THE BIOLOGY OF THE GREY GARDEN SLUG *DEROCERAS RETICULATUM* (MÜLLER),

by

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INTRODUCTION

The grey garden slug, Deroceras reticulatum (Müller), is of great economic importance in the Willamette Valley of Oregon. The versatile feeding habits and the adaptability of this species to its environment make it difficult to control. The grey garden slug is a serious pest to truck and field crops, small fruits, greenhouse plants and ornamentals. The mild winters and generally moist springs of western Oregon are particularly favorable for the growth and reproduction of the slugs. In the United States, damage occurs in the northern areas; the slugs being of little or no economic importance in the south. The literature concerning the grey garden slug is mainly from the United States, Canada, England, France, and Germany.

The grey garden slug was first described in Europe by Müller (48, p. 10) in 1773 but had been known in England since 1674. It was first reported in the United States in 1843 by DeKay (29, p. 20-21) near the seaports of Boston, New York and Philadelphia. Cocherell (20, p. 70-71) recorded this species near Portland, Oregon, in 1891.

Morphologically, the slugs present a peculiar situation. The bilateral symmetry characteristic of allied forms is lacking. The organs of respiration, circulation, excretion and reproduction are fully developed on the right side only. The corresponding organs on the left side are functionless and have no openings to the exterior. Slugs are hermaphroditic in nature, each individual being capable of laying eggs, and reach sexual maturity before attaining full growth.

Slugs have a soft, sensitive, moist skin provided with numerous minute glands from which a mucus slime is expelled. The slime has been found to be 98 per cent water (28, p. 181). This mucus is clear when exuded on objects over which the slugs glide but becomes turbid and sticky and is produced more copiously when the slug is disturbed by mechanical or chemical means. The milky color of the mucus exuded by a disturbed slug is said to be due to the presence of finely-divided calcium carbonate (61, p. 105). Production of the viscous slime is the slug's sole means of protection.

The primary objective of the present study was to clarify some of the contradictory observations found in the literature as to the biology of Deroceras reticulatum. The second objective was to develop a laboratory rearing method for the maintainance of a slug culture for toxicological studies.

NOMENCLATORIAL HISTORY

The grey garden slug belongs to the phylum Mollusca and to the class Gastropoda which includes both slugs and snails. This slug belongs in the sub-class Pulmonata, order Stylommatophora and in the family Limacidae, the members of which have no external shells.

Because of its wide distribution and varied coloration, the grey garden slug has been described under many generic and specific names. This has resulted in considerable taxonomic confusion. Cockerell (21, p. 175-176) cited 35 synonyms for Agriolimax agrestis. Pilsbry (52, p. 532-535) gave a rather complete synonymy of this group. He stated that the name Agriolimax, which has been used universally for the genus of field slugs, can be preserved as a generic name only by making it a "nomen conservandum", as there are several earlier names for members of the genus. Pilsbry favored the use of the earliest name, Deroceras, which was proposed in 1820 by Rafinesque (53, p. 10).

The grey garden slug was formerly known, and is still referred to by some workers, as Limax or Agriolimax agrestis L. The earliest name consigned to this species was Limax reticulatus by Müller in 1774 (48, p. 10); but according to Cockerell (20, p. 70-71), Limax reticulatus was a color variety of Agriolimax agrestis. Pilsbry stated (52, p. 538) that the true agrestis is a north European slug of Scandinavia, Finland and Northern Russia; and to his knowledge, does not occur in England or in the United States.

The scientific names currently used by various workers for the same species are Agriolimax agrestis, Agriolimax reticulatus and Deroceras reticulatum. The name Deroceras reticulatum (Müller), as recommended by Pilsbry (52, p. 532-535) and approved by the Entomological Society of America (22, p. 33), will be used in this study.

Determinations of specimens used in these studies were made by C. Dallas Hanna, California Academy of Sciences, as Deroceras reticulatum (Müller).

DESCRIPTION OF STAGES

The following descriptions of the stages of Deroceras reticulatum Müller have been modified from the descriptions of various authors including Pilsbry (52, p. 535-538), Carrick (15, p. 571-574), Taylor (61, p. 105-106) and Lovett and Black (43, p. 9-12).

The Egg

There is considerable variation in the size and shape of the eggs of Deroceras reticulatum, but those of the same clutch (Figure 13) are usually uniform. The volume of the egg fluctuates with changes of humidity, for the albuminous matter absorbs and gives up moisture readily. The normal egg is approximately 2 mm. long and 1.8 mm. in width. Spherical eggs, 1.6 mm. in diameter commonly occur. A few abnormal eggs are over 3 mm. long and 2 mm. wide. The above dimensions are of eggs rendered turgid by keeping them in contact with moist filter paper. The weight of the egg averages 6.4 mg. with a range of 5.2 to 7.8 mg.

The surface is roughly granulose, due to the presence of small calcium concretions embedded in the outer gelatinous coat. At each end of the egg is a short, tapering thread, or microphyle, and successive eggs are normally united by these threads to form a string of eggs.

The newly-laid egg is transparent. The inner structures of the egg can readily be seen when it is placed under water and viewed by transmitted light. Inside the outer membrane, there is an inner

membrane which surrounds a mass of faintly bluish albumen, the medium in which the embryo develops. This medium in a fertile egg contains a twisted membranous structure which Carrick (15, p. 563) called the remains of the sperm body.

The Immature Slug

The immature slug is very similar to the adult in form and coloration but differs, of course, in size. The newly-hatched slug is almost colorless. After a few hours, the eye peduncles show blackened areas; and the slug takes on a grayish-pink color. When the immature slugs begin feeding, there is a rapid transformation from a light to a dark coloration and the reticulation of the mature form appears. Sexual maturity is attained before the slugs reach maximum size.

The Adult

The grey garden slug (Figure 1) is the smallest slug commonly found in Oregon. It is rather stout, nearly cylindrical in form and somewhat keeled dorsally near the posterior extremity. The mature slug, when in motion, is about 35 mm. and up to 50 mm. in length. The mantle is concentrically striated and the rest of the integument has long, low tubercles. The upper surface is whitish, cream or flesh colored. It is either almost uniform or has gray flecks on the mantle and gray or blackish irregular markings or reticulations elsewhere. Sometimes the surface is without markings or, rarely, quite black. The sole is longitudinal, narrow, and is tripartite,

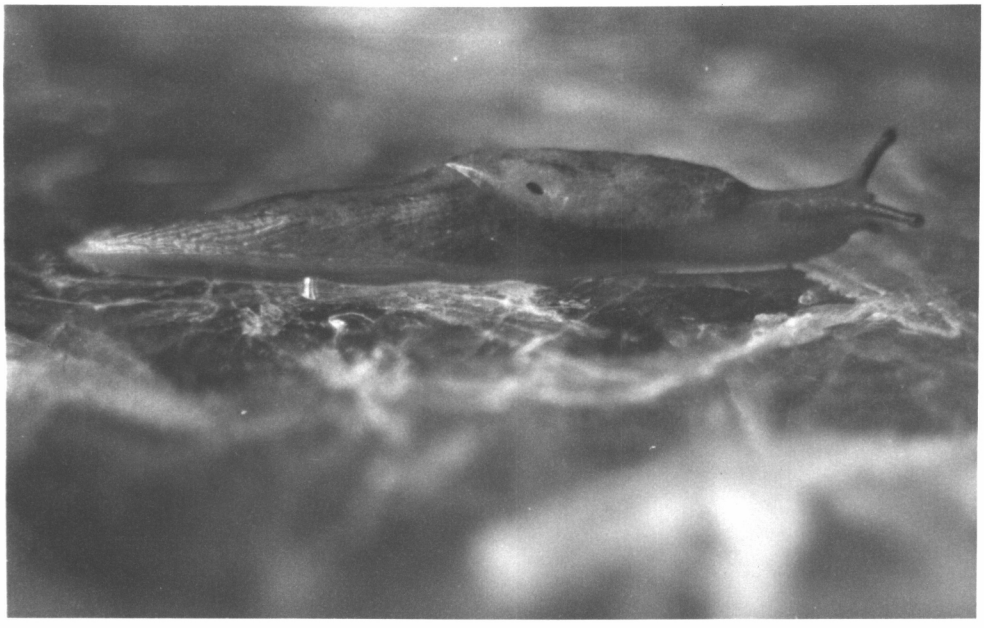

Figure 1

Deroceras reticulatum adult (X 3.6)

whitish or dirty yellow with the median field being gray. The mantle is prominent, fleshy and is more than one-third the total length of the body. The breathing pore is located on the right side of the posterior fourth of the mantle and is marked by a prominent cleft in the mantle. A characteristic of this species is that the breathing pore has a raised, pale border with no pigmented areas. The neck is slender and is usually of a light greenish-yellow color. The upper tentacles are black at the tip with an indistinct black line extending from the tip back through the neck to the anterior edge of the mantle.

The color markings are highly variable and have given occasion for a long list of varietal names, as compiled by Cockerell (20, p. 70-71). The normal color markings of the Deroceras reticulatum forms commonly found in the Willamette Valley are deep mottled gray, greenish gray flecked with brown or dark brown. Due to the great variation of color and pattern, Simroth (56, p. 312) contended that dark color was the normal reaction to cold climate, that high mountains gave black forms, dry deserts reddish-yellow and that southern temperatures produced lighter and brighter colored slugs. Exceptions to the temperature control theory appear to be numerous in our fauna since dark and light forms, with gradations in between, have been collected in the Willamette Valley in the same location.

Because of the unreliable nature of external characteristics and color, the leading authorities now base species determination largely on internal characters. Taylor (61, p. 105-106) gave an

excellent systematic description of the grey garden slug as follows:

"ANIMAL limaciform, with large but flattened tubercles; of a somewhat uniform whitish or pale ochreous ground color, but sometimes dull lavender or other tint, often mottled, speckled or reticulated with brown or black, and at times totally suffused with black; BODY somewhat compressed and keeled toward the tail; TENTACLES dark colored; SHIELD more than one-third the total length of the animal, rounded in front and behind, concentric striae not deep, with the nucleus on the right side and towards the rear; RESPIRATORY ORIFICE with a broad, usually unpigmented, raised ring which is cut anteriorly by the anal cleft; SOLE pale and longitudinally tripartite, the side areas sometimes darker, especially towards the tail; SOLE-FRINGE separated as usual from the body by a furrow, containing a row of elongate tubercles, upon which the body tubercles rest unconformably. MUCUS plentiful and viscous, often clear when crawling, but becoming milky white on irritation, due to innumerable particles of carbonate of lime. Length usually about 35 mm.

SHELL white, oblong-oval in shape, somewhat convex above and correspondingly concave below, usually rather thin; NUCLEUS distinct and placed towards the left side of the posterior margin of the shell; concentric lines of growth perceptible, margin membranaceous. Length, 4 mm; width, $2\frac{1}{4}$ mm.

