did people practise in the past as compared to now? For each of the important changes or trends, you should try to establish a general time frame for the events.

Decision analysis matrices

A decision analysis matrix is simple tool for obtaining an overview of the factors that influence decisions by farmers on their current actions of rodent

management. This activity is best done at a focus-group meeting. Ask the farmers to list the type of management actions they use to control rodents. This list should include occasional actions, including those that are only used in years when rat numbers are very high.

Once a basic action list is developed, ask the farmers—for each action—when it is taken, where it is taken (including scale of action), by whom is it done (individual male and/or female farmers; groups; the whole community), whether it is affordable (in terms of economic benefits versus cost), whether it is feasible (e.g. labour available at the right time; water available for early planted crops to attract rats), whether it is socially and politically acceptable (likely response of neighbours, the wider local community and the government), and whether it has any environmental impact (beneficial or adverse). An example is shown in Table 10.1. Enter the information into a large-format table that everyone can read. Encourage people to comment on the information at any stage during the process.

Once the table is complete, ask the focus-group participants to prioritise the current management actions. Which ones do they consider the most important overall for rodent management, and which ones are less important? The ensuing discussion about priorities will often provide important insights into why certain decisions are made by individuals or by the community as a whole.

Table 10.1 Decision analysis for San Jacinto/San Jose, Pangasinan Province, the Philippines, of current actions plus proposed use of a community trap–barrier system (CTBS). Note that the scale of most actions is currently at the individual farmer level.

Actions (what)	Timing (when)?	Who?	Where?	Feasible?	Affordable?	Socially OK?	Politically OK?	Environmentally OK?	Priority
1. Maintain cleanliness (banks, villages etc.)	Year-round	Farmer	Whole village	Yes	Yes	Yes	Yes	Yes	High
2. Rat hunt (dig/flood burrows)	Oct/Nov	Farmer	Major banks	Yes	Yes	Yes	Yes	Yes	Medium
3. Rat drive	Oct/Nov & Mar/Apr	Community	Major banks, long grass	Yes	Yes	Yes	Yes	Yes	Medium
4. Small dikes	Land preparation	Farmer	Small banks	Yes	Yes	Yes	Yes	Yes	High
5. Zinc phosphide	Before harvest	Farmer	Rice fields	?	If >5% loss	?	Yes	No	Medium
6. Racumin	Before harvest	Farmer	Rice fields	?	If >5% loss	?	Yes	No	Low
7. Biological control	Year-round	Farmer	Rice fields	Yes	Yes	Yes	Yes	Yes	High
8. Rat traps	Year-round	Farmer	Rice fields	Yes	Yes	Yes	Yes	Yes	Medium
9. Fumigation	Dry season after harvest	Farmer	Banks	Yes	Yes	No	Yes	Yes	Low
10. Crop timing	Planting	Community	Whole village	Yes	Yes	Yes?[a]	Yes	Yes	High
11. CTBS	2–3 weeks before main crops	Community	Whole village	Yes[b]	Yes	Yes	Yes	Yes	High

[a] Price of rice could drop if everyone harvests at the same time.

[b] Depends on the availability of early irrigation water.

Social mapping and wealth analysis

In many societies, there are obvious differences in livelihood status between individuals or between families. If your goal is to improve the livelihood security of all, or most, members of a community, then it is important that you try to understand the basis of these differences. Knowledge about the social and economic structure of a community should help you to develop new approaches that are appropriate to the resources of the wider community and which do not further disadvantage those who are already worst off.

The pattern of social organisation of rural communities is often highly complex, with a number of parallel systems based on ethnicity, systems of familial descent through either male or female lines, and systems of official accreditation based on government appointments (e.g. village headship, teachers). In addition, communities that have received people from other regions as a result of dislocation sometimes have an added historical element (poorer families have often arrived most recently). Many, though not necessarily all, of these factors may influence a family's degree of access to particular resources such as land, water or labour.

