

## Chapter 7

### The mass emergence of Earthworm *Pheretima* sp. (Oligochaeta: Megascolecidae) on Fine Day after Rainy Day

#### Introduction

Earthworm creeps out on the soil surface on the day of rain (Nishida 1950, Siraishi 1954, Ohfuchi 1947). Schwert (1980) said that in northern North America and Europe, the mass emergence of Lumbricids during periods of heavy rain fall or dew is a common, yet poorly studied, phenomenon. The mass emergence of *Pheretima* sp. (H-1) (Oligochaeta: Megascolecidae) frequently occurred in the experimental field of Kyushu University during rainy season of June and July. The mass emergence of earthworm, in many case, occurred on fine and higher temperature days after rain during the rainy season of June and July. Earthworms die with solar radiation in the way of marching on bare ground. Then, the mass emergence in summer causes the decrease of population density on those days.

No one obtains correct understanding about earthworm's mass emergence though this phenomenon recently had been paid attention up to now. Oofuchi (1947) enumerated the factor to stimulate earthworm's emergence as follows, (1) parasite, (2) natural enemy, (3) the change of soil temperature, (4) the decline of oxygen tension caused by rain fall, (5) the outbreak of harmful gases. Nishida (1950) said that the decline of oxygen tension in soil and the change of water content of soil might not be a stimulus which brought out the creeping out behavior of earthworm, but only the increase of CO<sub>2</sub> tension in soil followed with this behavior of earthworm. Nishida (1950) and Siraishi (1954) ascertained the negative chemotaxis of *Eisenia foetida* (Savigny) to CO<sub>2</sub> gas. The later author said it uncertain to explain the creeping out of the earthworm on the rainy day only by its chemotaxis to carbon dioxide. They did not explained why the CO<sub>2</sub> tension in soil air increased on fine days after rain. Moreover, the meaning of this phenomena in the population change have not discussed till this day.

Present chapter investigate the stimulus which induce the mass emergence of earthworm on fine days after rain and secondary the factors which break out harmful gases, and discuss the effect of the mass emergence of earthworms on the population change.

#### Method

##### 1. Counting the number of dead earthworm

Dead earthworm on bare ground were counted in the experimental filed of kyushu University through early summer (May - July) in 1971 and 1972. Figure 7-1 shows the location of the bare ground studied. On the clay sand in the surrounding of the bare ground studied, the experimental population was established for another purpose. Where, the migrating and dead earthworms were also observed in the gutter dug along the clay sand. The detail on the experimental population will be mentioned in later paper (Chapter 10).

## 2. Measurement of soil respiration

The daily fluctuation of CO<sub>2</sub> expiration from soil surface was measured on the clay sand in the experimental field. The measurement was carried out from May 8 to June 27, 1973 with the absorption method using disc of plastic sponge as absorbent holder (Kirita and Hozumi 1966, Kirita 1971 a-b). Kirita (1971 c) reported that the soil respiration on a beginning week of the measurement was higher than in ordinary day by the reason of soil disturbance. In consideration of his result, the apparatus was set up 14 days before the measurement beginning. Six of the apparatus were installed on the clay sand on April 24, 1973. 30 g dry wt litter of *Oryza sativa* L. and ten individuals of *Pheretima* sp. (H-1) being one gram wet weight were added on the soil surface in five apparatus. One apparatus which litter and the earthworm had not added was used as a control.

5 ml of 0.5 N KOH solution was used as absorbent of Carbon dioxide. The disc of plastic sponge containing KOH solution was suspended in a tin cylinder standing on soil surface for five hours (From 12 a.m. to 17 a.m.). The cylinders were closed with a lid and kept air tight during the measurement. After five hour suspension, the disc of plastic sponge were take out, and KOH solution was determined by 0.1 N HCL. At each measurement time, blank test was carried. An arrangement of blank test was as bellow, (1) the infiltration of KOH solution into plastic sponge, (2) the extraction of KOH solution from plastic sponge, (3) the determination of CO<sub>2</sub> content absorbed in handling time. The measurement value was corrected by the result of the blank test (Kirita 1971).

The temperature on soil surface of the study area was recorded on 12 o'clock of every measurement days. Water content of the soil in the study area was frequently measured. The number of dead earthworm were counted on bare ground around of the experimental field through the soil respiration measurement (Fig. 7-1).

## 3. Respiration of wormcast and of soil-litter mixture

The soil microorganism may cause soil respiration. Parle (1963 b) said that the number of soil organism in wormcast was abundant more than that in soil, and then oxygen up-take of wormcast was higher than that of soil. Fecal pellet of earthworm exists abundantly surrounding the habitat of earthworm. Then, the respiration rates of wormcast and of the soil-litter mixture materials containing soil and litter were measured.

