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## 25.6 Earthworms

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### 25.6.1 Introduction

Among the soil fauna, earthworms are perhaps the most widely recognized and, along with ants and termites, function as ecosystem engineers with significant effects on soil structure and processes (Lavelle and Spain, 2001; Wardle, 2002). For these reasons, earthworms have been intensively studied for their potential benefits in agriculture, waste management, and land reclamation. The scientific literature on earthworms dates back over 200 years to the taxonomic description of *Lumbricus terrestris* by Linnaeus (1758). The modern era of earthworm research, in the context of soil science, began with Darwin (1881) and a vast literature has accumulated since then. Reviews of the literature from the past several decades can be found in Satchell (1983), Lee (1985), Dindal (1990), Curry (1994), Hendrix (1995), Edwards and Bohlen (1996), Lavelle et al. (1999), Lavelle and Spain (2001), and Edwards (2004). This chapter draws from these and other works to give a brief overview of earthworm biology, ecology, methods of collection, and analyses of earthworm tissues in food-web studies or for advanced systematic and taxonomic work.

### 25.6.2 Basic Taxonomy

Earthworms are classified within the phylum Annelida, class Clitellata, and subclass Oligochaeta (increasingly, Crassicitellata). Seventeen families are usually recognized worldwide, making up the semiaquatic and terrestrial forms commonly known as earthworms (Jamieson et al., 2002). Approximately 3700 species of these megadrile oligochaetes have been described, and it is estimated that total global species richness may exceed 7000 (Reynolds, 1994; Fragoso et al., 1999; Lavelle and Laped, 2003). Earthworm families along with their biogeographic origins are listed in Table 25.13. This table also includes the family Enchytraeidae, which is discussed in Section 25.5. Although the taxonomy and systematics of earthworms is

**TABLE 25.13** Classification and Regions of Origin of Major Families of the Terrestrial Oligochaetes

Phylum: Annelida		
Class: Clitellata		
Subclass: Oligochaeta (Crassicitellata)		
Order: Haplotaxida		
Suborder: Enchytraeina		
Family:	Enchytraeidae	NH, SH
Suborder: Lumbricina		
Family:	Lumbricidae	NH—NA, EU
	Komarekionidae	NH—NA
	Sparganophilidae	NH—NA
	Lutodrilidae	NH—NA
	Megascolecidae	NH, SH—NA, SA, OC, AS
	Glossoscolecidae	SH—SA
	Eudrilidae	SH—AF
	Acanthodrilidae*	SH—AS, SA, AF
	Octochaetidae*	SH—OC
	Ocnodrilidae	SH—SA, AF, AS, MA
	Ailoscolecidae	NH—EU
	Hormogastridae	NH—ME
	Kynotidae	SH—MA
	Microchaetidae	SH—AF
	Almidae	SH—SA, AF, AS
	Biwadrilidae	NH—JA

*Source:* Summarized from Wallwork, J.A. 1983. *Earthworm biology*. Studies in biology No. 161, Institute of Biology, Camelot Press, Southampton, U.K.; Sims, R.W. 1980. A classification and the distribution of earthworms, suborder Lumbricina (Haplotaxida: Oligochaeta). *Bull. Br. Mus. Nat. Hist. Zool.* 39:103-124; Jamieson, B.G.M. 1981. Historical biogeography of the Australian Oligochaeta, p. 887-921. *In* A. Keast (ed.) *Ecological biogeography of Australia*. W. Junk, The Hague, the Netherlands; Reynolds, J.W., and D.G. Cook. 1993. *Nomenclatura Oligochaetologica, Supplementum Tertium*: A catalogue of names, descriptions and type specimens of the Oligochaeta. New Brunswick Museum Monograph Series No. 9. (Nat. Sci.). New Brunswick, NJ; Jamieson, B.G.M., S. Tillier, A. Tillier, J.-L. Justine, E. Ling et al. 2002. Phylogeny of the Megascolecidae and Crassicitellata (Annelida, Oligochaeta): Combined versus partitioned analysis using nuclear (28S) and mitochondrial (12S, 16S) rDNA. *Zoosystema* 24:707-734.

NH, Northern Hemisphere; SH, Southern Hemisphere; AF, Africa; AS, Asia; EU, Europe; JA, Japan; MA, Madagascar; ME, Mediterranean; NA, North America; OC, Oceania; SA, South America.