THE ALIMENTARY CANAL is triodromous, composed of the stomach tract and three intestinal coils; the ingestive TRACT is the shortest, the OESOPHAGUS is also short, and the voluminous CROP of a light-brown color, thin, and scarcely furrowed, having the long and much-indented SALIVARY GLANDS adherent to its sides. The last tract or rectum has, about midway, a short coecum on the right side generally directed backwards, and laid upon the upper surface of the crop. The DIGESTIVE GLAND is of an ochreous color, the right lobe extending quite to caudal end of the body, and in common with the whole intestinal mass has been subjected to a noticeable spiral twist in such a way as to indicate than an external shell if present would be a dextrally-coiled one.

The REPRODUCTIVE ORGANS have their orifice about two millimetres behind the right ommatophore; the OVOTESTIS is comparatively enormous, and laid upon the upper surface of the digestive gland, the acini being light-brown, with darker connective tissue; DUCT short and inflated, buff to creamy white, with a slender VESICULA SEMINALIS; ALBUMEN GLAND with few lobes, and of a light-brown or slate color; OVISPERMATODUCT broad above, narrow below; OVIDUCT with ample folds, free oviduct short, with a yellowish glandualr investment; SPERM DUCT slender above, more compact and broad below; VAS DEFERENS short, and entering penis-sheath laterally near the free-end; PENIS-SHEATH a broad, irregular, and medially constricted sac, with narrow outlet, distal-end with a clawlike digitate gland, which varies greatly in size and complexity each segment or digit having on its concave side a row of papillae. The lower half of the sheath contains a conical and fleshy SARCOBELUM or excitatory organ, which is often pigmented at the tip; the RETRACTOR is a short, broad band, arising from the lung floor, to the right of the mid-dorsal line, anterior to both kidney and pericardium, and is attached, not to the apex, but to the middle of the penis-sheath at the same side as the point of entry of the vas deferens; SPERMATHECA fusiform or claviform, attached closely to the base of the oviduct, stem slender, and opening into the narrow and thick-walled ATRIUM, at the junction of the oviduct and penis-sheath.

The MANDIBLE or jaw is crescentic in shape, with well and bluntly rounded ends; it is a millimetre or more in width, moderately arcuate, of a yellowish-brown color and smooth in texture but showing several distinct darker lines parallel with the upper and lower margin; median beak or rostrum not prominent, but somewhat acute, its vertical carina not well marked."

EXPERIMENTAL METHODS AND MATERIALS

Experimental Equipment

Most experimental investigations of the effect of temperature on the rate of development of an organism have been conducted with constant temperatures. No constant temperatures, however, exist in nature where animals are subject to continually varying temperatures owing to diurnal and seasonal fluctuations as well as to non-periodic changes in weather conditions. In view of this fact, developmental rates of Deroceras reticulatum, when subjected to constant and to fluctuating temperatures, were compared during the course of this study.

For the purpose of maintaining constant and variable temperatures during the experiments, two "Cenco Refrigerating Incubators"[1] and a rearing room equipped with refrigeration were used. The incubators (Figure 2) were General Electric refrigerators modified to accomodate a heating unit that consisted of a resistance wire which supplied the heat and was controlled by a Fenwal Differential Expansion Thermoswitch.[2] The heating units were equipped with a small electric fan which operated continuously for air circulation within the incubator. The desired temperature was pre-set on the thermo-switch, which maintained temperature by closing the contacts with decreasing temperature, thus activating the heating unit.

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1 Central Scientific Company, Chicago, Illinois

2 Manufactured by Fenwal Incorporated, Ashland, Massachusetts

Figure 2

Cenco Refrigerating Incubator

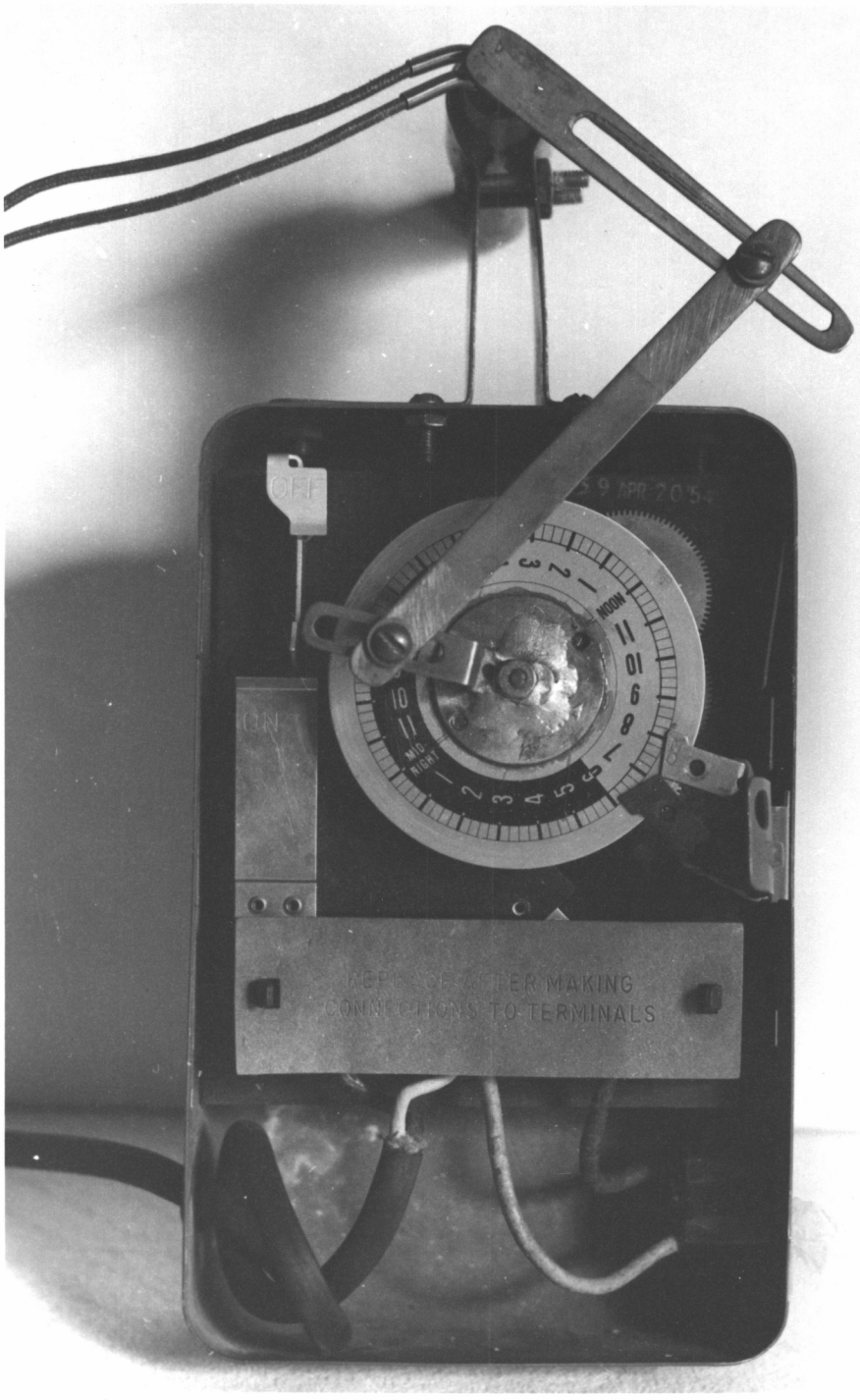
The usual approach to the technical problems of creating a fluctuating temperature condition has been to alternate two constant temperatures. The only published accounts consulted, in which a true fluctuation was attempted, were by Wishart (68, p. 78-82), Munger (49, p. 554-555) and Howe (34, p. 188-194); but the apparatuses they described were inadequate for this study with the exception of Howe's. This contrivance was modified by connecting the thermo-switch of the incubator heating unit with a 24-hour clock by means of rods (Figure 3). The cap of the thermostat, normally used to adjust the setting, was removed and replaced by a long metal arm. Regular movement of the arm back and forth by the 24-hour clock produced a cyclic temperature control. With the lengths of the clock arm, thermostat arm and connecting rod in a fixed position, only one curve with a fixed range could be obtained. This mechanism produced one full cycle of continuous change in temperature in a 24-hour period.

At the start of this study, one serious fault with the system was encountered in that the thermo-switch contacts, although having a currant rating of 10 amps—110 volts or 5 amps—230 volts AC, would stick and thus gave rise to erratic, high temperatures. This difficulty was corrected by introducing a relay into the system to carry the main current. After the above change, perfect daily cyclic temperatures were obtained throughout the study as shown in Figure 4.

The 10 X 12 foot rearing room was equipped with a one horsepower refrigeration unit that maintained temperatures of plus or minus 3°C.

Figure 3

Cyclic temperature control apparatus



This room was utilized to work out and improve rearing techniques. The bulk of the slug cultures were kept in this room at 17 to 23°C.

Temperatures were checked during the experiments with the use of bulb thermometers or recording thermographs. The thermographs were calibrated with a standard bulb thermometer. During the preliminary experiments, the relative humidity within the plastic crisper chests was checked by means of a direct-reading hygrometer.[3]

Field Collecting

The majority of the slugs used in this study were collected in fields and gardens of Eugene and Corvallis, Oregon. The slugs were collected during late afternoons, at night with a flashlight or early in the morning when the slugs were usually at the surface of the ground. During dark, cloudy, rainy periods, the slugs could be collected during most hours of the day. They were dropped into empty coffee cans by hand and transferred to laboratory dishes as soon as possible.

Termination Criteria

In the growth studies at various temperatures, it was determined that four to five weeks was sufficient time to establish the rate of increase or decrease in weight. With longer durations of time, the possibilities of slug mortality increased. In the experiments

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3 Manufactured by Serdex Inc., Boston, Massachusetts

concerning growth rates of slugs under different temperature conditions, there was often a high death rate which necessitated the early conclusion of many experiments. Due to a wide variation in growth rates among individuals of a random sample, the death of one or two of the heaviest slugs caused an abrupt drop in the mean weight figure of the slug group. Data secured on weight increases of slug groups suffering mortality was considered invalid.

Sources of Error

In the course of weighing the slugs, there was a slight decrease in weight due to evaporation and the production of slime. Since this error is equally applicable to all tests and groups, it was considered to be in the realm of allowable experimental error.

Rearing Methods

There has been a great demand for a system of rearing slugs under laboratory conditions. Biologists, parasitologists, toxicologists, entomologists, etc. have attempted to do experimental work with slugs; but due to the high mortality rate under laboratory conditions, their results have been, to a certain extent, erratic. These workers have had to depend on field-collected slugs which are abundant usually only in the fall and spring. Kozloff (38, p. 17-19), in doing experimental work with protozoa, reported that some uninfested field-collected slugs died within a week. Meggitt (45, p. 390-409), in trying to study the life history of the fowl tapeworm,

reported that he could not keep slugs alive in the laboratory long enough to do comprehensive studies. Reynolds (54, p. 48-53) reported similar experiences.

