BIRDS AND VIRUSES IN LOWER AMAZONIA

By Thomas E. Lovejoy

The number of different kinds of organisms in biological communities increases as one approaches the equator. Of all terrestrial environments in the tropics, the rain forest has the greatest diversity of species. For example, a sample of 1,000 trees made in our study area near Belem, Brazil, contained 156 species. This variety is reflected by almost every group of organisms. Over 450 species of birds have been recorded in the vicinity of Belem, and it is most likely that the list is not complete. Within a three-kilometer study area on the outskirts of the city of Belem, 66 species of ants have been found, with the inventory far from finished. More than half of all kniwn arthropod-borne viruses (arboviruses) occur in the Belem area.

The very variety, however, makes it difficult to do research on almost any problem, particularly on problems relating to the diversity itself. Unless he possesses genius or naivete, a scientist soon finds, for instance, that if he is going to learn to distinguish between the 450 species of birds he must relinquish the aspiration of mastering the 600 species of trees.

This, however, severely limits research possibilities. It is possible to describe the distribution and abundance of the various birds, but one can do little to determine the ecological factors involved. The diet of a bird has a great deal to do with where and in what numbers it is found. The study of this, however, again confronts one with hundreds of species of plants and a frightening number of insects.

The only solution to this is some sort of cooperative research program. In 1966 such a program was initiated in the Lower Amazon with the establishment of the Guama Ecological Research Area. The original concept was to fence off a three square kilometer tract of forest to prevent disturbance of an epidemiological program of the Belem Virus Laboratory. It soon became apparent to a number of scientists present in Belem that this provided an opportunity for coordinated research, where each research program would collect data in a form useful to the other researchers. Six such programs are under way or are about to begin: botany, vertebrate biology, epidemiology, a photosynthesis study, and a study of physical factors such as temperature and humidity.

Data gathered by each program are collected in a standard fashion. For each observation, its time and place within the research area are recorded. For recording the location of an observation, a grid system has been staked out in the forest in such a way that there is a number for every ten-meter square.

By this method any program can depend on any other. For example, the botany program, in the course of its studies of succession and forest

structure, has identified all the plants within certain portions of the research area. As a result, information on the vegetation of a ten meter quadrat is available to the entomology program if it is needed for studies of pollinating insects. Similarly, the data collected on army ant distribution by the entomology program in the course of their studies of social insects is recorded in reference to the quadrats. These data are thus available to me in studying the causes of antbird (Formicariidae) distribution. Data on bird distribution are available for studies of the natural cycles of arboviruses that occur in birds.