\* Taxonomic status in question (Jamieson et al., 2002, Jamieson, 2006).

now reasonably stable at the family and generic level, there has long been confusion and controversy associated with certain groups in terms of species names. This situation results in part from the remarkable variability in external physical characters for some groups (e.g., the lumbricid genus *Aporrectodea*) that are reliable for taxonomic diagnoses for other groups (e.g., the lumbricid genus *Lumbricus*). Likewise, the frequent occurrence of parthenogenetic (asexual) reproduction in some groups, with variable reduction of male reproductive parts that are also used for taxonomic diagnosis, has resulted in different morphotypes of a single species being named as several species (e.g., the megascolecid genus *Amyntas*; Gates, 1972). It is to be hoped that advances in genetic and molecular techniques will provide resolution for many of these problems, but this work is just under way (see Briones et al., 2009; Chang et al., 2009; Dupont, 2009). Further information on earthworm taxonomy and biogeography can be found in Reynolds (1977, 1994, 1995), Sims (1980), Jamieson (1981), Gates (1982), Hendrix (1995), James 1995, Edwards and Bohlen (1996), Sims and Gerard (1999), Omodeo (2000), Jamieson (2006), and Hendrix et al. (2008).

The families Lumbricidae and Megascolecidae are ecologically the most important in North America, Europe, Australia, and Asia, while the families Glossoscolecidae and Eudrilidae are prevalent in South America and sub-Saharan Africa, respectively. Species from several of these families have been introduced worldwide by human activities and now dominate the earthworm fauna in many areas. Such "peregrine" or "anthropochorous" species are highly successful in agricultural or otherwise disturbed areas and often show significant effects on soil processes (Lee, 1985; Edwards and Bohlen, 1996; Hendrix and Bohlen, 2002). Whether introduced earthworms displace native species or occupy areas devoid of native species due to disturbance is a subject of debate (Lee 1961; Kalisz and Wood, 1995). Earthworm invasion biology and ecology have become topics of keen interest recently (Bohlen et al., 2004; Hendrix, 2006; Hendrix et al., 2008).

### 25.6.3 Biology and Ecology

Earthworms are elongated, cylindrical, segmented invertebrates, ranging in length from a few millimeters to 1.4 m, such as the giant Australian *Megascolides australis*. They consist of a relatively simple, tube-within-a-tube body plan, the internal tube comprising the alimentary canal. The body segments are separated by septa and are filled with coelomic fluid that provides a dynamic, hydrostatic "skeleton" for locomotion. When fully hydrated in free water, earthworm body weight may consist of 80%–90% water, but under ideal soil moisture conditions water content is typically 65%–75% (Lee, 1985). Water is lost from the body as mucus secretions onto the body surface from epidermal gland cells. Respiration occurs through the moist integument, where blood in subcuticular capillaries absorbs oxygen that is transported throughout the body in a closed vascular system driven by a series of muscular heart-like structures. Earthworms are hermaphroditic, each individual carrying male and female reproductive organs, but a number of species display

parthenogenesis. During amphimictic reproduction, sperm is exchanged between two individuals, stored in spermathecae, and later released along with eggs into cocoons secreted by the glandular clitellum, a characteristic thickening along several anterior segments. Single or multiple hatchlings emerge from cocoons after a period of embryological development determined by species and prevailing environmental conditions. Further details of earthworm biology can be found in Wallwork (1983), Lee (1985), Edwards and Bohlen (1996), and Sims and Gerard (1999).

Earthworms occur worldwide in most areas where climatic conditions are favorable for at least part of the year (all but desert and polar conditions); temperature is the main controlling factor globally, whereas soil moisture strongly influences local patterns of abundance and distribution (Lee, 1985; Curry, 2004). Across this range of habitats, earthworms display a wide array of morphological, physiological, and behavioral adaptations to environmental conditions. During unfavorable periods (e.g., drought), many species are able to enter a temporary dormant state (aestivation or diapause) or produce resistant cocoons that can hatch when conditions improve.

The abundance of earthworms across habitats is highly variable depending on climatic and edaphic conditions, ecosystem type, and the degree to which the habitat has been altered, for example, by agriculture. Under otherwise suitable conditions, soil C concentration has been shown to be highly correlated with earthworm population density and biomass (Edwards, 1983; Hendrix et al., 1992). Earthworm density and biomass in a variety of habitats worldwide are presented in Table 25.14. Densities range from <10 to >2000 m<sup>-2</sup>, with the highest values occurring in grasslands (especially fertilized pastures) and the lowest in acid or arid soils (coniferous or sclerophyllous forest). Typical densities from temperate deciduous or tropical forests and certain arable systems range from ca. 100 to 400 m<sup>-2</sup>, depending on intensity and nature of disturbance. Earthworm biomass tends to track density, but biomass comparisons can be problematic due to different methods used by various investigators (Section 25.5.1). Because earthworm populations often show seasonal variation in abundance (especially in temperate regions), time of sampling also affects density and biomass estimates.