In 1954, Sivik (57, p. 129-130) reported a technique for rearing Deroceras reticulatum in a wooden box containing soil and covered with gauze. He did not state how long he could keep the slugs alive.

Various rearing containers were used during the course of the present study. Plastic crisper chests[4] (Figure 5) with dimensions of 10 1/2 x 14 x 4 3/4 inches were very effective for the stock cultures and for experiments that consisted of more than 15 slugs per group. Jars, one-pint size with metal screw-type tops, were used for experiments utilizing groups of less than 15 slugs each. For newly-hatched slugs, standard-size petri dishes were used. The plastic crispers were sterilized with Chlorox before each experiment.

Field-collected slugs were placed in the crisper chests with either sandy loam soil or Vermiculite.[5] All the soil that was used in the rearing cultures was autoclaved at 15 pounds pressure for three hours to eliminate soil organisms. Vermiculite retained moisture more satisfactorily than soil and also allowed the slugs to go beneath the surface.

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[4] Manufactured by Tri-State Plastic Moulding Company, Henderson Kentucky

[5] Manufactured by Vermiculite--Northwest, Inc., Portland, Oregon

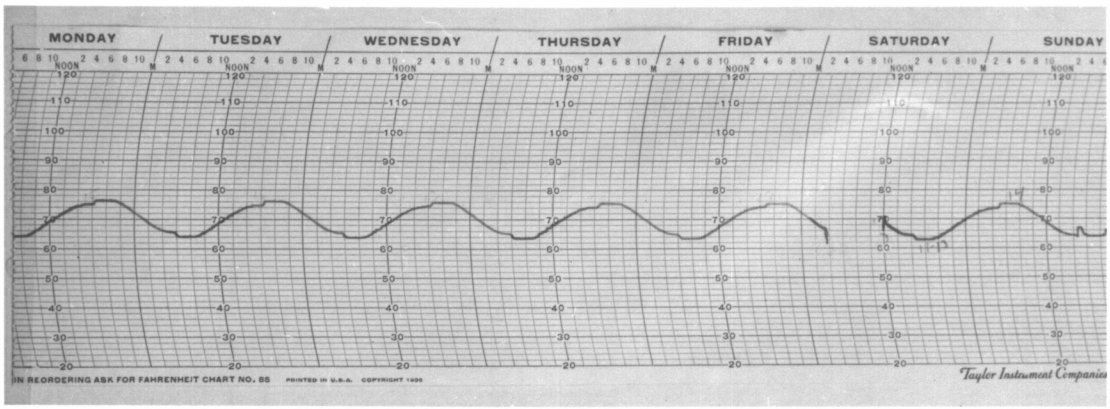
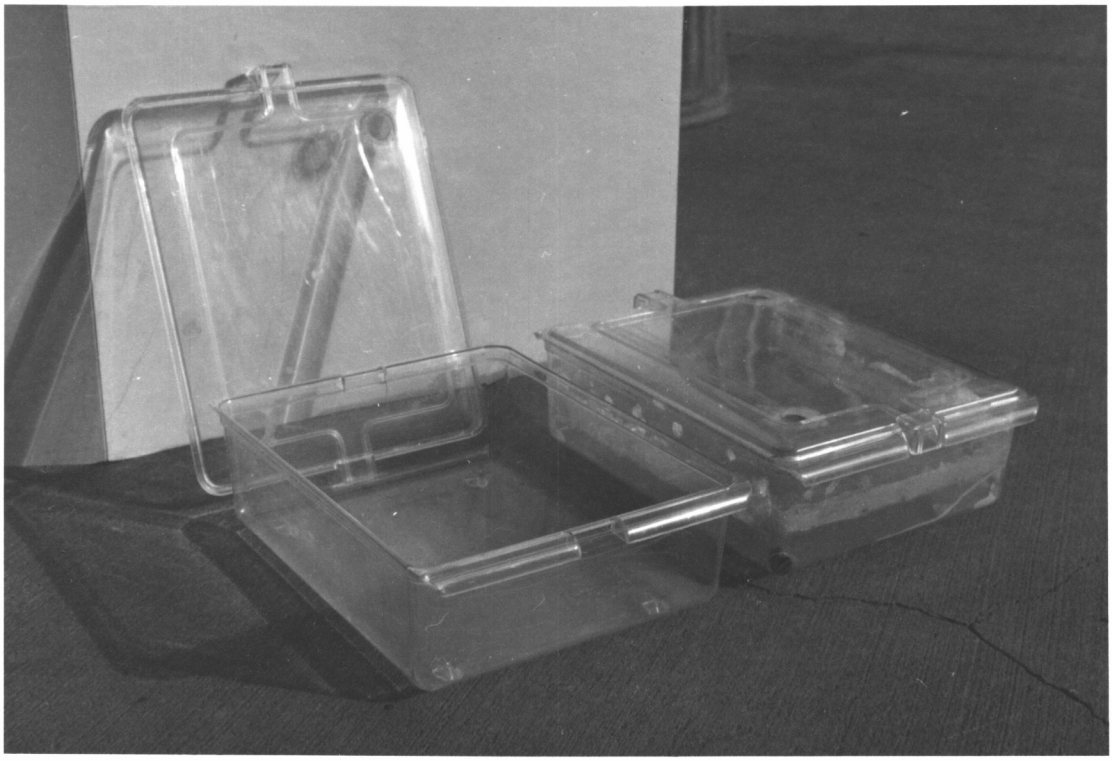


Figure 4

Thermograph chart showing daily cyclic temperatures

Figure 5

Plastic crisper chest rearing containers



Fungi and bacteria were usually present on slugs and their eggs brought in from the field. These organisms were particularly active and virulent in the rearing containers. The fungus which attacked the slugs was determined as a Fusarium sp. The fungus found attacking the eggs and preventing their development was determined as Arthrobotrys sp. Both of these determinations were made by the Botany and Plant Pathology Department, Oregon State College. According to Barnett (3, p. 1-60), Arthrobotrys is parasitic on some nematodes. Water containing 200 ppm Agri-mycin 100 (Streptomycin 15% and Terramycin 1.5%)[6] combined with 200 ppm of captan (N-trichloromethylthio—75% tetrahydrophtholimide) was added to the soil or Vermiculite. This treatment effectively prevented bacterial and fungus growth in the rearing cultures.

The transfer of bacteria and fungus from eggs to newly-hatched slugs was prevented by the use of chemicals. Eggs from the rearing containers were placed for three minutes in an aqueous solution containing 200 ppm of Agri-mycin 100 and 200 ppm of captan. This procedure also prevented bacterial and fungus growth during the incubation period of the eggs.

Although the slugs laid some eggs when placed on Vermiculite, soil of approximately 75 per cent moisture saturation proved more effective for egg production. The eggs in general cultures were collected on a weekly basis with a camel's hair brush. In some experiments, the eggs were collected on a daily basis.

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6 Charles Pfizer & Co., Inc., Brooklyn 6, New York

The slugs were fed outer leaves of head lettuce. The lettuce was replaced three times a week which kept them supplied with fresh food at all times. Any portions of the lettuce leaves left uneaten were taken out before the addition of fresh leaves.

The best growth of the slugs in the general rearing cultures was obtained at temperatures of 15 to 21°C. The relative humidity was maintained at 85 to 100 per cent.

The main difficulty encountered in attempting to rear this species was the presence of parasitic nematodes and protozoa in the field-collected slugs. The organisms multiplied rapidly when the slugs were kept under laboratory conditions. In the initial phases of this study, it was impossible to keep the slugs alive for more than a three-week period. Mortality usually started within two weeks after collection from the field. This period was prolonged by using Vermiculite instead of soil in the cultures and by transferring the slugs to a clean container every two weeks. The slugs were transferred with feather-weight type forceps to minimize the injury.

The nematodes were determined as Rhabiditis lambdiensis, Panogrolaimus sp., and Diplogaster sp. by Dr. H. J. Jensen, Associate Nematologist, Oregon Agricultural Experiment Station. These nematodes were normally found underneath the mantle of the slugs. The nematodes were occasionally observed protruding through an opening that had been made on top of the mantle. When abundant, the nematodes were observed also in the respiratory orifice.

These parasites were apparently able to reproduce within the slug since all stages could be found at any one time. Nematodes were also observed free-living in the soil of the rearing containers. Tremendous numbers of nematodes could be found in heavily-infected slugs. According to Steiner (59, p. 427-435), Rhabditis lambdiensis is commonly found in mushroom beds.

An attempt was made to surface-sterilize the eggs of Deroceras reticulatum in order to eliminate the transfer of nematodes and protozoa. All attempts at chemical sterilization failed. The egg albumen was denatured at low concentrations of ethyl and methyl alcohol, sodium hydroxide, Zephiran Chloride (benzalkonium chloride 12.8%)[7], and Chlorox. The concentrations used were too low to have any effect on the protozoa and nematodes present.

A method was finally developed which was very effective in eliminating the nematodes from the infected slugs themselves. Infested slugs were placed on a special agar-base medium for a two-week period. They were then transferred to the crisper chests which contained sterile soil or Vermiculite. The constituents of the medium used are shown in Table 1.

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7 Obtained from Winthrop Laboratories, New York, New York

TABLE 1

CULTURE MEDIUM USED AS FOOD FOR *DEROCERAS RETICULATUM*

Potatoes (only the autoclaved extract)	200 gms.
Powdered skim milk (Carnation)	50 gms.
Dextrose	200 gms.
Bel Ais salt mixture	100 ml.
Bacto yeast extract	10 gms.
Agar	20 gms.
Agri-mycin 100	2.68 gms.
Methyl-p-hydroxybenzoate	.375 gms. (.025 %)
Water	1 liter

The complete medium was compounded by combining three separate fractions. The first fraction consisted of agar combined with Bel Ais salt mixture. The second, a mixture of the appropriate dry ingredients: powdered skim milk, dextrose, and Bacto yeast extract. The third consisted of the potatoes of which only the extract was used. These three fractions were autoclaved separately at 15 pounds per square inch for 25 minutes. The fractions were then mixed well. Agri-mycin 100 and the methyl-p-hydroxybenzoate[8] were added after the autoclaving process.

Brust and Fraenke (11, p. 186-204) reported using methyl-p-hydroxybenzoate as a fungicide in their nutritional studies of

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8 Obtained from Eastman Kodak Co., Rochester, New York

the blow fly, Phormia regina. At a concentration of .025 per cent of methyl-p-hydroxybenzoate, all stages of slugs survived. At .05 per cent, there was approximately 45 per cent mortality, mostly of young slugs. All stages of slugs readily fed on this medium.

To determine if the slugs could subsist on this medium for any length of time, the following experiment was performed. Three groups of ten slugs each were placed in jars containing the agar-base medium. These slugs were kept in the rearing room at 17 to 23°C. The slugs were weighed on a weekly basis and the mean weights recorded. The results (Figure 6) show that the slugs decreased in weight when kept in the agar medium. A two-week period was found to be sufficient to eliminate the parasitic nematodes.