Wealth is generated and controlled within a traditional social system, but wealth can also alter the traditional balance. Increasing access to market economies can sometimes allow people to gain access to external funds (e.g. through the sale of craft materials) which can then be used to gain access to new resources such as hired labour and improved quality seed. In many societies, the systems of social and economic influence are going through a process of rapid change.

Social mapping and wealth analysis are two tools that can help you to understand the complex socioeconomic relationships within and between communities. The challenge in a new project is to quickly identify the most critical opportunities and constraints, but to do so without impinging on sensitive issues.

A good way to begin with social mapping is to ask specific questions about the community resource map. If a large canal passes through the cropping areas, it would be worth asking an open-ended question about usage of the irrigation water. For example—Who uses water from the canal to irrigate their crops? If the answer is that only some people do, then you can follow up with questions that are more probing—What percentage of farmers use the water? What is the relationship among the farmers in that group? A series of general questions about access to key resources should help you to build a general impression of how the community is structured. If possible, it is a good idea to test your ideas through a series of individual interviews, ideally with people representing the full socioeconomic spectrum.

Wealth analysis is a tool that can help you to understand the economic circumstances and capacity of various 'wealth groups' within a community. What is it, in terms of possessions or access to resources, that distinguishes the poorest members of the community from those who are moderately well off and those who are considered to be best off? A wealth analysis can also begin with small group discussions. You could start by asking the participants to each write down the economic attributes of the poorest and the richest families within the community. If possible, this should be done without reference to individual families. You can then assemble these results onto a larger sheet as a series of hypothetical gradients (e.g. possesses no livestock versus owns herd of water buffalo). Each of the key parameters can then be discussed in turn to identify what pathways might exist for someone who would wish to improve their livelihood status. Hypothetical discussions of this kind can reveal much about the socioeconomic dynamics of the community.

Problem-cause diagrams

A problem-cause diagram is a graphical representation of the causes and effects of a particular problem, as perceived by the members of the community. The diagram is typically developed by a focus group, with assistance from a facilitator.

You will need a large board, some cards and marker pens or crayons. The process starts with identification of a specific problem. Try to avoid making this too general (e.g. 'Rats'). In our experience, a more specific problem makes a better starting point. For example, 'Rats attack our dry season crop' or 'Rats eat our stored grain'. Write the problem on a card and stick it to the middle of the board. Then ask the focus group to identify the causes of this problem. Write each cause on a card and pin it above the problem. Oftentimes, focus-group members will be aware that the various causes are interrelated; these linkages should be discussed and indicated by connecting arrows (Figure 10.5a). When this discussion starts to become repetitive, ask the focus group to think about the effects or impacts of the problem. Write these on cards in the same manner and attach them below the problem (Figure 10.5b), again indicating any cross-links that the focus group are able to identify. Remember that this should be a representation of local perceptions of the problem, so be careful to avoid leading questions or adding your own causes, effects or linkages.