Within habitats, earthworms often show heterogeneous spatial distributions. "Single-tree-influence," spatial distributions of other controlling environmental factors (e.g., soil texture, moisture, or organic matter content), or intrinsic population characteristics (e.g., fecundity, body size, and dispersal ability) often result in aggregation or clumped distribution patterns of earthworm populations across landscapes. In addition, local habitat and feeding preferences of various earthworm species dictate their vertical distributions within the soil profile (Boetcher and Kalisz, 1991; Barois et al., 1999; Rossi et al., 2006). A categorization of earthworm life forms or functional types based on habitat and feeding ecology is presented in Table 25.15. These categories describe niche separation of earthworm species within a soil volume. Polyhumic, epigeic, and epiendogeic species utilize litter and organically enriched surface layers; poly-, meso-, and oligohumic endogeic species inhabit mineral soil

TABLE 25.14 Abundance and Biomass of Earthworms in Selected Habitats from Various Parts of the World

Habitat	Location	Collection Method	Earthworm Taxa	Abundance No. m <sup>-2</sup>	Biomass g m <sup>-2</sup> Fresh wt	
Sown pastures	New Zealand	Hand sorting	Lumbricidae	208-775	60-241	
				740-1235	146-303	
				690-2020	305 (mean)	
Sown pastures	South Australia	Hand sorting	Lumbricidae	460-625	62-78	
Sown pastures	South Africa	Hand sorting	Lumbricidae	72-1112	—	
Fertilized pasture	Argentina	Hand sorting	Lumbricidae, Megascolecidae, and Glossoscolecidae	27	—	
Pastures with heavy rates of fertilizers	Ireland	Hand sorting	Lumbricidae	400-500	100-200	
Old pasture	Sweden	Hand sorting	Lumbricidae	109	59	
Old pasture	England	Hand sorting	Lumbricidae	390-470	52-110	
Old pasture	Wales	Hand sorting	Lumbricidae	646	149	
Old pasture	France	Hand sorting	Lumbricidae	288	125	
Fallow	South Australia	Hand sorting	Lumbricidae	210-460	16-76	
Fallow	Wales	Hand sorting	Lumbricidae	226	79	
Cropland	South Australia	Hand sorting	Lumbricidae	20-25	2-2.5	
Cropland	Romania	Hand sorting	Lumbricidae	5-100	0.5-20	
Natural grassland	Romania	Hand sorting	Lumbricidae	200 (mean)	10-60	
Natural grassland	Wales	Hand sorting	Lumbricidae	22	8	
Natural grassland	Tennessee	Hand sorting	Lumbricidae	13-41	3.2-7.5	
Natural grassland	South Africa	Hand sorting	Glossoscolecidae	74	96	
Natural grassland	India	Hand sorting	Megascolecidae and Ocnerodrilidae	64-800	6-60	
Natural grassland	New Zealand	Hand sorting	Megascolecidae	250-750	—	
Tropical savannas	Ivory Coast	Hand sorting and washing/sieving	Megascolecidae and Eudrilidae	230	49	
Orchard	Netherlands	Hand sorting	Lumbricidae	300-500	75-122	
Orchards	Australia	Hand sorting	Lumbricidae	150	—	
Mulched and irrigated orchards	Australia	Hand sorting	Lumbricidae	2000	—	
Garden	Egypt	Hand sorting	Megascolecidae	420	153	
Gardens	Argentina	Hand sorting	Lumbricidae, Megascolecidae, and Glossoscolecidae	73	—	
Taiga	Finland	Hand sorting	Lumbricidae	17.4	2.8	
				Siberia	23.0	8.4
				USSR	3-7	—
Northern European and Asian coniferous forests	Finland	Hand sorting	Lumbricidae	14-68	—	
				Sweden	103-167	30-35
				USSR	12	—
				Japan	27-72	—
Spruce forest with lime topdressing	USSR	Hand sorting	Lumbricidae	1000	—	
European deciduous forests	England	Hand sorting	Lumbricidae	118-138	—	
				USSR	136	68.3
				Czechoslovakia	106	98.1
North American deciduous forests	Canada	Hand sorting	Lumbricidae	240-780	38-109	
				Tennessee	2-96	1.3-14
				Indiana	14-124	26.3-280.3
Dry sclerophyll forest	Australia	Hand sorting	Megascolecidae	7-38	1.3-25.5	
Wet sclerophyll forest				34-76	12.3-47.9	
Subalpine woodland				15-106	5.7-35.7	