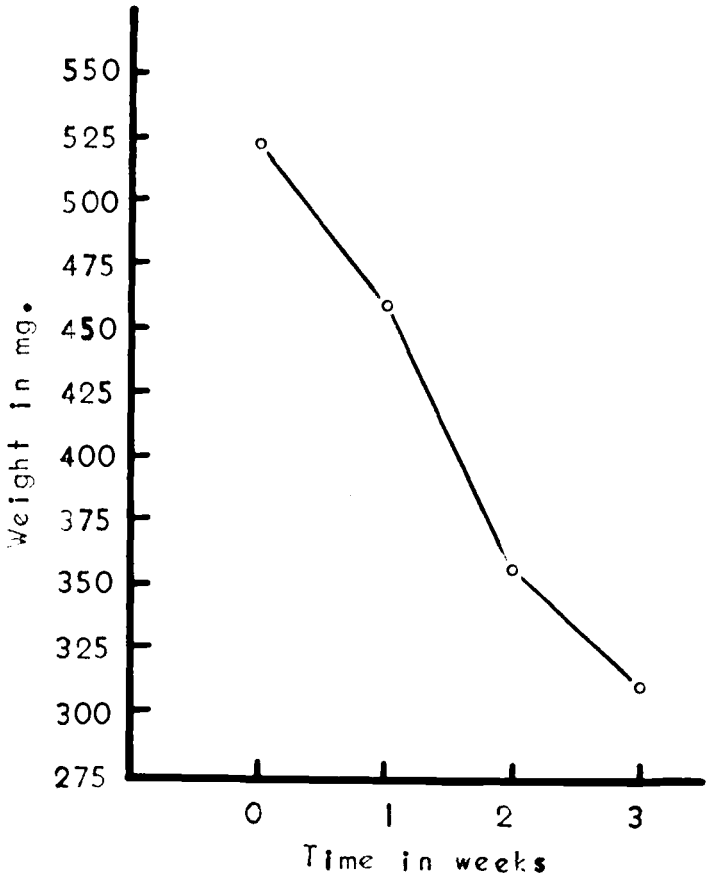
Some nematodes were isolated from the slugs and placed in petri dishes containing the above-mentioned medium. All the nematodes were dead after five hours. The checks contained the same medium but without the methyl-p-hydroxybenzoate. Nematodes in the check plates reproduced and increased in great numbers, practically covering the whole dish in the three-week period.

The protozoan found in Deroceras reticulatum was determined to be Tetrahymena limacis by Dr. E. Kozloff, Lewis and Clark College, Portland, Oregon. According to Kozloff (38, p. 17-19) (39, p. 204-208) (40, p. 75-79), these particular protozoa are not pathogenic but confine themselves to the digestive gland as mere symbiontes.

In the opinion of the writer, these protozoa are strongly pathogenic when abundant in great numbers under laboratory conditions.

Figure 6

Decrease in weight of *Deroceras reticulatum* feeding on agar medium (mean of 30 slugs)



The protozoa were observed on several occasions in the internal spongy tissue of live slugs and could be seen through the outer skin with the aid of a microscope. When abundant, they could be seen coming out through the genital aperature when the slug was immersed in water. This could be one of the reasons why the slugs ceased to lay eggs after a period of six weeks under laboratory conditions.

Both nematodes and protozoa were found in the same individuals, but one would predominate over the other in numbers. When the nematodes were eliminated from the slugs, by the method mentioned previously, the numbers of protozoa increased greatly. Reynolds (54, p. 48-53), in his studies with protozoa in slugs, indicated that infected slugs could live from 10 to 30 days under laboratory conditions. Stout (60, p. 211-215) and Thompson (66, p. 203-205) found that Deroceras reticulatum in nature was also infected by Tetrahymena rostrata.

Protozoa were not eliminated by the agar medium. Cleveland (18, p. 309-326) (19, p. 455-568) was successful in eliminating protozoa from various organisms by subjecting them to oxygen pressure. A modified version of Cleveland's method was tested during this study.

Two groups of ten slugs each were oxygenated for various periods of time. The results (Table 2) showed that Deroceras reticulatum withstood oxygen pressures up to thirteen hours. The slugs were motionless when taken out of the pressure chambers.

After a period of time, the individuals that withstood the treatment recovered and the rest succumbed.

TABLE 2

PER CENT MORTALITY OF DEROCERAS RETICULATUM AT OXYGEN PRESSURE OF 42 POUNDS PER SQUARE INCH
(20 slugs per treatment)

Number of Hours in Pressure Chamber	Per Cent Mortality
4	0
8	0
12	0
16	40
20	60
24	80
28	100

Since the exposures to oxygen pressures which were not fatal to slugs also failed to eliminate the protozoan parasites, this technique was abandoned.

BIOLOGICAL STUDIES

The Egg

Developmental Period

The published data on the duration of the egg stage of Deroceras reticulatum is not consistent. This is perhaps due to the slugs having been studied under different environmental conditions. Taylor (61, p. 107) stated that the egg period was from three to four weeks, and that it varied somewhat according to the weather. According to Hawley (31, p. 984), the average incubation period in the summer was 26.5 days. During the winter, at temperatures from 55 to 75°F, the average period was 37.3 days and ranged from 26 to 57 days. Carrick (15, p. 570) reported that the incubation period during the summer months was 22 to 36 days. During the winter, he recorded a period of 96 days. Lovett and Black (43, p. 15) recorded periods from 15 to 30 days. Binney (7, p. 148) reported a period of 20 days.

Effect of Temperature on Development. The time which elapses between oviposition and eclosion (incubation period) varies within wide limits according to the temperature. Carrick (16, p. 50) did some work on the incubation period of eggs at various constant temperatures. His results are summarized in Table 3.

TABLE 3

INCUBATION PERIOD (IN DAYS) OF EGGS
AT VARIOUS CONSTANT TEMPERATURES
(from Carrick)

Temperature	0°C	5°C	10°C	15°C	20°C	22°C	23°C	25°C
Minimum Period	—	98	53	25	15	—	—	—
Maximum Period	—	118	55	36	22	—	—	—
Average Period	—	105	54	29	18	—	—	—
Per Cent Mortality	100	0.5	17.7	15	37	100	100	100

Somewhat similar results were obtained by Bachrach and Cardot (2, p. 261).

During the studies conducted by the writer, over 60 observations were made consisting of 50 eggs per observation. The egg masses were washed in a water solution containing Agrimycin (200 ppm) and captan (200 ppm). They were then placed in petri dishes with moist filter paper and submitted to a constant temperature of 20°C. The minimum period for incubation was 11 days with a maximum of 21 days. The average period was 15.5 days.

A pair of slugs was observed mating and was separated afterwards and kept in individual containers at 21.5°C. One of the pair commenced laying eggs 15 days later. A few eggs from the original clutch hatched after an incubation period of five days and the rest after periods of seven and eleven days.

A wide variation has been observed in the rate of development of individual eggs of a single clutch all kept under the same

conditions. Rarely do all the eggs of a single mass hatch on the same day. As the time of development lengthens, the difference in the time of hatching of eggs of the same clutch increases correspondingly. Eggs of the same batch that remained unhatched after 12 days following the initiation of hatching failed to develop. Examination of these remaining eggs with a microscope revealed the absence of the sperm body characteristic of fertile eggs. In some egg masses, 45 per cent of the eggs were infertile.

During development, there appeared to be a progressive increase in the resistance which the eggs showed to extremes of temperature. When first laid, the egg is very susceptible to freezing and to temperatures higher than 22°C. Embryos which have almost completed their development can withstand a temperature of 0°C for several days and thereafter resume development at higher temperatures. According to Carrick (16, p. 49), the limiting temperatures between which normal development can take place are below 5°C and above 22°C. This fact facilitates the storage of the eggs in refrigerators for a long period of time. In the present study, approximately 1,000 eggs were kept in a refrigerator at 4°C for seven months and, when removed to 20°C temperature, commenced hatching within 24 hours.

Effect of Moisture on Development The eggs of Deroceras reticulatum possess no mechanism for accomodation to changes of humidity in their surroundings. The eggs desiccate and the embryos die unless contact is maintained with a moist surface. The water content of the eggs has been found to be 85 per cent (16, p. 46).

According to Binney (7, p. 6), eggs of Deroceras reticulatum have been completely desiccated for years and dried eight consecutive times in a furnace until they were reduced to an almost invisible minuteness. Upon exposure to humidity, these eggs regained their form and eventually developed normal embryos. He stated that in all these instances, the young developed in the same manner as other eggs not subjected to this treatment.

Lovett and Black (43, p. 13) reported that they desiccated several groups of eggs for a few week's period and obtained the same results as above. Carrick (16, p. 47) stated that these results were too fantastic for credence.

A similar experiment was performed during the present study in an attempt to elucidate this controversy. Eggs in dishes were kept in the rearing room at temperatures of 17 to 23°C and were allowed to become desiccated. The eggs were left in the petri dishes in a shriveled condition for five months. At the end of this period, water was added to the filter paper. The eggs regained their original form and turgidity, but no development of the embryos took place.

The writer agrees with Carrick. The low resistance of the eggs to dehydration is a fact which has impressed itself upon almost all who have experimented with them.

Eclosion

The form of the adult is attained before the young slug makes an attempt to emerge from the egg. The mantle is evident, and the heart can be observed contracting strongly. Both pairs of tentacles are apparent with prominent, pigmented eye-spots on the larger anterior pair. Movement of the embryo becomes more pronounced just before hatching takes place. The action of the radula can be clearly seen if the eggs are immersed in water and examined under the microscope. The radula is protruded well in front of the tentacles and is addressed to the membrane with an upward, steady stroking movement. As a result, the membrane bulges at the point attacked and eventually ruptures.

Since the development of the embryo in the egg is easily observed, much study has been given to this part of the life cycle by Mark, (44, p. 173-625), Kofoid (37, p. 35-118), Byrnes (12, p. 201-226) and Carrick (15, p. 574-594).

The Immature Slug

Upon emergence from the egg, newly-hatched individuals of Deroceras reticulatum may commence feeding upon the remains of the eggs or on intact eggs nearby (15, p. 571). Because of the limited food supply in proportion to the large number of slugs hatching from a single egg mass, these slugs appear gregarious. As they become older and more hardy, they migrate from the original center and spread out in search of other food. Cooke (23, p. 43)

reported that on hatching, the young slugs go into the ground for four or five days before feeding. The writer has noted similar habits of the newly-hatched slugs in the laboratory. The food in the containers did not show strong evidence of feeding for several days after the slugs hatched.

The young slug often secretes its slime in such large quantities that it is able to descend from plants to the ground on a thread of this material. Taylor (61, p. 109) stated that this slime thread is of the same nature as that of the trail of slime left by the slug wherever it crawls.