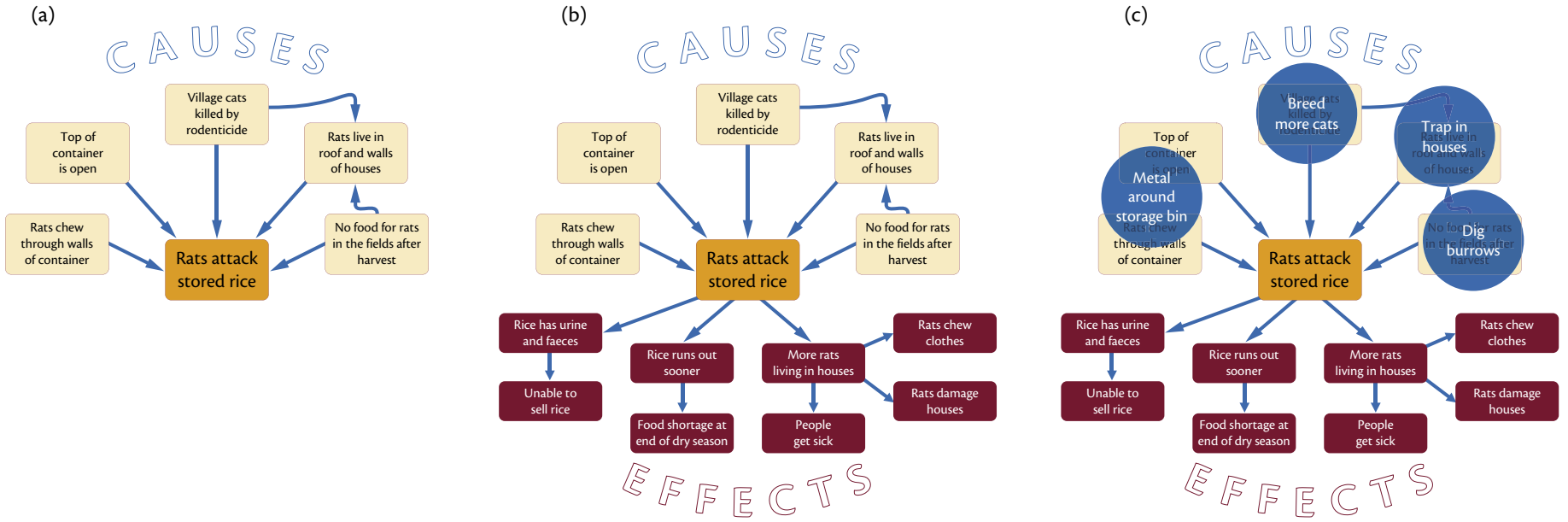

Figure 10.5 An example of a problem-cause diagram created by a focus-group around the problem 'Rats attack stored rice'. As a first step, the focus group have identified five possible causes of the problem (a), some of which are thought to be interlinked. Next, a range of impacts are identified (b), again with some perceived links. In (c), current actions are added to the diagram, the placement indicating the rationale behind each action.

Next, ask the focus group to indicate what they are currently doing to combat the specific problem. Write the current actions on cards and place these over the top of relevant causes (Figure 10.5c). As a final step, you could ask the focus group to think about any other possible actions that might have been tried but abandoned, or discussed but not tried. You could also ask the focus group to speculate as to why these other actions may not be appropriate. The completed problem-cause diagram can be shown to other groups and individual people within the community to gauge the level of representativeness of the focus-group perceptions.

Problem-cause diagrams are a useful method for exploring local knowledge and perceptions of how the agricultural and natural system works, and of finding out about current practices. They can also form a good starting point for subsequent discussions about the project. For example, you could use the diagrams to explain why a particular piece of ecological research is needed, or why certain experimental trials are being conducted. In the later stages of a project, the diagrams might also form a starting point for discussions about the possible benefits and pitfalls of potential new approaches to rodent management.

Individual, structured interviews and KAP questionnaires

The methods discussed above all begin with focus-group discussions as a way of gathering general information and forming a broad impression of the socioeconomic dynamics of a community. Although these activities can be run in ways that reduce the potential influence of one or two dominant individuals, they nonetheless rely on limited and possibly non-representative sampling of opinions within a community. One common means of increasing the sampling of community opinions is to use individual, structured interviews, based on a number of carefully framed but pre-set questions contained within a questionnaire.

One particular kind of questionnaire that has been used with some success in the field of rodent pest management explores the 'knowledge, attitudes and practices' or 'KAP' of a target community (see case studies under Further reading). The first group of questions in a typical KAP survey is designed to establish the basic socioeconomic profile of the respondent (sex, age, some basic wealth parameters). This is followed by questions that explore the respondent's knowledge of the scale and possible causes of rodent problems. Subsequent sections document the kinds of actions that are currently taken to combat these problems and the financial and other costs (actual and perceived) of these actions.

Finally, attitudes towards rodent problems and control measures are explored through questions that range from individual attitudes through to societal norms. Although all KAP surveys tend to follow a similar format, cropping systems are too diverse and cultural sensitivities too variable across the Asia–Pacific region to employ a standard questionnaire in all areas.