Ten pairs of wormcast and soil-litter mixture were collected in the experimental field in the period from May to June 1973. The pair of wormcast and soil-litter compound (of each was 100 g wt) was collected from one place at same time. The CO<sub>2</sub> expiration rate of pair materials was measured on sampling days with InfraRed Gas Analyzer (URAS). The respiration chamber was made from the glass tube 30 cm in length and 7 cm diameter. Each material was wrapped in filter paper and put in each chamber. The chambers were immersed into water bath. The measurement was carried out at 20 °C, 30 °C and 40 °C. Before the measurement, the materials were weighed. After the measurement, the materials were dried at 105 °C for 5 hours and weighed again. The age of the

wormcast collected was unknown.

#### **4. Microbial increase in earthworm intestine**

The present experiment follows the change in the microbial population in ingested material as it passes through the intestine of *Pheretima* sp. (H-1). Albium agar was used to count bacteria and actinomycetes. Earthworms were collected from the experimental field of Kyushu University, on 16 May, 26 May, 31 May, 14 June and 29 June 1973. The incubation was carried out on the day when the earthworm was collected. The earthworms were washed free from soil and batch of five were killed with brief immersion in water at 48 °C - 50 °C. Immediately the intestine of earthworm were dissected to three part: fore gut, Mid gut and hind gut. Samples of gut content of about 450 mg dry wt placed in 100 ml sterilized water. These were, violently up and down, shaken at 40 strokes by hand. A series of ten-fold dilution was prepared from this suspension with pipetting. Three samples were taken from each dilution into each culture medium.  $10^5$  fold,  $10^6$  fold and  $10^7$  fold dilutions were used for counting. The number of organisms was estimated from the total number of showing growth. The remained suspension was filtrated through filter paper. The soils in the suspension was collected, dried at 105 °C for 5 hr. and weighed. The observed number of colony was converted to the number per one gram dry wt soil.

#### **5. Responses of earthworm to CO<sub>2</sub> gas and N<sub>2</sub> gas**

This work follows the responses of the earthworm to CO<sub>2</sub> gas and N<sub>2</sub> gas. Figure 7-2 shows the apparatus for observation. The apparatus was composed of gas cylinder, flow meter, three way cock and response box. The response box was made up of two parts. One part was the gases diffusion chamber, the volume of which was 1250 ml, and other part was the observation chamber. To prevent the sudden gust of gas, the plastic boll which was porous was settled at the end of polyvinyl chloride tube in the gas diffusion chamber. The observation chamber was filled up with the fecal pellet which was used as the habitat of earthworm like wormcast in field. By filling up the chamber with fecal pellet, various gases can flow from the plastic boll to outside of the chamber through the layer of fecal pellet. As earthworm could move easily in the fecal pellet, the behavior of earthworm could be observed exactly. The fecal pellet used as wormcast were produced by *Pheretima* sp. (H-1) and preserved before the observation. The depth of the wormcast layer in chamber was 2 cm. The density of wormcast was 0.21 - 0.29 g dry wt cm<sup>-3</sup> and its humidity was 26.25 % in average. Test specimens were collected from the experimental field in May and June 1973. The average weight of test specimens was  $1.56 \pm 0.25$  g fresh wt. Five specimens were used per an observation. The wormcast and test specimens were replaced for every observation.

The procedure of experiment was presented as bellow. (1) Three-way cock was turned open to A site and the valve of gas cylinder was open for a sometime. Then, the gas flowed through the tube 1, 2, 3 and the plastic boll. (2) Three-way cock was handled to C site. After, the response box was sweeping by the stream of O<sub>2</sub> gas. (3) The fecal pellet was placed on the surface of

wormcast layer. The specimens were laid on the surface of wormcast layer. They creep into wormcast layer and came to a standstill there. (4) Five minute after the standstill of earthworm in wormcast layer, a three way cock was handled to B site, the valve of gas cylinder was open. The flow was settled at an appointed rate using a flow meter, a three way cock was handled to A site, the object gases flowed into the gas diffusion chamber for a settled time. Then, an appointed volume of gases flow into the box. After that, a three way cock was handled to B site (then, the excess gases flowed out from the observation system to an open air), and immediately the valve of gas cylinder was closed.

The observation on behavior of earthworm was begin just after the inflow of object gas into the diffusion chamber and continued for 22 minute. The part length of the earthworm's body appearing on the wormcast surface was determined with a ruler. The ratio of the part length of the earthworm's body appearing on the wormcast surface to the total body length was regarded as the index of response of earthworm to various gases, although the body length of earthworm was changeable.