Growth Rate At Constant and Fluctuating Temperatures

The rate of growth of Deroceras reticulatum in the field varies widely according to food, temperature and other factors. Growth rates reported in the literature of slugs reared under laboratory conditions, however, also vary widely. Lovett and Black (43, p. 15) estimated the period from egg to maturity to be from 90 days to one year. Theobald (64, p. 1-6) reported that the slugs reached sexual maturity in six weeks. Taylor (61, p. 107) cited one instance in which a slug mated and deposited eggs 66 days after eclosion. Carrick (16, p. 57) stated that Deroceras reticulatum reached sexual maturity in four to six months after hatching. The studies by the above authors could have been conducted at different temperatures or other environmental conditions and thus given rise to the reported differences in growth rates.

The mean weight of newly-hatched slugs in 100 observations was found to be 1.8 mg. with extremes of 1.3 to 2.1 mg. Three groups containing 20 newly-hatched slugs each were placed under constant temperature conditions at 20°C. The same number of slugs was placed under fluctuating temperature conditions of 17 to 24°C.

The results after 45 days (Table 4) showed no marked difference in mean weights of the slugs under the two temperature conditions. The test did demonstrate, however, the great variation in growth rates possible for individual slugs. The variation reported in the literature is not too surprising in view of the great variation in growth rates among individuals from eggs hatching the same day and kept under the same environmental conditions.

TABLE 4

GROWTH RATES OF DEROCERAS RETICULATUM UNDER TWO SETS OF TEMPERATURE CONDITIONS IN A 45-DAY PERIOD

	Constant Temperature 20°C	Fluctuating Temperatures 17 to 24°C
Mean Weight	24.8 mg.	26.1 mg.
Maximum Weight	83.3 mg.	73.0 mg.
Minimum Weight	4.4 mg.	1.8 mg.

Slugs in their earliest stages are very delicate and difficult to manipulate without causing injury, even when handled with a fine camel's hair brush. Because of their delicacy and proclivity toward weight loss through water evaporation, the writer decided to work with slugs that had attained at least 25 mg. in weight.

Slug activity in the field is associated with conditions of high humidity, and this association has led to an assumption that activity was in some way induced by damp conditions. Crozier and Pilz (24, p. 711-721) showed, however, that the speed of locomotion of Agriolimax compestris varied directly with temperature. Dainton (26, p. 25)(27, p. 165-187) showed that certain changes in temperature, and not conditions of high humidity, induced activity. By activity, she meant locomotion. She used constant and fluctuating temperatures in her experimental work with Deroceras reticulatum. Her results showed that activity was sharply stimulated by falling temperatures below 21°C and by rising temperatures above 21°C. Activity subsided as soon as the temperature was maintained constant at any value. Temperature changes in the reverse direction had no such effect. As at constant temperature, activity was low when the temperature was rising toward 21°C or falling from above this value.

As a result of her work, Dainton assumed that increased acquisition of food necessarily accompanied activity. To test her assumption and to work out a method for bringing slugs to maturity as rapidly as possible in laboratory rearing, the writer ran a series of experiments to determine the growth rates of Deroceras reticulatum at various constant and fluctuating temperatures.

Four groups, each consisting of 10 slugs, were kept in a temperature cabinet at a constant temperature of 20°C. The same number of slugs were placed in another temperature cabinet at

fluctuating temperatures ranging from 17 to 24°C. The slugs were kept at approximately 100 per cent relative humidity. These slugs were weighed by groups at weekly intervals. The data was recorded (Figure 7) as the mean weights of the total number of slugs used.

The results showed that the growth rates at the two temperatures were not markedly different. Food consumption by the more active slugs under fluctuating temperature conditions, however, was noticeably greater.

It was desirable to find out if the heavier food uptake by activated slugs could compensate for higher metabolic rates expected at higher fluctuating temperatures as compared to a constant temperature. Four groups consisting of 10 slugs each were placed at fluctuating temperatures of 20 to 27°C. The same number of slugs was placed at a constant temperature of 17°C. The mean weight of the slugs was recorded on a weekly basis for four weeks.

The results (Figure 8) indicated that there was a considerable difference in growth rates under the two temperature situations. In spite of the lower growth rate of the slugs at the higher and fluctuating temperature, these slugs consumed almost twice as much food as their counterparts at constant temperature. Thus it appeared that the higher food consumption did not even compensate for the higher metabolic rate of the more active slugs.

After comparing growth rates between constant and fluctuating temperatures, growth rates at various constant temperatures were studied. Five groups consisting of five slugs each were placed

Figure 7

Growth rates of Deroceras reticulatum at constant and fluctuating temperatures (mean weights of 40 slugs)

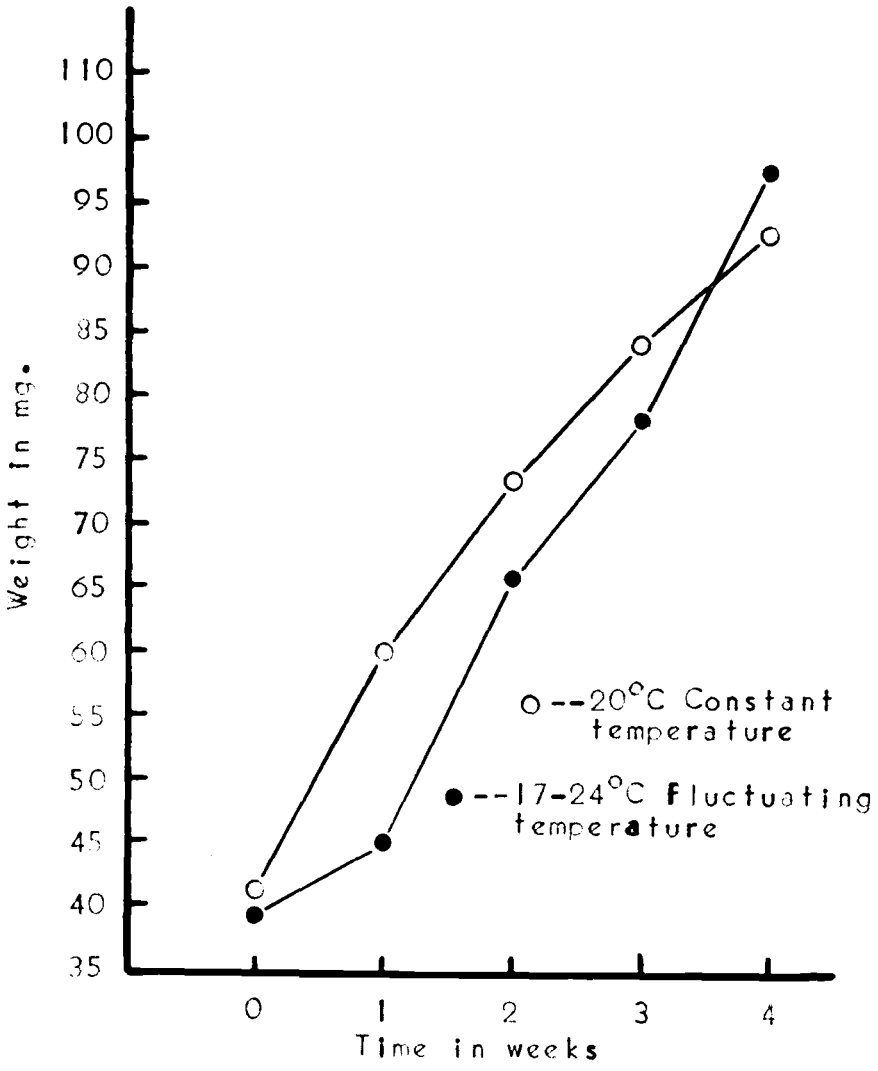
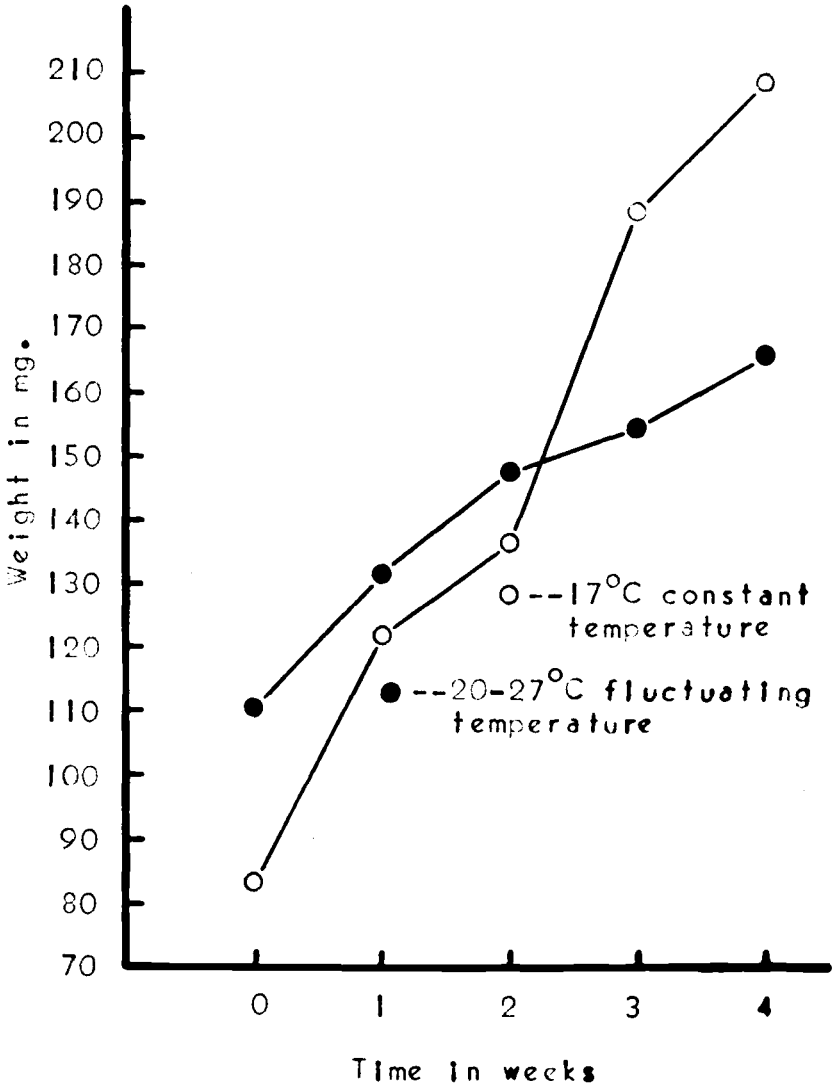


Figure 8

Growth rates of slugs at constant and fluctuating temperatures (mean weights of 40 slugs)



in each of the four temperature-controlled cabinets. The temperatures were held constant at 5, 10, 15 and 28°C respectively. The mean weights of the slugs were recorded over a four-week period.

