SN antibodies. Based on the occurrence of viremia and demonstrable SN antibodies, the rate of infection with WEE virus in Saskatchewan garter snakes in 1964 was not very different from that in frogs—39.3 percent for garter snakes and 31.3 percent for frogs. This study and an earlier one (*4*) show that in parts of the province the infection rate in garter snakes is high (at Pelly, 75 out of 160; at Keeler, 13 out of 16).

Five isolations obtained 162 to 168 days after the first bleeding of the snakes, without preliminary chilling, suggest that recurrence of viremia has a cyclical rhythm independent of the temperature of the environment. Garter snakes may, therefore, be overwintering hosts of the virus regardless of the latitude at which they occur. Isolation of WEE virus from naturally infected *Rana pipiens* establishes that species as a potential reservoir host for this virus.

In contrast to the earlier study (*4*), in which natural SN antibodies were found in garter snakes and leopard frogs only in restricted geographical areas, evidence, in this study, of WEE virus infection was found in snakes and frogs from all 12 localities where they were collected. Accordingly, from 1961 to 1964, evidence of natural infection with WEE virus in leopard frogs or garter snakes has been found in 18 different localities in the province, ranging from Elmore (49°00′N) to Onion Lake (53°43′N). Thus WEE virus infections appear to be widely distributed in garter snakes and leopard frogs in the agricultural area of Saskatchewan (*8*).

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8. A more detailed account of our studies, with confirmatory observations made in 1965 and 1966, is in preparation.
9. We thank Cynthia Boodram for technical help, and L. Thomas, Rocky Mountain Laboratory, Hamilton, Montana, and L. C. LaMotte, U.S. Public Health Service, Greeley, Colorado, for reviewing our manuscript. Supported by National Health and Welfare grant 607-7-59 and Defence Research Board grant 6801-27 (Ottawa).
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Respiration of a Forest Measured by Carbon Dioxide Accumulation during Temperature Inversions

Abstract. *Nocturnal accumulations of carbon dioxide during 40 temperature inversions in 1 year were used as an index of the metabolic activity of a forest. Rates of CO_2 production varied with temperature and with season. Spring and summer rates were 2 to 3 times higher than winter rates at the same temperature. Mean monthly temperatures, averaged over 15 years, were used with the curves of respiration on temperature to compute annual gross respiration of the Brookhaven oak-pine forest. The forest was estimated to have a yearly release of approximately 3400 grams of CO_2 per square meter, theoretically equivalent to 2104 grams of dry matter (carbohydrate).*

Although metabolism is one of the generalized functions of natural communities that has long been recognized to offer substantial diagnostic value, the difficulties of measuring it have limited its use, especially in terrestrial ecosystems. Those estimates of metabolism of terrestrial ecosystems that have been attempted have generally been based either on inference from detailed studies of structure (*1*), on measurements of CO_2 exchange rates of small enclosed segments (*2*), or on measurements of CO_2 flux (*3*).

Of these, measurement of CO_2 flux is an especially attractive technique because chambers are not required, and the problem of disturbing natural conditions is reduced to a minimum. The difficulties are substantial because elaborate instrumentation and a large number of measurements are necessary. Nevertheless, it has been applied successfully in various agricultural ecosystems (*3*). In forests where structure of the community is much more complex, the technique is even more difficult. We wish to report the successful measurement of nocturnal respiration of a forest by a simplification of the CO_2 flux method.

The forest is a relatively homogeneous, but diminutive and open, oak–pine stand on level glacial outwash sands of central Long Island, New York. The principal trees are white oak (*Quercus alba*), scarlet oak (*Q. coccinea*), and pitch pine (*Pinus rigida*). The larger oaks were 45 years old and 5 to 10 m tall at the time of this study. A few scattered pines were 100 years old and 12 to 15 m tall, but most were 10 m or less and the same age as the oaks. Blueberries (*Vaccinium angustifolium, V. vacillans*) and the black huckleberry (*Gaylussacia baccata*) form a shrub stratum. Soils are well drained, sandy, and podzolic.

An infrared gas analyzer was used to measure carbon dioxide concentrations at various heights in the forest during local, low-level, temperature inversions. Rates of increase of CO_2 concentration, integrated over that depth of the atmosphere influenced by the forest, gave a rate of CO_2 production which was correlated with temperature and was assumed to be a measure of the rate of respiration of the community. Temperature was measured to within less than $\pm$ 0.5°C with aspirated thermocouples. A complete set of measurements, including both CO_2 concentration and temperature, was recorded automatically at 15-minute intervals on punched tape. Sampling points were at eight heights on each of two towers in the forest (one 16 m tall, the other 21 m tall). Additional data taken at six heights on a 125 m tower about 2 km from the experimental forest clarified the gross structure of the CO_2 profile during these inversions, showing that the CO_2 build-up was restricted to heights less than 25 m.