The information from KAP surveys can be used to assess various parameters—such as the severity of existing rodent problems, the perceived efficacy of current management actions, and the society's preparedness to try new kinds of actions. Because of the quantitative nature of the information, data from KAP surveys also can be used to compare the impact of rodent management actions on individual and societal attitudes—either by doing a 'before and after' comparison within treatment communities (where new rodent management practices are implemented) or comparing treatment communities with control communities (no change in practices).

KAP surveys usually aim to sample 100 or more respondents, with unbiased representation of males and females, and a good cross-section of 'wealth groups'. These ideals may not be possible in all societies. Wide consultation is needed before the design and implementation of a KAP survey and it is always advisable to do a 'pre-test' of a new questionnaire to make sure that the questions are appropriate in both subject matter and wording.

Pre-tests are also useful in determining how long the survey will take to conduct. Wherever possible, the survey questions and possible responses should be translated into the respondents' first language to reduce any potential for misinterpretation.

Some useful lessons already learned

Several recent rodent management studies in Southeast Asia have included an economic assessment of the various inputs (costs or investments) and outputs (benefits or outcomes), as well as a sociological assessment of the implementation of various methods by community members. These studies have produced some useful insights that you might wish to keep in mind throughout the development of a new project.

Key socioeconomic factors that affect adoption of new methods

Some of the key socioeconomic factors that are likely to influence the economic viability and sustainability of a particular rodent management strategy are listed in Table 10.1. This list is not exhaustive, but it might be a good starting point for consideration.

Examples of short-term and longer-term costs, benefits and constraints are given in Table 10.2. Short-term costs and benefits are relatively easy to quantify. Moreover, by assigning a monetary value to produce and labour, these factors usually can be expressed in terms of a common 'currency'. Similarly, the short-term constraints are usually easy to identify through many of the methods discussed above (e.g. seasonal calendars, wealth analysis).

The long-term costs and benefits of any action are far more difficult to assess. In part, this is due to the difficulty of predicting the long-term or large-scale impacts of rodent management. For example, it may be reasonable to suggest that a reduction in rodents within the fields and village environment would lead to a reduction in rodent-borne diseases such as leptospirosis, and to an increase in the health and fertility of livestock. However, it may be difficult to be assign a monetary value to improvements in human health and even more so for improvements in wider environmental health.

Another very important issue in the assessment of costs and benefits is the concept of **risk**. This in turn relates to the twin concepts of variability and

Table 10.2 A typical list of short and longer-term costs, constraints and benefits of rodent control. Not all of these factors are equally important in every situation and there may be other significant factors apart from those listed here.

	Short-term	Longer-term
Potential costs	• Financial cost of materials • Labour required for actions • Time invested in any associated social activity ('transaction' costs)	• Environmental costs (e.g. impact on non-target species)
Potential constraints	• Other demands on money • Other demands on time • Inability to coordinate actions	• Changing economic or political context • Inability to maintain necessary social structures
Potential benefits	• Increase in agricultural production • Improvement in the quality and value of harvested produce • Reduction in the postharvest loss of stored foods • Reduction in the level of contamination of stored foods • Value that can be assigned to captured rats	• Long-term benefits to human or livestock health (e.g. reduction in the impact of rodent-borne diseases) • Long-term benefits to environmental health (e.g. reduction in chemical use)

predictability. **Variability** is a normal element of all ecological systems, although both the scale or size and the degree of regularity of the changes differ greatly between systems. **Predictability**, the degree to which such variations can be forecast, can relate to either the scale or the regularity of the changes. For example, ecological changes associated with monsoonal flooding are probably quite predictable in terms of timing, but highly irregular in terms of severity. On the other hand, ecological changes that relate to wildfire activity might be predictable in terms of scale but much less predictable in terms of timing.

The economic importance of these concepts can be appreciated from an Australian rodent management example (see Box 10.2). In this case study, farmers can choose from a range of strategies that vary in their degree of associated risk.

The importance of community action and common property resources

In most situations, rodent management will be most effective if appropriate actions are taken over large areas and in a coordinated manner. Where this is not done, there is a real danger that any local impact on rodent numbers will be rapidly and literally overrun by dispersal of excess animals from any adjacent area where numbers remain high.