The experiment were made in the period between late May and early June 1973, under a dark room with only a dark room lamp to avoid the external light that test specimens might respond to. The room temperature was ranged between 18 °C and 21 °C. In the preliminary experiment, earthworms received a death blow by the continuously exposing of gaseous carbon dioxide, on the other hand, showed no behavior response to the inflow of a finite volume of Nitrogen gas. Then, the observation were made in the inflow of 2.0 l, 1.5 l, 0.667 l, 0.5 l, 0.25 l and 0.125 l ( $15 \text{ l h}^{-1} \times$  an pointed second) of gaseous carbon dioxide, and in the continuously inflow of Nitrogen gas ( $30\text{-}80 \text{ l h}^{-1}$ ).

## Result

### 1. Daily change of the number of death individual

The daily change of the number of death earthworms that had died on the bare ground was shown in Figure 7-3 with the change of the daily change of soil surface temperature and the percipitation.

In 1971, Earthworm's death individual was observed on the fine day after rain for the period from June 7 to July 20. However, the death individual was not observed though there was a rainfall frequent for one week from June 1 to June 6. The most abundant death individuals were observed on June 13. It rained until about eight o'clock of this day, and became fine weather afterwards. The temperature of soil surface reached 24°C around nine o'clock. The movement of the earthworm began to be observed after 11 o'clock. Twenty earthworms were killed by the sun ray on bare ground or its environs by 17 o'clock. Earthworm's mass emergence was not observed though the rainfall was often, was also comparatively high soil temperature (24°C average) for the period from June 15 to June 28. For the period from July 8 to July 19, Small number of death individuals was

observed on the day of fine after small rain.

In 1972, Mass emergence of earthworms on fine days after rain was observed for period from 11 May to 18 July. The day of the mass emergence beginning in 1972 was about 30 days earlier than the case of 1971. Soil surface temperature on May 11 was low (19.5 °C). Nevertheless, several death individuals were observed on this day. A few death individuals were observed for the period from May 11 to May 30. A lot of death individuals were observed for the period from May 31 to June 15. It rained until about nine o'clock on June 8, and became fine weather afterwards (21.3 °C of soil surface temperature at 12 o'clock). 21 death individuals of *Pheretima* sp. (H-1) and five death individuals of *Pheretima vittata* (Goto et Hatai) was observed in bare land and its surround on this day. Some death individuals were observed on the 11th and June 12. Earthworm's neither mass emergence nor death were observed though it was high temperature and rainy for the period from June 16 to June 26. On June 30, it rained until eight o'clock, but become fine by ten o'clock afterwards, and the soil surface temperature rise. At ten o'clock, earthworm appeared on soil surface and litter, and earthworm showed the symptom of mass emergence. 20 individuals were killed by the sunray and 19 individuals died in pool till 17 o'clock of this day.

The above mentioned results can be summarized as bellow. (1) Earthworm's mass emergence is observed for the period from May to July every year. Earthworm's mass emergence is always generated on the fine day after a heavy or a middle rainfall. (2) On the cloud day after rain with low temperature, few individuals creep out over the soil surface and few individuals die. (3) For a week after the mass emergence, only few earthworms dead in spite of the fine day after rain. (5) The number of dying earthworms after mass emergence was far more abundant in 1972 compared with 1971. Moreover, earthworm's mass emergence started 30 days earlier in 1972 of 1971.

The supplemental observation on the mass emergence was made for the experimental population established on the clay sand in 1973. Figure 7-4 shows the result on the supplemental observation. On June 19, it was warm and fine after rainy day. 6 death individuals of earthworm were observed on this day. It is equally true in 1973 for mass emergence of earthworm to occur usually on fine days after rain.

## **2. Daily fluctuation in soil respiration**

Figure 7-4 shows the daily fluctuation of soil respiration. The soil respiration rate was 1225.3 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> on May 14. It was rainy day and low soil temperature on May 16 (18.5 °C). The soil respiration on May 16 was 621.9 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>. On May 18, it was fine day after rain. The soil respiration rate on this day attained to 1215.8 mg CO<sub>2</sub> m<sup>-2</sup>. This rate continued until May 21 (three days after rainy day). After that, the rate decreased to 591.2 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup> until May 26. The water content of soil decreased from 31.1 % to 24.4 % in the period from May 16 to May 24. On May 29, it was two days later rainy day and the water content of soil was 31.7 %. The soil respiration on this day showed the higher rate (1147.4 mg CO<sub>2</sub> m<sup>-2</sup>h<sup>-1</sup>). This higher rate maintained