It has been recognized for several years that carbon dioxide concentrations in the atmosphere follow a diurnal course related to the rates of photosynthesis and respiration of the plant cover. The CO_2 concentrations usually increase at night, especially close to the ground, and decline during the daytime. A diurnal course is recognizable, under certain conditions, as high as 100 m above the ground (*4*).

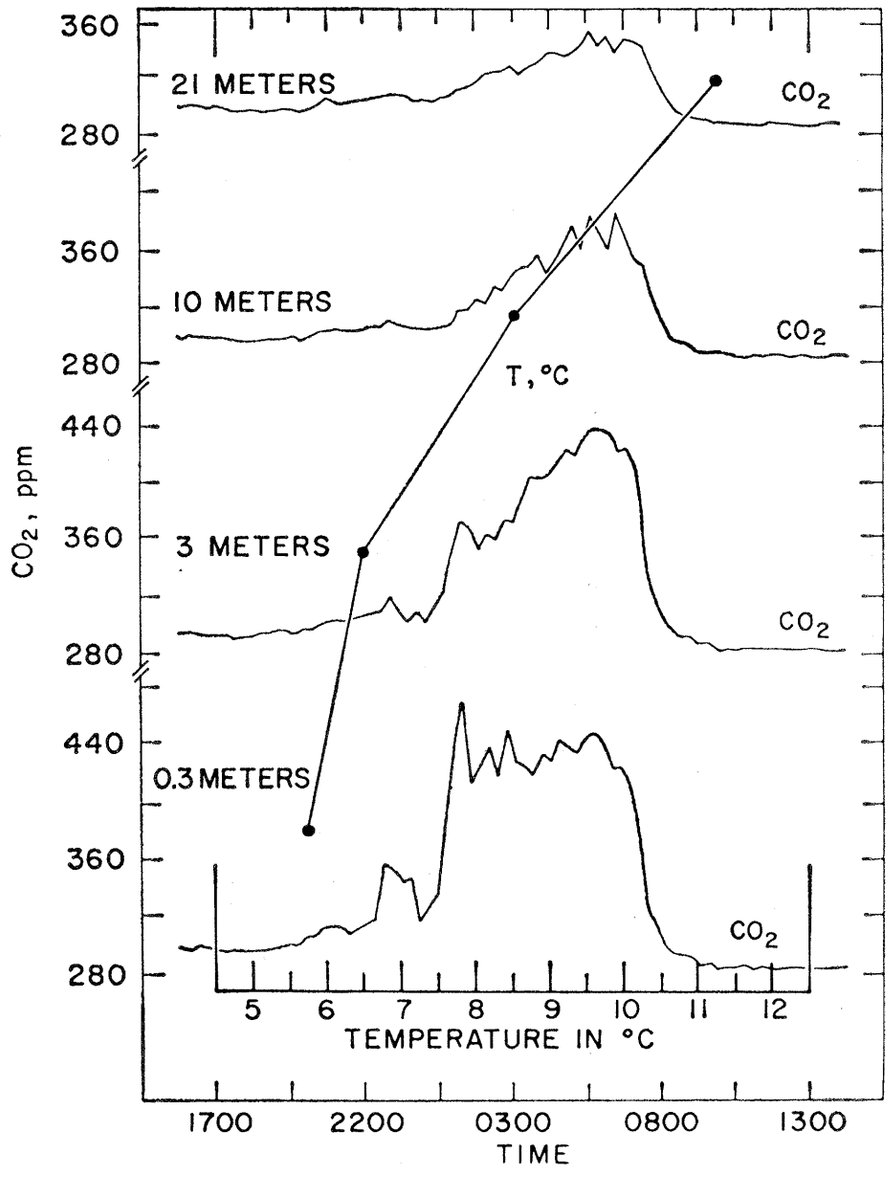


Fig. 1 (top left). Carbon dioxide concentrations at various heights in the Brookhaven forest, 11–12 June 1965. Such accumulations occur frequently but are useful for measurement of respiration only when there is temperature inversion similar to that shown for 0300 on 12 June.