The application of rodent management over large areas is relatively straightforward in broadacre crop production systems where one farm owner or manager is not only responsible for deciding how and when to act but also has control over all the necessary equipment and budgets. However, across most of Southeast Asia and the Pacific, the situation is very different—the land is typically owned and managed by numerous smallholders and there may be various social and historical factors that make it difficult for people to work collectively towards effective rodent management.

Box 10.2 The economics of house mouse management in Australia

The introduced house mouse (*Mus musculus*) undergoes periodic outbreaks or 'plagues' in some wheat-growing regions of south-eastern Australia, with an average interval of seven years between these events. During plague years, mice cause huge crop losses and other damage to property. However, at other times, mouse numbers are low and they do little damage. Both the severity of plagues and the interval between them vary at any one location.

In normal years, the cost of mouse control outweighs any potential benefit in terms of damage reduction. During plague years, the reverse is always true, but the best benefit-to-cost ratios are achieved when mouse control is started early, before mouse numbers get too high. Farmers now have access to predictions based on probabilistic models, but there is a significant element of risk associated with following these predictions—the models give wrong results (either false alarms or failure to predict a plague) around 30% of the time.

Farmers who live in areas affected by mouse plagues have three main options:

- they can apply mouse control every year—this may well avoid all future plagues, but in six years out of seven they will be wasting their money
- they can apply mouse control only during the predicted plague years, but in the knowledge that these will be in error approximately 30% of the time
- they can reject mouse control altogether and hope that the losses during plague years are offset by the money saved by not applying any control.

The economic consequences of each risk management strategy can be calculated using an individual farmer's potential costs and losses.

The situation may be further complicated where the rodent management system either involves the use of a shared materials or equipment, or else depends on farmers making a contribution of money or labour to activities that will lead to shared benefits. Sociologists use the term **common property resource** in such cases where users share the 'rights' and 'benefits' of resource use, and also share the 'duties' of resource management.

One such system that has been tested in various socio-cultural contexts in Southeast Asia is the community trap–barrier system (CTBS; see Box 10.3). This system was designed and tested in lowland irrigated rice-growing systems in several Southeast Asian countries. Typically, in these regions, farmers own or manage landholdings of 1 ha or less. However, a CTBS unit set up within the boundaries of one farmer's field can be effective in reducing rat numbers and crop damage over a total surrounding area of around 10–15 ha. Hence, many families potentially share the benefits of a CTBS and might be reasonably expected to share in the material and labour costs of installing and maintaining the CTBS.

One study of the CTBS as a common property resource in the Mekong Delta region of Vietnam identified a range of sociological constraints and opportunities for sustainable application of the CTBS. Foremost among these were the social relationships and associated systems of obligation among CTBS participants, and the nature of existing institutions that emphasise cooperation, such as integrated pest management (IPM) clubs.

The role of these social and institutional factors need to be considered in each new socio-cultural context and with a keen awareness of the wider political and economic environment, including the likelihood of change.

Box 10.3 The community trap–barrier system (CTBS)

The CTBS is a physical method of rodent control that was developed to control rat damage in lowland irrigated rice systems in Indonesia, Malaysia and Vietnam. The major pest species in these systems is the rice-field rat (*Rattus argentiventer*) which times its breeding activity in these systems to match the growth and maturation stages of the rice crop. The efficiency of the CTBS system is currently being evaluated in Indonesia, Vietnam, Philippines and Cambodia, with good results to date.

The CTBS consists of a square or rectangular barrier system (typically with each side measuring 50–100 m) that encloses a lure crop (typically, a rice crop planted 2–3 weeks ahead of the surrounding cropping area). Rats are attracted to the early maturing lure crop and are captured in multiple-capture traps placed at entry points along the barrier. By drawing adult rats out of the local population before they start breeding, the rate of population increase of the remaining population is lowered, thereby avoiding the high rat densities typical of unmanaged fields. Empirical studies of crop damage around CTBS units suggest that each unit may be effective in protecting a surrounding area of 10–15 ha. In a large, uniform cropping area, CTBS units ideally would be positioned in a way that achieves overlap between the individual 'halos of protection' of each unit.