The results obtained (Figure 9) show that the slugs at temperatures of 10 and 15°C were of almost comparable weight after the third week. The growth rate was somewhat low at 5°C. The slugs at 28°C decreased in weight after their initial increase.

During the course of the present study, there was a noticeable variation in growth rates by the slugs in a given group. An experiment was performed to study the individual variations in growth which might be expected in a random sample of young slugs. Twenty-five slugs were taken out of a stock culture. They were four months old and had been kept at a constant temperature of 20°C. These twenty-five slugs were placed in a temperature-controlled cabinet at fluctuating temperatures of 17 to 24°C. The slugs were weighed individually on a weekly basis. The mean, minimum weights were recorded. The growth rates during an eight-week period were plotted in Figure 10. The decrease in weight after the sixth week cannot be explained. These results show the tremendous individual growth variation that can be attained from eggs that have hatched the same day.

Survival Without Food

Immature slugs can withstand extended periods without food providing other conditions are favorable. Lovett and Black (43, p. 13) reported keeping nine slugs for one month without food.

Figure 9

Growth rates of Deroceras reticulatum
at constant temperatures
(mean weights of 25 slugs)

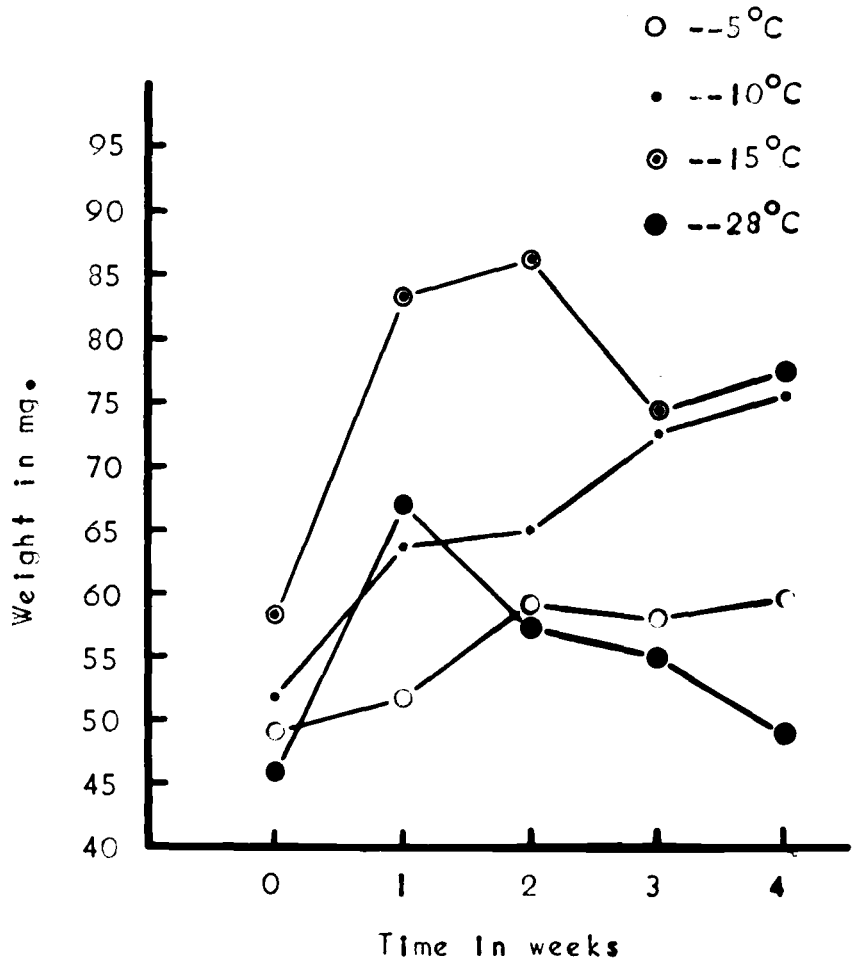
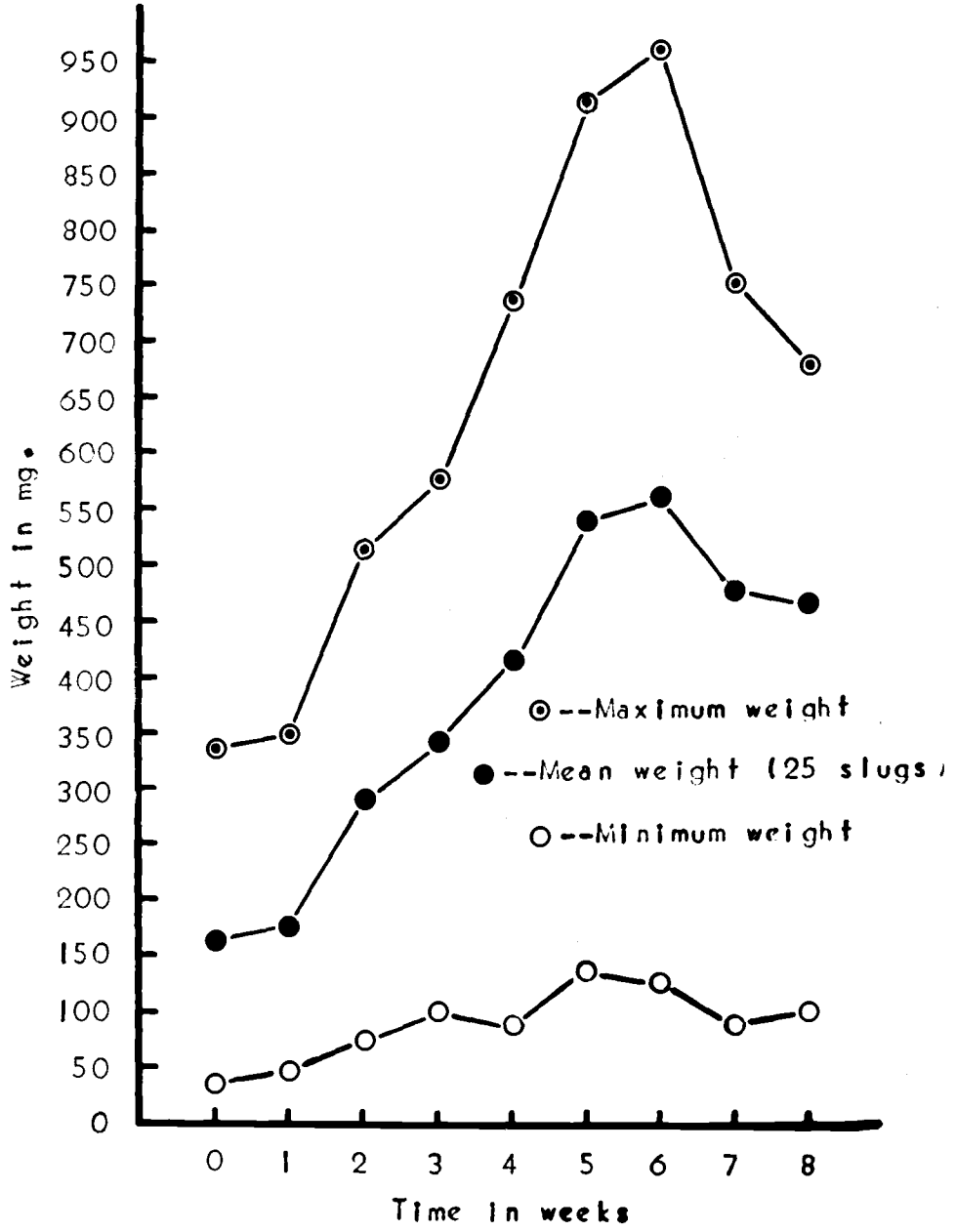


Figure 10

Variation in growth rate of Deroceras reticulatum at fluctuating temperatures of 17 to 24°C



To explore the possibilities that slugs can withstand starvation for a long period of time, week-old slugs were placed in jars with unwashed river-bottom sand. Five groups containing ten slugs each were placed under a constant temperature of 20°C. These slugs were deprived of food during the duration of the experiment. One group remained alive without food for five months.

The Sexually Mature and Adult Slug

Seasonal Activity Under Field Conditions

The activity of grey garden slugs appears to be governed, not by any definite annual cycle, but by the prevailing condition of weather. Aestivation occurs only when conditions are too hot and dry for slug activity. This state of conditions seldom persists for any length of time in the Willamette Valley of Oregon. During the summer, it is extremely difficult to find slugs in unirrigated fields where they were known to be abundant earlier. The first period of wet weather in the fall is immediately followed by their appearance in large numbers. There is no known period of true hibernation in the course of the life cycle, and hard frost or snow is necessary to induce cessation of activity.

There are conflicting statements in the literature in regard to the breeding season of Deroceras reticulatum. Taylor (61, p. 107) stated that the breeding season is throughout the year. Lovett and Black (43, p. 12) stated all seasons but particularly spring and

early summer. Hawley (31, p. 987) stated that breeding is in the fall. Carrick (15, p. 47) stated that it takes place throughout the year.

Field observations of the writer indicate that the reproductive period is not confined to one particular season of the year, but breeding takes place whenever conditions are suitable. The most abundant egg production takes place during the fall and spring months. In the laboratory, egg production was readily induced at any time of the year.

Food Preference

Deroceras reticulatum has such a wide range of food plants that it is classified as almost omnivorous. Some of its food hosts have been reported as cabbage, potatoes, eggplant, lettuce, beans, lima beans, peas, corn, strawberries, gooseberries, cucumbers, melons, cauliflower, wheat, turnips, beets, carrots, radishes, celery, clover, oats, dahlia, dandelion, dock, chicory, tobacco, hops and tomatoes (31, p. 982). This species of slug feeds on many ornamental shrubs and vines, and it finds abundant food in sod land and in lawns. Cooke (23, p. 31) mentioned as possible foods beetles, mayflies and dead slugs. Lovett and Black (43, p. 14) added sow bugs, earthworms and aphids to this list. Taylor (61, p. 109) recorded an instance in which Deroceras reticulatum killed and ate slugs of the species Deroceras campestris when the two were placed in the same container. Lebour (41, p. 393-395) found that they eagerly devour the proglottides of Moniezia, a tapeworm

of sheep. Ingram (36, p. 34-35) reported an observation of Deroceras reticulatum feeding on pillbugs, Armadillidium vulgare. It would seem, therefore, that, while the slugs usually prefer a vegetable diet, they will feed readily on food of animal origin.

Method of Feeding

Deroceras reticulatum does not eat in the same manner as does a biting insect, for its feeding apparatus is very different. According to Taylor (61, p. 106), the jaw is a concave, chitinous process attached to the roof of the pharynx. In the center, it bears a tooth with finely serrate edges which helps in tearing the food apart. Opposed to this, on the floor of the pharynx, is a flexible plate made up of many small, sharp teeth and known as the radula. The radula is supported on the muscular tongue and can be moved forward and backward. By the combined use of the jaw and radula, small particles of food are torn from the plant and are then passed on to the stomach. The radula works by undulating movements, carrying the food as by an endless belt back to the oesophagus. Cooke (23, p. 33) stated that the teeth of the radula are sharp enough to break the skin of the human hand if the slug is permitted to use this organ for a short time in one place.

