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POPULATION DYNAMICS OF THREE HAEMOSPORINA OF BLUE GROUSE (*DENDRAGAPUS OBSCURUS*)

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled POPULATION DYNAMICS OF THREE HAEMOSPORINA OF BLUE GROUSE (*DENDRAGAPUS OBSCURUS*) submitted by RAYMOND ANTHONY ALLAN in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

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Abstract

Population dynamics of *Leucocytozoon bonasae* Clarke 1935, *Leucocytozoon* sp. and *Haemoproteus mansoni* White and Bennett 1979, gametocytes, in wild and captive blue grouse (*Dendragapus obscurus*) from Hardwicke Island, British Columbia, were investigated from 1980-1982. Analysis was based on the assumption that microparasites exist in a patchy environment, and that differences in patch parameters can be measured and compared.

Patterns in round and elongate gametocyte populations of *Leucocytozoon* indicated separate origins for these gametocytes. One species produced elongate gametocytes and was classified as *L. bonasae*, while round gametocytes were designated as *Leucocytozoon* sp..

Time available for transmission (transmission window width) varied between microparasites; this may influence gametocyte population behaviour. *Leucocytozoon* sp. and *L. bonasae* were transmitted to captive chicks for approximately 5-9 weeks, whereas *H. mansoni* transmission occurred over a much shorter period (3-4 weeks). Relapse infections in both captive and wild blue grouse had constant monthly prevalence for *L. bonasae* and *Leucocytozoon* sp.. By contrast, relapse prevalence increased throughout this period for *H. mansoni*; in addition, the intensity also showed a similar rise throughout relapse. Patency in infrapopulations of *H. mansoni* was 5-11 weeks for primary, and 11-26 weeks for relapse infections. Peaking times for gametocyte intensity

was 1-4 weeks for primary, and 5-11 weeks for relapse infections. No such differences in the above parameters were detected in the other two parasites. Data indicate that a precise peaking time is selected for in microparasites with narrow transmission windows, but not in parasites with wide transmission windows. A model was proposed to explain the evolution of dual population behaviour during primary and relapse periods in *H. mansoni*.

Blue grouse were a patch for these microparasites. Between-patch dynamics were measured by year to year fluctuation in prevalence of each parasite in wild chicks, and gave an indicator of transmission effectiveness. Prevalence of *L. bonasae* was constant and high (>90%) in all three years, while prevalence of the other two microparasites was variable and lower during the same period. Within-patch dynamics were gauged by comparing primary and relapse gametocyte output within each microparasite population. This gave a measurement of the dependability of the vertebrate host for reproduction of the parasite. During primary and relapse, output of *L. bonasae* was equivalent, but in *Leucocytozoon* sp. relapse output was much lower than primary output. *H. mansoni* had higher output during relapse.

Comparison of decline in *Leucocytozoon* sp. and *H. mansoni* primary gametocyte populations during the first three weeks of infection indicated more rapid loss of the former. Normal loss of uninfected erythrocytes, as measured

by depletion of chromium 51 label, was similar to loss of *H. mansoni*, but less than decline of *Leucocytozoon* sp. gametocyte populations. This was taken as further evidence for the undependable association between *Leucocytozoon* sp. and blue grouse.

By combining the two environmental parameters- transmission effectiveness and dependability of the vertebrate host for reproduction of [redacted]asite, a habitat templet model was developed to explain [redacted]ife history strategies of microparasites. Blue grouse-parasites fit three categories, *L. bonasae* was K-selected, *Leucocytozoon* sp. was relatively r-selected and *H. mansoni* was relatively A-selected. Differences in life history characteristics were proposed on the bases of the model.

Acknowledgements

'Properties belong to interactions not to independently existing things.'

This has been a good experience. Thanks to the efforts and interests of others, I feel I've made strides in many directions.

I particularly want to congratulate the members of my committee for showing diligence, patience and fortitude. They were, Dr. Jerome Mahrt, Dr. John Holmes, Dr. Fred Zwickel, Dr. D. A. C. Craig and my external examiner Dr. R. Beaudoin.

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Field data were collected on the lands of Crown Zellerback of Canada Ltd, and Bendickson Contractors Ltd.

Grouse were housed in Alberta at Biological Animal Services at Ellerslie. I appreciate all the hard work of the Ellerslie personnel. Cleaning caecal droppings takes perseverance. Without you help this long study would have been impossible.

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I. GENERAL INTRODUCTION

Birds that inhabit areas where ornithophilic biting flies coexist may become infected with intracellular blood parasites (Fallis *et al.*, 1974); these microparasites (Anderson and May, 1979) belong to the suborder Haemosporina which includes the genera *Haemoproteus*, *Leucocytozoon* and *Plasmodium* (Levine *et al.*, 1980). Some of these microparasites are pathogenic to birds (Fallis *et al.*, 1974; Garnham, 1977; Khan and Fallis, 1968; Kocan and Clark, 1966), and thus their importance has stimulated considerable research.

Research has included surveys of bird species for blood parasites, determination of life cycles, and investigations into pathology of infection (Fallis *et al.*, 1974). There is however, little known about the population dynamics of any avian Haemosporina. Few researchers have applied general ecological principles to their findings which is understandable as most of the research was done prior to the development of ecological thinking in parasitology.

Infrapopulations of parasites, as defined by Esch *et al.* (1977), can be sampled in series from one host, or once from many individuals in a host population. Repeated sampling of many infrapopulations should provide insight into general population trends of each microparasite within a single host population.

The present study evaluates the life strategies of three blood microparasites in a population of blue grouse

(*Dendragapus obscurus*) by analysis of population dynamics in naturally infected captive and wild birds. Before this was undertaken, circumstantial evidence from population dynamics of round and elongate gametocytes of *Leucocytozoon* was used to evaluate how many *Leucocytozoon* species were present. The three parasites are *Leucocytozoon* sp., *L. bonasae* Clarke 1935 and *Haemoproteus mansoni* Castellani and Chalmers 1910 (see revision by White and Bennett, 1979).

Gametocyte population behaviour in *Haemoproteus mansoni* was compared during primary and relapse periods in Chapter III, and the width of the transmission window was discussed in connection with gametocyte output. A model was proposed to account for the evolution of population behaviours during primary and relapse periods. Primary gametocyte populations of *Leucocytozoon* sp. and *H. mansoni* were also compared in Chapter V; the relationship of these changes to erythrocyte longevity and dependability of the vertebrate host for the reproduction of the parasite were discussed. In Chapter IV population dynamics of the three microparasites were assessed. Although this study was of short duration (three years), it was assumed that the chosen parameters will influence the evolution of microparasites. A model for selection processes in microparasites was developed in the final discussion by applying ideas from non-parasitic systems. Predictions about life history characteristics of microparasites were made from the model.

General life cycle of Haemosporina

A basic understanding of the life cycle of these microparasites is essential for a comprehension of life history patterns. Sporozoites are introduced into the avian blood stream by biting flies (Bennett and Fallis, 1960; Fallis and Bennett, 1961,1966; Herman and Bennett, 1976). Once in the blood stream, sporozoites seek target organs, and then reproduce asexually (merogony) within specific cells. The cycle of merogony may be repeated several times, and then finally produce male and female gametocytes. This sexual stage occurs in the peripheral blood, and represents overall survival and reproductive output within the avian host. Gametocytes are then ingested in the blood meal of an ornithophilic fly. Fertilization occurs and is followed by asexual reproduction (sporogony). Sporozoites move to and are stored in the salivary glands ready to be transmitted when the fly feeds. *Leucocytozoon* and *Haemoproteus* overwinter in the tissues of infected birds; relapse of infection, as evident from the reappearance or increase in gametocyte intensity, may occur in the following spring (Fallis *et al.*, 1974).

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II. Differentiation of two *Leucocytozoon* species in blue grouse

Abstract

Population dynamics of round and elongate gametocytes of *Leucocytozoon* in wild and captive blue grouse from Hardwicke Island, British Columbia, were studied from 1980-1982. Blue grouse (*Dendragapus obscurus*) chicks were sampled weekly throughout each transmission season. In naturally infected chicks, three patterns were detected: (1) round gametocytes, (2) elongate gametocytes only, and (3) round and elongate gametocytes. Patterns did not change in 60 captive birds sampled for 2.5 years. Prevalence of round and elongate gametocytes in wild blue grouse chicks were different in two of the three years. Reexposure of yearlings already infected with only elongate gametocytes to simuliids resulted in round gametocyte infections in six of the eight birds. Timing of round and elongate gametocyte appearance during primary infections was synchronous only 50% of the time, and population behaviour of the two gametocytes was very different. Results indicated that round and elongate gametocytes were from different species of *Leucocytozoon*. The elongate gametocytes were identified as *L. bonasae* Clarke, 1935, and round gametocytes were designated as *Leuocytozoon* sp.. This is the first record of sympatric species in *Leucocytozoon*. *Leucocytozoon bonasae* produced elongate gametocytes only in captive blue grouse during a

2.5 year period. This was evidence for a different life cycle than has already been described in *Leucocytozoon* species.

Introduction

Leucocytozoon is one of the more common intracellular microparasites in tetraonids.

The gametocyte stage of *Leucocytozoon* species occurs in the blood as two distinct morphological types, round and elongate. The production of these different morphological forms of gametocytes is a function of the specific life cycle for a given species of *Leucocytozoon*. Such morphological variation is related to the origin of merozoites and deformity of the host cell (Fallis *et al.*, 1974). Two life cycles have been proposed, one which produces a sequence of both round and elongate gametocytes (Chernin, 1952a and b; Desser, 1967; Fallis *et al.*, 1956; Fallis *et al.*, 1951; Fallis *et al*, 1974; Kocan and Clark, 1966) (Figure 1), while in the other life cycle only round gametocytes are produced (Khan and Fallis, 1970)(Figure 2). *Leucocytozoon simondi* in ducks, and *L. danilewskyi* in saw-whet owls, produce a sequence of round and elongate gametocytes during primary infections, whereas *L. dubreuili* in robins, and *L. fringillinarum* in grackles, produce only round gametocytes. Extensive field and laboratory studies with *L. simondi* have defined the relationship between round and elongate gametocytes in that species (Fallis *et al.*,

Figure 1. Life cycle of Leucocytozoon simondi in ducks (Fallis et al., 1974). RES* = Reticuloendothelial system

LIFE CYCLE of L. SIMONDI

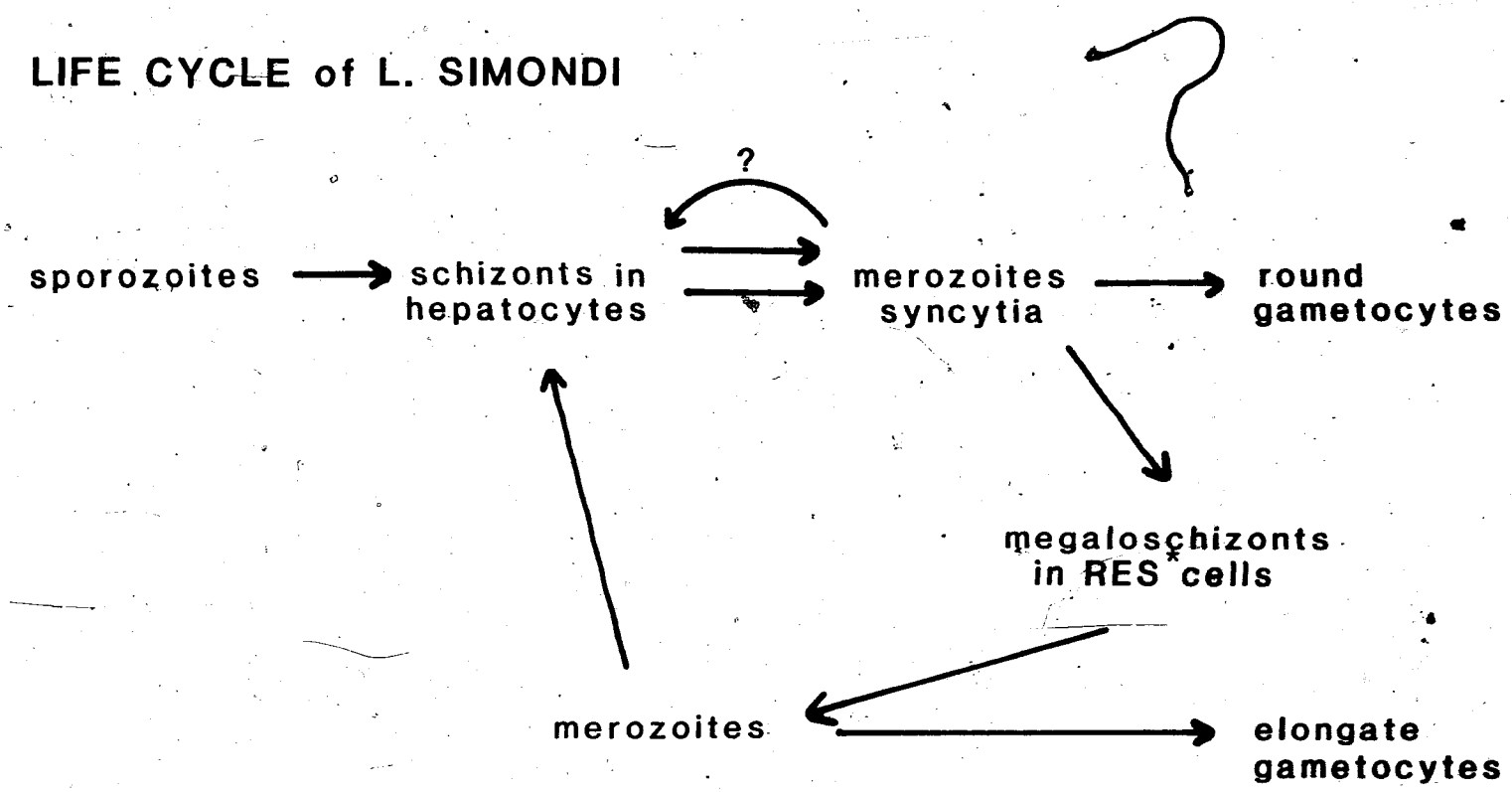
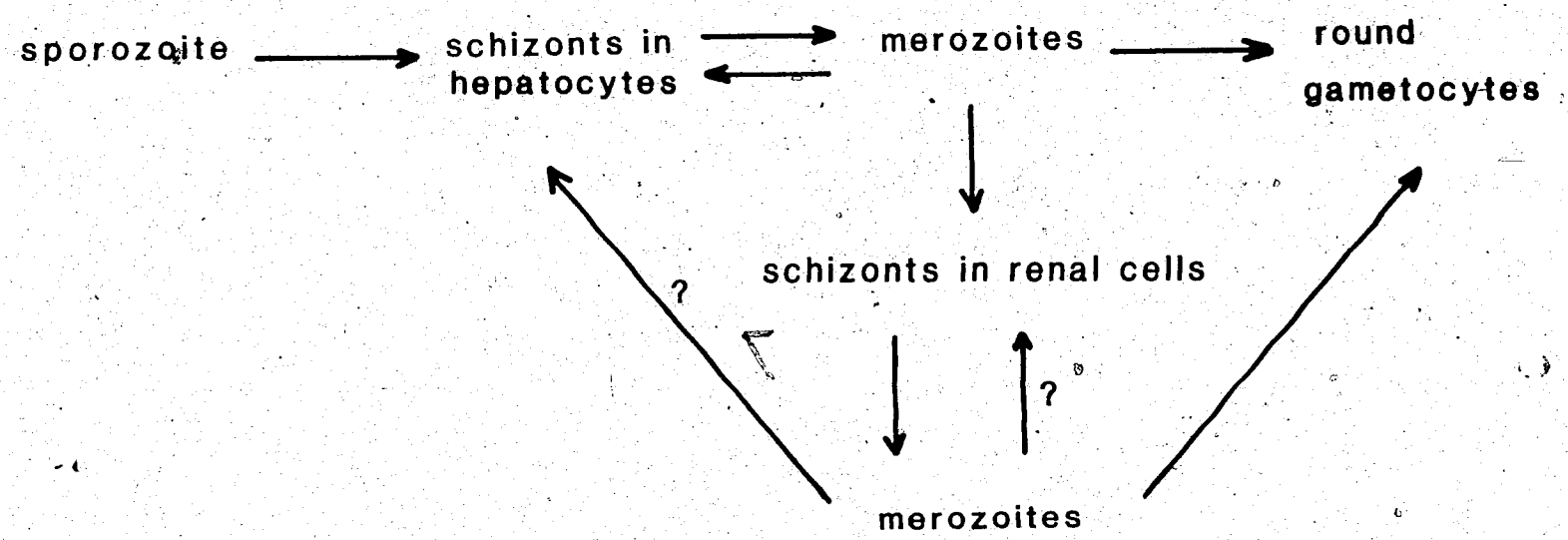


Figure 2. Life cycle of Leucocytozoon dubreuili in robins (Fallis et al., 1974).

LIFE CYCLE of L. DUBREUILI



1974). Transfer of megaloschizonts, the tissue stage precursor of elongate gametocytes, into clean ducks produced both elongate and round gametocytes of *L. simondi* (Yang *et al.*, 1971).

In their review, Fallis *et al.* (1974) indicate that there is much to be learned in order to refine our knowledge of taxonomy and biology of the *Leucocytozoon*. Bennett and Campbell (1975) reexamined the morphometric characteristics of *Leucocytozoon* gametocytes used to describe species and found round and elongate gametocytes easily distinguishable. However, measurements of round gametocytes from different species revealed significant overlap, which implied synonymity. The authors cautioned trying to distinguish species on this basis.

In the original description of *L. bonasae* in ruffed grouse (*Bonasa umbellus*) only elongate gametocytes were described (Clarke, 1935); however, other researchers have reported both round and elongate gametocytes from ruffed, hazel (*Tetrastes bonasia*), black grouse (*Lyrurus tetrix*), capercaillie (*Tetrao urogallus*) (Borg, 1953; Fallis and Bennett, 1958; Newman, 1968). This difference in gametocyte type could have several interpretations. Presumably earlier authors regarded round and elongate gametocytes as morphological variants of the one species. This interpretation has been strengthened by a reported absence of sympatric species in *Leucocytozoon*. In addition, cross transmission of other *Leucocytozoon* species from

non-tetraonids to tetraonids have been unsuccessful (Bennett, 1960; Fallis and Bennett, 1962; Fallis *et al.*, 1954; Solis, 1973).

Observations on which gametocyte types are produced must be interpreted carefully, as methodologies vary. Numbers of hosts sampled, number of samples from an individual host, and time of year when sampled in relation to transmission season may affect data and conclusions. Repeated sampling over an extended period is required before results can be a true reflection of the type of gametocytes produced. Only then can distinctions about life cycles and species characteristics be made.

In this study evidence from population dynamics of round and elongate gametocytes in blue grouse (*Dendragapus obscurus*) help to elucidate the relationship of these two gametocytes. Prevalence patterns from year to year and gametocyte infrapopulation dynamics through time were evaluated. Three patterns of gametocyte production were present in the grouse population. The objective was to determine if these patterns were the expression of one or more than one species of *Leucocytozoon*.

Data from captive and wild blue grouse naturally infected with *Leucocytozoon* were used to evaluate prevalence levels, timing in appearance of gametocytes during a primary infection, gametocyte population profiles, and intensities and reexposure of grouse to vectors. These data show conclusively that the patterns observed are best explained

in terms of two sympatric species of *Leucocytozoon*. Taxonomic designations were suggested and the involvement of population analysis in *Leucocytozoon* research were discussed. In addition, there were implications of another life cycle for *Leucocytozoon* species in which only elongate gametocytes were produced.

Materials and Methods

Wild blue grouse were sampled for blood parasites on a study area on Hardwicke Island (50° 28'N, 125° 48'W), located off the east-central coast of Vancouver Island, British Columbia. A 460 ha area was the main study site for an intensive population ecology research project (Jamieson and Zwickel, 1983). The vegetation consisted of regrowth western hemlock forest in different stages of succession. Birds were captured and bled during field observation by using the location and capture methods of Zwickel and Bendell (1967). Blood from a brachial vein puncture was smeared on duplicate slides which were identified by bird band number. A total of 1490 birds were sampled between April and August 1980 to 1982. Samples were 713, 528 and 249 in 1980, 1981 and 1982 respectively.

Chicks up to two weeks old, as determined by the method of Redfield and Zwickel (1976), were captured during the last week in June and the first week in July 1981 and 1982, at Adams River (24 Km south west of Hardwicke Island), on Vancouver Island. Adams River was chosen as a collection

site because the grouse on Hardwicke Island were part of an ongoing population study and were unavailable for collection. Broods were located with an English pointer dog, and subsequently caught by hand, or with a handheld fishing net. Patagial wing tags with the addition of numbered plastic streamers were used for identifying individuals, until the chicks were large enough to receive numbered metallic leg bands. Chicks were transported from Adams River to Hardwicke Island in well ventilated polystyrene coolers. Before commencing the trip, wheat heated to 40° C and packed in tinfoil was placed in the bottom of the container to provide warmth for the chicks while in transit.

An aviary was built on Hardwicke Island (Figure 3). A section of the aviary was equipped with a brood house which was heated by an under-floor propane heater. A metal cone, 60 cm in diameter, was suspended from the roof of the brooder and positioned with its rim 6 cm above the netting floor, to provide a heat trap under which the temperature remained at approximately 40° C. For the first few days, until the chicks became familiar with their surroundings, they were locked in the brooder. After a week, chicks were free to roam the aviary and returned to the brooder when cold. The heater was no longer necessary once the chicks were a month old.