CTBS units require regular maintenance to ensure that the fence and traps are not compromised. Ideally, the cost of materials and the tasks of constructing and managing each CTBS unit are shared by all of the people who derive benefit from the unit. In some parts of Southeast Asia, the commercial value of captured rats provides added incentive for daily checking and regular maintenance of the CTBS.

Further reading

Heong, K.L. and Escalada, M.M. 1999. Quantifying rice farmers' pest management decisions: beliefs and subjective norms in stem borer control. Crop Protection, 18, 315–322.

Horne, P. and Stur, W. 2003. Developing agricultural solutions with smallholder farmers: how to get started with participatory approaches. ACIAR Monograph No. 99. Canberra, Australian Centre for International Agricultural Research, 119 p.

Kenney, A.J., Krebs, C.J., Davis, S., Pech, R.P., Mutze, G.J. and Singleton. G.R. 2003. Predicting house mouse outbreaks in the wheat-growing areas of south-eastern Australia. In: Singleton, G.R., Hinds, L.A., Krebs, C.J. and Spratt, D.M., ed., Rats, mice and people: rodent biology and management. ACIAR Monograph No. 96. Canberra, Australian Centre for International Agricultural Research, 325–328.

Kleindorfer, P.R., Kunreuther, H.C. and Shoemaker, P.J.H. 1993. Decision sciences: an integrative perspective. Cambridge, Cambridge University Press, 470 p.

Martin, A. and Sherington, J. 1997. Participatory research methods—implementation, effectiveness and institutional context. Agricultural Systems, 55, 195–216.

Morin, S.R., Palis, F.G., Chien, H.V., Chi, T.N., Magsumbol, M. and Papag, A. 2003. A sociological perspective on the community-based trap–barrier system. In: Singleton, G.R., Hinds, L.A., Krebs, C.J. and Spratt, D.M., ed., Rats, mice and people: rodent biology and management. ACIAR Monograph No. 96. Canberra, Australian Centre for International Agricultural Research, 380–388.

Norton, G.A. 1976. Analysis of decision making in crop protection. Agro-ecosystems, 3, 27–44.

Norton, G.A. and Pech, R.P. 1988. Vertebrate pest management in Australia: a decision analysis/systems analysis approach. Melbourne, CSIRO Publications, 67 p.

Röling, N.G. and Wagemakers, M.A. 1998. Facilitating sustainable agriculture: participatory learning and adaptive management in times of environmental uncertainty. Cambridge, Cambridge University Press, 318 p.

Singleton, G.R., Sudarmaji, Tuat, N.V. and Douang Boupha, B. 2001. Non-chemical control of rodents in lowland irrigated rice crops. ACIAR Research Notes No. 26. Canberra, Australian Centre for International Agricultural Research.

Sudarmaji, Singleton, G.R., Herawati, N.A., Djatiharti, A. and Rahmini 2003. Farmers' perceptions and practices in rat management in West Java, Indonesia. In: Singleton, G.R., Hinds, L.A., Krebs, C.J. and Spratt, D.M., ed., Rats, mice and people: rodent biology and management. ACIAR Monograph No. 96. Canberra, Australian Centre for International Agricultural Research, 389–394.

Tuan, N.P., Williams, S.J., Brown, P.R., Singleton, P.R., Tan, T.Q., Hue, D. T., Ha, P.T.T and Hoa, P.T. 2003. Farmers' perceptions and practices in rat management in Vinh Phuc province, northern Vietnam. In: Singleton, G.R., Hinds, L.A., Krebs, C.J. and Spratt, D.M., ed., Rats, mice and people: rodent biology and management. ACIAR Monograph No. 96. Canberra, Australian Centre for International Agricultural Research, 399–402.

Walters, C. 1986. Adaptive management of renewable resources. New York, MacMillan, 374 p.

Walters, C.J. and Holling, C.S. 1990. Large-scale management experiments and learning by doing. Ecology, 71, 2060–2068.