until June 1. After 1 June, the rate decreased slowly and attained to  $666.6 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  on June 4. The water content of soil decreased from 31.7 % to 28.8 % in the period from May 29 to June 4. On June 6, it was rainy day. The soil respiration showed the lower rate of  $646.6 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  on this rainy day. On June 7, it was fine day after rain, the soil respiration rate increased to  $957.1 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ . On June 18, it was fine day after small rain. The soil respiration on this day showed the higher rate ( $1263.2 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ ). After, the rate decreased from  $1263.2$  to  $656.6 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  in the period from June 18 to June 25. The water contents of soil decrease from 29 % to 25 % in those days. On June 26, it was rainy day when soil moisture was 30 % and soil surface temperature was  $22.1 \text{ }^\circ\text{C}$ . The rate on this day was  $423.7 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ . This rate was the lowest value through the measurement time.

The above results can be summarized as bellow. (1) In rainy days, soil surface temperature is low and soil moisture is high. The soil respiration rate in those day is low (May 16, June 6 and June 26). (2) On fine days after rain, it is warm and soil moisture is high. The soil respiration rates in those days are high (the periods from May 18 to May 21, from May 28 to June 1 and from June 6 to June 7, and June 18). (3) On fine day of several later day after rain, soil moisture is low. The soil respiration rates in those days are low (the periods from May 23 to May 26, from June 3 to June 4 and from June 20 to June 25).

The soil respiration rate fluctuated between  $424.0 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  and  $1304.0 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ . The average rate was  $857.12 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ . Otherwise, the respiration rate of the control cylinder fluctuated between  $104.0$  and  $601.28 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ . Its average rate was  $409.6 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$ .

Ten earthworms of *Pheretima* sp. (H-1) each being 1 g fresh wt and 30 g dry wt litter were laid on the soil surface in the observation cylinders on April 24, 1974. Its soil surface had consisted of the layer of 1.0 cm - 1.5 cm wormcast by May 8.

The respiration of ten earthworms of 1 g fresh wt at field temperature was estimated at  $124 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  (chapter 3). This rate is attained to 20 % of the soil respiration rate inner the cylinders. Many dead bodies were found on the soil surface of inside of cylinder before May 8. Then, the contribution of ten earthworms to respiration might decrease with time pass. This mean that the excess respiration was caused largely by the microbial population contained in wormcast.

Tiba and Tutumi (1967) reported  $200\text{-}500 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  of respiration rate of the forest in Japan. Kirita (1971 d) reported  $600\text{-}800 \text{ mg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$  of respiration rate of evergreen forest in summer. The measurements in the present study were made in the daytime from 12 o'clock to 17 o'clock, but not through a day. The higher rate in the present measurement may be partly due to the time zone with high temperature in daytime, in present study.

### 3. CO<sub>2</sub> expiration rate of the soil-litter mixture and the wormcast

Figure 7-5 shows the correlation between the CO<sub>2</sub> expiration of wormcast and that of

soil-litter mixture. CO<sub>2</sub> expiration was higher in wormcast than in soil-litter mixture. Average CO<sub>2</sub> expiration rate of wormcast was attained to several fold of that of soil litter mixture.

Water content of materials may be an important factor controlling the soil respiration rate. The water content ranged from 6.0 to 36.3 % in wormcast and from 3.9 to 24.5 % in soil litter mixture. Figure 7-6 shows the correlation between soil respiration rate and water content. Following equations represents the relations

$$Y1 = 1.282 X - 7.665 \quad N=10 \quad r=0.736 \quad \text{at } 40 \text{ } ^\circ\text{C} \quad (7-1)$$

$$Y1 = 0.267 X + 12.27 \quad N= 6 \quad r=0.287 \quad \text{at } 30 \text{ } ^\circ\text{C} \quad (7-2)$$

$$Y1 = 0.48 X - 3.096 \quad N= 4 \quad r=0.7781 \quad \text{at } 20 \text{ } ^\circ\text{C} \quad (7-3)$$

$$Y2 = 0.639 X - 3.11 \quad N=10 \quad r=0.916 \quad \text{at } 40 \text{ } ^\circ\text{C} \quad (7-4)$$

$$Y2 = 0.45 X - 2.85 \quad N= 6 \quad r=0.9878 \quad \text{at } 30 \text{ } ^\circ\text{C} \quad (7-5)$$

$$Y2 = 0.27 X - 2.043 \quad N= 4 \quad r=0.8717 \quad \text{at } 20 \text{ } ^\circ\text{C} \quad (7-6)$$

where, X is the water content (%), Y1 is the CO<sub>2</sub> expiration rate of the wormcast (CO<sub>2</sub> g<sup>-1</sup> dry wt h<sup>-1</sup>), and Y2 is the CO<sub>2</sub> expiration rate of the soil litter mixture. Soil respiration rates were largely depended on the water contents in both materials except the rate of wormcast at 20 °C. The grades of linear equation at 40 °C and 20 °C was two fold in wormcast than in soil litter mixture. These results mean there are another factors besides water content to control the rate of soil respiration. The regression coefficient of wormcast between soil respiration and water content showed the unreliability compared with those of soil litter mixture. The low reliability of the equations of wormcast might be due to the age of cast. Perhaps, the age of cast might have an important effect upon the microbial density. However, the age of the wormcast could not be determined.