Newly captured chicks were fed a mixture of ground turkey starter (21% protein), chopped lettuce and hard boiled eggs (Lance *et al.*, 1970). Eggs were removed from the

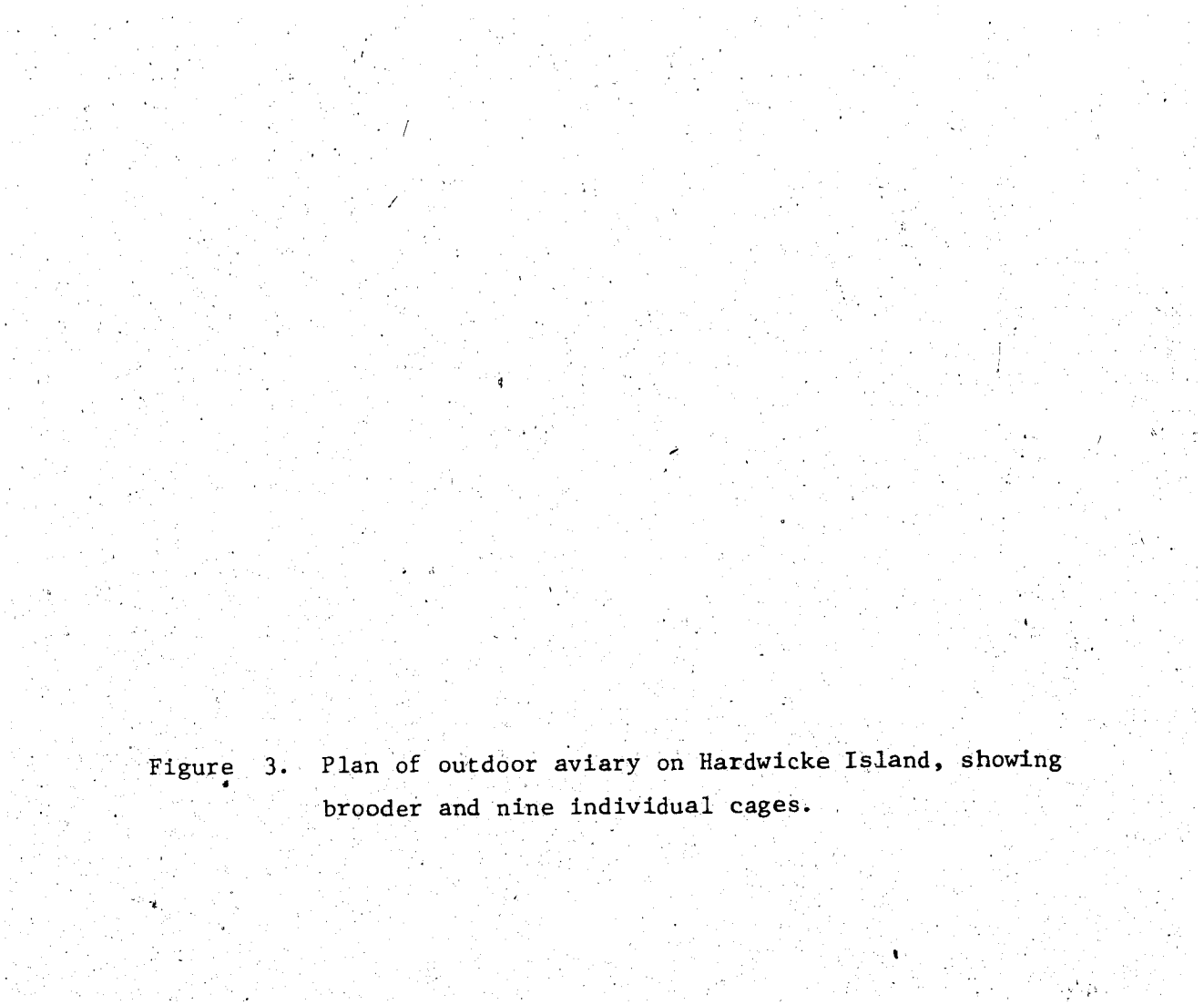
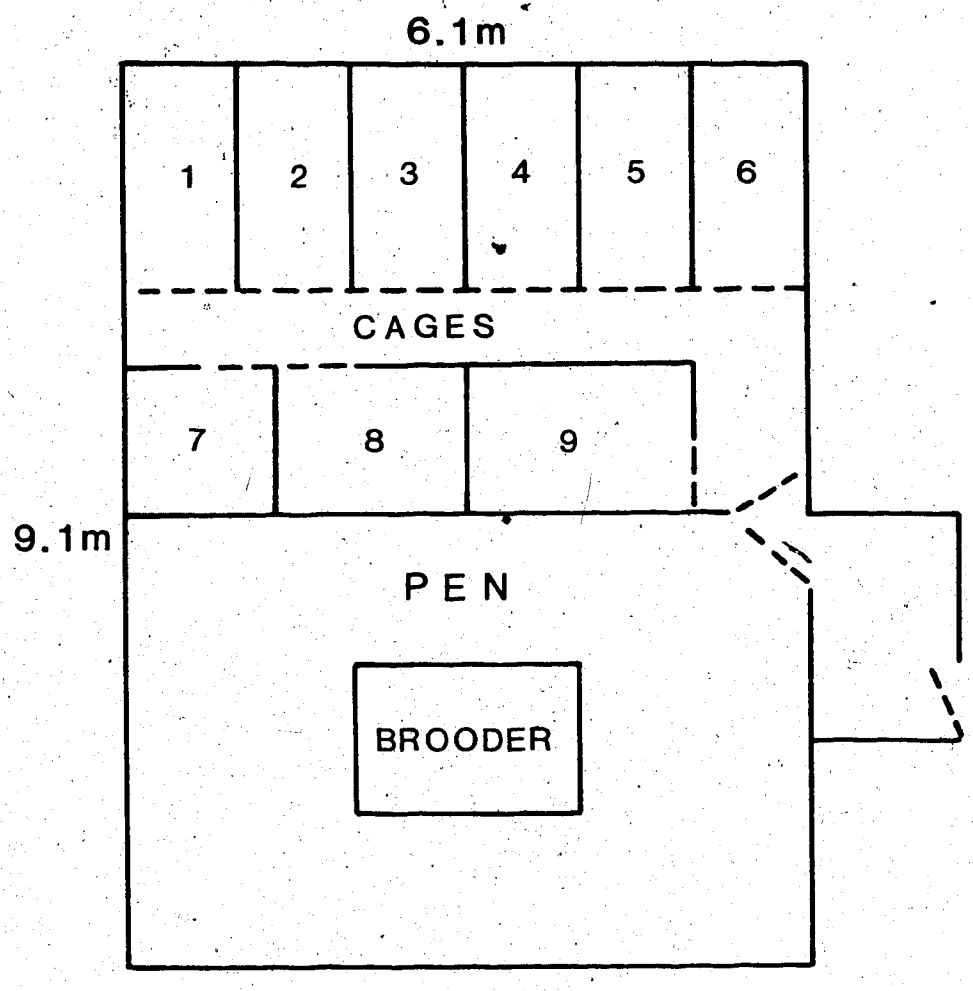


Figure 3. Plan of outdoor aviary on Hardwicke Island, showing brooder and nine individual cages.


6.1m
1
2
3
4
5
6
CAGES
7
8
9
9.1m
PEN
BROODER

diet after the first week and by August the young birds ate only turkey starter. Fresh water was provided and the soil floor of the aviary scraped clean every day. For ease of disposal, a galvanized metal sheet under the brooder served to collect faeces. Mold growth on faeces was a problem, therefore careful cleaning was essential in order to reduce the possibility of aspergillosis.

At the end of August, 38 chicks in 1981 and 36 chicks in 1982 were shipped by air, in well ventilated wooden boxes, to Biological Animal Services, at Ellerslie, Alberta. Different age classes were housed separately in large indoor rooms (4.9 m^2). In each room, nest boxes, perches, turkey finisher (17% protein) and water were supplied. Each room had independent light controls which, unless otherwise stated, were set during the summer for 8D - 16L and in the winter at 16D - 8L. Temperature was held constant at 25° C.

Captive grouse were bled weekly using the method described for wild grouse. Diurnal fluctuations in peripheral *Leucocytozoon* numbers are known to occur (Gore and Noblet, 1978; Roller and Desser, 1973). To minimize the possible effect of these fluctuations on gametocyte intensity in the peripheral blood a standard sampling schedule was undertaken; sampling was 2-3 hr. after [illegible]ise in the outdoor aviary (Hardwicke Is.), and 2-3 hr. after the artificial lights were switched on in the indoor aviar[illegible] (Alberta).

Birds were weighed every week, as weight loss was the best barometer of health problems. Birds showing a dramatic weight loss were isolated in small cages, but not removed from the room unless a heavy infection of aspergillosis was suspected. Rooms were scraped daily and disinfected weekly. When chicks in the winter of 1981 contracted aspergillosis, the above cleaning schedule was sufficient to relief this problem. During the winter of 1982, birds became infected with the enteric bacterium *Clostridium colinum*, which was controlled by administering Neotetracycline or Tetracycline in the drinking water.

In May 1982, 11 yearlings from the 1981 cohort were returned by air to Hardwicke Island from Alberta. They were housed in individual cages equipped with shelters, in the aviary. Turkey finisher and water were provided and the cages were cleaned every second day. During the summer of 1982, eight birds from the same cohort remained in Alberta and were kept indoors in fly-free conditions at Biological Animal Services, at Ellerslie.

Blood slides were fixed in absolute methanol for two minutes, air dried and stained with Giemsa (Harleco, Gibbstown, N.J. 08027 U.S.A.) buffered to pH 6.8. After staining for one hour, slides were rinsed with buffered water, air dried, and then examined microscopically. Prevalence of parasites was determined by a five minute search under 100x and 400x magnification. Intensity of *Leucocytozoon* species is often quoted per 10,000 RBCs

(Williams, Mahrt and Zwickel, 1980); however, in this study intensities were so low that counts of 150,000 RBCs were required to monitor very mild infections. A modification of Van Riper's method provided estimates of RBC numbers (Van Riper, 1982). Microscope fields at 400x were classified according to the density of RBCs. The average number of RBCs per field was calculated from a count of 10 fields at a particular subjective classification, i.e., very dense (no spaces between RBCs, but little overlap) to few (very patchy, with half of field empty). The number of fields required to give a total of 150,000 RBCs was determined. To minimize error, all intensity data were collected by one observer. Morphometric measurements of round and elongate gametocytes were also determined.

Results

Round and elongate gametocytes

Figure 4 illustrates the generalized shapes of round and elongate gametocytes. The morphometric dimensions diameter 1 and diameter 2 of round and elongate gametocytes were different (Table 1). Elongate gametocytes were elliptical, and thus diameter 2 was significantly larger than in round gametocytes; in addition, elongate gametocytes had cytoplasmic tails which were absent in round gametocytes. No intermediate forms were present.

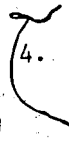
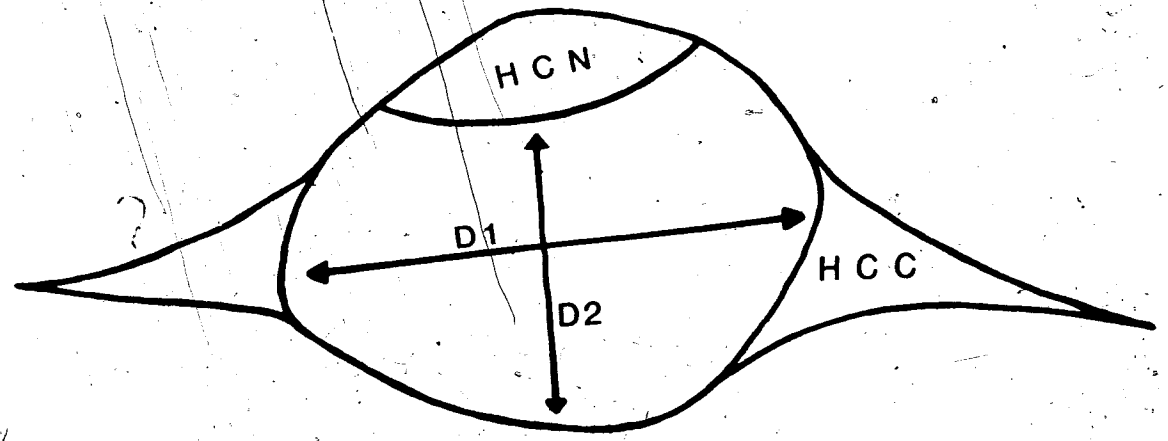
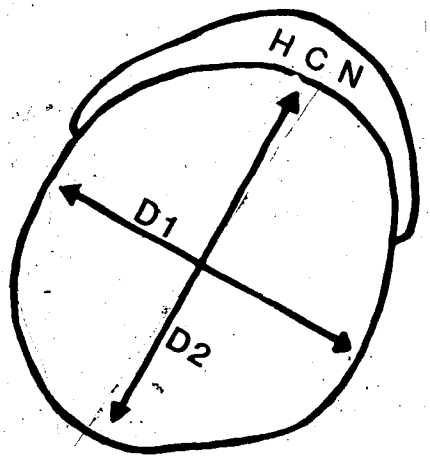


Figure 4. Generalized shape of round and elongate gametocytes, indicating diameter 1 (D1) and diameter 2 (D2).



ELONGATE GAMETOCYTE



ROUND GAMETOCYTE

D 1 = diameter 1

D 2 = diameter 2

HCN host cell nucleus

HCC host cell cytoplasm

TABLE 1. DIMENSIONS OF ROUND AND ELONGATE GAMETOCYTES FROM PERIPHERAL BLOOD SMEARS OF BLUE GROUSE.

DIMENSION	MEAN ± SD (um)	SAMPLE SIZE	T	P
Diameter 1*				
Round	10.8 ± 0.8	50	24.4	0.001
Elongate	16.9 ± 1.5	50		
Diameter 2*				
Round	10.3 ± 1.0	50	3.3	0.002
Elongate	9.4 ± 1.4	50		

* = see Figure 4.

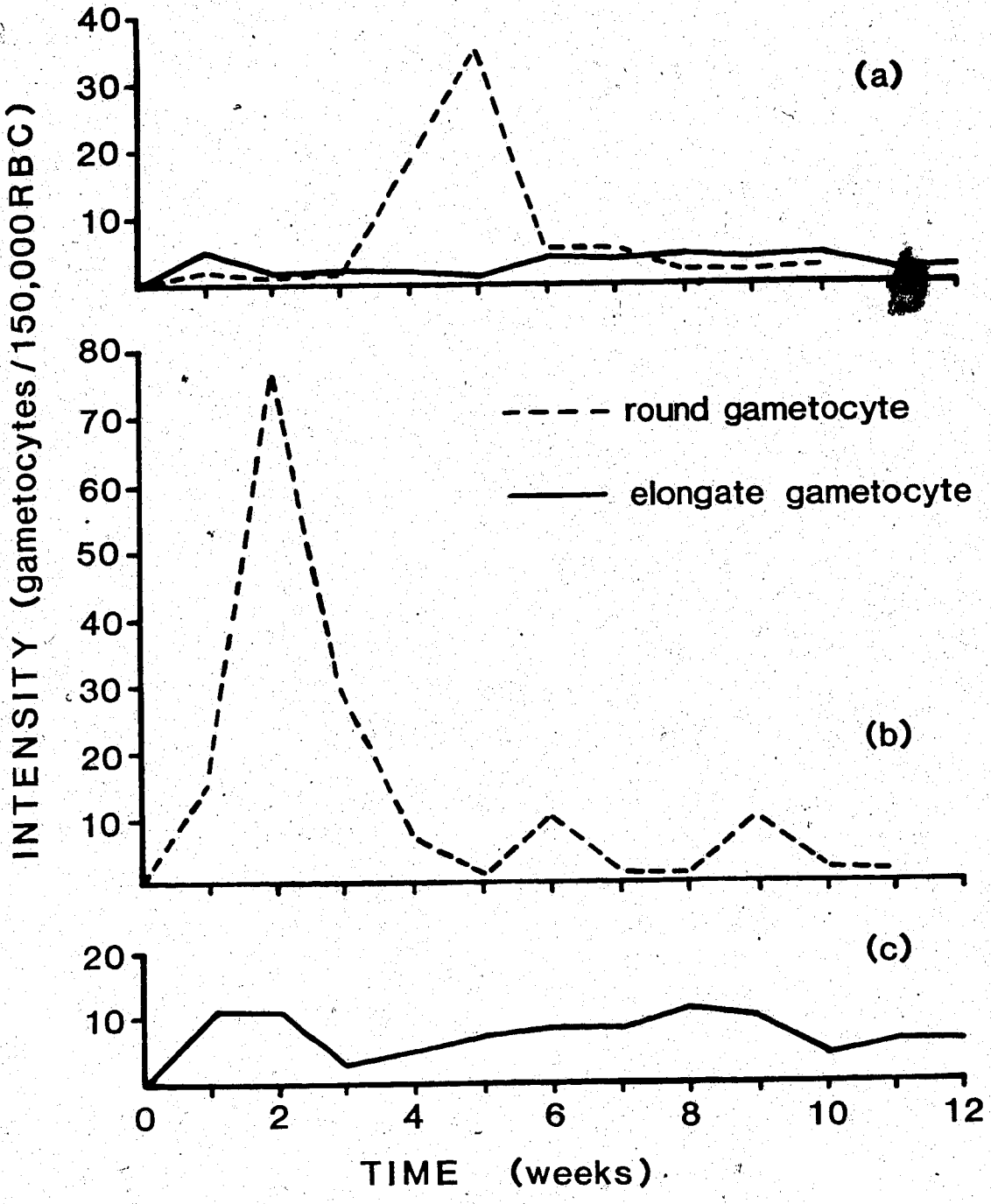
Patterns of infection

Primary infections of *Leucocytozoon* in captive chicks were followed by sampling blood on a weekly basis. Infrapopulations from chick cohorts 1981 and 1982 fell into three patterns. The number of birds exhibiting each pattern varied from year to year. In pattern (a) both round and elongate were present (Figure 5a). Twenty-one birds had this pattern, four(14%) in 1981 and 17 (46%) in 1982. In pattern (b) only round were recorded in a primary infection; bird 93 in 1982 was the only example (Figure 5b). Pattern (c) was exhibited by 37 birds which produced exclusively elongate, 24 (86%) in 1981 and 13 (43%) in 1982 (Figure 5c). Within individual birds these patterns were highly consistent. Fifteen (a), one (b) and 17 pattern (c) infrapopulations were followed for over one year; there were no deviations from these patterns. In all, 2800 blood samples from 60 captive grouse, over a two and a half year period, adhered to three patterns. Although there were few repeated samples from individual wild birds, these patterns were observed in all three years as well as in the three age classes: chicks, yearlings and adults.

Prevalence of round and elongate gametocytes in chicks

Prevalence of elongate in captive chicks was high, 74% in 1981 and 84% in 1982, and did not vary significantly between years ($X^2=0.58$, $p>0.05$). Similarly, high prevalence was observed in the field, by the end of August the range

Figure 5. Primary infrapopulation profiles in three blue grouse chicks with (a) round and elongate gametocytes, (b) round gametocytes, and (c) elongate gametocytes.


INTENSITY (gametocytes/150,000RBC)
40
30
20
10
(a)
80
70
60
50
40
30
20
10
round gametocyte
elongate gametocyte
(b)
20
10
(c)
0
2
4
6
8
10
12
TIME (weeks)

was 87-99% infected between years (Table 2).

Prevalence of round in captive chicks differed between years, with 11% in 1981 and 50% in 1982 ($X^2=14.0$, $p<0.05$). Prevalence in wild chicks also varied from year to year. By August it ranged from 65-96% ($X^2=19.31$, $p<0.05$).

Captive chicks had different prevalences of round and elongate within the same year (1981: $X^2=31.5$, $p<0.05$) (1982: $X^2=9.06$, $p<0.05$). Prevalence of round and elongate in wild chicks was different in 1980 and 1981, but similar in 1982 (1980: $X^2=10.07$, $p<0.05$) (1981: $X^2=4.13$ $p<0.05$) (1982: $X^2=1.29$, $p>0.05$).

Reexposure experiment

Eight yearlings previously infected with only elongate gametocytes were reexposed to black flies in their second summer. Six of the eight developed round infections coincidental with primary round infections in captive chicks. No significant difference occurred between peak round intensity in chicks and reexposed yearlings (Mann Whitney test, $p>0.05$). Elongate gametocytes were found in these reexposed yearlings throughout the summer. Control birds from the same cohort produced elongate gametocytes while in fly-free conditions at Biological Animal Services at Ellerslie. They did not produce round gametocytes.

Asynchrony in developing infections

TABLE 2. PREVALENCE AT END OF FIELD SEASON FOR ROUND AND ELONGATE GAMETOCYTES OF LEUCOCYTOZOON IN WILD AND CAPTIVE JUVENILE BLUE GROUSE.

	PREVALENCE (N) Wild juveniles (15-30 g.)			PREVALENCE (N) Captive juveniles*	
	1980	1981	1982	1981	1982
Round	65(64)	72(61)	96(68)	11(36)	50(38)
Elongate	89(64)	87(61)	99(68)	74(36)	84(38)

* captive chicks sampled on the 22nd. of August of both years.