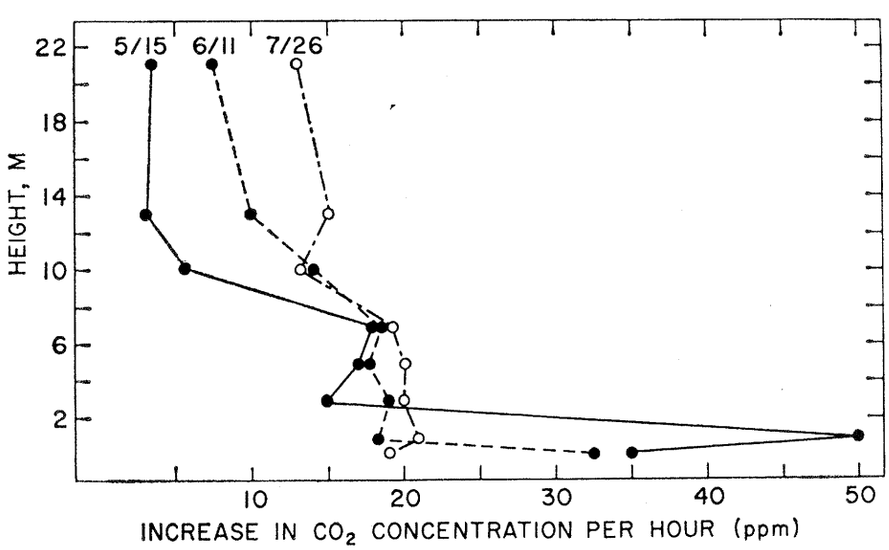
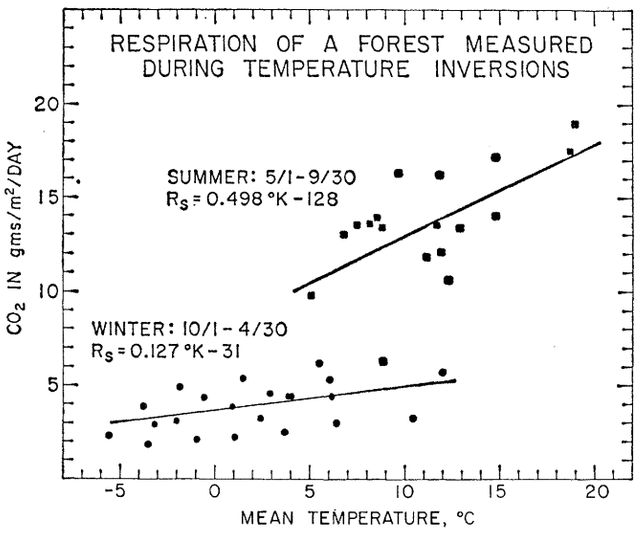


Fig. 2 (bottom left). The hourly increase in CO_2 concentrations at various heights during temperature inversions in the Brookhaven forest.

In forests, the diurnal pattern of CO_2 concentrations at various heights often approximates that shown in Fig. 1. During daylight when the normal lapse rate exists there are usually 290 to 300 ppm CO_2 in the air at all elevations up to 21 m. At night there is often an accumulation with time, the greatest accumulation occurring close to the ground. When the air is still, as it may be during an inversion, the rate of CO_2 accumulation at each elevation is a measure of the rate of CO_2 production by the forest. Care must be taken in determining that there was a stable atmosphere. Although the CO_2 profiles shown in Fig. 1 are quite common, they are useful in the present context only when there is a marked temperature inversion, such as the one illustrated for a night in June when the temperature at 0.3 m was 5°C less than at 21 m.

When an inversion occurs, the rate of CO_2 accumulation can be estimated from the slopes of the curves of CO_2 concentration (Fig. 1) at different heights within the forest; the rates can then be plotted against height as shown in Fig. 2. Rates of CO_2 production are universally higher near the ground than at the top of the forest, but it is significant that even at 21 m there was an appreciable increase with time in CO_2 levels. It is also interesting that at 4 to 6 m there was an inflection, indicating a concentration of CO_2 production at this height. There appears to be a reasonable basis for this in that there is a preponderance of trees 3 to 6 m tall in the somewhat diminutive forest studied.