Survival Without Food

The mature slug can survive extended periods without food as do the immature forms. In order to compare the rate of decrease

in weight by the slugs in the absence of food under two sets of temperatures, the following experiment was performed. Field-collected slugs were fed for one week and then were not fed during the period of this experiment. Ten of these slugs were kept at 20°C constant temperature. The same number of slugs was kept at 17 to 24°C fluctuating temperature. The slugs were weighed on a weekly basis for a period of five weeks. The mean weights of the two groups of slugs are shown in Figure 11.

The results showed that the rates of decrease under the two sets of temperatures were very similar. The experiment was terminated after the fifth week because of slug mortality due to parasites.

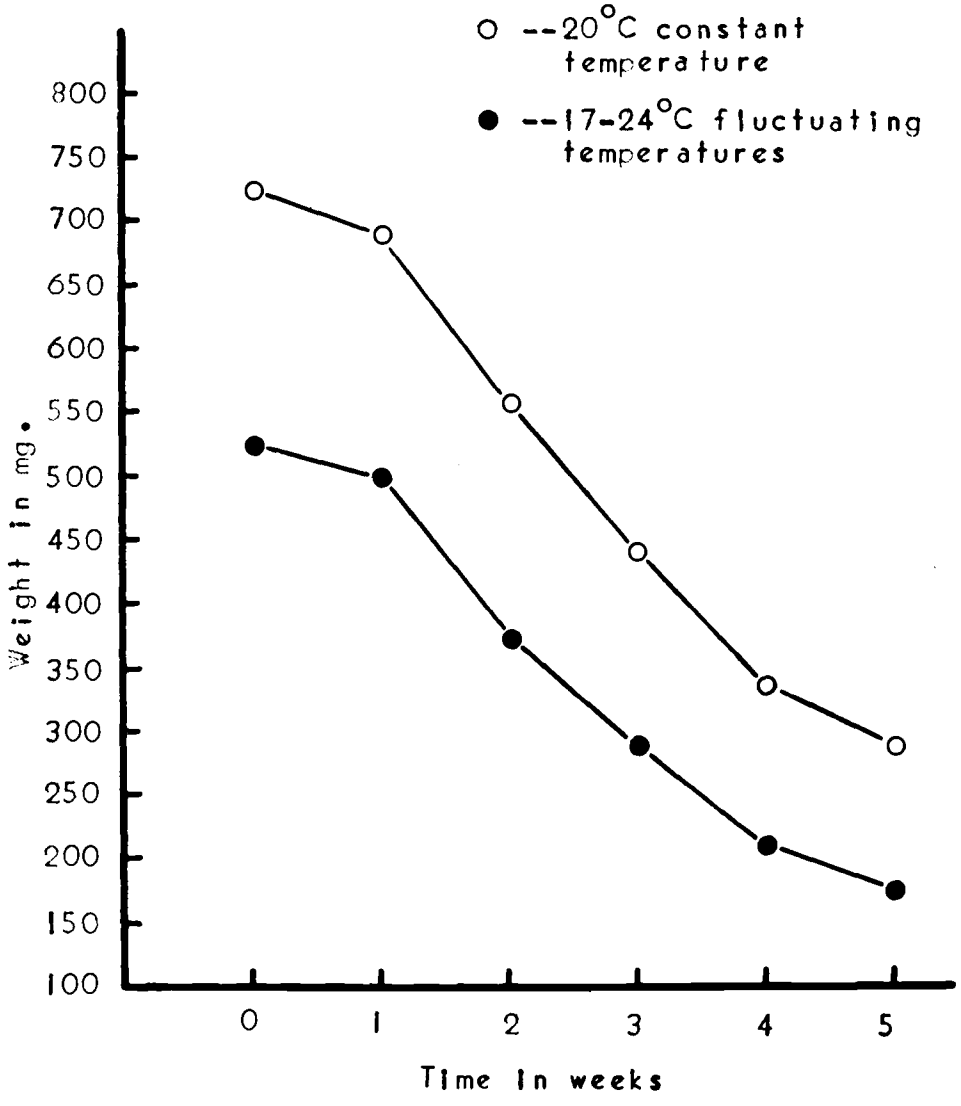
Soil Moisture Preference

Slugs possess no known mechanism for accomodation to changes of humidity in their habitats. They frequent moist, shady places and retire into seclusion to avoid excessive loss of water during the day or periods of drought. The body of Deroceras reticulatum has been found to consist of over 80 per cent water (16, p. 46).

To determine the water content of the soil most attractive to slugs, a narrow tray containing sandy loam soil was utilized. The tray was two inches wide and eighteen inches long and contained a three-quarter inch layer of soil. The tray was slanted with the lower end immersed in water. In this way, the soil was kept flooded at the lower end; and there was a complete gradation to almost air-dry soil at the upper end.

Figure 11

Decrease in weight of Deroceras reticulatum under starvation conditions



Ten slugs were left in the closed tray overnight. The following morning they were found congregated in a narrow band of soil which proved to contain 68 per cent water. Moisture percentage was computed by drying the soil at 200°F for 24 hours. Difference in weights between damp and dry soil gave the per cent of soil-water content. Carrick (16, p. 47) stated that Deroceras reticulatum will favor soil which is 64 per cent water content.

The Effect of Humidity and Light on Activity

Slugs are normally active at night; but daytime activity can sometimes be observed in overcast, showery weather in the Willamette Valley. In view of this fact, it seems reasonable to suppose that the activity of slugs is a response to high humidity. Dainton (28, p. 165-170) showed that no such response existed.

Preliminary experiments in this study, using various saturated salt solutions (58, p. 67-68) (13, p. 173-175) to control the relative humidity, showed no obvious differences in growth rates at various humidity levels. For this reason, humidity was not controlled during the growth rate studies but was kept at approximately 100 per cent relative humidity.

Observations on slug activity in the laboratory indicated that the normal nocturnal activity was not necessarily associated with the onset of darkness. On the contrary, it continued under conditions of nocturnal illumination. The onset of illumination is accompanied by a short burst of activity. This effect of light in initially stimulating activity was confirmed by Dainton (28, p. 188-194)

in a study of the effect of illumination on slugs. According to Dainton, illumination of any individual results in a short burst of activity provided that the animal is dark-adapted. She stated that dark-adaptation by Deroceras reticulatum takes more than one hour but less than two hours. Light-adaptation is more rapid and is complete well within one hour. Crozier and Wolf (25, p. 83-92), in a study of phototropism in Agriolimax campestris, also found that dark-adaptation was much slower than light-adaptation. All of the available evidence indicates that once the slugs are adapted to light, their activity is not affected by illumination. This fact was used in the studies on the effect of various temperatures on growth rates as described previously.

Maturation Period

The longevity of Deroceras reticulatum is, at the present, somewhat of a mystery. Only estimations have been published. Ellis (30, p. 54) estimated over twelve months. Taylor (61, p. 107) reported it to be less than eighteen months. Lovett and Black (43, p. 15) stated that it is from eighteen months to two years. Theobald (63, p. 201-211) stated that this species may live several years. Cooke (23, p. 39) reported that slugs of this species are usually full grown by the middle of the second year and die during the first part of the third year. Taylor (61, p. 107) cited one instance in which a slug was full grown in eighty-two days and lived for eighteen months. Hawley (31, p. 988) gave a figure of eighteen to twenty months for the life span of Deroceras reticulatum.

A single individual of Deroceras reticulatum, observed for a period of 13 weeks, gave information on the weight of slugs approaching maturity (Figure 12). This slug had been kept isolated four months from the time of eclosion at a constant temperature of 20°C. It was then placed in a temperature-control cabinet with fluctuating temperatures of 17 to 24°C. The results showed that the slug reached maturity between the nineth and eleventh week of observation. Thus this particular slug was about six months old at the time of reaching maturity. The size of the slug at maturity was 50 mm. long and 9 mm. wide when extended. Since there is a great variation in individual growth rates as shown previously (Figure 10), these results should not be accepted as an average period for Deroceras reticulatum to reach maturity. These results (Figure 12), however, demonstrated the growth rate possible for this species.

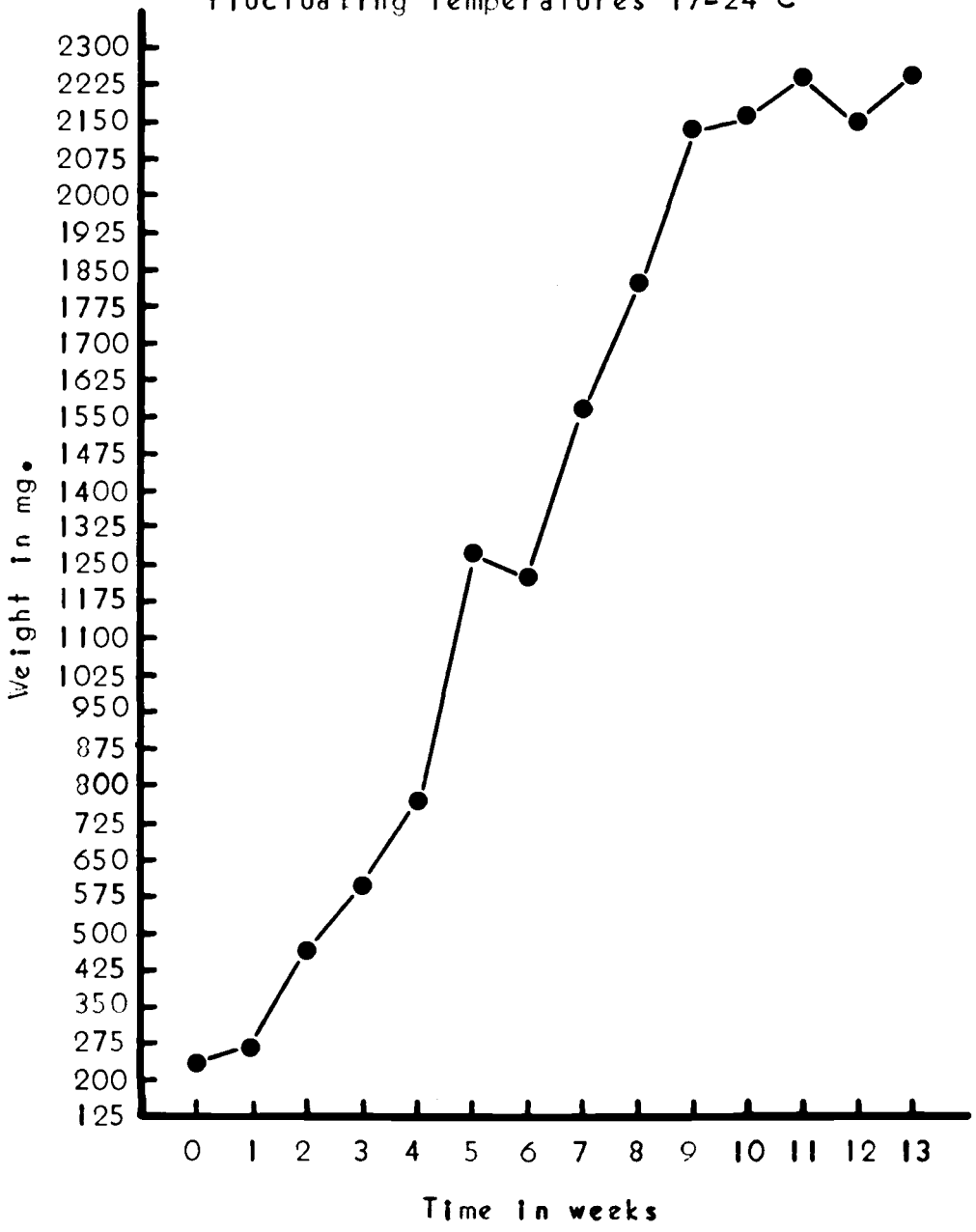
Mating

It has been previously noted here that Deroceras reticulatum is hermaphroditic, both male and female sex organs being in the same individual. Whether or not the slugs are capable of self-fertilization is not definitely known. Theobald (64, p. 1-6) stated that their reproductive organs mature at different times. Robson (55, p. 12) reported that Agriolimax agrestis has been reared for two generations by self-fertilization.