#### 4. Micro organisms in earthworm intestine

Bacteria in different sections of the gut were counted in 25 samples of *Pheretima* sp. (H-1) in group of five. The result is summarized in Table 7-1. Bacteria increased 3-5 fold during the passage of materials through the intestine of *Pheretima* sp. (H-1) (Table 7-1).

Parle (1963 a) reported that actinomycetes and bacteria, but not fungi increased rapidly during the passage of food through the earthworm gut and filamentous fungi and yeast increased rapidly after the cast was produced. He said that the wormcast produced have more plentiful flora of microbial population than the control soil do so. Moreover, Parle reported that oxygen up take by cast material was still considerably higher than that of soil after 50 days. In the habitat of *Pheretima* sp. (H-1), the wormcast showed the higher rate of respiration than the rate of soil litter mixture (Fig. 7-6 and Table 7-1). The higher respiration rate of the wormcast presented in the habitat of *Pheretima* sp. (H-1) might be due to the bacteria increased during the passage of material through earthworm intestine and, perhaps, of fungi and east which might increase after the cast was produced.

Parle (1963a) reported that bacteria and actinomycetes were up to 103 time in gut of *Lumbricus terrestris* L. than in surrounding soil. Otherwise, bacteria increased to 3-5 time during the

passage of material through the gut of *Pheretima* sp. (H-1). The difference in the increasing rate of microbial population between two species may be caused by the difference in passing time of material through the gut of each earthworm species. The material remains in the gut of *L. terrestris* for about 20 hr. (Parle 1963a) and in the gut of *Pheretima* sp. (H-1) for about 4-5 hr. Gut turn over rate of *Pheretima* sp. (H-1) was calculated as the ratio of maximum weight of gut content to fecal pellet production per a day. Parle (1963a) considered that the length of time the food remains in the gut of *L. terrestris* would not allow the break down of cellulose by microbial action and the organism present did not help earthworm to ingest its food. It is equally impossible for the bacteria in *Pheretima* sp. (H-1) to help the digestion of earthworms because of shorter time of the food remain of this species than that of *L. terrestris*.

##### **5. The response of earthworm to various gases**

Figure 7-7a,b show the response of earthworm to Carbon dioxide gases. In the experiment with 2.0 l of CO<sub>2</sub>, a precipitous action of earthworm occurred in 2 -3 minute later the opening of flux. 80 % of specimens abruptly creep out over the wormcast and rush out in 3 - 14 minute later of opening (Fig. 7-7a). In the experiment with 1.5 l of CO<sub>2</sub>, the rushing out individual appeared at 3 minute later and continued till 10 minute later of opening. The peak of the rushing out individuals appeared at six minute later of opening (Fig. 7-7a). In the experiment with 1.0 l CO<sub>2</sub>, the rushing out individuals appeared in 5 - 11 minute later after opening (Fig. 7-7a). In the experiment with 0.8 l CO<sub>2</sub>, only one individual among 5 test specimens creep out abruptly and rush out (Fig. 7-7a). Other individuals smoothly creep out over the wormcast for a while and creep into the wormcast layer again (Fig. 7-7b). In the experiments with 0.5 and 0.25 l CO<sub>2</sub>, only 4 individuals (among 40 individuals) or 1 individuals (among 20 individuals) rush out from the chamber, respectively (Fig. 7-7a). Other earthworms smoothly creep out, and expose their body on for a while and crawl in to cast layer (Fig. 7-7b). In the experiment with 0.125 l CO<sub>2</sub>, only one among 10-specimen creep out smoothly and crawl into wormcast again. Other specimens showed no behavior response to this concentration ((Fig. 7-7b). At the end of each observation, the activity of earthworm was examined by poking the body of earthworm with a wood lot. The earthworms remaining in cast layer, among the specimens exposed with 2.0, 1.5 and 1.0 l CO<sub>2</sub>, did not moved by poking. The individuals remaining in cast layer, among the specimens tested with 0.667, 0.5, 0.25 and 0.125 l CO<sub>2</sub>, were vigor by poking. The above results reveal the negative chemotaxis of *Pheretima* sp. (H-1) to CO<sub>2</sub> gas and the harmfulness of this gas to earthworm.