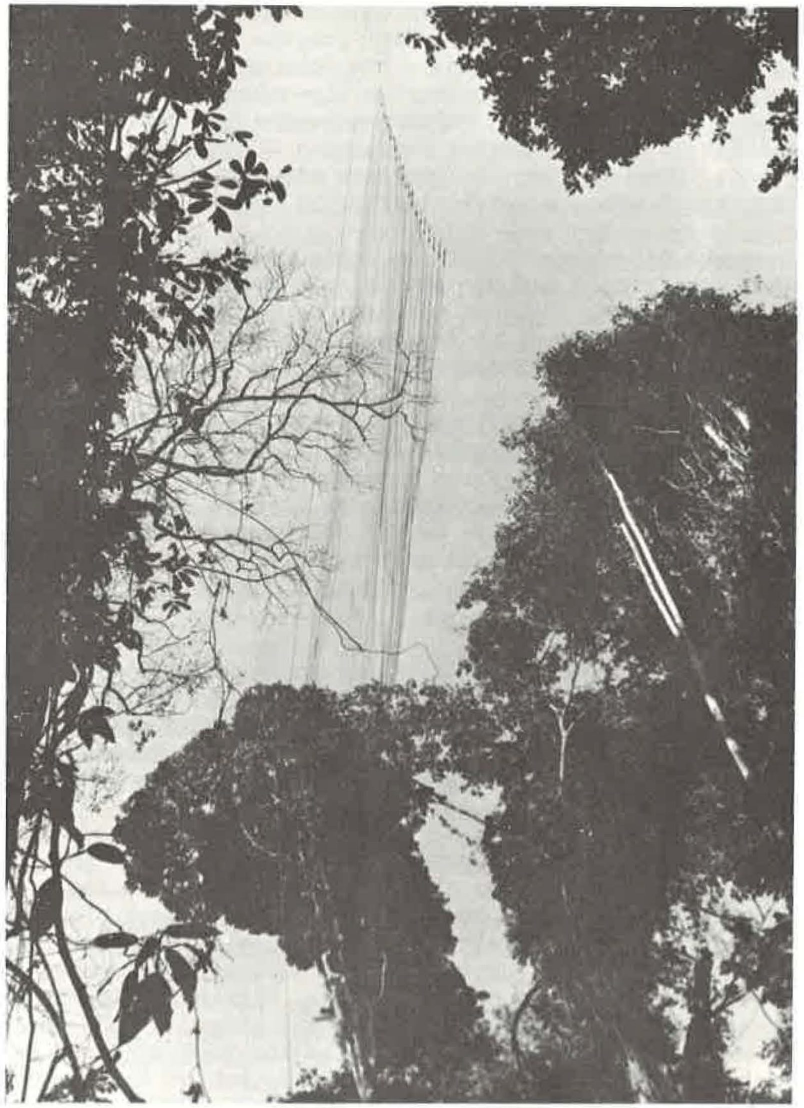
Before the establishment of the Guama Ecological Research Area or of coordinated research, I had planned to do a research project in that very tract of forest. The advent of the Area de Pesquisas Ecologicas do Guama (APEG) as the area is known in Brazil, considerably affected my plans. It did not change them, but it enabled me to plan on doing a great deal more.

The plan began when Philip S. Humphrey, then Curator of Birds at the Smithsonian Institution, suggested that I go to Belem for the summer of 1965 to learn how ornithology and ecology are important to the study of arboviruses. I had recently explored the idea of combining ornithology and epidemiology in my graduate work. The summer in Belem would enable me to observe and participate in such an interdisciplinary study, for Dr. Humphrey was planning a summer of work in cooperation with the Belem Virus Laboratory.

One of the goals of the work in the summer of 1965 was to collect information on the birds and the viruses of the forest canopy. There are a large number of birds typical of the rain forest canopy but they have always been difficult to study. Dr. Humphrey and his research assistant, David Bridge, had worked out a scheme for using mist nets at any height in the forest.

The high net system is inexpensive and simple, with the key ingredient being a professional tree climber. Such a climber can pass a rope over a branch of a tree, and another rope over a branch of another tree. The two ropes are joined together at ground level and then hoisted up. Attached to this rope is a continuous nylon line which passes through a set of rings which act as a pulley system. Mist nets attached to this nylon line (by a system using shower curtain rings) can be hoisted up and down like sails by pulling the line, while the rope stays up in position and supports the entire rig. A full description of the technique will appear soon in Bird-Banding (Humphrey, Philip S., David Bridge and Thomas E. Lovejoy, "A Technique for Mist-Netting in the Forest Canopy").

The high nets worked out well in practice and yielded many interesting birds, including two Band-rumped Swifts (*Chaetura spinicauda*). Of the 47 species caught in the canopy nets, 14 were caught only in these nets and not in those at ground level. I gradually realized that I could use these nets to get information on the vertical stratification of birds and



High net rig mentioned on preceding page, taken from almost directly below it.

viruses carried by them. This I made the core of my plans for a thesis project. Although possibilities of coordinated research at the APEG have allowed me to expand the project, stratification remains its nucleus.

The information on stratification of the bird species will be derived from the height of the shelf of the net in which birds are caught. There will be nets at all heights from the canopy to the ground. When a bird is captured, the time and height of capture will be recorded and before it is released, it will be banded, and then a blood sample will be taken to be used for virological studies at the Belem Virus Laboratory.