During primary infection the sequence of appearance of gametocytes in the peripheral blood of birds with both types of gametocytes can be evaluated from weekly samples taken during the transmission season. Table 3 presents the timing of elongate in relation to the appearance of round. Elongate were observed up to three weeks in advance or four weeks post appearance of round. Synchronous timing occurred in approximately 50% of cases. Secondary elongate infections in reexposed yearlings were only occasionally synchronized with round (Table 3).

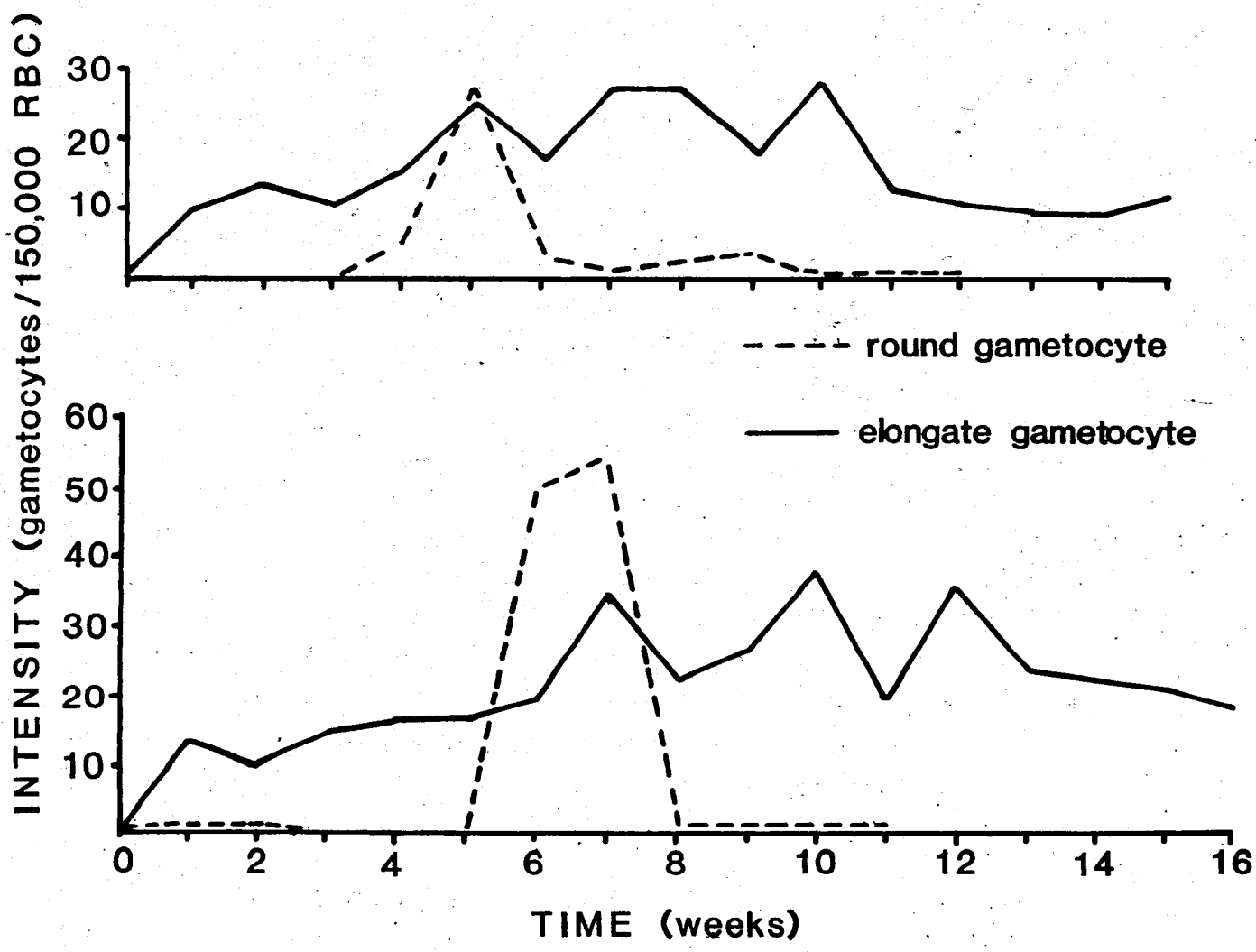
Primary profile

During the first 10 weeks of a primary infection, round and elongate populations grew and declined in distinctly different manners (Figure 5). Round populations rose exponentially, reached a peak and declined to a chronic level. In contrast, some elongate populations rose gradually to a high, and then declined over many weeks to a lower level; some infections were persistently low throughout the primary infection with no appreciable rise. The difference between round and elongate gametocyte population behaviour (refers to the trend in the gametocyte population profile through time) was consistent, and not due to low intensity elongate being compared with high intensity round infections (Figure 6). In addition, population behaviours were not subject to change as a result of pattern or sequence in appearance of the gametocytes; likewise, age of the host had

TABLE 3. TIMING IN APPEARANCE OF ELONGATE IN RELATION TO ROUND GAMETOCYTES IN CAPTIVE BLUE GROUSE.

	No. of weeks in advance of round			Same time	No. of weeks post appearance of round			
	3	2	1	0	1	2	3	4
Primary (juveniles)	2	1	6	10	1	0	0	1
Reexposed	0	2	2	2	0	0	0	0

Figure 6. Primary infrapopulation intensity profiles in two blue grouse with round and elongate gametocytes.


INTENSITY (gametocytes/150,000 RBC)
30
20
10
round gametocyte
elongate gametocyte
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
TIME (weeks)

no effect.

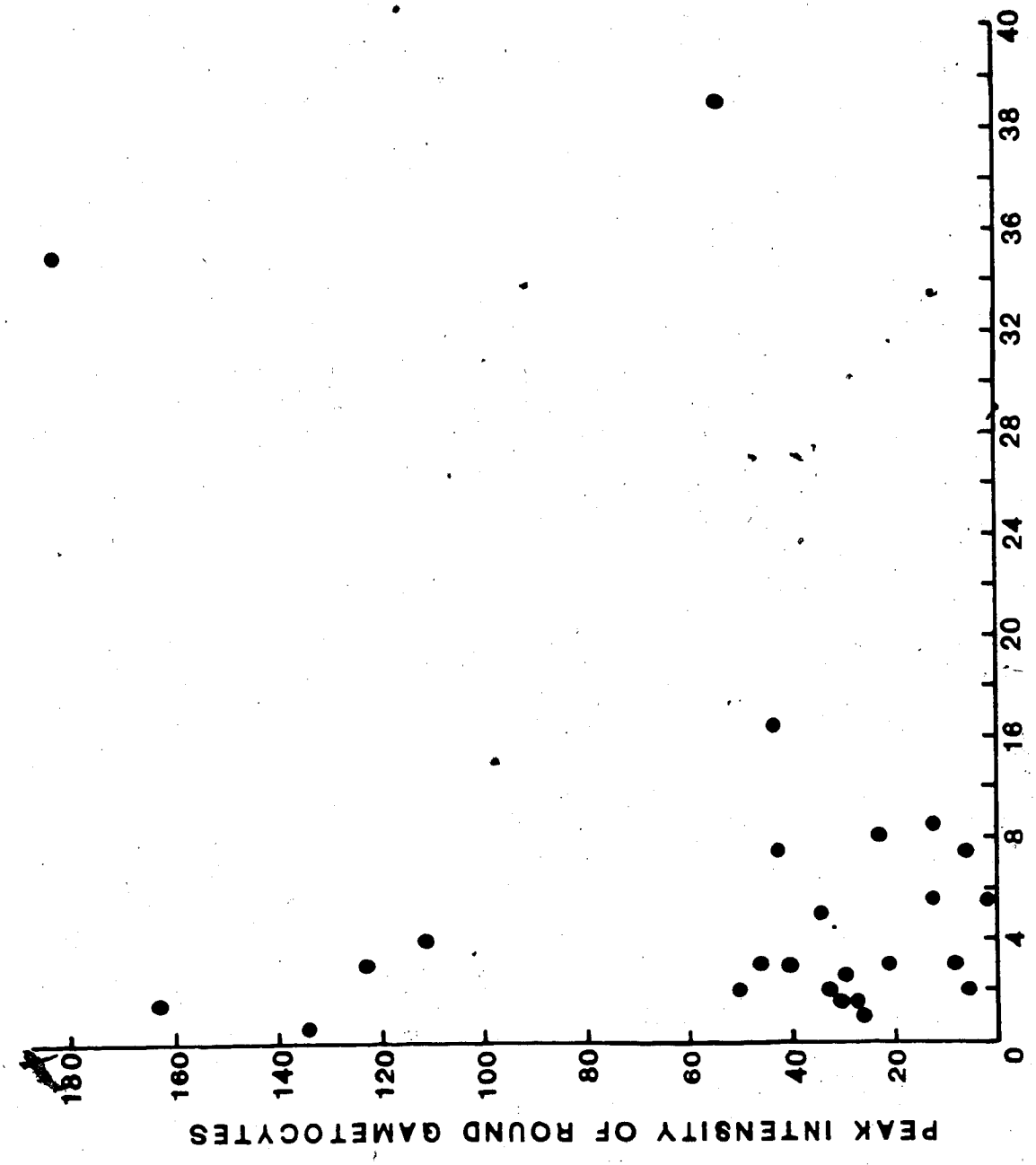
A regression analysis was performed with peak round on peak elongate gametocyte intensity in birds with pattern (a) (Figure 7). The regression was not significant, and therefore there was no relationship between peak intensities of the two types of gametocytes during primary infections in captive grouse ($t=1.21$, $p>0.05$).

Discussion

Three patterns in the type of gametocyte produced during a primary infection were observed in captive blue grouse. Such variation in the expression of the gametocyte stage within a single population of vertebrate hosts suggests a different interpretation than has previously been reported in *Leucocytozoon*. In Clarke's original description of *L. bonasae* in ruffed grouse, only elongate gametocytes (pattern (c)) were reported (Clarke, 1935). However, later observations on ruffed grouse and other tetraonid species indicated two types of gametocytes, round and elongate (pattern (a)) (Borg, 1953; Fallis and Bennett, 1958; Newman, 1968). In this study the observed two types of gametocytes were consistently morphologically different.

No other studies of *Leucocytozoon* have shown evidence for more than one pattern of gametocyte production within a single host population at one geographical location. However, different species of *Leucocytozoon* express independently each of the three patterns observed in this

Figure 7. Peak intensity of round gametocytes (Gametocytes/150,000 RBC) versus peak intensity of elongate gametocytes (Gametocytes/150,000 RBC).


PEAK INTENSITY OF ROUND GAMETOCYTES
0
20
40
60
80
100
120
140
160
180
PEAK INTENSITY OF ELONGATE GAMETOCYTES
0
4
8
16
20
24
28
32
36
38
40

study. *Leucocytozoon simondi* in ducks and *L. danelewskyi* in saw-whet owls produce both types of gametocytes, as in pattern (a) of blue grouse (Desser, 1967; Khan, 1975; Yang *et al*, 1971). *Leucocytozoon dubreuili* and *L. fringillinarum*, produce round gametocytes only, as in pattern (b) (Khan and Fallis, 1970). Elongate gametocytes only, pattern (c), were observed in *Leucocytozoon* infections of Falconiformes and Galliformes (Fallis *et al.*, 1974). The life cycle for *Leucocytozoon* species exhibiting pattern (c) has not been determined.

In blue grouse all three patterns were present at the same time, and in the same host population. In addition, the proportion of captive birds exhibiting each pattern varied from year to year. Two hypotheses can explain these results. They are: (i)two species are present, one produces round gametocytes only (pattern (b)), and one produces elongate gametocytes only (pattern (c)), with dual infections resulting in pattern (a) or (ii)one species is present and produces all three patterns.

Superficially each pattern seen in blue grouse was similar to that seen in other *Leucocytozoon* species; however a more detailed analysis will describe the pertinent differences. As pattern (a) has two types of gametocytes, it should follow predictions made from other *Leucocytozoon* species with pattern (a).

The timing in appearance of round and elongate gametocytes is a consistent feature of other *Leucocytozoon*

species with pattern (a). During a primary infection of *L. simondi* or *L. danelewskyi* variation in the developmental time, after introduction of sporozoites, resulted in round gametocytes appearing in the peripheral blood first, and then three days later elongate appeared (Desser, 1967; Fallis *et al*, 1951; Khan, 1975). This was a consistent feature of these infections.

Desser (1967) artificially infected six ducks with *L. simondi* and followed development of infection over 14 days. Approximately equivalent peak intensities of round and elongate were produced during a primary infection. One could hypothesize that one species of *Leucocytozoon* producing morphologically distinct gametocytes should produce comparable peak levels of each gametocyte during individual infections. A correlation should exist between the peak levels of each gametocyte observed in an infection. Desser (1967) also observed equivalent population behaviours of round and elongate in experimentally infected ducks, as both populations rose and fell exponentially; natural infections follow a similar development (Fallis *et al.*, 1951).

Evaluation of pattern (a) in blue grouse using the above predictions of: (1) timing in appearance of round and elongate is consistent, (2) equivalent peak intensities of round and elongate are produced during a primary infection, and (3) equivalent population behaviours are observed in round and elongate, produced contrasting results. Analysis of population dynamics for round and elongate gametocytes in

blue grouse indicated that pattern (a) in this system did not conform to pattern (a) observed in other *Leucocytozoon* species. The two gametocytes in blue grouse were not linked in origin. Timing in appearance of elongate in relation to round gametocytes during primary infections in captive blue grouse chicks with pattern (a) was highly variable (Table 3). This variability was observed in chicks from 1981 and 1982 cohorts, and also in yearlings reexposed in 1982. Such variation in sequence of development of gametocyte populations in grouse may incorporate variation in development, but such large divergences are most likely a consequence of different transmission systems vectoring different *Leucocytozoon* species. In addition, elongate appeared first in 9 of 21 cases which was the opposite sequence to that reported for *L. simondi* and *L. danelewskyi*. No relationship exists between peak intensities of round and elongate in primary infections of blue grouse; the regression of peak round on peak elongate intensity was not significant(Figure 7). Primary infections of round and elongate in blue grouse develop in completely different manners independent of pattern, age of grouse, intensity or sequence in appearance of gametocytes. In *L. simondi*, similar population behaviours do not confirm the relatedness of round and elongate; but conversely, totally different population profiles of the two gametocytes in blue grouse indicate different selective pressures acting on the populations. Wild fluctuations in the timing of appearance

of gametocytes, disjointed primary peak intensities, and contrasting population profiles were opposite to that observed in other species of *Leucocytozoon* with pattern (a). I therefore provisionally accept Hypothesis (i) as pattern (a) was not a result of a single species, but the coexpression of two independent species. The three patterns can be explained in terms of two independent entities.

Can the three patterns of gametocyte production in blue grouse be the expression of one species? If all patterns were the result of a species varying production of gametocyte types in response to varying resistance of individual hosts, one would not expect population profiles of each gametocyte to differ with respect to each other but to differ with respect to each host. This was not the case. The two gametocyte population behaviours were consistently different, and were not altered by individual hosts.

Six birds with primary elongate gametocyte infections were reexposed in their second summer. They all developed round infections at the same time as chicks. Reexposure resulted in a change in pattern. This was the only occasion when a pattern changed. The development of round gametocyte populations in reexposed yearlings of equivalent intensities to that of chicks, implies no difference in resistance to round between birds, with or without round after a first exposure. Absence of round gametocytes in chicks and mature birds was a consequence of lack of contact with the vector. Moreover, each infrapopulation showed high fidelity to a

particular pattern; in 2800 samples there were no unexplained switches from one pattern to another. Absence of irregular occurrences in these infection patterns indicated tight control, and lack of variable expression on the part of the parasites.

In addition, data from wild blue grouse supported hypothesis (i) and refuted hypothesis (ii). Prevalence of elongate and round in chicks was different in two of the three years. This was most likely the result of different transmission levels for each gametocyte type; it was not a consequence of changing responses of one parasite and/or changes in resistance of the grouse population. It is unlikely that either parasite or hosts population genetics would change so dramatically between years. Moreover, captive chicks exhibited a similar trend with large differences in prevalence of round, but little change in prevalence of elongate between years. Changes in transmission levels of two parasites was the only logical explanation for these observations.

In conclusion, the three patterns in gametocyte output can be adequately explained in terms of two sympatric species. Difference in prevalence and timing in appearance of the two gametocytes must be a consequence of different levels and timing of transmission. A lower prevalence of round gametocytes in some years was not due to differential resistance of hosts.

Differences in the type of gametocyte produced by *Leucocytozoon* species have been noted in Canada geese infected with *L. simondi* (Desser and Rychman, 1976). Only round gametocytes were produced in some geese, while others produced both types of gametocytes. Recycling the infection from geese with only round gametocytes through ducks produced a normal cycle, i.e. round first and elongate second. Desser *et al.* (1978) concluded that absence of elongate gametocytes in some goose infections was the result of strain differences. Such an explanation for the *Leucocytozoon*/blue grouse system will not suffice. Pronounced differences in the behaviour of the two gametocyte populations and the resilience of these differences regardless of intensity of infection, suggests different evolutionary forces shaping each parasites life strategy.

I suggest the following taxonomic designation for these species. The species producing elongate gametocytes conforms to Clarke's original description and remains as *L. bonasae*, while round gametocytes will be referred to as *Leucocytozoon* sp.. This *Leucocytozoon* may be a vertebrate host generalist, and therefore may be present in other bird species from Hardwicke Island. This will be discussed further in succeeding chapters.

Single infections of *L. bonasae* in captive grouse produced elongate gametocytes for up to 2.5 years. This suggested a third type of life cycle for *Leucocytozoon*, one

in which only elongate gametocytes are produced. Although elongate gametocytes have been recorded from other species (Greiner and Kocan, 1977), extensive monitoring of infections will be required to provide sufficient evidence to confirm the absence of round gametocytes at any stage in the life cycle. This study provides such evidence.

Sympatric species are a newly described phenomenon for *Leucocytozoon* spp.. *Leucocytozoon* sympatry may occur in other grouse as both types of gametocytes were present (Borg, 1953; Fallis and Bennett, 1958; Newman, 1968). Are these sympatric species or one species? The study of infrapopulation dynamics may prove fruitful for future studies of *Leucocytozoon* not only as a tool for population biologists, but also as an additional method of unravelling taxonomic problems.

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III. Influence of transmission window width on population behaviour of three microparasites

Abstract

Leucocytozoon sp., *L. bonasae* and *Haemoproteus manson*i infections in captive blue grouse were monitored weekly throughout primary and relapse periods. Chicks became naturally infected with *L. bonasae* and *Leucocytozoon* sp. during 5-9 weeks of exposure, whereas *H. manson*i was transmitted during a much shorter period. Relapse infections in both captive and wild blue grouse had constant monthly prevalence for *L. bonasae* and *Leucocytozoon* sp.. By contrast, prevalence increased throughout the relapse period for *H. manson*i. The intensity also showed a similar rise throughout the relapse period. The patent period in infrapopulations of *H. manson*i was 5-11 weeks for primary, and 11-26 weeks for relapse infections. Likewise, peaking time was 1-4 weeks for primary, and 5-11 weeks for relapse infections. No such differences were detected in the other two parasites. A precise peaking time was selected for in microparasites with narrow transmission windows, but not in parasites with wide transmission windows. Gametocyte production for a longer period during relapse than during primary periods satisfies constraints imposed by a seasonal

vector on microparasite transmission to vertebrate hosts. A model was proposed to explain the evolution of dual population behaviour during primary and relapse periods in *H. mansoni*.

Introduction

The reappearance or increase in intensity of gametocytes of microparasites in chronically infected vertebrate hosts is recognized as an adaptation of the parasite for transmission via a seasonal vector to the next generation of hosts. A spring rise in prevalence and intensity (relapse) of blood protozoons has been documented for *Leucocytozoon* (Chernin, 1952; Desser *et al.*, 1968), *Haemoproteus* (Alverson and Noblet, 1977; Cowan and Peterle, 1957; Dorney and Todd, 1960; Khan and Fallis, 1969) and *Plasmodium* (Applegate, 1970 and 1971; Farmer, 1962).

A number of factors influence reproductive output throughout primary and relapse periods. The dependability of the vertebrate host for the reproduction of the parasite has a strong influence on the intensities of gametocytes observed during reproductive periods (primary and relapse) (Chapter IV). The amount of time vectors are available for transmission within a season may vary between species of microparasite. Such variations could result in discernible differences in the population behaviour of microparasites. Vectors can be divided into two extreme classes, with respect to seasonal timing: pulse vectors transmit a

parasite for a short period, whereas non-pulse vectors are available over a much longer period. These vectors may have different selective effects on the parasite population with respect to the timing of reproductive output during primary and relapse periods.

The gametocyte population profiles of three microparasites, *Leucocytozoon* sp., *L. bonasae* and *Haemoproteus manson*i, which infect blue grouse were compared over time. Gametocyte output was related to the width of the transmission window (vector pulsing). In *H. manson*i, the time of peak intensity (peaking time) and patent period varied between primary and relapse periods. This was not observed in the other two parasites. An evolutionary model was developed to explain these different population behaviours.

Materials and Methods

Captive chicks were reared in an outdoor aviary on Hardwicke Island, and sampled for blood every week (Chapter II). All captive birds were shipped via air freight to Edmonton at the end of August 1981 and 1982. The techniques for determination of prevalence and intensity of *L. bonasae* and *Leucocytozoon* sp., in wild and captive blue grouse were the same as previously described (Chapter II). The prevalence of *H. manson*i was measured as above; however, since intensities were much higher than those of *Leucocytozoon* spp., the intensity was based on 30,000 RBC.