(continued)

**TABLE 25.14 (continued)** Abundance and Biomass of Earthworms in Selected Habitats from Various Parts of the World

Habitat	Location	Collection Method	Earthworm Taxa	Abundance No. m <sup>-2</sup>	Biomass g m <sup>-2</sup> Fresh wt
Gallery forests	Ivory Coast	Hand sorting and washing/sieving	Megascolecidae and Eudrilidae	70–103	3.4–6.8
Tropical forest	Nigeria		Eudrilidae	34	10.2
Tropical forest	Nigeria	Hand sorting	Eudrilidae	61.7	2.5
Lowland dipterocarp forest	Sarawak	Hand sorting	Moniligastridae and Megascolecidae	37–92	0.7–1.3
Lower montane forest			Moniligastridae and Megascolecidae	55	3.1
Upper montane forest			Moniligastridae and Megascolecidae	47–108	1.8–2.7
Upper montane low forest			Megascolecidae	2–24	0.2–2.1

Source: Reproduced with permission from Lee, K.E. 1985. Earthworms: Their ecology and relationships with soils and land use. Academic Press, Sydney, Australia.

**TABLE 25.15** Ecological Strategies of Earthworms

Epigeic (litter dweller)—Mesophage; detritivore Lives in and consumes litter; small size; uniformly pigmented (e.g., <i>L. rubellus</i> , <i>Bimastos</i> spp., <i>Dendrobaena octaedra</i> , <i>Dendrobaena rubida</i> , <i>Eisenia foetida</i> , <i>Amyntas</i> spp.)
Endogeic (subsoil dweller)—Microphage; geophage; (epiendogeic or hypoendogeic; oligohumic, mesohumic, or polyhumic) Lives in horizontal, branching burrows in organomineral layer; consumes soil; small to large in size; weakly pigmented (e.g., <i>Aporrectodea caliginosa</i> , <i>Octolasion cyaneum</i> , <i>Diplocardia</i> spp., <i>Pontoscolex corethrurus</i> )
Anecic (topsoil dweller)—Macrophage; detritivore Lives in deep vertical burrows, casting on surface; emerges at night to draw down organic matter (plant residue, etc.); large as adults (200–1100 mm); brown pigment anteriorly and dorsally (e.g., <i>L. terrestris</i> , <i>Allolobophora longa</i> )

Source: Lee, K.E. 1959. The earthworm fauna of New Zealand. Department of Scientific and Industrial Research Bulletin No. 130. Wellington, New Zealand; Bouché, M.B. 1977. Stratégies lombriciennes. In U. Lohm and T. Persson (eds.) Soil organisms as components of ecosystems. Ecol. Bull. (Stockholm) 25:122–133; Lavelle, P. 1983. The structure of earthworm communities, p. 449–466. In J.E. Satchell (ed.) Earthworm ecology: From Darwin to vermiculture. Chapman and Hall, London, U.K.; Barois, I., M. Brossard, P. Lavelle, J. Tondoh, J. Kanyonyo, A. Martínez, J. Jiménez et al. 1999. Ecology of earthworm species with large environmental tolerance and/or extended distribution, p. 57–85. In P. Lavelle, L. Broussard, and P. Hendrix (eds.) Earthworms management in tropical agroecosystems. CABI Publishing, New York.

within the rhizosphere and beyond; and anecic species exploit both the surface litter as a source of food and the mineral soil as a refuge. Lee (1985) summarizes data showing that within a particular soil, commonly less than a half-dozen earthworm species are found. The species in a given earthworm association often effectively partition the soil volume according to the functional categories mentioned above. Furthermore, the activities of earthworms within these categories influence biogeochemical processes in various ways. For example, epigeic species facilitate the breakdown and mineralization of surface litter, whereas anecic species incorporate organic matter deeper into the soil profile and enhance aeration and water infiltration through burrow formation (Lee, 1985; Shipitalo and Le Bayon, 2004).