The continuously flowing N<sub>2</sub> gases (30-80 l h<sup>-1</sup>) did not brought the violently behavior of earthworm (Fig. 7-8). The 30 l h<sup>-1</sup> flowing rate of N<sub>2</sub> gas brought no behavior response of earthworms for 18 minute. With the 80 l h<sup>-1</sup> flowing rate of nitrogen gas, test specimens creep out smoothly over wormcast at five minute later. The number of the earthworm staying over wormcast increased with time passed. 37 % of total length was on the surface of the wormcast at 18 minute

later after the beginning. After the observation, all test specimens were very vigor. The above results showed that Nitrogen gas was harmless for earthworm. Some behavior response of animal observed at the higher flowing rates ( $40-80 \text{ l h}^{-1}$ ) was due to the strong current of Nitrogen gas.

### Discussion

#### 1. The factor bringing about the mass emergence of earthworm

The probable stimulus for mass emergence of earthworm were parasite, natural enemy, the change of soil temperature, the decline of oxygen tension in soil and the outbreak of harmful gases (Ohfuchi, 1947).

The inner parasite ameba and the outer parasite as like the diptera reported by Nakamura (1968) did not present on the surface of the dead bodies collected on the experimental field. A mole, the most important natural enemy for earthworm, was destroyed with traps by January 1971. After, they had never appeared in the experimental field. Then, parasite and natural enemy may not induce the mass emergence of earthworm.

The mass emergence of earthworm occurred usually on fine days after rain, when soil temperature was arising gradually (Fig. 7-3). Then, the striking of the low temperature is not a factor to cause mass emergence of *Pheretima* sp. (H-1). However, the author observed many dead bodies of *Pheretima heterochaeta* (Michaelsen) on bare ground of a certain field on frost day Nov. 1973. Then, the striking of low temperature may be able to induce the mass death of some earthworm species.

The mass emergence of *Pheretima* sp. (H-1) appeared on fine days after rain but did not on rainy days (Fig. 7-3). The continuously inflow of  $\text{N}_2$  gas gave a slight and reversible effect on earthworm (Fig. 7-8). Earthworm is enable to live for several days in water or under pure  $\text{N}_2$  gas (Ohfuchi 1947). Then the decrease of  $\text{O}_2$  tension caused by rainfall was not a stimulus to cause the mass emergence of earthworm.

The soil respiration rate increased on fine days after rain (Fig. 7-4). The mass emergence of earthworm in field begun to occur mainly on four - five hour later of end of rain.  $\text{CO}_2$  gas, even with low tension, induced a creeping out behaviour of earthworm (Fig. 7- 7a, b). The high concentration of this gas killed earthworm in a few minutes in the experiment of chemotaxis of *Pheretima* sp. (H-1). Then, as mentioned by Nishida (1950), the increase of  $\text{CO}_2$  tension of soil air on fine day after rain must be the most important stimulus to cause the mass emergence of earthworm.

#### 2. How concentration of $\text{CO}_2$ gas induce the behavior response of earthworm

In the experiment of chemotaxis of *Pheretima* sp. (H-1), earthworms showed some behavior response to the lowest concentration of gaseous carbon dioxide. Some individuals showed the shock response as like jumping to less than 20 % concentration of  $\text{CO}_2$  gas ( $0.25 \text{ l CO}_2$ ). In these concentration of  $\text{CO}_2$ , the peak of response appeared in the time between 8 and 13 minute later after opening. After the peak of behavior response, some earthworms creep into the wormcast again. The

specific gravity of CO<sub>2</sub> gas is 1.5 fold of that of air. CO<sub>2</sub> gas was poured into the box with a very slow rate (0.5 l minute<sup>-1</sup>). The gas was sending out from the porosity plastic ball in the experiment of chemotaxis of earthworm to CO<sub>2</sub> gas. Then, CO<sub>2</sub> gas might accumulate first on the bottom of the diffusion chamber. After, the gas might diffuse to the outer air through the wormcast layer at a very slow rate. The CO<sub>2</sub> concentration at the bottom of wormcast layer would attain to the peak at several minute later opening. Then, the CO<sub>2</sub> concentration at the bottom of wormcast layer in the observation chamber may be less than the concentration estimated from the ratio of the poured CO<sub>2</sub> gas to the volume of diffusion chamber. Siraishi (1954) reveal that *E. foetida* showed some behavior response by exposing CO<sub>2</sub> gas diluted 16 fold with air (6 % concentration) directly to the earthworm's body. Then, *Pheretima* sp. (H-1) would show some response to CO<sub>2</sub> gas far less than 10 % concentration by exposing the gas directly to earthworm's body.