The studies in 1965 showed a drop in capture rates on successive netting days, indicating that the capture operation was disturbing the natural behavior patterns. To reduce this problem in my study, I will net birds in three separate parts of the APEG reserves, using each for only two days every three weeks. Each of these locations will be in different types of rain forest, making it possible to compare stratification patterns in the three environments: high ground rain forest (known as mata de terra firme), varzea forest, and igapo forest. The varzea and igapo are two very different types of swamp forest. The igapo is a forest permanently flooded with "black water". The varzea is found only on the banks of the rivers and is flooded by "white water" during the rainy season. Near the sea, it is also slightly flooded twice daily by tidally induced fluctuations in river level.

In 1965 we also noted disturbance of the behavioral patterns of birds when we made preparations for installing high nets. A certain amount of clearing and trimming is necessary to provide a clear space for hauling the net up and down. As I will operate 60 high nets, the disturbance factor made it necessary to do the preparation of the sites in advance. This was done in June 1965 and January 1967.

The remainder of the work other than the capture-recapture program falls into two categories: the ecological and the epidemiological. Both, however, are related to the problem of stratification.

The epidemiological aspect of the project concerns learning about the natural cycles of 18 arthropod borne viruses, including Eastern Encephalitis, Western Encephalitis and Yellow Fever. Jungle Yellow Fever is not known to occur in birds, but is known to be a virus of the canopy. Canopy birds have not been investigated for Yellow Fever infection and although I do not expect to find it, even the negative information will be important.

The presence of absence of virus activity can be ascertained by subjecting the blood specimens from the capture program to various laboratory tests and procedures. A portion of each blood specimen (only 0.2 c.c. is needed from each bird) will be used for virus isolation. The plasma is injected into a baby mouse and if it sickens, virus can usually be isolated from its blood and tissues, and then identified.

Another portion of the blood will be tested for the presence of antibodies to the various viruses. There is a swift test for this known as the haemaglutination-inhibition (HI) test, but it is not always completely accurate, as other substances than antibodies can produce false positive results. Whenever possible, therefore, the results of the HI test will be confirmed by a more lengthy, elaborate and accurate test, the neutralization test. As in the HI test, identification of the antibodies is based on the antigen-antibody reaction. Antibody and the probable virus that the antibody was produced to protect against are injected into mice. If the mice do not become ill, it indicates in essence that the antibody was produced against that particular virus.

The method of banding and blood-sampling will allow me to use these tests to follow case histories of individuals. The first blood samples taken from an individual bird may show no history of virus infection. Blood samples taken subsequently may demonstrate viremia and ensuing antibody production. Long series of blood samples may contain evidence of more than one virus infection and as might be expected, birds with longer life spans may tend to have histories of more virus infections.

The information on the activities of the various viruses in bird populations will shed light on only a portion of the natural cycle. The epidemiological program, directed by John P. Woodall, will give us knowledge of virus activity in the other organisms involved in the cycles: the vectors. South of the Amazon the main vectors of arboviruses are mosquitoes; ticks are rare. The epidemiology program will make collections of mosquitoes at the same times, places and heights, as the bird captures. At the laboratory the collections will be identified and virus isolation will be attempted.

Thus the epidemiological portion of my project will determine virus activity in the avian hosts and the vectors. This may suggest natural cycles for the various viruses but it will not prove them. The study will only provide circumstantial evidence of the sort that an infected species of mosquito occurs at the same time and place as an infected bird species. We will not know whether transmission occurs when and wherever the birds are roosting at night. Nor will we know if the suspected mosquito species actually feeds on the hypothetical host. All we will know is that the suspected vector has the opportunity to transmit the particular virus because it occurs at the right place and time.

In addition to the epidemiology of viruses, that of blood parasites of birds will be studies. A thin blood smear will be made from the drop of blood that remains in the syringe after the sample is taken from the bird. The smears will be examined by the Wellcome Parasitology Unit in Belem. The brief study in 1965 suggested possible negative correlations between virus activity and distribution of blood parasites.