For *H. mansoni* infections the patent period was the number of weeks gametocytes were present at a level of ≥ 1/30,000 RBC.

By comparing parasite size within erythrocytes, *H. mansoni* gametocytes were classed as young, medium or mature. Young gametocytes ranged in size from just visible to occupying < 1/3 of the erythrocyte, medium occupied > 1/3 to < 2/3, and mature occupied > 2/3 of the cytoplasm of the erythrocyte.

Blood samples were collected from wild blue grouse from April to August. It was important to know if parasites seen in the peripheral blood of wild mature grouse, prior to the appearance of gametocytes in wild chicks, were either relapsing populations, or new infections plus relapsing populations. Since black flies are known vectors for *Leucocytozoon* (Fallis and Bennett, 1958; Fallis and Wood, 1957; Roller and Desser, 1973) and *Culicoides* are vectors for *Haemoproteus* (Fallis and Bennett, 1960; Fallis and Wood, 1957; Greiner *et al.*, 1978), it was important to determine if transmission occurred to older age classes before chicks became infected. Thus, the following procedures were conducted. Eleven yearlings from the 1981 cohort, were used in host baiting experiments from May to August on Hardwicke Island. Nine were infected with *L. bonasae*, three had concurrent *H. mansoni* infections. Only one yearling had previously contracted a *Leucocytozoon* sp. infection. Two yearlings were uninfected. Four infected control yearlings

were kept in fly free conditions, and monitored throughout the summer. Of these, two had *L. bonasae* and two were infected with *H. mansoni*. These control birds were housed with clean birds, none of which became infected.

Black fly trapping experiments were performed using sentinel blue grouse in cylindrical cages, 61 cm in diameter and 30 cm high, with 2.5 cm mesh net. Three cages were placed at separate sites, two in open vegetation beside the outlet stream from Little Lihou Lake on Hardwicke Island, and one in more closed vegetation also close to the stream. Little Lihou Lake was chosen for these experiments, because earlier sampling for black fly larvae and pupae found this site was good habitat for a variety of black fly species (Mahrt, 1982). After blue grouse were exposed to flies for 30 minutes, a large covering cage (90 cm in diameter and 45 cm high), with fine fly proof netting (holes 190 um square) was placed over each cage. After 20 minutes any flies which had been on the sentinel birds were retrieved using an aspirator. Flies were classified as engorged or unengorged.

Programs in MIDAS were used for statistical analysis (Fox and Guire, 1976). Parametric statistics were utilized where variables could be transformed to obtain homogeneity of variance. Otherwise, non-parametric statistics were applied.

Results

Table 1 gives a chronology of appearance of the three parasites in captive and wild grouse chicks. In all years no parasites appeared until the very end of June.

Two clean yearlings exposed in the outdoor aviary, on Hardwicke Island, from mid May did not become infected with *L. bonasae* or *Leucocytozoon* sp. until July. The intensity of *L. bonasae* in nine previously infected yearlings declined throughout the exposure period, until a sharp rise in intensities occurred in some birds on July 21, 1982, and at a later date in others. This was taken as evidence of reinfection. These higher intensities were sustained until December. A similar rise in intensities did not occur in control birds held in fly free conditions.

During the same exposure period, gametocytes of *Leucocytozoon* sp. were not detected in ten uninfected yearlings until July 28, 1982. This was coincidental with *Leucocytozoon* sp. infections in captive chicks. In addition, host baiting experiments were unsuccessful until July 7, 1982, when both engorged and unengorged black flies were caught.

No new *H. mansoni* infections occurred in eight uninfected yearlings used in host baiting experiments until August 24; *H. mansoni* infections appeared in captive chicks on the same date. Thus, relapse in the three microparasites was the period from early spring until patency began in chicks. Relapse was only measured until transmission began;

TABLE 1. CHRONOLOGY OF FIRST APPEARANCE OF *LEUCOCYTOZOON* SP., *L. BONASAE* AND *HAEMOPROTEUS MANSONI* IN WILD AND CAPTIVE BLUE GROUSE CHICKS.

PARASITE	SOURCE	DATE OF FIRST APPEARANCE		
		1980	1981	1982
Leucocytozoon sp.	wild	7/11*	7/07	7/20
	captive	-	8/05	7/28
L. bonasae	wild	6/30	7/01	7/20
	captive	-	7/15	7/21
H. mansoni	wild	7/30	8/23	8/16
	captive	-	9/02	8/24
Sampling began	wild	5/28	6/27	6/25
	captive	-	7/07	7/14

* = month/day

after that point intensity data from mature wild birds could include gametocyte output from newly acquired infections.

Transmission period in captive chicks

Thirty-eight and 36 captive chicks were naturally exposed in 1981 and 1982, respectively. As birds were shipped to Edmonton at the end of August, no further opportunity for transmission occurred after this move. In 1981, chicks were sampled weekly beginning July 7. The first patent infections of *L. bonasae* occurred on July 15 (1981), and new infections appeared every week until September 9, a period of nine weeks. In 1982 sampling began on July 14, with new infections detected for eight weeks, beginning July 21.

New infections of *Leucocytozoon* sp. were recorded over a shorter period. Patent infections first occurred on August 5 in 1981 and July 28 in 1982. They continued to appear for five and six weeks in 1981 and 1982, respectively. During 1982 there were substantial increases in the numbers of birds infected (Chapter II).

Haemoproteus mansoni was not present in blood smears from captive chicks until the beginning of September, 1981 and the end of August, 1982. In both years, new infections were clumped around a single week. In 1981 and 1982, wild chicks first became patent with *H. mansoni* from 15-30 August which was very similar to captive chicks. Captive chicks were removed from contact with vectors at the end of August.

As the prepatent period from *Haemoproteus* spp. was approximately 10-14 days (Fallis and Bennett, 1960; Khan and Fallis, 1971) then captive chicks were exposed to vectors for two to three weeks. Further transmission to wild chicks did occur in September of 1981 (Chapter IV), thus the transmission period calculated from observations on captive chicks (2-3 weeks) will underestimate the transmission window.

Intensity of *H. mansoni* in wild blue grouse

Wild blue grouse did not show changes in geometric mean intensity during primary (July, August) or relapse period (April, May, June) in either *L. bonasae* or *Leucocytozoon* sp. (Chapter IV); however, *H. mansoni* did show dramatic changes. Table 2 presents the geometric mean intensity of *H. mansoni* in wild blue grouse. In 1981 intensity of primary infections peaked in September and declined by October (Kruskal-Wallis test $H=6.55$, $p<0.01$). A peak in September and decrease in intensity by October was also noted in captive chicks infected in the same year (Figure 1). In all years, relapse geometric mean intensity in mature birds rose from April to July (Table 2), and intensities were lower in August, 1981 ($H=6.158$, $p<0.01$). Although the same pattern was observed in 1982, small sample size precluded detection of a sigificant difference ($H=3.48$, $p\geq0.05$).

Relapse prevalence in wild blue grouse

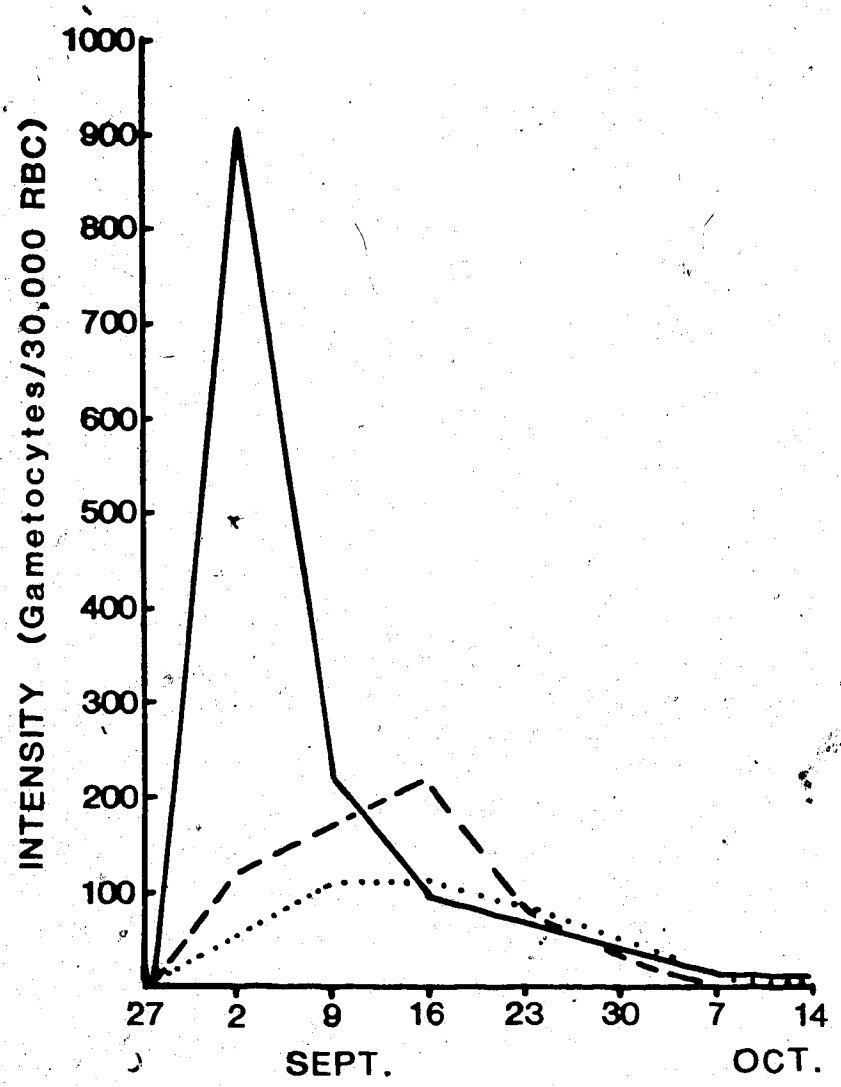
TABLE 2. GEOMETRIC MEAN INTENSITY OF HAEMOPROTEUS MANSONI DURING PRIMARY AND RELAPSE PERIODS IN WILD BLUE GROUSE.

PERIOD	MONTH	GEOMETRIC MEAN INTENSITY+ (N)		
		1980	1981	1982
Primary (juveniles)	August	816(10)	160(6)	609(35)
	September	-	646(4)	-
	October	-	29(6)	-
Relapse (mature birds)	April	2(10)	-	-
	May	8(38)	8(11)	6(7)
	June	220(60)	328(19)	309(33)
	July	2816(22)	1386(19)	1009(8)
	August	-	204(6)	204(5)

+ = Geometric mean intensity expressed as Gametocytes/30,000 RBC.

- = data missing

Figure 1. Profiles of primary infections of Haemoproteus mansoni in three captive blue grouse chicks from 1981.


INTENSITY (Gametocytes/30,000 RBC)
1000
900
800
700
600
500
400
300
200
100
27
2
9
16
23
30
7
14
SEPT.
OCT.

In all three years there were no differences in prevalence of *L. bonasae* in mature wild birds between relapse months, April, May and June (X^2 test for months, $p>0.05$) (Figure 2). With the exception of May, 1982, the monthly relapse prevalence was above 90%. *Leucocytozoon* sp. relapse monthly prevalence never rose above 84% (Figure 3). Within each year there were no significant differences between months (X^2 test for months, $p>0.05$). In all years the relapse prevalence of *H. mansoni* had significant rises from April through May to June (X^2 for months, $p<0.05$). There were no significant differences between June and July in any year (X^2 for months, $p>0.05$) (Figure 4).

Comparison of primary and relapse *H. mansoni* infections

Gametocyte intensities of *H. mansoni* peaked within the first four weeks of primary infection (peaking time); the patent period lasted from 5 to 11 weeks, but for the majority of infections it was 6-8 weeks.

After subsidence of *H. mansoni* primary infections, gametocytes were absent from smears until May - June of the following year. Profiles of three relapsing populations are presented in Figure 5. Intensities rose and fluctuated sharply before declining. Nine captive birds had relapses in the summer following primary infections. These birds accounted for 100% of the sample with *H. mansoni*, as the others died during the winter from various causes which were unrelated to *H. mansoni* inf[illegible]

Figure 2. Relapse prevalence of Leucocytozoon bonasae in mature wild blue grouse sampled from April-June, 1980-1982. Sample size given on top of each bar.

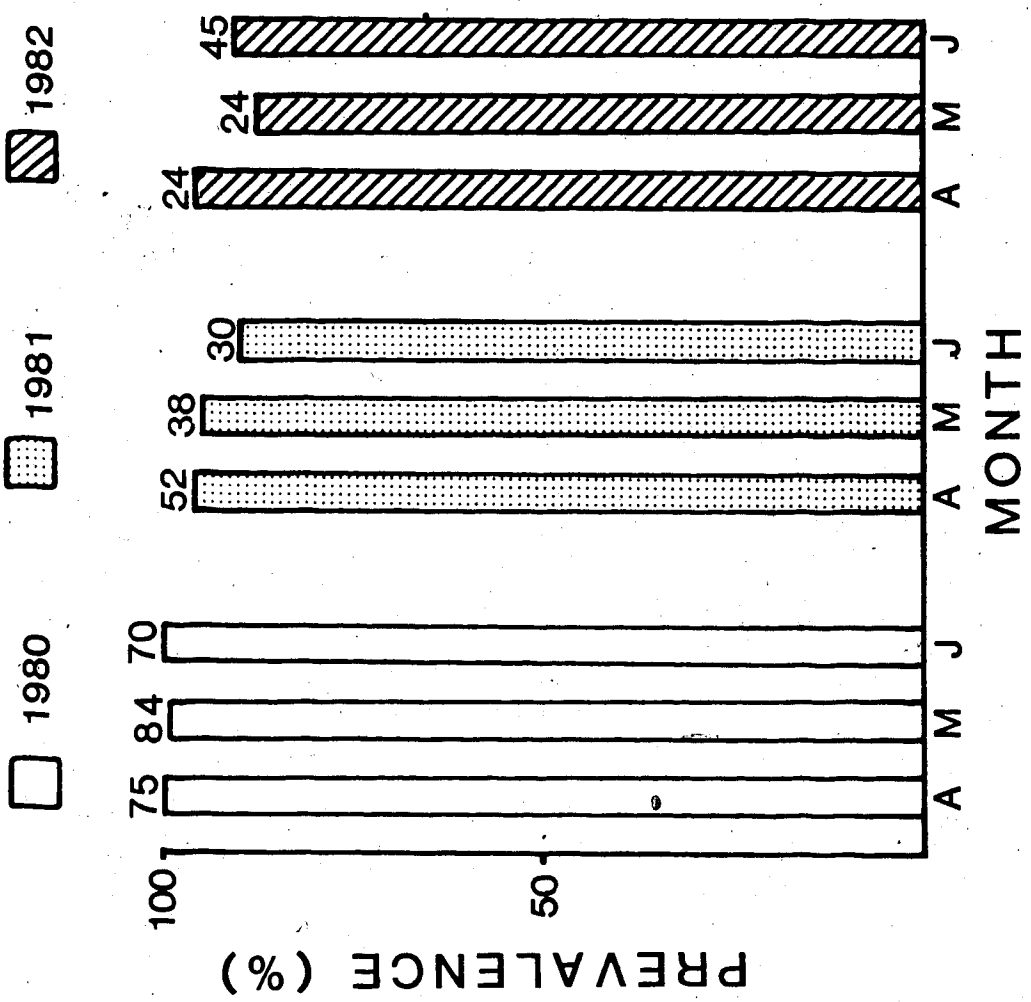

1980
1981
1982
70
84
75
30
38
52
45
24
24
A
M
J
MONTH
100
50
PREVALENCE (%)

Figure 3. Relapse prevalence of Leucocytozoon sp. in mature wild blue grouse sampled from April-June, 1980-1982. Sample size given on top of bar.

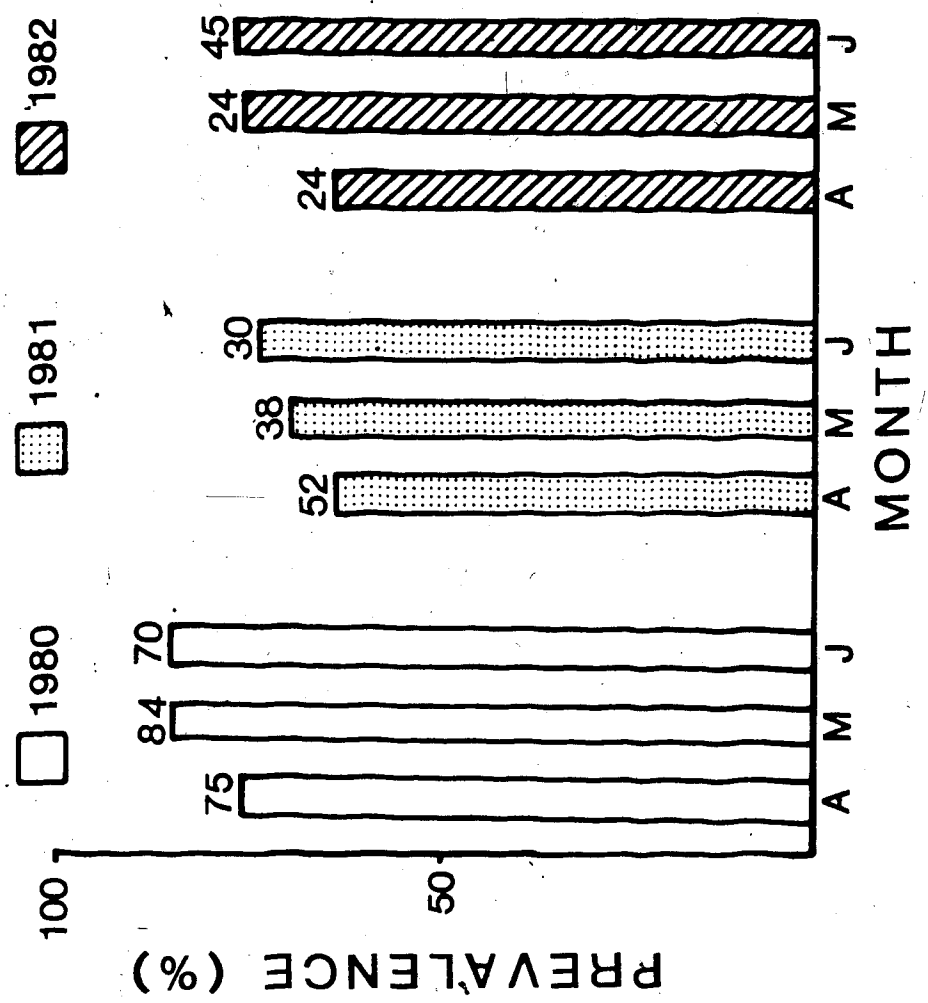

1980
1981
1982
70
84
75
30
38
52
45
24
24
A
M
J
MONTH
100
50
PREVALENCE (%)

Figure 4. Relapse prevalence of Haemoproteus mansoni in mature wild blue grouse sampled from April-July, 1980-1982. Sample size given on top of bar.