For management of earthworms in agroecosystems, Lee (1991, 1995) recommends that “target earthworm communities” consist of

one or more anecic/epigeic species that make deep vertical burrows and that cast on the surface and bury residues, and one or more endogeic species that feed belowground on dead roots and organic matter and that make horizontal burrows. Diverse assemblages of earthworms may more effectively exploit soil resources and influence a wider array of processes, such as organic matter turnover, in addition to soil structural properties, than a single species.

## 25.6.4 Importance to Soil Processes

Where earthworms are abundant, they can exert significant influence on soil processes through effects on organic matter and nutrient cycling, and on soil structure. These topics are reviewed in Lee (1985), Hendrix (1995), Edwards and Bohlen (1996), Lavelle et al. (1999), Lavelle and Spain (2001), and Edwards (2004).

### 25.6.4.1 Organic Matter Dynamics and Nutrient Cycling

Effects of earthworms on organic matter and nutrient cycling are closely linked with the life form and feeding ecology of earthworms (Table 25.15). Epigeic species typically live in the O and upper A soil horizons where, through feeding and casting activities, they mix mineral soil and plant litter, fragment organic particles, inoculate them with microbes, and thereby accelerate organic matter decomposition rates. Anecic forms pull surface litter into their burrows, thus transporting organic material deeper into the soil profile. They cast on the soil surface, mixing organic and mineral particles in the litter layer. The activities of both epigeic and anecic earthworms produce “mull” soil horizons, defined as those in which organic matter is intimately incorporated into the upper mineral soil of a well-developed A horizon overlain with litter or humus layers <2 cm thick. The extreme case is termed “vermimull,” in which the Ah horizon is granular and characterized by strong organomineral complexes consisting of earthworm casts (Green et al., 1993). Endogeic earthworms feed within the soil on organic matter and microbes associated with the rhizosphere or mineral soil. As mentioned previously, they are termed oligohumic, mesohumic, or polyhumic,

depending on the level of organic enrichment of their substrate. Casts and burrows of endogeic earthworms are also sites of increased microbial activity and organic matter decomposition, and the presence of these worms has been shown to positively influence the availability of nutrients in some soils (e.g., Callaham and Hendrix, 1997; Chapuis-Lardy et al., 2009). Mineralization of organic matter in earthworm casts and burrow linings produces zones of nutrient enrichment compared to bulk soil. These "hot spots" (the "drilosphere") are often sites of enhanced activity of plant roots and other soil biota (Beare et al., 1995; Lavelle and Spain, 2001). Indeed, most of the effects that earthworms have on soil organic matter and nutrient dynamics in ecosystems are mediated through their interactions with other soil biota and in particular soil microbiota, as earthworms have been shown to influence the size and composition of microbial communities (e.g., Svensson and Friberg, 2007; Pawlett et al., 2009), as well as the activity of microbial communities in terms of both total soil respiration and gaseous losses of nitrogen (Speratti et al., 2007).

#### 25.6.4.2 Soil Structure

Soil structure is affected by earthworms principally through production of casts, which form stable aggregates upon and within the soil, and formation of burrows, which produce macropores that may increase water infiltration and aeration within the soil. Casts are produced by ingestion of mineral and organic particles, mixing, organic enrichment, and microbial stimulation in the gut, and egestion of the material as a slurry or as discrete pellets (depending on earthworm species), which harden into stable aggregates. Mechanisms of cast stabilization include organic bonding of particles by polymers secreted by earthworms and microbes, mechanical stabilization by plant fibers and fungal hyphae, and stabilization due to wetting and drying cycles and age-hardening/thixotropic effects (Tomlin et al., 1995). Earthworm casts are usually enriched with plant available nutrients and thus may enhance soil fertility.

Earthworms create burrows of various sizes, depths, and orientations, depending on species and soil type. Burrows tend to be similar in diameter to that of the body, ranging from 1 to >10 mm diameter and constituting among the largest of soil pores (Lee, 1985; Tomlin et al., 1995; Shipitalo and Le Bayon, 2004). Geophagous species (Table 25.15) may form networks of variously oriented macropores, as the earthworms consume the soil and cast behind them as they burrow. Although such networks may form continuous pores for some depth, casting within the burrows may impede free water movement. Anecic earthworms may create vertical burrows that can form continuous macropores to depths of >1 m. Such burrows are often highly stable because their walls are lined with organic matter drawn in or secreted by earthworms, and they tend to have higher bulk density than surrounding soil. Continuous macropores resulting from earthworm burrowing may greatly enhance water infiltration by functioning as by-pass flow pathways through saturated soils (Lee, 1985; Tomlin et al., 1995; Shipitalo and Le Bayon, 2004). These pores

may or may not be important in solute transport depending on antecedent soil water, nature of the solute, and exchange properties of the burrow linings (Edwards et al., 1990, 1993).