The ecological portion of my project will be concerned with determining factors affecting bird distribution and abundance. The data collected in the summer of 1965 suggest that some species occupy different levels of the forest at different times of the year. This implies that some ecological factor, such as food supply, is affecting bird distribution.

Studies of the food habits and food supply of the birds will be a major part of the ecological studies. The first portion of the study will involve the snalysis of the stomach contents of 3,000 bird specimens collected in the Belem region. These analyses will indicate certain plants as common food species. J. Murca Pires, director of the botany program, will be studying flowering and fruiting cycles, and thus will be able to provide data on the distribution and variation of the plant foods.

The study of variation of the food supply of the insectivorous birds will be limited. The stomach content analyses may suggest a few common food species whose variation in availability may be followed if it is not too difficult to do so. Conversely, the analyses will help the entomologists to assess the importance of bird predation on insects. The entomology program, under the direction of Domiciano Dias, will provide more easily gathered data on the distribution and phase of the army ants, for studies of the distribution of the antbirds and other ant-following birds.

Another portion of the ecological studies will attempt to assess the effect of various physical factors on bird distribution. Temperature, relative humidity, and cloud cover will be measured by the program studying physical parameters. The general activity cycles of birds, which will be derived from the data on times of captures, may correlate with variations in cloud cover or temperature. In the varzea forest environment, measurements of the degree and time of flooding will be taken. Data from 1965 suggest that jacamars and kingfishers are present only at the time of day when the varzea is flooded. How the permanent flooding of the rainy season may affect these distributions is unknown.

I will also explore the possibility that density of foliage may correlate with certain distributions. When birds are released after banding, I will make a record of the routes they take, and try to correlate the routes taken by various species with the various foliage densities in the release area.

All the data gathered in the ecological studies will be directly relevant to the epidemiological studies. Increasing emphasis has recently been placed on the basic similarity of epidemiology and ecology, and this work may provide a good example of how important ecology is in virus studies. Variation in food supply, for instance, may bring a bird species into strata of the forest where the virus it carries can spill over into human populations.

All of the planned work will produce vast amounts of data for analysis.

For instance, approximately 15,000 bird captures are anticipated, and to each capture much additional data will be attached. To cope with this I shall keep the data in a manual punch card system which will be kept up to date week by week during the field work. This will provide a continually up to date assessment of the situation, enabling me to follow up on any suggestive trends such as a developing epidemic in one group of birds. Final analysis of the data will be done with the aid of automatic data processing.

At the end I will have some basic information about virus cycles and avian natural history in a tropical rain forest. If all goes well, the data may be able to contribute towards the solution of some basic questions. Tropical rain forest birds lay fewer eggs than their non-forest relatives, and Alexander Skutch has suggested that they can successfully raise more young than they hatch (Ibis 91: 430-455. 1949). He suggested that predation, or perhaps disease, rather than food supply, may limit the numbers of rain forest birds. The virus and blood parasite data may shed some light on this, although the effect of a viremia on a bird is unknown.

David Snow, working on manakins (Pipridae) in Trinidad found surprisingly low adult mortality, on the order of 10% (Zoologica 47: 65-104 and 183-198. 1962). Such mortality data is known for few other rain forest birds. If I am able to band most of the populations of some species, it should be possible appreciably to increase the number of species for which mortality data is known.

I plan to carry out my program for 18 months. This should show whether the rain forest birds have annual cycles or whether the environment is so constant, as for some tropical sea birds (N.P. Ashmole, 1963, Ibis 103b: 297-364), that the birds are not tied to an annual cycle. It may be possible to answer other questions as well. Perhaps the data may even provide a clue to the tantalizing riddle of tropical species diversity.

Dept. of Biology, Yale University, New Haven, Conn. 06520

Unflooded depression in the varzea swamp forest