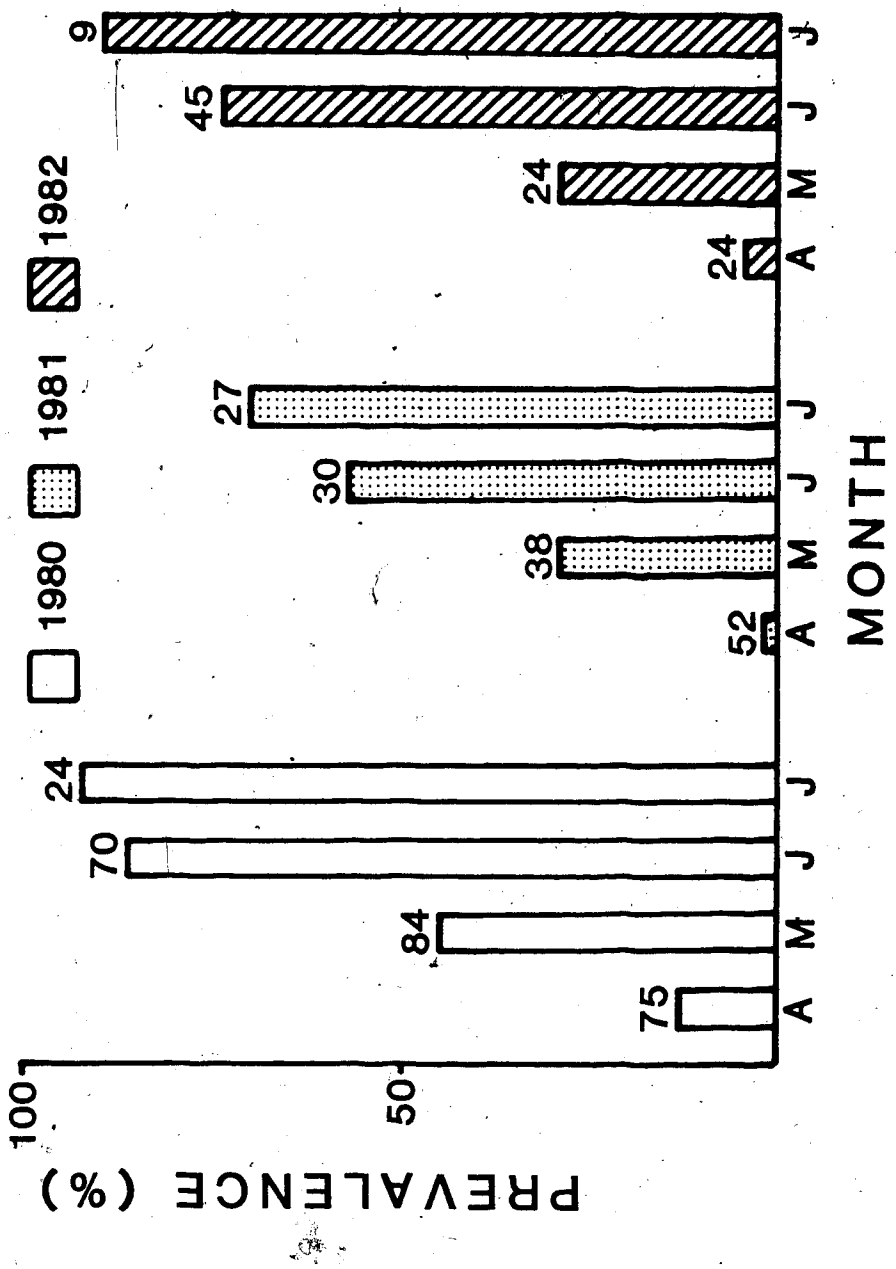
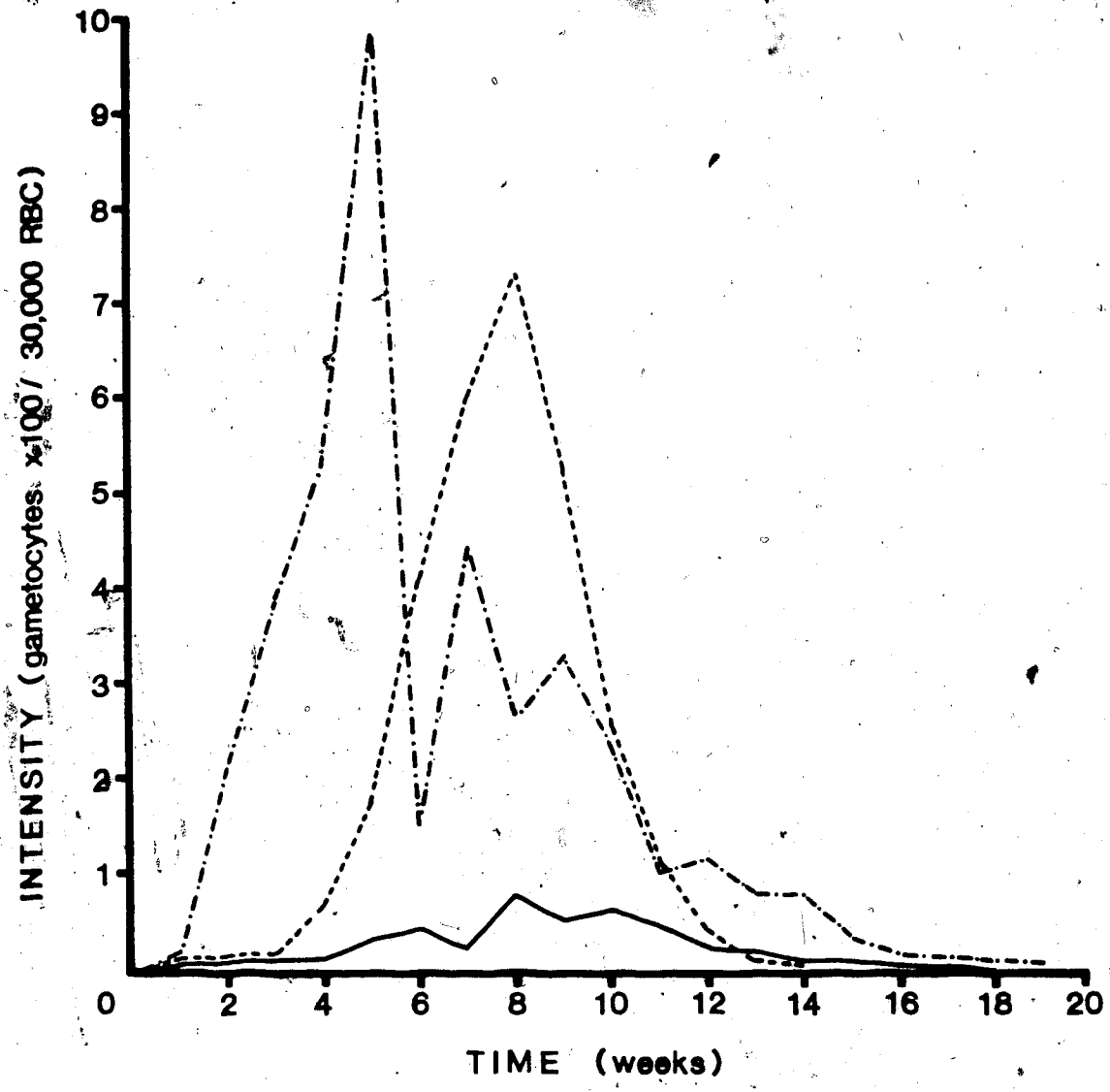

PREVALENCE (%)
100
50
MONTH
A M J J
75 84 70 24
A M J J
52 38 30 27
A M J J
24 24 45 9
1980
1981
1982

Figure 5. Profiles of three relapse infections of Haemoproteus mansoni in captive blue grouse.


INTENSITY (gametocytes x100/ 30,000 RBC)
10
9
8
7
6
5
4
3
2
1
0
2
4
6
8
10
12
14
16
18
20
TIME (weeks)

The ranges in patent period and peaking time for each infrapopulation was documented (Table 3). Primary infections displayed much shorter patent periods and much earlier peaking times than first and second relapses (Table 3). In contrast, there was little difference in patent period or peaking time between first and second relapse. Peaking times ranged between 5 and 11 weeks during relapses and only 1 to 4 weeks in primary infections. Distict patent periods were not observed in either *L. bonasae* or *Leucocytozoon* sp.. Gametocytes were produced throughout the winter. As calculation of the peaking time depended on a defined patent period it was not calculated for these parasites.

Age structure of *H. mansoni* gametocyte populations

The age of *H. mansoni* gametocytes from infrapopulations were estimated by size of gametocytes in relation to the size of the infected erythrocyte. The mean percentage composition of age classes (young, medium and mature) from 13 primary infections changed dramatically over six weeks (Figure 6). During the first week of patency approximately equivalent proportions of each age class were present. During the second week of infection, and thereafter, the majority of gametocytes were mature and by the fifth and sixth week mature gametocytes composed >90% of the populations.

This sequence in gametocyte age composition was not observed in relapse populations. The percentage of young

TABLE 3 . RANGE IN PATENT PERIOD AND PEAKING TIME OF *HAEMOPROTEUS MANSONI* FROM PRIMARY, RELAPSE AND SECONDARY RELAPSE INFECTIONS IN INDIVIDUAL CAPTIVE BLUE GROUSE.

BIRD	PATENT PERIOD (NO. OF WEEKS)			PEAKING TIME (NO. OF WEEKS)		
	PRIMARY	1st RELAPSE	2nd RELAPSE	PRIMARY	1st RELAPSE	2nd RELAPSE
00	5	14	-	2	8	-
38	6	17	18	3	9	8
65	8	18	-	2	6	-
67	6	11	-	4	8	-
69	10	19	-	2	8	-
75	11	26 *	-	2	11	-
83	6	13	-	2	5	-
90	6	14 **	11	3	-	6
92	8	22	19	1	6	5

data not available, * Bird died before infection subsided, ** data lost so start of infection could only be estimated, therefore figure for patency is an estimate.

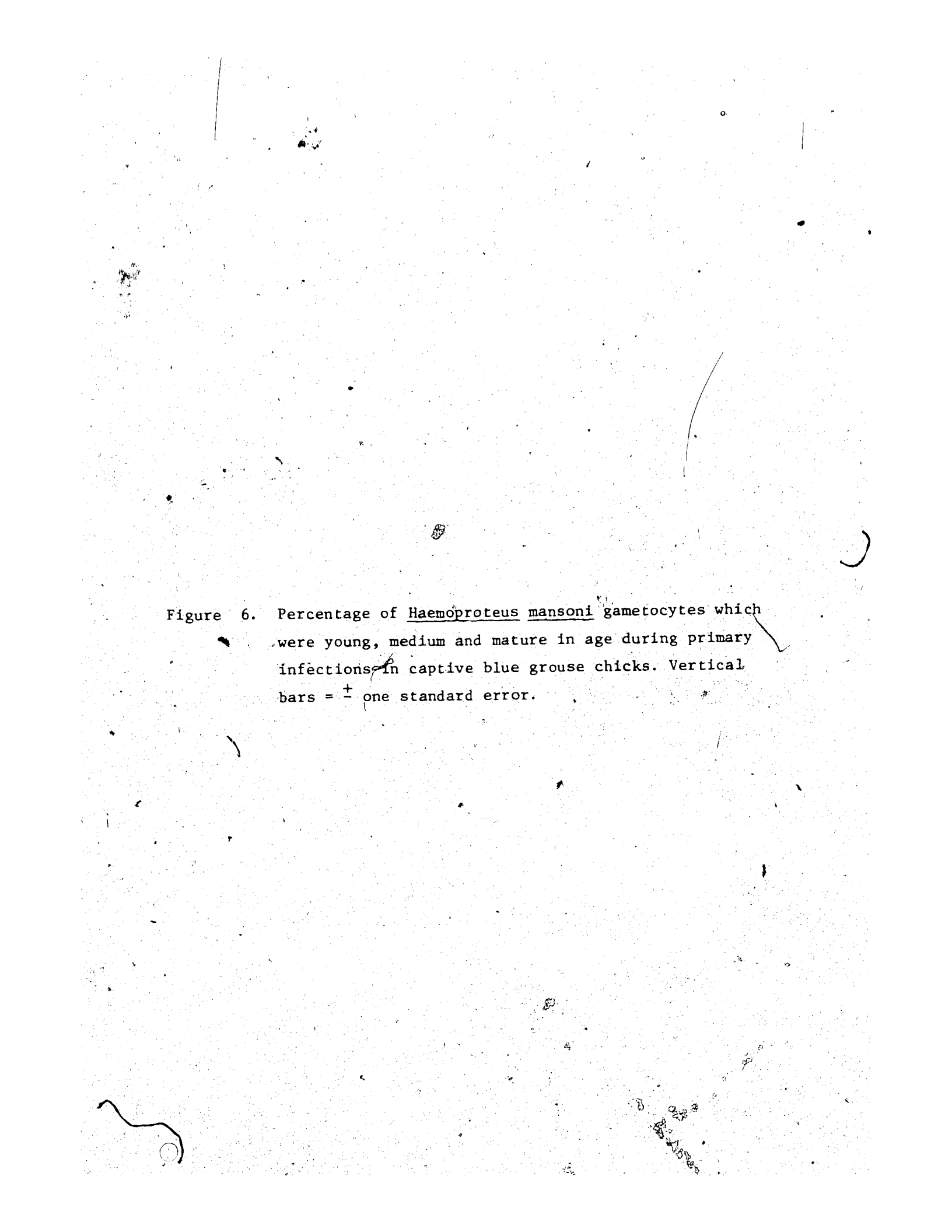
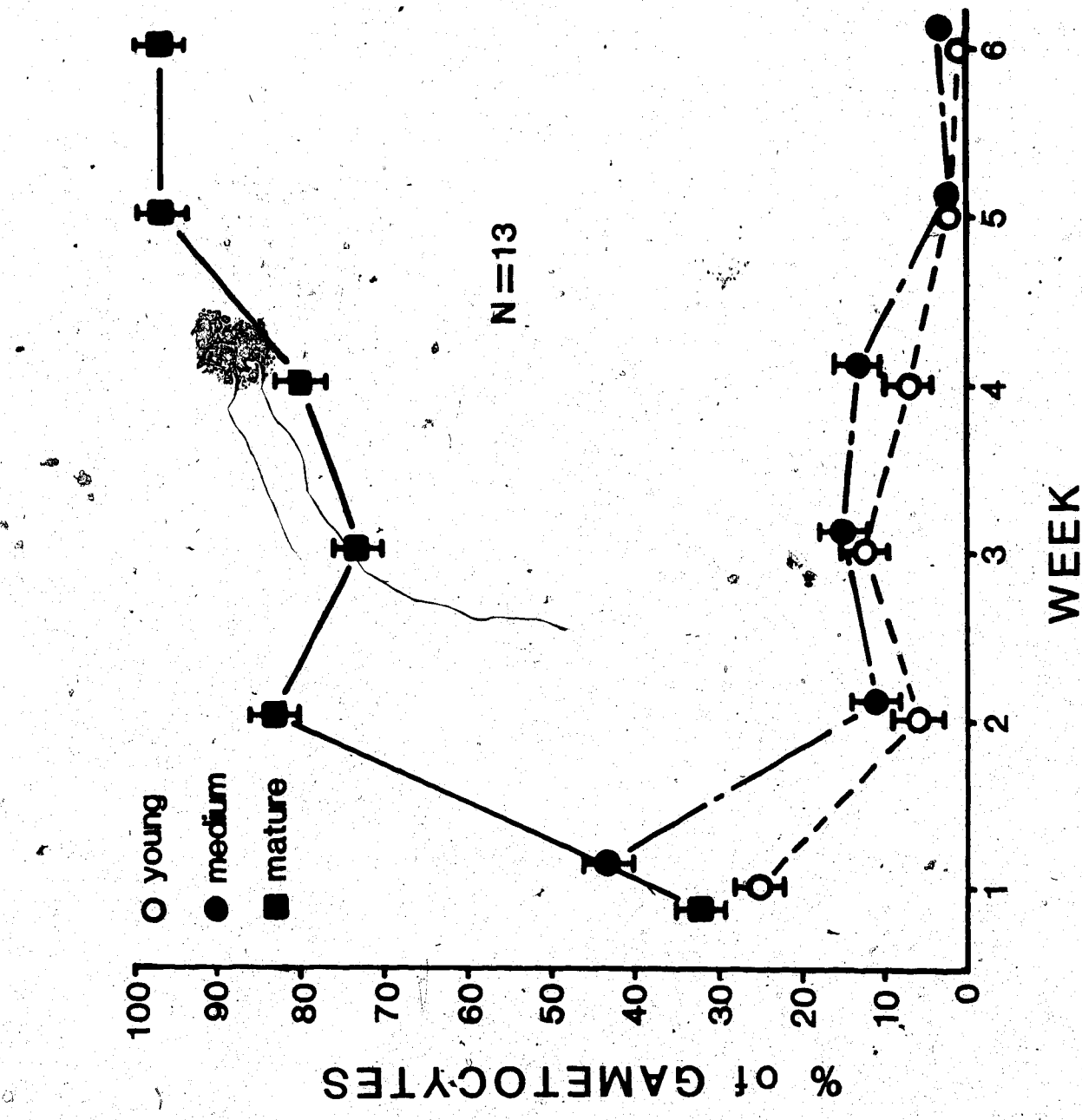


Figure 6. Percentage of Haemoproteus mansoni gametocytes which were young, medium and mature in age during primary infections in captive blue grouse chicks. Vertical bars = ± one standard error.


% of GAMETOCYTES
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
WEEK
N=13
O young
● medium
■ mature

gametocytes was determined for 10 relapse infections. No consistent pattern was observed, as wide fluctuations occurred. However, there were certain common features in relapse populations that were very different from primary infections. During the first eight weeks of relapse, 76% of observations from 10 infrapopulations exhibited >10% young gametocytes; in primary infections this occurred only on the first and third week of infection (Figure 6). In addition, 24% of samples had >30% young gametocytes during the same period; but in primary infections this was rare even in the first week of patency. The percentage of young gametocytes in a relapsing infrapopulation (bird No. 65) is presented in Figure 7. In general, young gametocytes were present for longer than eight weeks in a relapse infection, but were in substantial numbers for only one week in primary infections.

Discussion

In order to complete their life cycles blood microparasites must be transferred from one vertebrate host to another (grouse to grouse). The vertebrate host can be viewed as a patch for the microparasite; each patch has a finite space and time available (all patches eventually die from various causes) (Price, 1980). In most climates, vectors transmit microparasites to vertebrate hosts during limited time periods (usually summer months). Thus a microparasite should coordinate its output of gametocytes (the infective stage for the vector) in order to optimize

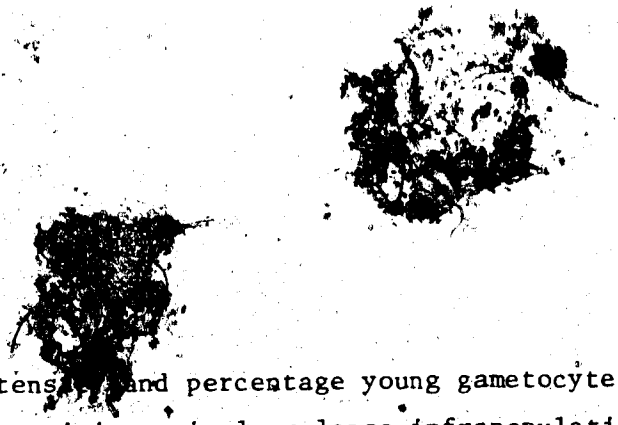
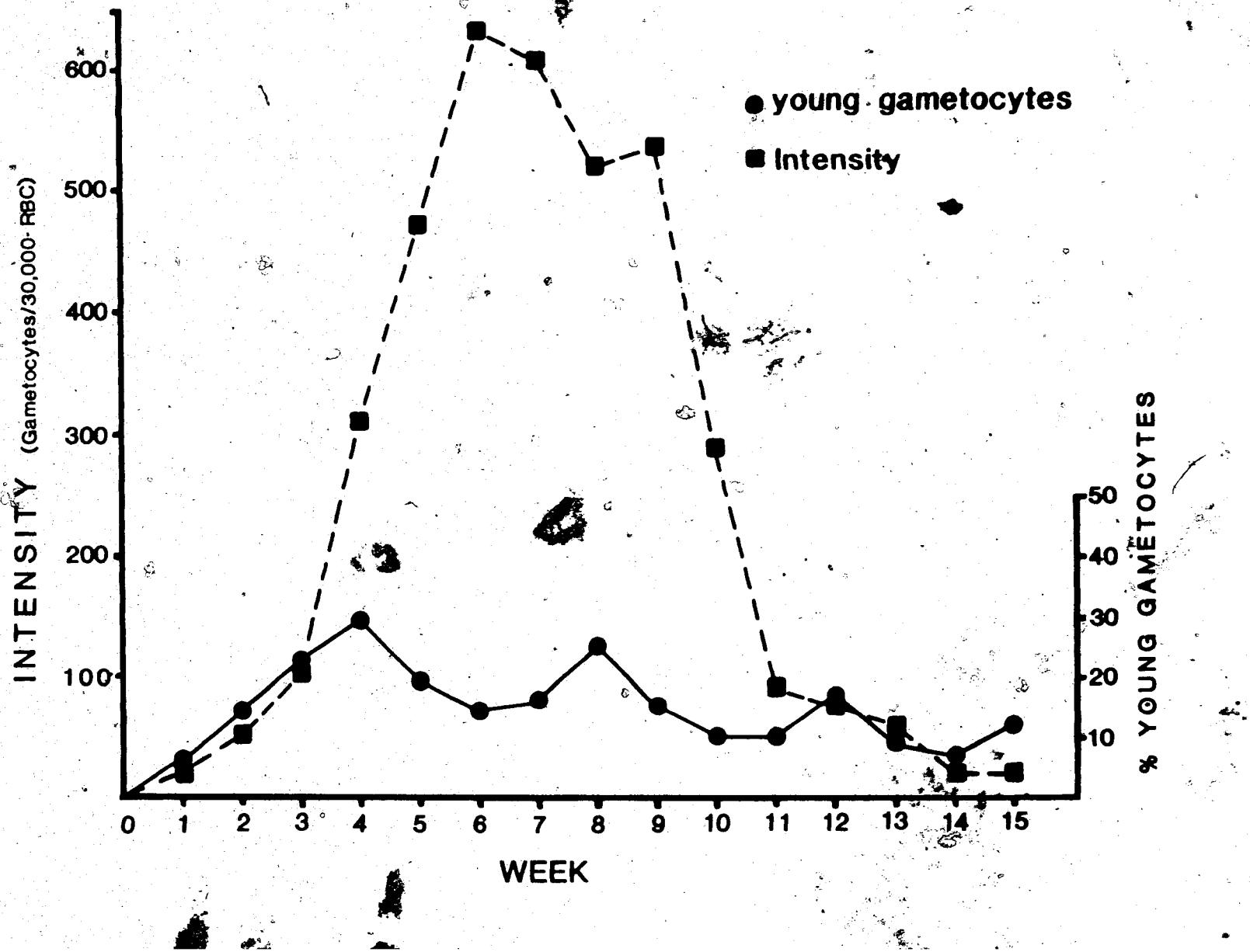


Figure 7. Intensity and percentage young gametocytes of Haemoproteus mansoni in a single relapse infrapopulation from a captive blue grouse.


young gametocytes
Intensity
INTENSITY (Gametocytes/30,000 RBC)
% YOUNG GAMETOCYTES
WEEK
600
500
400
300
200
100
50
40
30
20
10
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

transfer to new uninfected vertebrate hosts (grouse chicks).