### 25.6.5 Methods

#### 25.6.5.1 Earthworm Collection

Techniques for field sampling of earthworms are reviewed in Lee (1985) and Edwards and Bohlen (1996). Unless otherwise given, methodological details and specific reference citations can be found in these works. Collection techniques are passive, behavioral, and indirect (Table 25.16).

Hand digging and sorting, is the most commonly used and probably the most reliable method for quantitative sampling of earthworms. The technique involves digging pits of known dimensions (e.g., 25 × 25 × 25 cm), breaking the soil by hand, and collecting all earthworms found. Often the collected specimens are immediately preserved in 70% ethanol or 5% formalin for later counting and identification (see Fender and McKey-Fender, 1990; Schwert, 1990, for details of preservation and preparation of specimens for identification).

Washing and sieving is an elaboration of hand sorting, in that the soil is dispersed in water (or a dispersing agent), poured through a sieve or nest of sieves, and the earthworms and cocoons hand picked from the sieve contents. Mechanized approaches to washing and sieving are described by Bouché and Beugnot (1972). Flotation of sieve contents in a high density solution, such as 1.16–1.20 SG MgSO<sub>4</sub>, is an additional means of separating earthworms and other soil fauna from more dense soil particles.

A number of factors influence efficiency of hand sorting, including species and body size of earthworms (i.e., seasonal phenology and population demography), root density (especially in grasslands), soil type, and a "human factor," related to training of personnel and time spent on each sample (Schmidt, 2001a; Jiménez et al., 2006).

Several approaches have been taken to extracting earthworms from soil based on their behavioral response to certain stimuli. A number of chemical irritants have been used, including HgCl<sub>2</sub>, KMnO<sub>4</sub>, formalin and, more recently, mustard. Aqueous solutions of 0.165%–0.550% formalin have been used commonly and shown to be effective on *L. terrestris* when applied in three sequential doses of 18 L m<sup>-2</sup> but formalin may be less effective on other species (Satchell, 1969; Callaham and Hendrix, 1997; Schmidt, 2001b). Aqueous extract of mustard has shown earthworm extraction efficiency similar to that of other chemical extractants and has come into favor because of its minimal effects on human health and low phytotoxicity compared to formalin (Gunn, 1992). Effectiveness of any chemical extractant varies with earthworm species and activity, temperature, soil porosity, and soil water content, saturated soils being less likely to transmit extractant solutions deep into the soil. Comparisons with hand sorting should be done before adopting chemical extraction techniques for quantitative sampling.

TABLE 25.16 Descriptions of Methods for Collecting Earthworms

Method	Description	Advantages	Disadvantages
<i>Passive</i>			
Hand sorting	Known volume of soil cut with spade or corer, broken and worms removed by hand	Simple, reliable in the field; low cost	Laborious; may not collect deep burrowing species, small earthworms and cocoons
Washing and sieving	Known volume of soil cut with spade or corer, soaked in dispersant/preservative, and washed through sieve(s) by hand or mechanical device	Higher recovery of cocoons and small individuals	Laborious; may not collect deep burrowing species
Flotation	Material from hand sorting or washing/sieving floated in high density solution (e.g., MgSO <sub>4</sub> )	Separates earthworms from soil and plant debris; cocoons and small individuals collected	Laborious; may not collect deep burrowing species
<i>Behavioral</i>			
Chemical extraction	Soil saturated with chemical irritant (e.g., 0.2% formalin) causing earthworms to emerge onto soil surface	Simple; effective on deep burrowing anecic species	Not effective on all species, in all soils or under all conditions
Heat extraction	Soil blocks or cores suspended under heat lamps in water into which earthworms migrate	Effective on dense root mats	Not effective on all species; inconvenient for field use
Electrical extraction	Metal rods inserted into soil and connected to AC electrical source	Useful for selective or comparative sampling	Highly variable; not convenient in the field; dangerous
Mechanical vibration	Stake or rod inserted into soil and vibrated with bow or flat iron	Simple; useful for selective or comparative sampling	Not effective on all species
Trapping	Pitfall or baited traps placed in soil and sampled at desired intervals	Simple; useful for selective or comparative sampling	Not effective on all species
Mark-recapture	Individuals tagged, released, and population sampled at intervals	Useful for estimating population density, dispersal, and mortality	Laborious
<i>Indirect</i>			
Cast counting	Surface castings enumerated and identified	Simple	Not a quantitative estimate of population density

