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Collection and rearing of earthworms

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Abstract
Earthworm experimentation requires a source of supply. This may come from field-collection, purchase from a supplier, or from breeding of stock. The mode of obtaining the animals may be determined by the type of experimentation, but must not compromise the experiment. Typical collection methods employ digging and hand-sorting of soil, addition of a vermifuge, application of an electrical current to the soil or combinations thereof. Each collection method has advantages and may target particular groups of earthworms more successfully than others. Rearing earthworms in the laboratory may be viewed as difficult but if control of factors such as soil type, moisture, temperature, food supply and stocking density are in place, it can be straightforward. Culture design will be determined by the experimental objectives.

Keywords: Earthworm, sampling, cultivation

Earthworm collection:

It is often desirable to quantify earthworm number or biomass in a given habitat, or seek to collect them. A few species show their presence by surface casting (e. g. *Aporrectodea longa*) or creation of middens (e. g. *Lumbricus terrestris*) but most require some form of intervention due to their totally subterranean existence. To this end, various techniques have been developed to enable earthworm collection. Digging is the simplest, as it requires only a spade and quadrat for density calculations, but may detect only epigeic earthworms and horizontal burrowing (endogeic) species. Adults of deeper burrowing (anecic) species may be missed unless a hole is dug to several metres.

An alternative is the application of a vermifuge, which when poured on to the soil drives earthworms to the surface acting as a skin irritant when contacted in their burrows (direct application e. g. via a syringe to *L. terrestris* burrows may be very effective). Various chemicals have been used, with a dilute solution of formaldehyde (formalin) recognized as a standard (ISO, 2002), but as this has been reported as carcinogenic further options have been sought. EICHINGER et al, (2007) also suggested that there are severe negative effects to other soil fauna, soil respiration and vegetation cover if formaldehyde is applied.

GUNN (1992) used a suspension of table mustard in water, but tests (e.g. BUTT, 2000) have shown that mustard powder (50 g in 10 litres water) is both cheaper and more effective. GUNN (1992) made no comparison with efficiency of formalin extraction. More recently use of “hot” mustard has been compared directly with hand sorting alone (LAWRENCE and BOWERS, 2002) with the former giving a more consistent index of earthworm abundance across a range of soil types. As the type of mustard used may also affect results, ZABORSKI (2003) utilized an extract derived from mustard seed (AITC) for earthworm collection. PELOSI et al, (2009) suggested that AITC is a reliable and

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promising chemical expellant whether or not used in combination with hand-sorting.

Another collection method is application of an electrical current to the soil. For example, one technique employs 8 steel electrodes pushed into the soil in a circular pattern with an area of 0.2 m^2. A 12 v battery can provide a number of voltages, with a range of "switching frequencies". This method is attractive as little or no damage is done to the area sampled and only fallen leaves and overgrown vegetation need be removed prior to sampling to assist earthworm detection. To date only limited work has been undertaken with this method, specifically in agricultural soils (e.g. SCHMIDT, 2001; EISENHAUER and SCHEU, 2008).

Having collected earthworms from the field there is a need to care for them. If a vermifuge has been used, they need to be thoroughly washed in clean water and then, as with all collected earthworms, allowed to burrow into soil. This will permit removal of any damaged/dying individuals from the soil surface. On return to the laboratory the animals also need to be acclimated to the appropriate experimental conditions.

In short: The type of collection may be influenced by the desired earthworm species and prior knowledge of distribution patterns can make targeted collection less taxing on resources. If laboratory conditions permit, collection may be an infrequent occurrence if stocks can be maintained and bred.

Rearing earthworms:

The term "vermiculture" is often found in the literature but usually refers to the cultivation of epigeic earthworms grown in an organic matter substrate with no soil. Here, the focus is on earthworms kept in a matrix of soil with a surface application of organic matter or in a mixture of the two substances.

Rearing soil dwelling earthworms under controlled conditions requires an understanding of their needs. However, many species can exhibit a degree of plasticity in behaviour, so general maintenance does not necessarily require extremely large containers. *L. terrestris*, for example, does not need access to a vertical burrow and can be bred in pots which may be only a few cm in depth (e. g. BUTT et al, 1994). Nevertheless, the focus of the given experiment may dictate the type of experimental set up that is required. Generally, pot size should be kept to a minimum as space requirements may be limiting. Relatively inexpensive containers of various sizes are now easily obtainable and adapted to earthworm culture by provision of pin-sized air holes in the sealable lid (to prevent escape).

Major considerations are: substrate (soil and food); temperature; moisture; light; earthworm density and species composition. These abiotic and biotic factors have been reviewed by LOWE and BUTT (2005) but are worthy of brief individual consideration.

A standard soil may be useful and a loam may be suitable for most species, depending on pH and physical requirements. Often soil from a particular field site, suitably sterilized/amended is required due to experimental objectives. The type of food (organic matter) provided may also be dictated by the experiment or a standard (amended horse or cow) manure may be used. Amendment may include drying and rewetting or freezing or combinations thereof to remove ammonia, resident earthworms, competitors or predators. Positioning of the organic matter may also be crucial. Surface application, mixing with the soil or layering may be appropriate.

Optimum temperatures will be species specific, but for temperate earthworms may fall within the range of 10-20^{o}C. The moisture content of the soil/substrate may need to be determined from experience or perhaps related to field capacity. These two factors are linked and will be influenced by container type. For example where open surfaces are required, e.g. when observing surface mating of *L. terrestris* (NUUTINEN and BUTT, 1997) frequent spraying of the soil surface may be needed. Illumination can also be important here. Constant darkness will limit water loss, enhance earthworm activity, but may not mimic desired field conditions (although most earthworms are totally subterranean).

The density at which earthworm are maintained may be critical for experimental outcomes. To replicate field measurements might be advised but if maximum (re)production is desired, then this may be increased. Results with *L. terrestris* and *Allolobophora chlorotica* have suggested 3-5 adults (15-22g), or 10 adults (3-4g) per litre respectively.

Also if earthworm communities are under scrutiny then the interactions of different species may be critical and the ecological groupings to which the given earthworms belong needs to be known (LOWE and BUTT, 2004).

Should individual earthworms require recognition among a group, tagging of animals is now an option (e.g. BUTT and LOWE, 2007). This allows e.g. for mating, separation and monitoring.

In all experiments, requirements of the life stage(s) of the earthworms should be considered e.g. cocoons may only need to be kept moist e.g. in a Petri dish at a given temperature and food is not a concern until hatching. Provision of a soft filter paper may also mean that immediate feeding thereafter is not needed.

Conclusions:

Objectives of the research will drive the collection/rearing protocol but earthworm maintenance is a vital part of the process. Much can still be learned of earthworm biology, their effects on soil properties and roles in ecosystems, through relatively simple, yet elegant experimentation. Provision of earthworms of known origin / age / reproductive status / exposure will prove extremely valuable.

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