Gametocyte output can be divided into two periods: (1) primary output which results from an initial infection (usually appears 10-14 days after a vector has transmitted sporozoites), and (2)relapse output which occurs in a succeeding season after primary infection. Although a microparasite overwinters in a vertebrate host, the gametocyte output may be small or non-existent throughout the winter months until just prior to the next transmission season. Why are such patterns observed in gametocyte output?

Vertebrate hosts may actively suppress parasite reproduction by an immune reaction. The cost to the parasite of producing gametocytes at a time of year when vectors are absent may be high, and result in suppression of parasite reproduction at the critical transmission period in the life cycle (reducing between-patch transfer). It would be beneficial for the parasite to produce gametocytes only when vectors are available (summer months). Different timing in gametocyte production during primary and relapse periods were observed in the blue grouse-microparasite system. The evolution of these responses will be discussed.

The time period in which vectors transmit parasites can be referred to as the transmission window. Variation in the width of the transmission window will be discussed in relation to timing of reproductive output of the three microparasites. Unlike the continuously present gametocytes of *L. bonasae* and *Leucocytozoon* sp., *H. mansoni* had very

distinct primary and relapse periods with no gametocytes present from the end of primary infection (October) until the beginning of relapse infection in May.

Before contrasting month to month relapse prevalence and intensity of these microparasites, an explanation of the criterion for separating field data into relapsing populations and primary infections is required. Samples were collected from wild mature birds (yearlings and adults) from April to August. Chicks became patent with *L. bonasae* and *Leucocytozoon* n. sp. in July, but *H. mansoni* was not patent until August. Reexposure and host baiting with yearlings, from mid May onward in 1982, did not result in any new parasitic infections until the chicks were also becoming patent. Therefore, on the strength of a negative result from exposure and host baiting experiments, I have assumed that infections present in mature grouse prior to new infections appearing in the chicks were relapsing parasite populations.

Prevalence and intensities of *L. bonasae* and *Leucocytozoon* sp. in wild blue grouse were consistent from month to month throughout the relapse period (April-June) (Figures 2,3) (Chapter IV). By contrast, in all years, the prevalence and intensity of *H. mansoni* increased during the relapse period (April-July) (Figure 3, Table 2). Such striking synchrony in population behaviour of gametocytes between years suggested that time was an important factor in the evolution of population dynamics in this particular parasite. This was not the case for the other two parasites.

Dorney and Todd (1960) observed a similar phenomenon in ruffed grouse from Wisconsin. Rapid increases in intensity of *Haemoproteus* occurred from April to June, whereas only slight rises were detected in *Leucocytozoon* throughout the same period.

Differences in timing of vector transmission may provide insight into the timing patterns of these parasites. Blue grouse chicks were the best age class to provide evidence as to when vectors were transmitting each parasite. Wild chicks do not become patent with *H. manson*i until the end of the summer (Chapter IV). Likewise, captive chicks became patent with *H. manson*i at the beginning of September, with most of the infections appearing in the peripheral blood in the same week. As captive chicks were removed from vectors at the end of August (1981), such similar intensity profiles between captive and wild chicks (Table 2, Figure 1) supported the conclusion that vectors were probably absent or their numbers diminished after August. I propose that the vector for *H. manson*i is a pulse vector, providing a short transmission window.

Both *L. bonasae* and *Leucocytozoon* sp. are transmitted by a non-pulse vector system, with wide transmission windows. Chicks in the aviary contracted *L. bonasae* over a 8-9 week period. As a fixed number of chicks were being monitored for infection, there would be a lower probability of detecting new infections as more birds became patent. Therefore, the transmission period obtained by this method

may underestimate the transmission window. *Leucocytozoon* sp. was transmitted to captive chicks for 5-6 weeks. The transmission window width may be less than for *L. bonasae*.

The transmission of *Leucocytozoon* spp. by non-pulse vectors (simuliids) and *Haemoproteus* spp. by pulse vectors (*Culicoides*) has been recorded in previous studies (Bennett and Fallis, 1960; Fallis and Bennett, 1961, 1966; Herman and Bennett, 1976). Fallis and Bennett (1966) found more than one species of black fly responsible for the long transmission window of *L. simondi*. Likewise, two species of black fly were involved in transmission of *L. bonasae* to ruffed grouse (Fallis and Bennett, 1958, 1962), and to blue grouse (Woo, 1964). Each ve[illegible]pecies was dominant at a different time during the transmission period (asynchronous vectors).

A pulse vector will exert greater selection pressure than a non-pulse vector on a parasite to increase gametocyte output at an appropriate time of year. *Haemoproteus mansoni* illustrated this situation. Non-pulse vectors would not create such powerful selective pressures. Both *Leucocytozoon* sp. and *L. bonasae* exemplified this state, as prevalence and intensity did not increase as the transmission season approached.

The timing of reproductive output in *H. mansoni* was different during relapse and primary periods. Gametocytes of *H. mansoni* were present twice as long during first and second relapse in comparison to primary infections; this was

the result of invasion of erythrocytes by *H. mansoni* merozoites over a longer period of time (Figure 7). Reproductive activity over time had increased in all relapse infections. In addition, the peak intensity during first and second relapse was much later in relation to patency, than in primary infections. This shift in peaking time was independent of the intensity attained during the peak. In general, data from captive and wild grouse infections agree. These population behavioural differences between relapse and primary infections of *H. mansoni* may be the result of selection on the parasite to maintain maximum reproductive output during periods when vectors were available which should maximize benefit (transmission), and minimize cost (vertebrate host immune response to parasite).

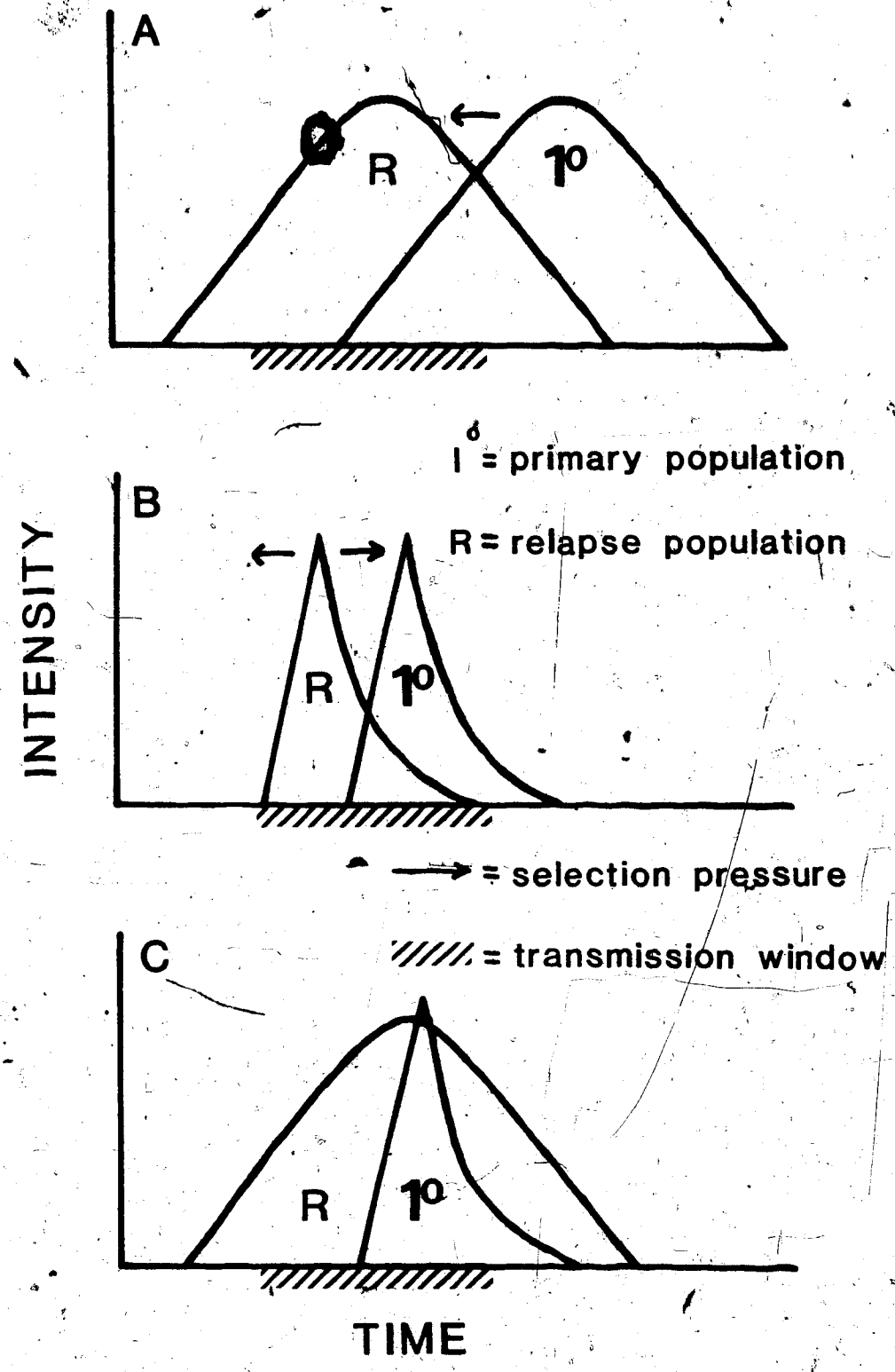
How could this dual behaviour in primary and relapse populations have evolved? A model to account for changes in peaking time and patent period, for *H. mansoni* in blue grouse is presented in Figure 8. I will assume that the dichotomy in population behaviour could have evolved from parasite populations with originally either type of population profile (primary or relapse). There are two possible evolutionary routes to the present population behaviour observed during primary and relapse periods. In Figure 8a both primary and relapse populations display the characteristics of the present relapse populations. The relapse population in Figure 8a peaked at the central [illegible] in the transmission window; selection would favour this

Figure 8. Model of proposed evolution of Haemoproteus mansoni gametocyte population behaviours during primary and relapse periods.

A = Hypothetical situation with both primary and relapse populations displaying current relapse population profiles observed in this study.

B = Hypothetical situation with both primary and relapse populations displaying current primary population profiles observed in this study.

C = Current gametocyte population profiles of H. mansoni.


A
R
1°
1° = primary population
R = relapse population
B
INTENSITY
R
1°
= selection pressure
= transmission window
C
R
1°
TIME

optimum. Primary infections would become patent during the transmission period, but would not peak until well after transmission had ceased; this would occur well into October. Strong selection would act on primary populations (Figure 8a), to favour primary populations that peaked more rapidly, and thus had a higher probability of contributing to subsequent generations of parasites within the same cohort of chicks. As sporozoites of haemoproteids take only four to eight days to develop in *Culicoides* (Fallis and Bennett, 1960), and an additional ten day prepatent period in the vertebrate host before gametocytes are present in peripheral blood, transmission could occur to the same generation of chicks within that season (transmission period lasts approximately three weeks). Populations would behave as observed in this study (Figure 8c). Relapse populations would have a centrally orientated peaking time and a long patency, primary infections would have a shorter patency and earlier peaking time. I assume that an immune response by blue grouse selects against the alternative strategy of producing gametocytes at a constant level from one transmission season to the next.

The second possible evolutionary route assumes that population behaviour during both primary and relapse exhibited characteristics of the primary infections in this study (Figure 8b). Both primary and relapse populations had short patent periods, and peaking time was early. Through evolutionary time, relapse populations with a broader more

normal population distribution would contribute more to subsequent generations. Thus selection would result once again in (c) (Figure 8c).

Although the selective pressure for different population behaviours is a consequence of the interaction of vectors and parasite, the behaviour would be expressed while in the vertebrate host. Thus, caution must be exercised when attempting to explain parasite behaviour in terms of the vertebrate host without considering the vector(s).

Other studies on Haemosporina showed similar patterns and variations on the patent period and peaking time. Fallis and Bennett (1960) noted sharp peaks and rapid declines in gametocyte intensities during primary infections of *H. mansoni* in ruffed grouse. Applegate (1971) followed infrapopulations of *Plasmodium relictum* in English sparrows and found short patent periods and peaking times during primary infections, whereas relapse infrapopulations had long patent periods and later peaking times. This was similar to my observations on *H. mansoni* in blue grouse.

Other studies of *Leucocytozoon* spp. indicated that they exhibited short patency during primary infections and longer patent periods during relapse periods. Prolonged patency during relapse was noted in both *L. dubreuili* in robins, and *L. danilewskyi* in saw-whet owls. Primary infections lasted two weeks, and relapse infections lasted three months (Khan and Fallis, 1970a,b).

In *Leucocytozoon* spp. the peaking times were highly variable, and did not exhibit the patterns observed in *H. mansoni* and *P. relictum*. *Leucocytozoon simondi* in ducks, had a short burst of primary gametocyte production that lasted two weeks (Desser, 1967; Desser and Ryckman, 1976; Fallis *et al.*, 1951), and intensity peaked in one week. Gametocytes of *L. simondi* were present throughout the winter at low levels, but intensities increased sharply in February and remained high until at least May (Chernin, 1952; Khan and Fallis, 1968); erratic fluctuations in intensity made it impossible to determine the peaking time. By contrast, *H. mansoni* had gametocyte populations with a centrally orientated peak and intensities declined rapidly after the peak. In addition, the estimated peaking times for *Leucocytozoon* spp. were out of phase with the vector appearance (Chernin, 1952; Khan and Fallis, 1968). Gametocytes of *L. bonasae* peaked in February/March in captive blue grouse, however it was not known if this was reflective of infections in wild blue grouse populations; transmission in the wild occurred in July. This contrasts with *H. mansoni* relapse populations in blue grouse, which peaked in July and August, the period when transmission would occur. Coordination of peaking time with vector availability will be selected for in parasites with pulse vectors, but may not be a powerful selection force for non-pulse vectored parasites. However, a long patent period during relapse was a more general response to seasonality in transmission, and it was expressed by

parasites with short and long transmission windows.

Microparasite infections no doubt elicit an immune response from the host. Therefore, a prudent parasite should limit gametocyte output to periods when vectors are available. As primary infections are the result of cycling through the vector, the transmission window for gametocytes produced from primary infections will be shortened by the development time of the parasite in the vector, and the prepatent period in the new vertebrate host (i.e., for *Haemoproteus* spp., four days to develop in the vector plus ten days prepatent period in the vertebrate host). Thus a short patent period during primary infection, and a longer patent period during relapse infection, would be advantageous in reducing the costs associated with gametocyte production.

What mechanism enables these parasite populations to change their behaviour? If the population behaviour of *H. mansoni* were reversed, with primary infections longer than relapse infections, it would be easy to hypothesize that an immune response by the host resulted in premature curtailment of reproduction during relapse. This would imply that in population regulation of *H. mansoni*, the parasite was a passive element. In the *H. mansoni*/grouse system such an immunological explanation will not suffice. *Haemoproteus mansoni* may avoid the immune response by switching antigenic expression throughout relapse, in much the same manner as *Plasmodium* (Brown, 1977; Brown *et al.*, 1968). Thus, the

relapse population profile may represent the accumulated output of many antigenically different *H. manson*i populations.

A single morph changing its immunological expression could explain these observations. Consistent timing in the development of relapse *H. manson*i infections in both wild and captive grouse, even though both groups were living under different light and physical environments, suggested very rigid control of parasite reproduction. Such robustness may be a consequence of a fixed internal biological clock in the parasite, and not a response to physiological changes in the vertebrate. This hypothesis could be tested by careful manipulation of the vertebrate host and experimental infections at different times of the year.

Evidence from this study and others suggests that the transmission window width or vector pulsing is a vital parameter in shaping the population behaviour of microparasites in vertebrate hosts. Pulse vectors promote precise peaking times in gametocyte output, whereas non-pulse vectors do not create selective pressure for rigid timing. Thus, on a continuum from pulse to non-pulse vectors, selection pressure for precise peaking time should decrease. Long patency during relapse and short patency during primary infection suggested a general response to a seasonal transmission window. Wide windows may result in continuous gametocyte presence as in *L. bonasae* in blue grouse, while shorter windows promote absence of gametocytes

for extended periods of time and a defined relapse period, as in *H. manson*i. In addition, the cost to a parasite, associated with gametocyte production, may influence gametocyte population behaviour.

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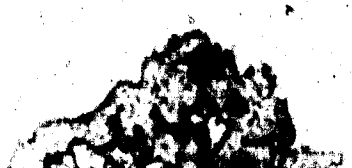

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IV. Population dynamics of three microparasites of blue grouse

Abstract

Population dynamics of three intracellular blood microparasites, *Leucocytozoon bonasae*, *Leucocytozoon* sp. and *Haemoproteus manson*i were investigated to determine pertinent features in the microparasite environment which influenced population dynamics. Infrapopulations from naturally infected captive and wild blue grouse were sampled. Prevalence of each parasite in wild grouse chicks was used to estimate the effectiveness of the transmission system from year to year (between-patch dynamcis). *Leucocytozoon bonasae* had ≥90% prevalence in blue grouse chicks every year; however, the prevalence of the other two parasites was lower, and fluctuated from year to year. *Leucocytozoon bonasae* had an extremely effective transmission system, whereas the other two parasites did not. The dependability of blue grouse for the reproduction of each parasite was compared by examining survival and reproductive output of infections during primary and relapse periods (within-patch dynamics). Survivorship was similar between parasites. Data from wild and captive blue grouse indicated that *L. bonasae* had equivalent reproductive output during primary and relapse periods, whereas *Leucocytozoon* sp. output was much reduced from the primary period. *Haemoproteus manson*i had higher output during relapse. The

two environmental parameters - effectiveness of transmission and dependability of the vertebrate host for reproduction of the parasite - were different for the three microparasites in blue grouse.

Introduction

Price (1980) indicated that in the ecology of parasitic organisms, "the effective environment for a parasite is the patch where it is situated and another patch that it or its progeny must reach in order to find new hosts. Thus our ecology must become one of within-patch dynamics and between-patch dynamics rather than one ecological overview of the many patches." How can between-patch and within-patch dynamics be measured and compared between species of parasites?

Esch *et al.* (1977) outlined several factors that interplay in determining a host's acceptability for a parasite: the rate of parasite development, life expectancy of the parasite and host, reproductive potential of the parasite, defence system of the host, pathogenicity of the parasite, and variability and transmissibility of the reproductive stages of the parasite. The last of these factors, transmission, clearly measures between-patch dynamics; the rest measure the relationship with the vertebrate host, or within-patch dynamics.

This study investigates the between-patch and within-patch dynamics of three intracellular microparasites,

Leucocytozoon sp., *L. bonasae* and *Haemoproteus manson*i, in blue grouse (*Dendragapus obscurus*) on Hardwicke Island, British Columbia. The two parameters used were effectiveness of transmission (between-patch dynamics) and dependability of the vertebrate host for reproduction of the parasite (within-patch dynamics).

Materials and Methods

Captive chicks were reared in an outdoor aviary on Hardwicke Island, and sampled for blood every week (Chapter II). The techniques for determination of prevalence and intensity of *L. bonasae*, *Leuocucytozoon* sp. and *H. manson*i in wild and captive blue grouse were the same as previously described (Chapter II,III).

Two captive blue grouse were held in fly free conditions at Biological Animal Services, Ellerslie, Alberta, for a period of two years. Total gametocyte production during the relapse period, February to July, was calculated by addition of weekly counts throughout this period. Statistics were applied as described in Chapter III.

Results

Prevalence and intensity of *L. bonasae*

The prevalence of *L. bonasae* in wild chicks showed a similar pattern in each year. Prevalence increased

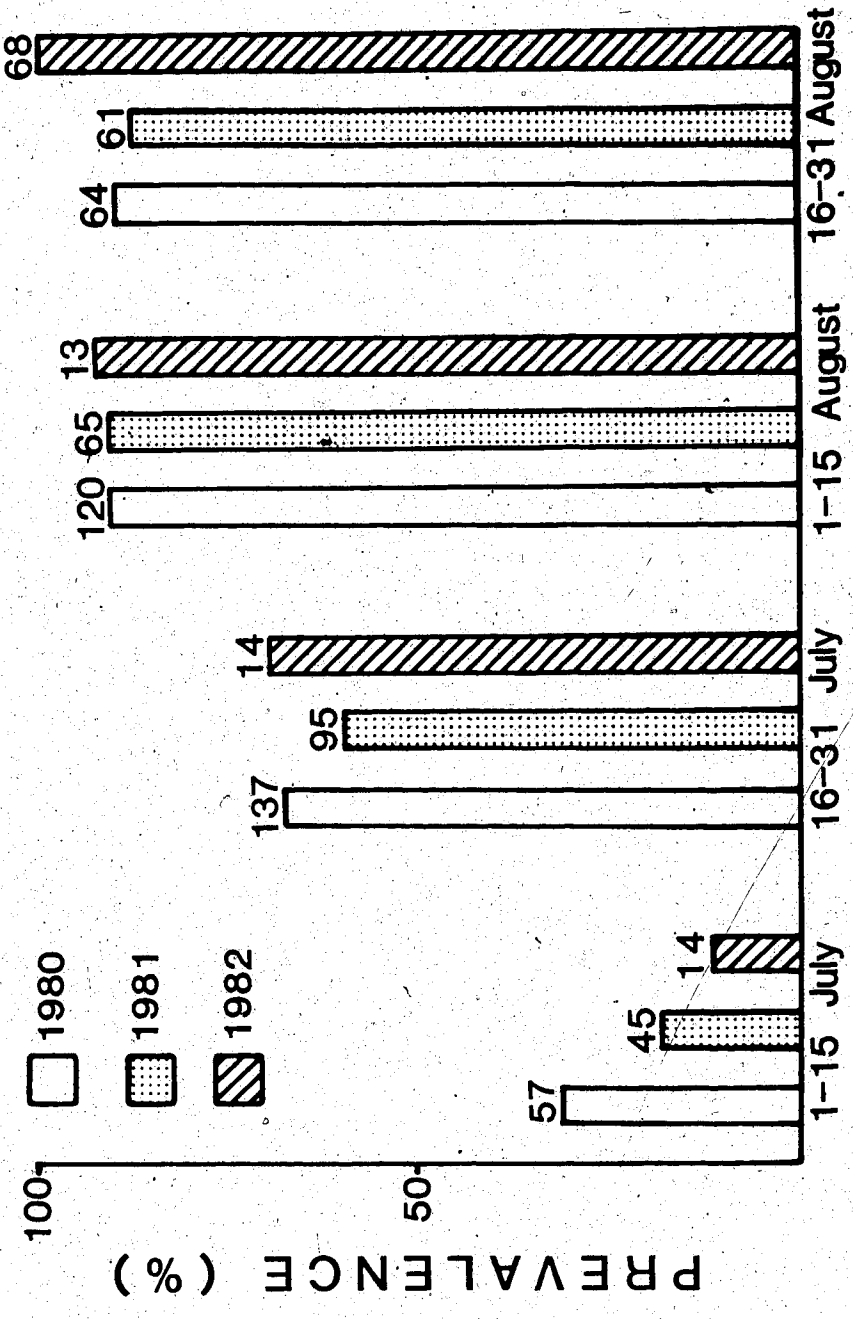
dramatically from the first two weeks in July through the first two weeks in August (Figure 1). Prevalence, in all years, was high with ≥90% of wild chicks infected by early August. However, prevalence in late August of 1981 and 1982 was significantly different ($X^2=10.74$, $p<0.05$).