### 3. CO<sub>2</sub> concentration in soil

Now, we have a few knowledge on CO<sub>2</sub> concentration in soil. Extreme limits of CO<sub>2</sub> concentration in soil range between 0.01 and 11.5 % (Satchell 1967). Nishida (1950) reported 2.29 - 2.65 % CO<sub>2</sub> tension in 10 cm depth soil on several hours later rain and 1.29 - 1.7 % on other day. He guessed 4.6 - 5.3 % of CO<sub>2</sub> in 10 cm depth soil air with the reason of the mixing of out air in the collected air. The rate of oxygen diffusion through water is only about one ten-thousandth as fast as the rate through air (Clark 1967). On and after rainy day, soil spaces are saturated with water and vapor. The diffusion rate of various gases in soil is lower in wetter season than in dry season. Then, the CO<sub>2</sub> gases produced by soil microorganism are caught by soil water particularly under wetter season. Then, the higher soil respiration rate on fine days after rain may induce the accumulation of large quantity of CO<sub>2</sub> in soil water, on these days than other days. The CO<sub>2</sub> concentration of soil air on those days may be enough to cause the mass emergence of earthworm (5-10%).

### 4. Why is the soil respiration high in the day of the rainy rising fine?

The soil respiration rate on fine days after rain attained to two fold of the rate on dry days or rainy days. On fine days after heavy rain, the high respiration rate was kept for four or five days. The respiration rate decreased with the decrease of water content of soil on fine days after small rain (Fig. 7-4).

Kirita (1971 d) listed the factors which might causes the increase of soil respiration rate on fine days after rain as follow (1) Rain fall push the soil air containing the high concentration of CO<sub>2</sub> gas, (2) Rain fall stimulate the activity of soil microorganism.

If rainfall push up the carbon dioxide gas accumulated in soil air, the soil respiration rate would increase on rainy days. However, the soil respiration rate on rainy days or dry days were only half the rate on fine days after rain (Fig. 7-4). These results mean that pushing out the accumulated CO<sub>2</sub> gas did not cause the increase of soil respiration on fine days after rain.

Following equation can represent the soil respiration rate in this field

$$\text{CO}_2 \text{ mg m}^{-2}\text{h}^{-1} = 0.012384 X_1 \times 1.3589X_2 \times 1.22484 \quad (7-7)$$

Where  $X_1$  is moisture content of soil and  $X_2$  is soil temperature. This formula show that the soil respiration rate depend on moisture and temperature condition. Witkamp (1966) pointed out that the soil respiration highly related to the microbial density in addition to moisture and temperature. The activities of microbial organism depend largely on temperature and moisture (Witkamp 1966). Then, the higher rate of soil respiration and the higher  $\text{CO}_2$  concentration in soil air on fine days after rain is due to the increase of microbial activity in those days. The half of the soil respiration in the habitat of earthworm due to the respiration of wormcast (Fig. 7-4), and the higher respiration rate of wormcast was caused by the activity of bacteria increased during the passage of material through the earthworm's intestine and, perhaps, of fungi and east increased after the cast produced, in addition to the larger water holding capacity of wormcast (Fig. 7-5 and Fig. 7-6, and Table 7-1). These result may mean that the soil respiration is much larger in the surrounding area of earthworm's habitat than in other area. The wormcast observed in study area attained to the maximum of 7440 g dry wt  $\text{m}^{-2}$  in June. This mean that the soil respiration rates or the  $\text{CO}_2$  concentration of soil air in the earthworm's habitat are most high on fine days after rain in June. The estimated days of the peak rate of the soil respiration coincides the occurrence days of mass emergence of earthworm. These mean that the occurrence of mass emergence of earthworm highly related to the quantity of wormcast, besides weather conditions.

##### **5. Meaning of mass emergence and large amount of the death in the population study**

The mass emergence of *Pheretima* sp. (H-1) on fine day after rain was followed by the density decrease of this species. The decrease rate of the density between late May and July was more higher than in other periods (Chapter 1). These results show that the mass emergence on fine days after rain and the death of individuals after mass emergence were most important mortality factor for earthworm: *Pheretima* sp. (H-1) in area D.

The population metabolism of *Pheretima* sp. (H-1) was most active in early summer (Chapter 1, 3). In area D, the litter consumption of litter feeders: *Pheretima* sp. (H-1) and *Ph. vittata* was attained to 1612.1 g dry wt  $\text{m}^{-2}$  by May (Chapter 1 and Chapter 4). Otherwise, annual primary production was 1176.5 g dry wt  $\text{m}^{-2}$  (Chapter 1 and Chapter 4). On clay sand area in the experimental field, there were 105.6 of maximum density of *Pheretima* sp. (H-1) per  $\text{m}^2$  and 456 g dry wt of the decaying straw mats per  $\text{m}^2$  on Feb. 15, 1972. Where, the decaying straw mats disappeared by late April 1972 (Chapter 11). These results may mean that the severe food shortage for earthworms would occur at least before early June.