Sources: Summarized from Lee, K.E. 1985. Earthworms: Their ecology and relationships with soils and land use. Academic Press, Sydney, Australia. Edwards, C.A., and P.J. Bohlen. 1996. Biology and ecology of earthworms. 3rd edn. Chapman and Hall, New York.

The heat extraction method is a modification of that used for enchytraeids (see previous section). Soil cores or blocks are placed in pans of water, exposed to heat from overhead light-bulbs, and earthworms are collected from the water after several hours. This technique was more effective than hand sorting or formalin extraction on small earthworms in dense root mats (Satchell, 1969). As with hand sorting, it is not effective on deep burrowing, anecic species such as *L. terrestris*.

Mechanical vibration employs a rod or wooden stake driven into the soil, vibration for a few minutes with a bow or flat piece of metal (e.g., an automobile leaf spring), and collection of earthworms that emerge onto the soil surface. *Diplocardia mississippiensis* are routinely collected by fishermen in north Florida, with this method (termed "grunting") and Hendrix et al. (1994) used it for sampling *Diplocardia* populations in that region. Mitra et al. (2008) analyzed seismic signal characteristics of vibrations generated by the technique and found that numbers of worms emerging were positively correlated with signal strength. In general, vibration techniques may not be effective on many groups of earthworms, for example, lumbricids (Reynolds, 1973) and are probably not suited to quantitative measurements of population density. However, they may be useful for selective or comparative sampling of certain earthworm populations.

Electrical extraction of earthworms involves inserting metal rods into the soil, connecting them to a source of alternating current and collecting earthworms that come to the soil surface. Different voltages and amperages have been used with varying degrees of success; effectiveness of the technique is highly dependent on soil water content, electrolyte concentration, and temperature (Lee, 1985). As with mechanical vibration, the soil volume sampled is not known and therefore this method has been considered best suited for qualitative or comparative sampling. However, a comparative analysis by Schmidt (2001b) found that the "octet method" of Thielemann (1986) extracted higher earthworm numbers than the formalin method and gave community size and species composition estimates comparable to that of the hand sorting method. Schmidt (2001b) concluded that the octet method may be reliable and especially useful in situations requiring minimal soil disturbance. It must be cautioned that all electrical methods are potentially very dangerous and should only be used with extreme care.

Two earthworm-trapping techniques have been described. Pitfall traps (open-top containers buried level with the soil surface and containing a fixative solution, such as picric acid; see Section 25.4) may be useful for sampling surface-active species in diurnal or seasonal studies (Callaham et al., 2003b). Arrays of

traps are installed and sampled at 12, 24 h, or longer intervals. Baited traps, such as perforated clay pots containing manure or other attractants and inserted into the soil may also be useful for collecting certain species. As with other behavioral methods, trapping is probably highly selective and best suited for qualitative or comparative sampling.

Mark, release, and recapture techniques have been widely used to study population dynamics of animals, including earthworms. Large numbers of individuals of desired species are collected, marked (e.g., with brands or nontoxic dyes), and released into the population of interest. Sampling over time and distance from the target site, and enumeration of tagged relative to untagged individuals, yields information on dispersal, mortality, and population density. Dyes (Mazaud and Bouché, 1980) and radioisotopes (Bastardie et al., 2003) have been employed to mark earthworms. González et al. (2006) recently evaluated the use of a fluorescent elastomer injected into *Pontoscolex corethrus* and were able to trace the marker for four months in populations incubated in field enclosures. Likewise, other studies (Butt and Lowe, 2006; Butt et al., 2009) showed that these elastomers did not affect the growth rates or cocoon production in *L. terrestris* and could be detected in the coelomic cavities of seven different earthworm species for up to 2 years (but sometimes was detected only after dissection). For earthworm species that cast on the soil surface, such as *Aporrectodea longa*, numbers and identity of castings may be a useful index of population activity. Because casting is dependent on soil temperature and moisture, this technique is highly variable and not a quantitative estimate of population density.