The presence or absence of *L. bonasae* in captive blue grouse held in fly free conditions was assessed during the first relapse period (April-August) after primary infection in the previous summer; 100% of the 27 infections relapsed, included was one bird which had one gametocyte present during the primary period of 1981; it relapsed in July of 1982 with a similar intensity.

The mean monthly intensity of *L. bonasae* in captive birds is plotted in Figure 2. The patterns for chick cohorts 1981 and 1982 were very similar, as were those for yearlings reexposed to infection in 1982. In all, the peak of primary infection occurred in August and September. All cohorts had lowest intensities in December, with a subsequent rise to a peak in February and March, after which intensities declined for the remainder of the season (April - July).

A signed rank test on all birds with primary and relapse infections did not reveal any significant difference between primary and relapse peak intensities ($Z=0.26$, $p>0.05$). A linear regression was calculated for peak intensity during relapse on primary peak intensity (Figure 3); 60.8% of the variance in intensity could be accounted for by this regression ($t=6.45$, $p<0.001$). In general, birds

Figure 1. Prevalence of primary infections of Leucocytozoon bonasae in wild blue grouse chicks from Hardwicke Island, B. C., 1980-1982. Sample size given on top of bars.


PREVALENCE (%)
100
50
1980
1981
1982
57
45
14
137
95
14
120
65
13
64
61
68
1–15 July
16–31 July
1–15 August
16–31 August

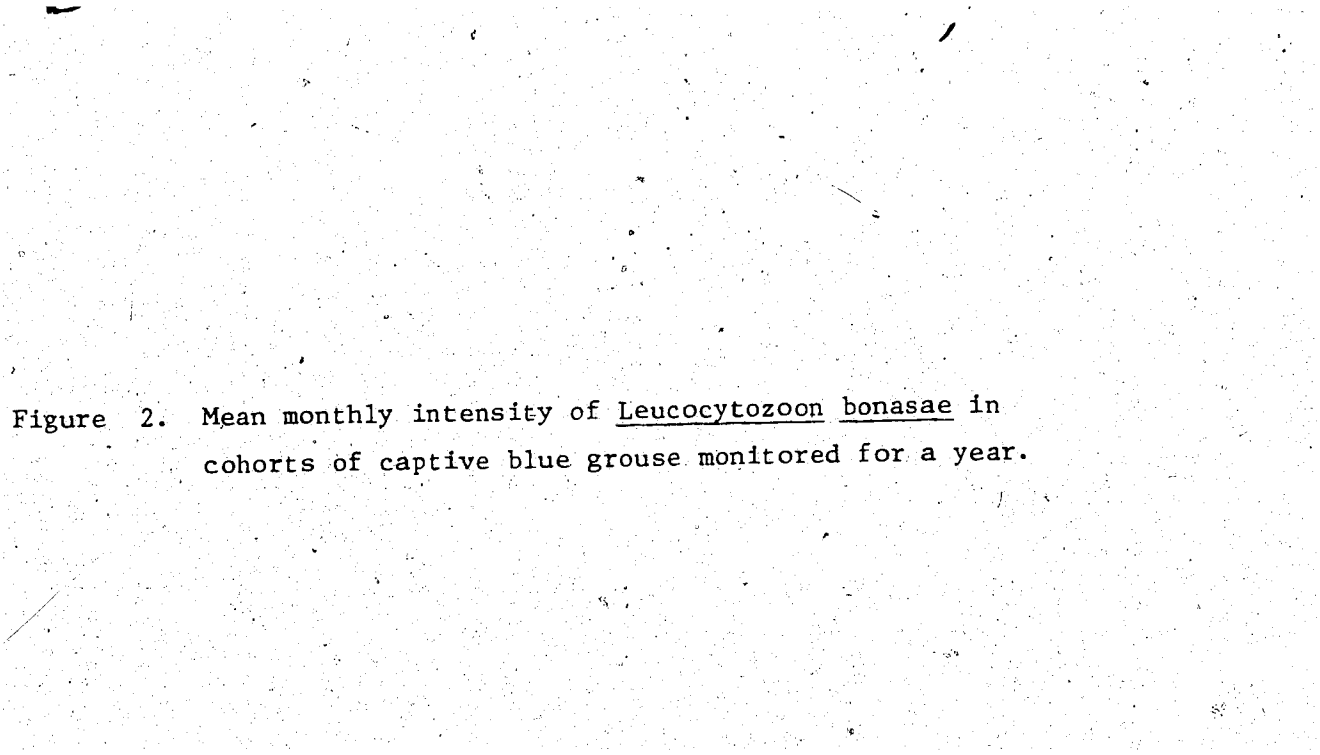
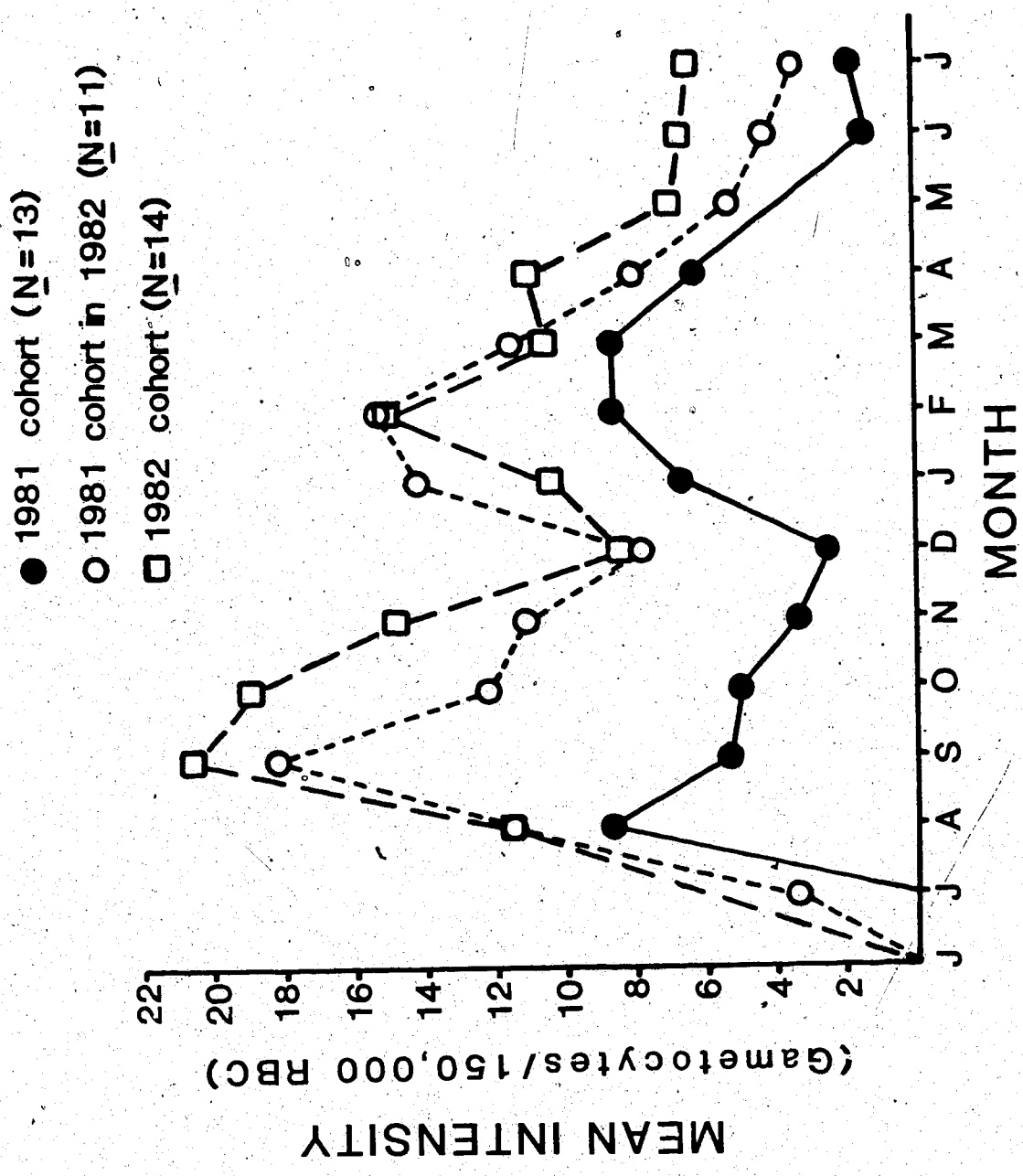


Figure 2. Mean monthly intensity of Leucocytozoon bonasae in cohorts of captive blue grouse monitored for a year.


● 1981 cohort (N=13)
○ 1981 cohort in 1982 (N=11)
□ 1982 cohort (N=14)
MEAN INTENSITY
(Gametocytes/150,000 RBC)
22
20
18
16
14
12
10
8
6
4
2
J
A
S
O
N
D
J
F
M
A
M
J
J
MONTH

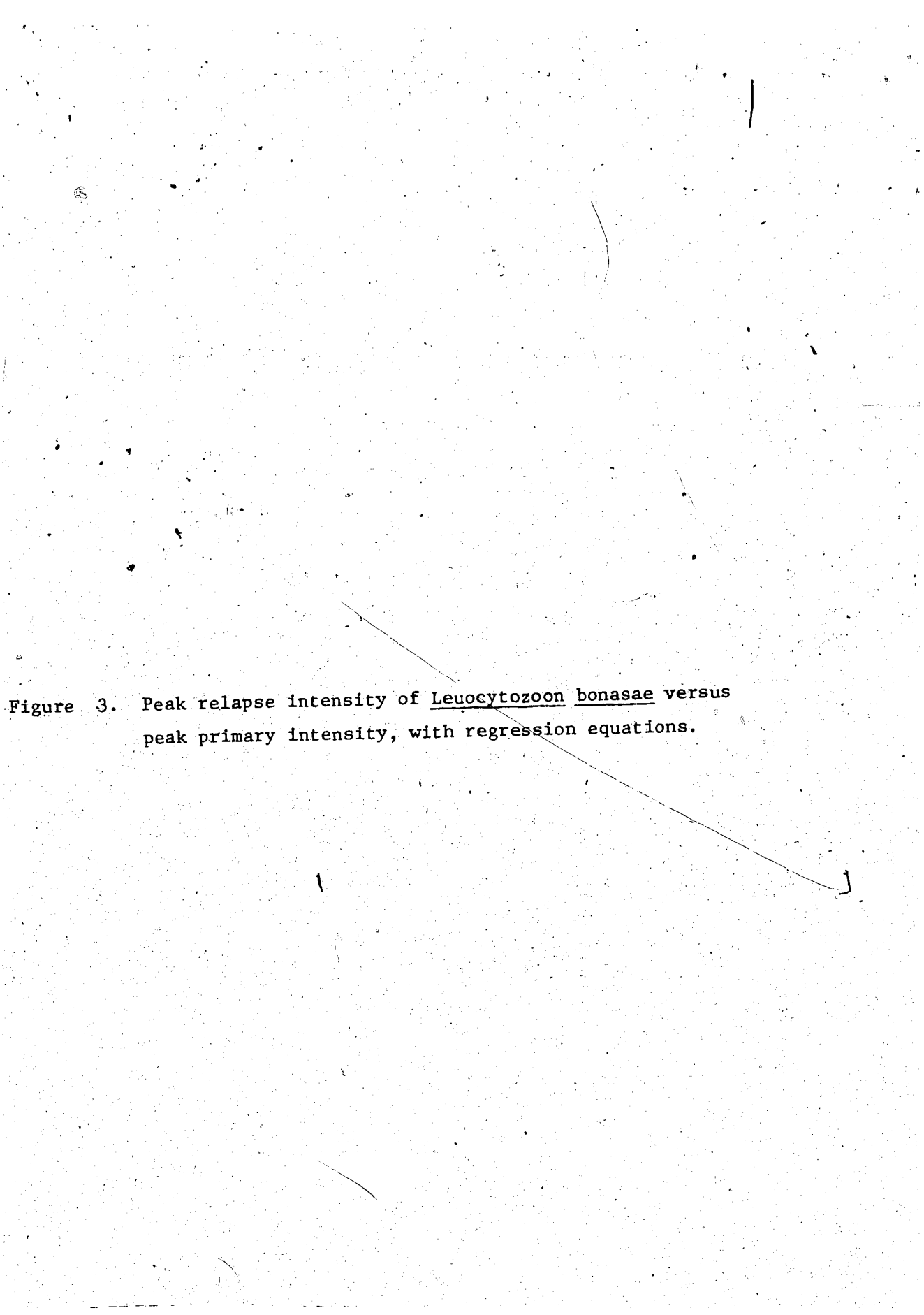
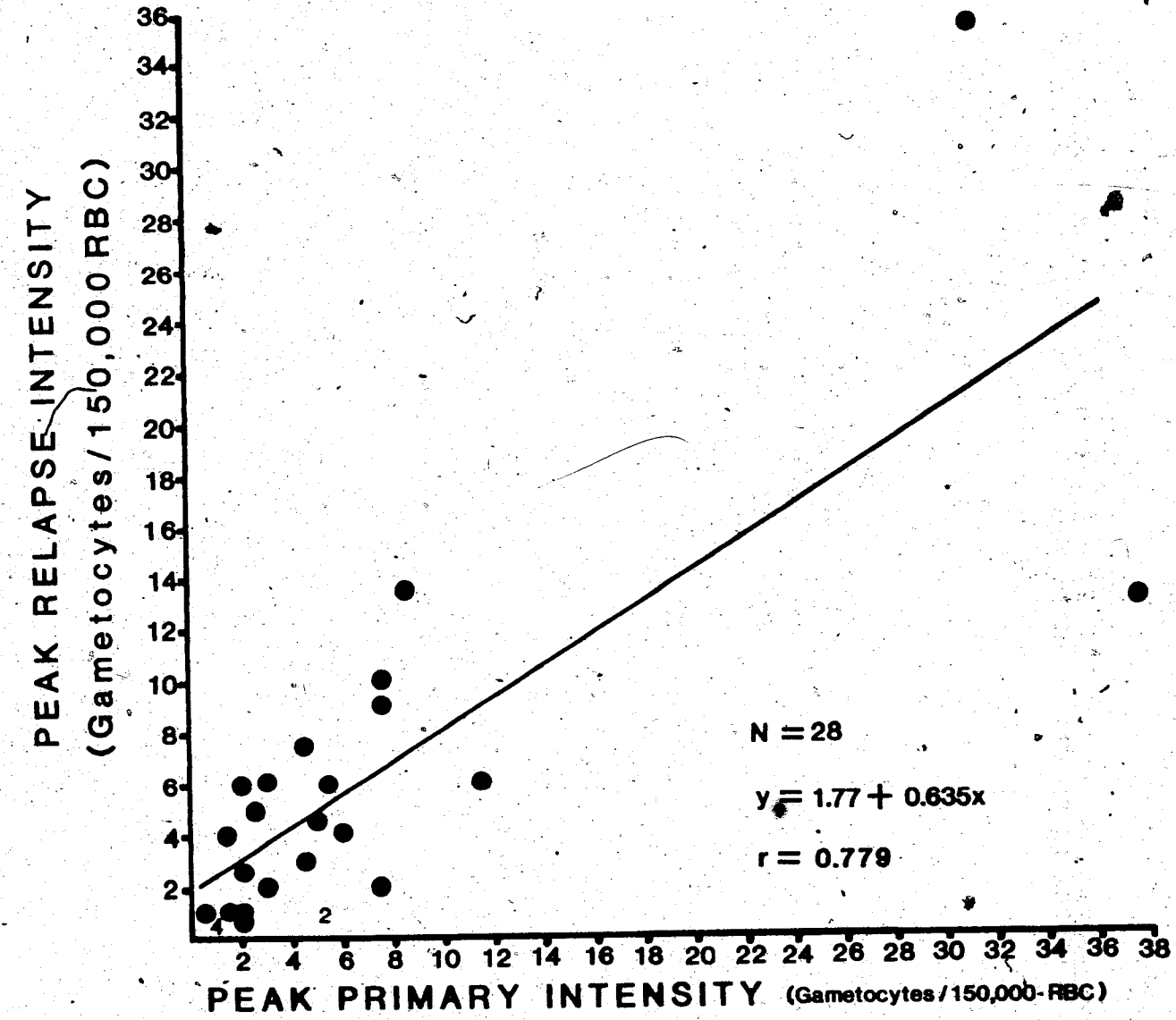


Figure 3. Peak relapse intensity of Leuocytozoon bonasae versus peak primary intensity, with regression equations.


PEAK RELAPSE INTENSITY
(Gametocytes/150,000RBC)
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
PEAK PRIMARY INTENSITY (Gametocytes/150,000-RBC)
N = 28
y = 1.77 + 0.635x
r = 0.779

with ≥5 gametocytes/150,000 RBCs peak intensities had gametocytes present every week throughout the first year of infection.

The primary geometric mean intensities of *L. bonasae* in wild chicks are presented in Table 1. In any year there were no significant differences between the months of July and August. In addition, primary intensities did not differ between years (ANOVA for years, $p>0.05$). Blue grouse had *L. bonasae* present when returning to the breeding grounds in February and March, but the limited sample in February/March was too small to include in the analyses. During the relapse months of April, May and June there were no significant differences in intensities between months in any year (ANOVA on months, $p>0.05$).

Primary geometric mean intensities did not differ from relapse intensities within the same year in 1980 and 1981, but primary intensities were higher in 1982 ($F=12.3$, $p<0.001$) (Table 1). There were no significant differences between years in intensities for adults ($F=2.25$, $p>0.05$) or yearlings ($F=1.16$, $p>0.05$) .

Prevalence and intensity of *Leucocytozoon* sp.

Prevalence of *Leucocytozoon* sp. in wild chicks is presented in Figure 4. As with *L. bonasae*, prevalence was very low in early July and climbed to a peak in August. In the latter half of July, the prevalence of *Leucocytozoon* sp. was much lower than that of *L. bonasae*, with significant

TABLE 1. GEOMETRIC MEAN INTENSITY OF LEUCOCYTOZOON BONASAE DURING PRIMARY AND RELAPSE PERIODS IN WILD BLUE GROUSE.