The increase in CO_2 content at heights in excess of 10 m was not as closely related to the structure of the vegetation. During the inversions to which this study was restricted, CO_2 concentrations (measured on the 125 m tower) usually declined with increasing height to 25 m, and remained approxi-



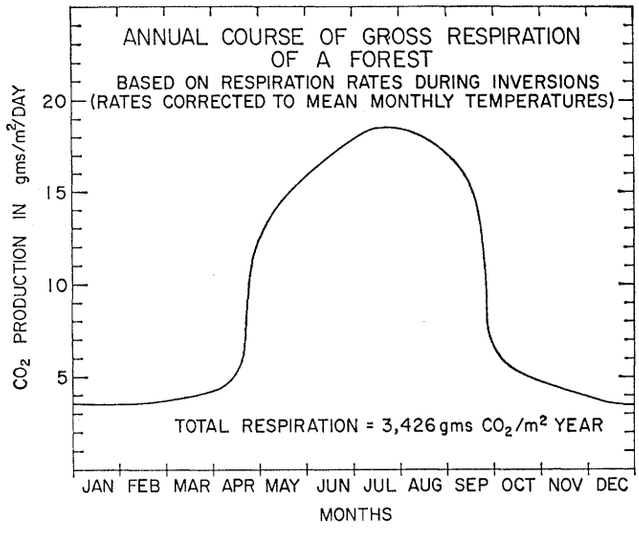


Fig. 3 (left). Respiration rates of the Brookhaven oak-pine forest plotted against mean temperature (the mean of the average temperatures at 10 m and at 3 m, measured every 15 minutes during the inverson). R_s = rate of respiration in grams of CO_2 per square meter per day. Fig. 4 (right). The annual course of CO_2 exchange of the Brookhaven oak-pine forest based on the 15-year mean monthly temperatures.

mately constant at greater heights. The fluctuations in CO_2 content at 125 m which did occur were not correlated during temperature inversions with concentrations below 25 m. It seems reasonable that the two air masses, the one below 25 m and the one above 25 m, be considered substantially independent of each other. Accordingly, when measurements are restricted to inversions, we have assumed that CO_2 accumulation due to respiration is limited in this forest to heights of 25 m or less; calculations of CO_2 production have arbitrarily been cut off at that height.

The hourly rate of CO_2 production by the entire community was calculated by integrating hourly rates of CO_2 accumulation during documented inversions over the eight heights at which CO_2 was sampled. These rates, plotted against average temperature (the average of the mean temperature measured at 15-minute intervals at 10 m and at 0.3 m during the inversion), showed that there are two distinctly different rates of CO_2 production at each temperature, with a higher rate in summer than in winter (Fig. 3). The transitions are abrupt, occurring in approximately 2 weeks during late April and September. The spring transition occurs about 4 weeks prior to the opening of buds on the trees; the fall transition occurs about the time that the leaves of the deciduous species begin to fall.

These curves can be used to calculate the annual course of gross respiration of the forest by applying to the appropriate curve the mean temperatures (averaged over the 15 years of meteorological records at Brookhaven National Laboratory) during the year. The transition from winter to summer rates was assumed to occur on 1 May, and the transition from summer to winter rates, on 1 October. Using monthly mean temperatures, the annual course of CO_2 production (gross respiration) appears as shown in Fig. 4. The skewness of the curve is due to the skewness of the distribution of mean monthly temperatures, and not to any measured shift in intrinsic rate of respiration during the summer, although such shifts in the respiration rates of tree stems may indeed occur (*5*). It is clear from this curve that in winter daily rates of gross respiration range between 3 and 4 g of CO_2 per square meter per day; in summer rates range to 18 to 20 g/m² per day. Integrating the rates over a year of average temperatures gives a total CO_2 production of 3426 g/m² (34.26 T/ha per year), assuming that there is no difference between light and dark respiration (*6*).

If 1 g of CO_2 evolved is equivalent to 0.614 g of dry matter catabolyzed, an approximation when carbohydrate ($C_6H_{10}O_5$) is the substrate, the annual loss of dry matter through respiration by this forest (including roots, soil organisms, organic matter, and aboveground plant parts) is about 2104 g/m². An extremely detailed series of measurements of growth in the same forest indicates that net annual production of dry matter by trees, shrubs, and ground cover is 1146 g/m² per year (*7*). Since net production equals gross production less the losses due to respiration, gross production of the Brookhaven oak-pine forest would be estimated by this technique at about 3250 g of dry matter per square meter per year. These data approximate the ranges observed in forest plantations in Denmark, and are predictably different from observations of a tropical rain forest in Ivory Coast (*8*). There total production was slightly less (900 g/m² per year), and total respiration and gross production were substantially more (4350 and 5250 g/m² per year). These differences reflect the difference in structure, the diminutive oak–pine forest having less than one-half the respiration of the tropical forest.