In addition to measurements of earthworm density (i.e., numbers per unit area), it is desirable to have estimates of earthworm biomass in most quantitative studies. Specimens from field collections contain varying amounts of gut contents that affect body mass measurements and therefore it is necessary to remove this material either by allowing live earthworms to void their guts (e.g., overnight in moist paper towels) or by dissecting and removing gut contents from preserved specimens. "Fresh weight" or "wet weight" measurements face two further problems: First, earthworms under field conditions contain varying amounts of water, and second, preserved specimens lose differing amounts of fresh mass in different preservative fluids (e.g., profuse mucus secretions in formalin; Lee, 1985). These factors must be considered in comparative studies across sites or seasons, or when different preservation techniques are used. For most purposes, it is best to convert live weight into dry weight, which may be accomplished by directly drying gut-voided specimens (e.g., freeze drying) or by using allometric relationships or regressions that relate body length or wet weight to dry weight (Lee, 1985; Hale et al., 2004), or oven dried weight to ash-free dry weight (Callaham et al., 2003a). Isotopic studies of earthworm feeding ecology require measurement of carbon mass, while also maintaining specimens for taxonomic identification. Live specimens can be killed instantly in boiling water and divided into a posterior half for gut clearance and freeze drying for chemical analysis, and an anterior half for preservation in

formalin or ethanol for morphological taxonomic study (e.g., Hendrix et al., 1999).

Finally, for those interested in molecular techniques for evaluating the presence of earthworms in environmental samples, new techniques are rapidly being developed for detection of earthworm DNA or other proteins, and these hold great promise for rapid analysis of samples with high resolution information for diversity of organisms in samples. These techniques include using whole soil DNA extractions followed by sequencing and comparison against clone libraries (as in Wu et al., 2009), as well as more specific applications such as those in Juen and Traugott (2006) where DNA was used to identify the different prey items (including earthworms) in the gut of a soil predator.

In summary, digging and hand sorting or washing are probably the most reliable means of sampling earthworms. However, no single method will be adequate to sample earthworm populations in all situations. Combinations of methods will probably achieve reasonable results. For example, formalin or mustard solution can be applied to the bottom of pits previously excavated for hand sorting, to extract deep burrowing anecic forms not sampled by digging (Edwards and Bohlen, 1996). Combinations of various methods may be useful in other situations.

#### 25.6.5.2 Identification

Many earthworm species in the family Lumbricidae can be identified from external body characteristics if the specimens are sexually mature. Taxonomic keys by Reynolds (1977), Schwert (1990), and Sims and Gerard (1999) are useful for the common lumbricids found worldwide. Reynolds (1977) also includes a key to North American Sparganophilidae, a limicolous or semi-aquatic group.

Most earthworms other than the Lumbricidae require dissection for accurate taxonomic identification. Knowledge of position and characteristics of sexual organs, the gut and associated glands, and other structures is required. The procedures must be done carefully and require a degree of skill and practice. At the family level, several keys are available: Jamieson (1988, 2000) contain a key and diagrammatic comparison of characteristic internal structures of most families, including the Megascolecidae of Australia; Edwards and Bohlen (1996) review major characteristics of the families; Sims and Gerard (1999) provide keys and species descriptions for seven families found in Great Britain; Fender and McKey-Fender (1990) give keys to the families of North America and the genera of Megascolecidae from western North America; James (1990) provides a key to the genus *Diplocardia*, a group of megascolecids found mostly in eastern North America, including Mexico and the Caribbean. Righi (1971) describes the Glossoscolecidae in Central and South America. Many of the works cited in Gates (1982) include keys and species descriptions for families and genera found in North and Central America.

The taxonomy and systematics of earthworms, as with many other taxa, is currently undergoing considerable revision as advances in molecular biology prompt reevaluation of accepted

phylogenies and taxonomic relationships (e.g., Jamieson et al., 2002; Marotta et al., 2008). Although these approaches have great promise for providing clarity to taxonomic problems that have plagued earthworm nomenclature for decades, it is clear that much work remains and that even with DNA-based approaches, some controversy will remain (see Chang et al., 2008). Internet resources provide the best means of keeping track of these revisions and the development of new taxonomic keys (e.g., <http://zipcodezoo.com/animals>, <http://www.discoverlife.org>, [http://species.wikimedia.org/wiki/Main\\_Page](http://species.wikimedia.org/wiki/Main_Page), and <http://tolweb.org/tree>).

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