The start of mass emergence was 30 days later in 1971 than in 1972 (Fig. 7-3). The maximum biomass of the 1971 year population was equivalent to 50 % of the maximum biomass of the 1972 year population (Chapter 1). These mean that the size of mass emergence related to the size of population metabolism and to the degree of food shortage.

The mass emergence was observed mainly on first fine day after rain, although the high respiration rate of wormcast maintained for several days (Fig. 7-3 and 7-4). Few individuals were killed by the sun ray though the day of clear weather after a rain came within several days again after mass emergence of a large scale was observed (Fig. 7-3). These facts show that the mass emergences occur usually after the relatively long period of dry condition. The occurrence of mass emergence may relate to the Assembly State of earthworms besides the increase of soil respiration on fine days. Under the dry condition of soil, earthworm would conform an aggregation to avoid dryness in narrow area. In there, earthworms piled up the more quantity of fecal pellets and build up the thicker wormcast. The wormcast have high water holding capacity and showed relatively low respiration rate under dry condition (Fig 7-5). Then, the thicker wormcast may be a relatively favorable habitat of earthworms under dry condition. Namely, the individuals in thicker wormcast enable to avoid dryness but are under severe food shortage in the thicker and dry wormcast. On fine days after rain, soil respiration or CO<sub>2</sub> concentration becomes to be larger in wormcast than in surrounding soil. Then the wormcast become to be unfavorable habitat for earthworm in addition to the severe food shortage condition of earthworm. The above mentioned inference may explain the mass emergence occurred after long period of dry condition. This means that the appearance of the mass emergence highly related to the character and quantity of wormcast. Wormcast have a high water holding capacity and show a higher respiration rate on fine days after rain. Moreover, wormcast were abundant in the surrounding area of the earthworm habitat. As earthworms produced wormcast, the accumulations of fecal pellets as wormcast mean the biological conditioning of the earthworm's habitat. Then, the decrease of density after the mass emergence was regarded as self-regulation.

By the decrease of population density, earthworm can avoid more or less the food shortage. The population may control themselves to adaptive density by this dispersion. Wormcast decayed with rainfall and other factor (Chapter 9). The survivor in wormcast might gain the food from the decayed wormcast (Chapter 9). If the emigrating and surviving individuals can gain the newly habitat, this species would extend their distribution area. The above mentioned process is summarized as Fig. 7-9.

### Summary

1) The observation on mass emergence of *Pheretima* sp. (H-1) were carried out on the bare land in the experimental field of Department of Biology Kyushu University in the period from May to July for two years 1971 and 1972. The mass emergence of *Pheretima* sp. (H-1) occurred frequently on fine day after rain. Several individuals was killed by solar radiation on bare land. Few individuals died on other days.

2) The response of *Pheretima* sp. (H-1) to harmful gases was examined under experimental condition. Earthworm showed the abruptly creeping out behavior even to the small increase of CO<sub>2</sub> tension. They showed no behavior response by the continuously exposing of pure Nitrogen gas.

3) The soil respiration rate on fine day after rain was two fold of the rate on rainy days or dry days. The increase of soil respiration rate in soil air on fine days after rain was the factor inducing the creeping our behavior of earthworm.

4) Bacteria increased to 3-5 fold during the passage of materials through the intestine of *Pheretima* sp. (H-1). It was inferred that the wormcast had more plentiful flora of microbial population than the control soil.

5) Worm cast showed higher soil respiration rate and larger water holding capacity than those of control materials. The higher respiration rate of wormcast might be due to the high microbial activity besides the high water holding capacity of the materials. The warm and wet condition on fine days after rain stimulated the activity of microbial populations contained in wormcast and then the soil respiration rate increased.

6) CO<sub>2</sub> expiration rate was higher in wormcast (earthworm's habitat) than in other areas. By the reason that earthworm piled up the pellets on soil surface most abundantly in early summer, soil respiration in earthworm's habitat might be highest in this season through a year. This might be the reason why mass emergence of earthworm occurred only in this season.

7) Earthworm adjusted their density to the adaptive density by the dispersion of individuals on fine days after rain. As the biological conditioning of the earthworm's habitat caused the mass emergence of earthworm, the mass emergence of earthworm on fine days after rain was regarded as the self regulation.

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## Part One Chapter seven

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