An attempt by the writer was made to determine whether or not Deroceras reticulatum was capable of self-fertilization. A number

Figure 12

Growth rate of Deroceras reticulatum at fluctuating temperatures 17-24°C



of slugs were isolated from each other soon after eclosion to prevent mating. Some slugs died before reaching sexual maturity. The ones that survived laid a few eggs, but the eggs did not develop. These eggs were examined and revealed the absence of the sperm body which is characteristic of fertile eggs.

The act of mating was observed on several occasions under field and laboratory conditions by the writer. Miles (46, p. 451-455) stated that mating takes place between 4 and 6 a.m. Under Oregon conditions, this is not entirely the case. The writer has, on many occasions, observed mating during the day in wet, heavy-overcast weather. Under laboratory conditions, mating takes place at any time during a 24-hour period. Field-collected slugs have been observed to mate under laboratory conditions up to three weeks from collection date. The reason for no further mating is not known.

On several occasions, the writer observed the sexual union of Deroceras reticulatum. The prolonged fondling and excitatory gestures with the tentacles which Taylor (61, p. 107) described did not take place. The two slugs, coming from opposite directions, approached each other on the surface of the soil. Neither seemed to sense the other until they were almost touching. They then took up positions with their right sides, which possess the genital openings, in close proximity to each other. Soon the excitatory organ, or sarcobelum, was extruded and used for caressing. They crawled around and around each other, in a caressing manner, on a patch of slime about an inch in diameter. This prelude to mating

was termed the nuptial "round dance" by Pilsbry (52, p. 537). After this behavior had continued for twenty to thirty minutes, the sarcobelum of each slug enlarged very greatly, the two organs twisted around each other and there was a great discharge of slime. At this time, each everted the conical, pointed penis and almost at once found the genital aperture of the other. The period of union lasted for approximately thirty to forty-five seconds. In regards to the actual transfer, Taylor (61, p. 107) stated, "The seminal element, mixed with mucus and worked up into a little ball, is transferred bodily, the forerunner of a true spermatophore." The entire process occupied less than 35 minutes. When they separated, the slugs moved apart with the penes well extended and kept them that way for several minutes.

These observations agree with Taylor (61, p. 107) who reported that the act of pairing usually occupies not more than half an hour with the final consummation lasting a few seconds. Carrick (15, p. 566), however, observed that the union lasted over a quarter of an hour. Heath (33, p. 22-24) stated that the act of mating lasts several hours. Hawley (31, p. 986) stated that this period lasts just over three-quarters of an hour.

The period between mating and oviposition is difficult to determine. Taylor (61, p. 108) quoted records of the interval lasting from five to twenty days. Lovett and Black (43, p. 15) gave figures of twelve to forty days. Carrick (15, p. 568) reported a ten to sixteen day period.

Eleven to fifteen day intervals between mating and oviposition were observed during the present study. These cases involved isolated slugs kept at a constant temperature of 20°C. The disparity in times recorded by various authors could be brought about by the difficulties in knowing whether the slugs had mated previous to the observed copulation.

Oviposition and Fecundity

In the field, egg masses of Deroceras reticulatum are placed almost indiscriminately in any damp situation. The eggs are usually deposited in cavities of the soil, usually within the top four inches. Eggs can be deposited during the summer provided the land is under irrigation. The mild winters, which usually prevail in Oregon, do not restrain egg production.

Eggs were readily deposited under laboratory conditions provided the slugs were furnished with damp soil. An experiment was performed to determine the range of the soil-water content suitable for oviposition. Field-collected adults of Deroceras reticulatum were kept for three weeks in a plastic crisper container which had a plastic-screen false bottom. Water was added below the screen to provide high humidity in the container. Fresh food was continuously available. No eggs were laid under these conditions. They were then placed in groups of five in glass jars containing four inches of loose sandy loam soil. The soil had moisture contents of 10, 25, 50, 75 and 100 per cent saturation, respectively. The records of oviposition are given in Table 5.

TABLE 5

OVIPOSITION OF DEROCERAS RETICULATUM IN SOIL AT VARIOUS SATURATION PERCENTAGES

Day	Saturation Per Cent 10	25	50	75	100
	(Number of eggs laid by five slugs)				
1st	—	—	32	41	—
2nd	—	—	48	72	—
3rd	—	45	62	36	—
4th	—	—	—	51	41
5th	—	56	23	—	—
Total No. of Eggs	0	101	165	200	41

Results of the experiment indicated that soil moisture content of approximately 75 per cent was preferred for oviposition. Garrick (16, p. 47) obtained somewhat similar results and stated that normal egg development will not take place in soils below 10 per cent saturation or in soil close to 100 per cent saturation.

The depth at which egg masses were laid in the loose soil in the above experiment varied according to the amount of moisture present. They were placed deeper at a lower soil saturation per cent. At 25 per cent, they were placed from 1½ to 3 inches deep; at 50 per cent, from 1 to 1½ inches deep; at 75 and 100 per cent soil saturation, the egg masses were laid on the surface of the soil.

The eggs were passed out singly through the genital aperture and laid at regular intervals with short pauses between each egg. They were laid in clutches or masses (Figure 13) ranging from eight to 54 eggs.

The fecundity of individual slugs under field conditions is difficult to determine since it may vary with climatic and other factors. Under laboratory conditions, larger egg masses than those observed by the writer in the field were usually obtained. During these studies, field-collected slugs ceased laying eggs after six weeks in the laboratory. The cause of this was attributed to parasitism of the slugs by nematodes and protozoa.

The total number of eggs laid by individual slugs is, of course, variable. Taylor (61, p. 107) recorded 776 eggs laid in one season. Miles, Wood and Thomas (47, p. 37-38) estimated 1,000 eggs per functional female. Carrick (15, p. 570) estimated 500 eggs per year. Lovett and Black (43, p. 16) cited a case in which one specimen deposited 612 eggs in one year. Theobald (63, p. 1-6) estimated that Deroceras reticulatum may deposit from 500 to 800 eggs per year. Pilsbry (52, p. 537) estimated the number to be more than 700 eggs per year. No record of total egg deposition by individuals was kept in the course of this study.

Figure 13

Egg clutch of Deroceras reticulatum (X 15)

SUMMARY

The primary purpose of this study was to study the biology of Deroceras reticulatum under laboratory conditions. A secondary purpose was to develop laboratory rearing methods by which a grey garden slug colony could be maintained for toxicological and other studies.

Nomenclatorial history and description of the stages of the invertebrate are presented.

Rearing of Deroceras reticulatum in the laboratory was only partially successful due to the presence of various parasitic organisms. The fungus which attacked the slugs was identified as a Fusarium sp., and the fungus which attacked the eggs was an Arthrobotrys sp. The slugs were also found to be heavily parasitized by nematodes and protozoa. The nematodes were identified as Rabiditus lambdiensis, Panagralaimus sp., and Diplogaster sp. The protozoan was identified as Tetrahymena limacis. Rearing methods and materials including diet, rearing containers, temperature, soil moisture, method for eliminating the parasitic nematodes and other factors were given.

The minimum egg incubation period obtained at 20°C was eleven days with a maximum of 21 days. The average period was 15.5 days.

The eggs were found to desiccate and the embryos died unless contact was maintained with a moist surface.

The eclosion process was observed. The embryos were observed using their radulas to procure their escape from the eggs.

The newly-hatched slugs differ very little in their habits from the full-grown form. The immature slugs would not feed for a period of four to five days after eclosion. There was essentially no difference in growth rates under constant and fluctuating temperatures, providing the constant temperature was at the mean value of the fluctuating temperatures. The mean weight of the immature slug right after eclosion was 1.8 mg., and it was possible for slugs to attain a weight of 2225 mg. at maturity.

There was a higher degree of activity at fluctuating temperatures than at a constant temperature.

There was higher food consumption at fluctuating temperatures as compared to a constant temperature, but the growth rates were essentially the same. This was probably due to a higher metabolic rate at the fluctuating temperatures.

It was determined that a maximum growth rate could be attained at temperatures of 10 to 22°C. It was shown that there was a tremendous variation in growth rates among individuals of the same age.

Experiments on starvation showed that slugs could survive at least five months without food under laboratory conditions.

It was shown that oviposition by the grey garden slug is governed, not by any definite annual cycle, but by the prevailing environmental conditions. Egg production was readily induced in the laboratory at any time of the year.

The slugs were shown to prefer a soil moisture content of 68 per cent. There was no obvious difference in growth rates at

various levels of relative humidity. Dark-adapted slugs gave a short burst of activity upon illumination. Once the slugs were adapted to light, their activity was not affected by illumination.

It was shown that Deroceras reticulatum can attain maximum growth within six months under laboratory conditions. The maximum weight attained was 2225 mg. The size of a mature slug was 50 mm. long and 9 mm. wide when extended.

There was no evidence of self-fertilization by the slugs during the course of these studies. Under laboratory conditions, mating took place at any time during a twenty-four hour period. Field-collected slugs continued to mate for about three weeks after being subjected to laboratory conditions.

The mating process of Deroceras reticulatum was observed in the laboratory. The period between mating and oviposition was found to be from eleven to fifteen days.

Moist soil or Vermiculite was necessary to induce oviposition. A soil-water content of approximately 75 per cent saturation was necessary for maximum oviposition. The eggs were placed deeper in soils of lower moisture content. Field-collected slugs ceased laying eggs after six weeks in the laboratory.

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