There is no doubt that CO_2 accumulation during temperature inversions can be used as a criterion of the metabolism of terrestrial communities. While the techniques of measurement are relatively complex, they are less complex than other methods and disturb natural conditions little, if at all. During inversions, there may be fluctuations in CO_2 concentrations above the forest canopy, but these fluctuations do not appear to be related in any simple way to the accumulation of CO_2 within the forest. Respiration rates of the forest we have studied vary with temperature and season, with summer rates

generally 2 to 3 times higher than winter rates at any temperature. The transition from winter to summer rates and back again in this instance was very abrupt, occurring during a 2-week period in late April or early May, and in late September; this corresponded to transitions to and from dormancy. A curve or set of curves of respiration rates on temperature can be used to calculate the annual course of CO_2 exchange of the entire ecosystem, including above- and below-ground plant parts and animals, by applying it to records of temperature. These are often available as means over several years, enabling one to calculate rates of respiration which are averages, as opposed to single or short-term measurements. It is hoped that the technique can be adapted for wide application in the study of terrestrial ecosystems (*9*).

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Multiple Gene Loci for the Monomeric Hemoglobin of the Hagfish (Eptatretus stoutii)

Abstract. *The fact that members of the subclass* Cyclotomata *possess monomeric hemoglobin molecules has been known for some time. Electrophoresis of hemolysates from 12 hagfish* (Eptatretus stoutii) *revealed five hemoglobin phenotypes with four to six distinct zones of hemoglobin. Each zone is believed to represent a monomer containing one heme group on a single polypeptide chain with a molecular weight of approximately 18,000. It is postulated that these monomers are controlled by genes at four loci.*

The present study on the hagfish has been undertaken to estimate the number of gene loci for hemoglobin polypeptides, and to determine if all the hemoglobin molecules exist only in the monomeric form. It has been postulated that the genes for hemoglobin and the gene for myoglobin of vertebrates evolved from a common ancestral gene (*1*). While myoglobin molecules remained monomeric, consisting of a single polypeptide chain attached to one heme group, the hemoglobin molecule of higher vertebrates, from teleost fish to mammals, is a tetramer. The tertiary structure of each subunit of the hemoglobin molecule, however, has been shown to be nearly identical with the single polypeptide myoglobin (*2*). In this respect, previous reports (*3*) which have indicated the monomeric nature of hemoglobin molecules of the hagfish have been viewed with special interest. Previous works, however, have not established whether the hagfish produces more than one kind of hemoglobin polypeptide.

Twelve live specimens of the hagfish (*Eptatretus stoutii*) were used (*4*). All exceeded 30 cm in body length. Seven were gravid females, and two were males, as confirmed by the presence of spermatozoa in testicular squashes. The sex of the remaining three was not readily determinable. Blood samples of 2 to 3 ml were collected in acid citrate dextrose solution directly from a major blood vessel on the upper ventral area. From twice-washed erythrocytes, a freeze-thaw hemolysate of 1 : 2 dilution was prepared in distilled water. Nuclei and stroma were removed by centrifugation at 3000 rev/min for a period of 10 minutes.

The monomeric nature of all the hemoglobin molecules of the hagfish was confirmed by the following experiments. Thin-layer gel filtrate was used to estimate the molecular weight of hemoglobin. A layer of 0.250 mm of superfine Sephadex G-75 was spread on glass plates of 20 by 20 cm. The Sephadex had previously been equilibrated for 3 days with 0.1*M* phosphate buffer, *p*H 7.4. After the plates were spread, they were placed in a chromatographic chamber at an angle of approximately 10° and brought to equilibrium. The hemolysates were run in duplicate for at least 8 hours with a series of three marker proteins of known molecular weight. The marker proteins were monomeric cytochrome 12,000, soya bean trypsin inhibitor 21,500, and lactoperoxidase 80,000. In addition to the hagfish hemolysate, the hemolysates of man as well as the rainbow trout (*Salmo irideus*) were also added. The distance migrated was then plotted against the log of the molecular weight. In order to keep the integrity of the thin-layer plate, a piece of Whatman No. 1 paper was gently smoothed over the surface of the plate. The plate was then sprayed with the benzidine reagent solution in order to localize hemoproteins. After the localization of hemoglobin spots, the paper which was removed from the plate was quickly dried in a warm oven. The location of hemoglobin spots was marked and the position of the other proteins was determined, by using the naphthol blue black solution. A mean molecular weight value of 18,000 was obtained on the hagfish hemoglobin, while both the human and trout hemoglobins gave molecular weights in the range of 60,000. Next, the area containing the hagfish hemoglobin was scraped from the plate. The scraped material, moistened with a small amount of distilled water, was inserted into a slot in the starch-gel plate and subjected to the electrophoretic procedure, together with the original hemolysate. All the bands originally present in the hemolysate were identified in the eluate.

The fact that each monomeric hemoglobin molecule of the hagfish contains one heme group was ascertained by determining the heme/protein ratio.