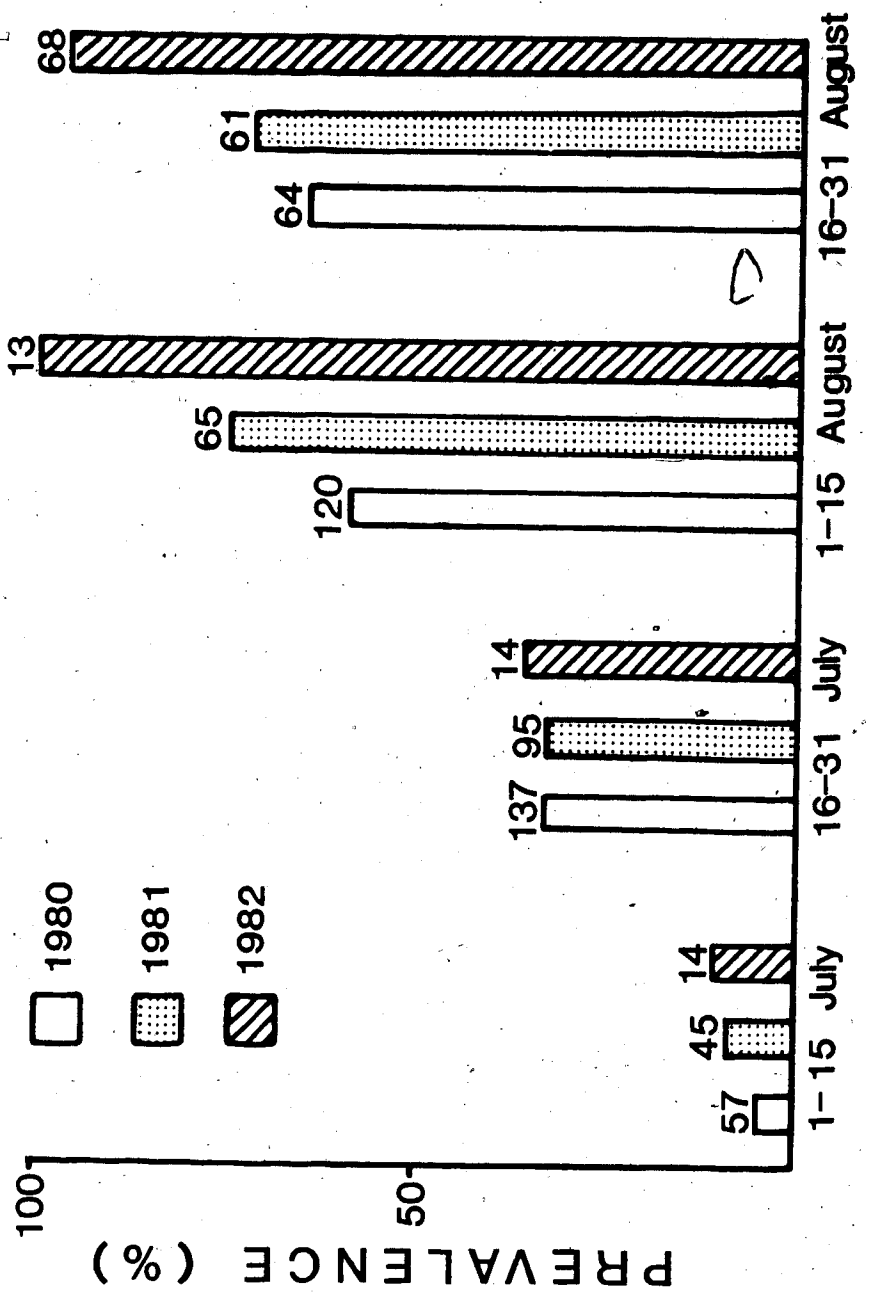
PERIOD	MONTH	GEOMETRIC MEAN INTENSITY+ (N) 1980	1981	1982
Primary	July	14.5(102)	11.8(70)	10.5(11)
	August	13.3(172)	15.3(114)	18.0(80)
Relapse	April	13.6(72)	13.0(50)	9.7(22)
	May	12.9(83)	13.8(36)	8.9(20)
	June	12.1(67)	15.3(27)	10.0(38)
Primary	All[a]	13.7	14.1	16.7***
Relapse	All	12.9	13.8	9.6

+ = Geometric Mean Intensity expressed as Gametocytes/150,000 RBC.

*** = Primary and relapse intensities (all months) significantly different at p 0.001 by ANOVA (Sokal and Rohlf, 1969).

a = Geometric Mean Intensity of all primary months.

Figure 4. Prevalence of primary infections of Leucocytozoon sp. in wild blue grouse chicks from Hardwicke Island, B. C., 1980-1982. Sample size given on top of bars.

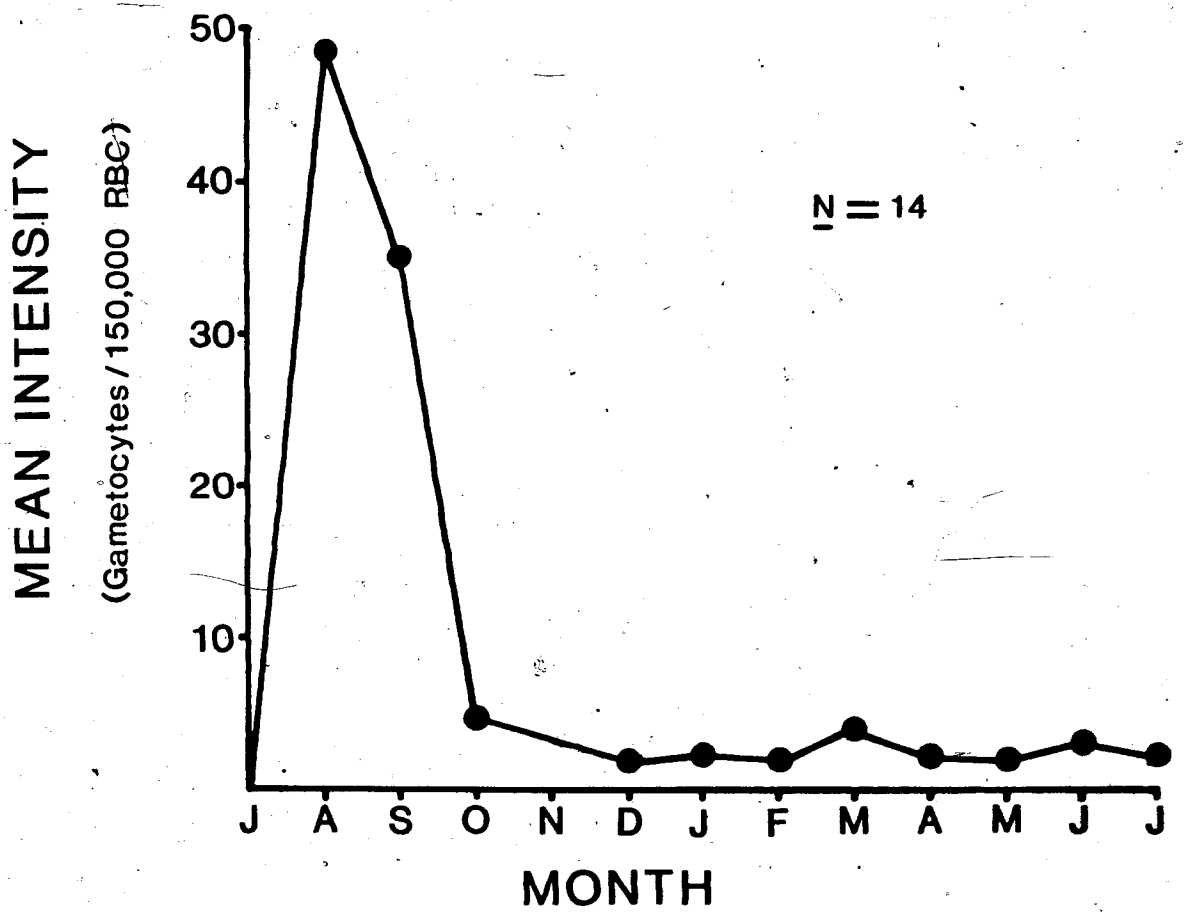

PREVALENCE (%)
100
50
1980
1981
1982
57
45
14
137
95
14
120
65
13
64
61
68
1–15 July
16–31 July
1–15 August
16–31 August

differences in 1980 and 1981 (X^2 =32.25, $p<0.05$; X^2 =13.25, $p<0.05$). *Leucocytozoon* sp. prevalence in late July was similar between years (X^2 for years, $p>0.05$), but by early August prevalence ranged from 59% in 1980 to 100% in 1982 (X^2=10.47, $p<0.05$). These differences persisted into late August (Chapter 1). Therefore, prevalence patterns were different between years in *Leucocytozoon* sp.

The presence or absence of gametocytes in captive blue grouse held in fly free conditions was assessed during the first relapse period (April-August) after primary infection in the previous summer. One bird did not show gametocytes in the spring or summer following primary infection; however, all others were positive (14 of 15).

The mean monthly intensity of *Leucocytozoon* sp. in 14 captive grouse was plotted for a complete year (Figure 5). Population profiles for yearlings and chicks were similar. Gametocyte populations rose rapidly during the initial infection period, reached a peak and then crashed to a low level in October. For the remainder of patency mean intensities remained low and never showed indications of recovery to the peak primary level. In all birds, after the initial peak and crash, gametocytes were recorded only sporadically for the remainder of the infection. Any increase in gametocyte intensity above 1/150,000 RBCs was sustained for longer than one week in only one bird, where it was maintained for four weeks. Transitory peaks were rarely higher than 4/150,000 RBC. A linear regression was

Figure 5. Mean monthly intensity of Leucocytozoon sp. in captive blue grouse monitored for a year.


MEAN INTENSITY
(Gametocytes / 150,000 RBC)
50
40
30
20
10
N = 14
J A S O N D J F M A M J J
MONTH

not significant for peak intensity during relapse on primary peak intensity, indicating no relationship between these parameters (Figure 6).

Data on primary *Leucocytozoon* sp. infections in wild chicks are presented in Table 2. The geometric mean intensities did not differ from July to August, or from year to year (ANOVA's for months and years, $p>0.05$). Relapse infections in mature birds, from April to June, were similar between months (ANOVA, $p>0.05$). The geometric mean relapse intensities did not differ from year to year ($F=2.23$, $p>0.05$). Within the same year, relapse intensities in mature birds were significantly lower than primary infections in chicks (Kruskal-Wallis test 1980: $H=25.3$, $p<0.001$; 1981: $H=39.4$, $p<0.001$; 1982: $H=46.1$, $p<0.001$). There were no significant differences in intensities between years for adults ($F=1.28$, $p>0.05$) or yearlings ($F=2.45$, $p>0.05$).

Prevalence and intensity of *H. manson*i

*Haemoproteus manson*i first appeared in wild chicks in August. Prevalence in August 1980 and 1981 was very low, whereas in August 1982 it was significantly higher ($X^2=70.8$, $p<0.001$) (Figure 7). In 1980 and 1982, data from wild chicks were not collected after the end of August; therefore, the prevalence in these years may have been underestimated. In 1981 blood samples were collected in September; prevalence was 39% (N=18), which was not significantly different from August of 1982 (58%) ($X^2=2.0$,

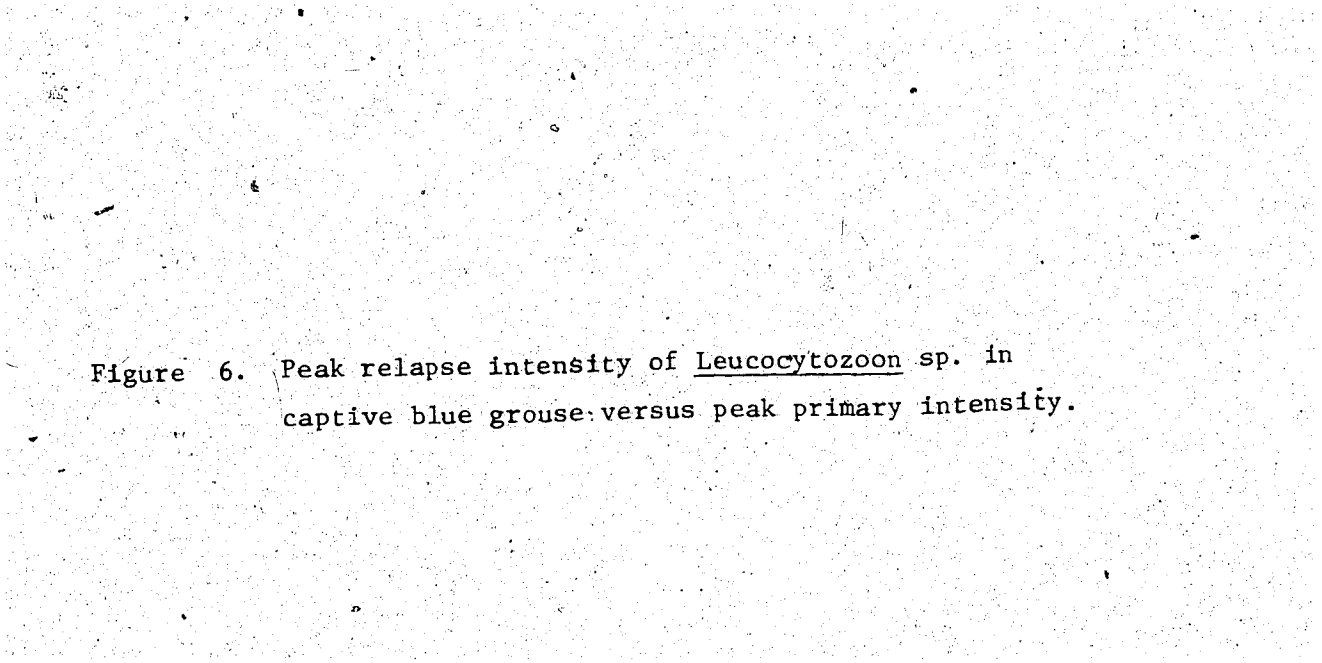


Figure 6. Peak relapse intensity of Leucocytozoon sp. in captive blue grouse versus peak primary intensity.

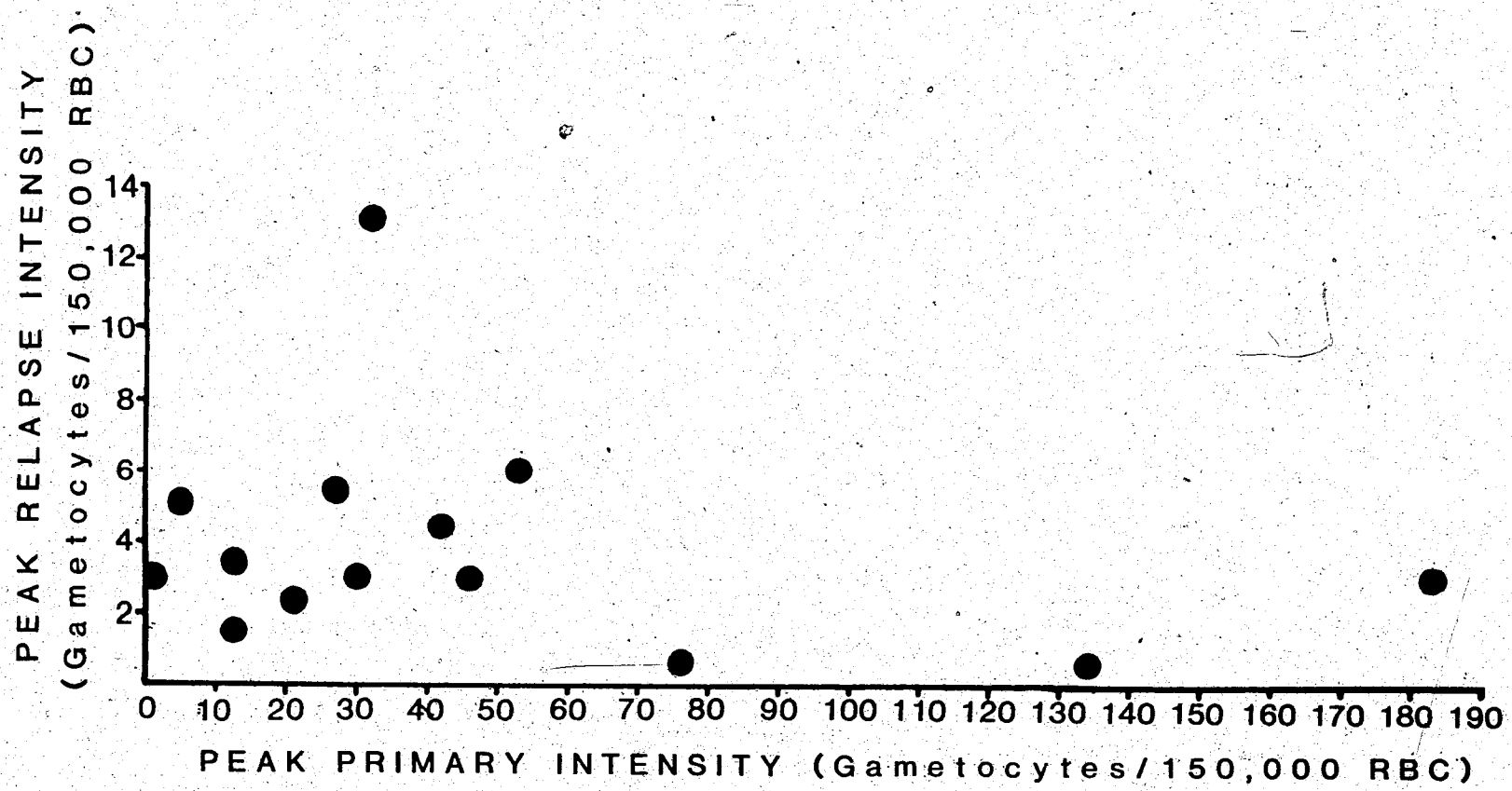

PEAK RELAPSE INTENSITY
(Gametocytes/150,000 RBC)
14
12
10
8
6
4
2
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190
PEAK PRIMARY INTENSITY (Gametocytes/150,000 RBC)

TABLE 2. GEOMETRIC MEAN INTENSITY OF *LEUCOCYTOZOON* SP. DURING PRIMARY AND RELAPSE PERIODS IN WILD BLUE GROUSE.

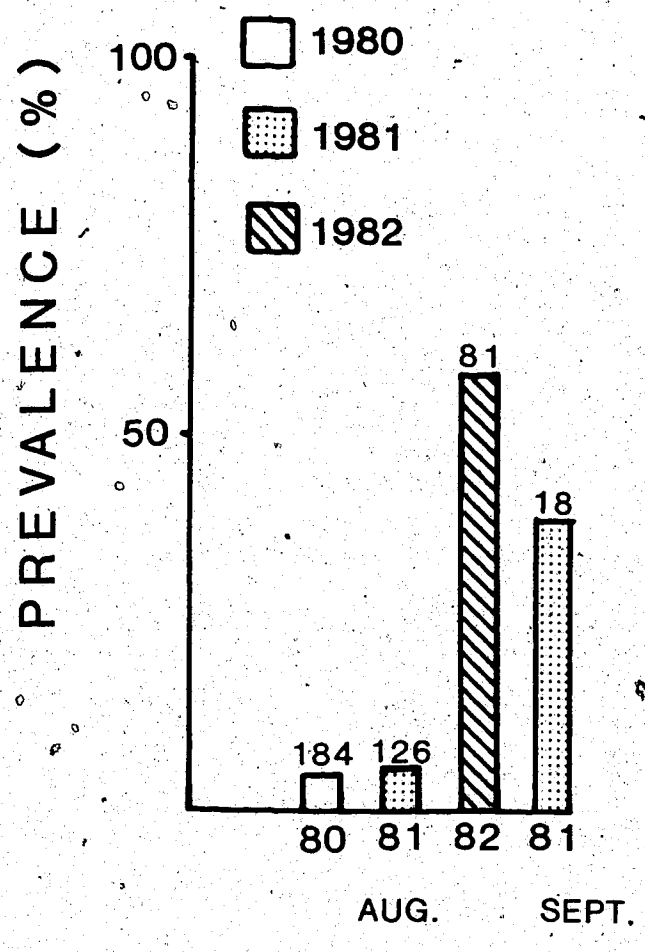
PERIOD	MONTH	GEOMETRIC MEAN INTENSITY+ (N) 1980	1981	1982
Primary (juveniles)	July	8.5(44)	11.6(30)	8.5(6)
	August	13.7(104)	17.6(99)	16.9(79)
Relapse (mature birds)	April	6.2(54)	4.3(33)	3.4(14)
	May	4.8(70)	4.7(25)	3.4(17)
	June	5.9(57)	6.1(22)	4.7(32)
Primary	All[a]	11.9***	13.9***	15.8***
Relapse	All	5.5	4.9	4.0

+ = Geometric Mean Intensity expressed in Gametocytes/150,000 RBC.

*** = Primary and relapse intensities (all months) significantly different at p 0.001 by Kruskal-Wallis.

a = Geometric Mean Intensity of all primary months.

Figure 7. Prevalence of primary infections of Haemoproteus mansoni in wild blue grouse chicks from Hardwicke Island, B. C., August 1980-1982 and September 1981. Sample size given on top of bars.


PREVALENCE (%)
100
50
1980
1981
1982
184
126
81
18
80
81
82
81
AUG.
SEPT.

$p>0.05$).

Ten of the chicks from 1981 and nine chicks from a 1982 cohort became infected with *H. manson*i during the exposure period in the outdoor aviary on Hardwicke Island. One of nine uninfected yearlings from the 1981 cohort became infected with *H. manson*i when reexposed in 1982. Examples of three primary infections with different intensities are plotted in Figure 8. Intensities peaked within the first three weeks, and then crashed. Primary peak intensities ranged from 10 to 1892 gametocytes/30,000 RBCs.

The presence or absence of *H. manson*i in captive blue grouse was assessed during the first relapse period (May-October) after primary infection in the previous summer; 100% of the 10 infections relapsed. Table 3 lists the peak primary, relapse and secondary relapse intensities observed in these birds. Four birds from the 1981 cohort survived captivity to relapse a second time. Two yearlings (Nos. 90,92) from the 1981 cohort were returned to Hardwicke Island in 1982, and may have been reinfected. All other birds were held in fly free conditions throughout the entire relapse period. The rank order of peak primary and peak first relapse intensity did not change in captive birds (Kendall coefficient of correlation, $p>0.05$). In six birds relapse peak intensities were higher, three were lower, and in one bird essentially the same as primary peak intensities (Table 3). In secondary relapse infections, peak intensities increased in one bird while decreasing in three others from

Figure 8. Intensity profiles of three primary infections of Haemoproteus mansoni in blue grouse chicks.

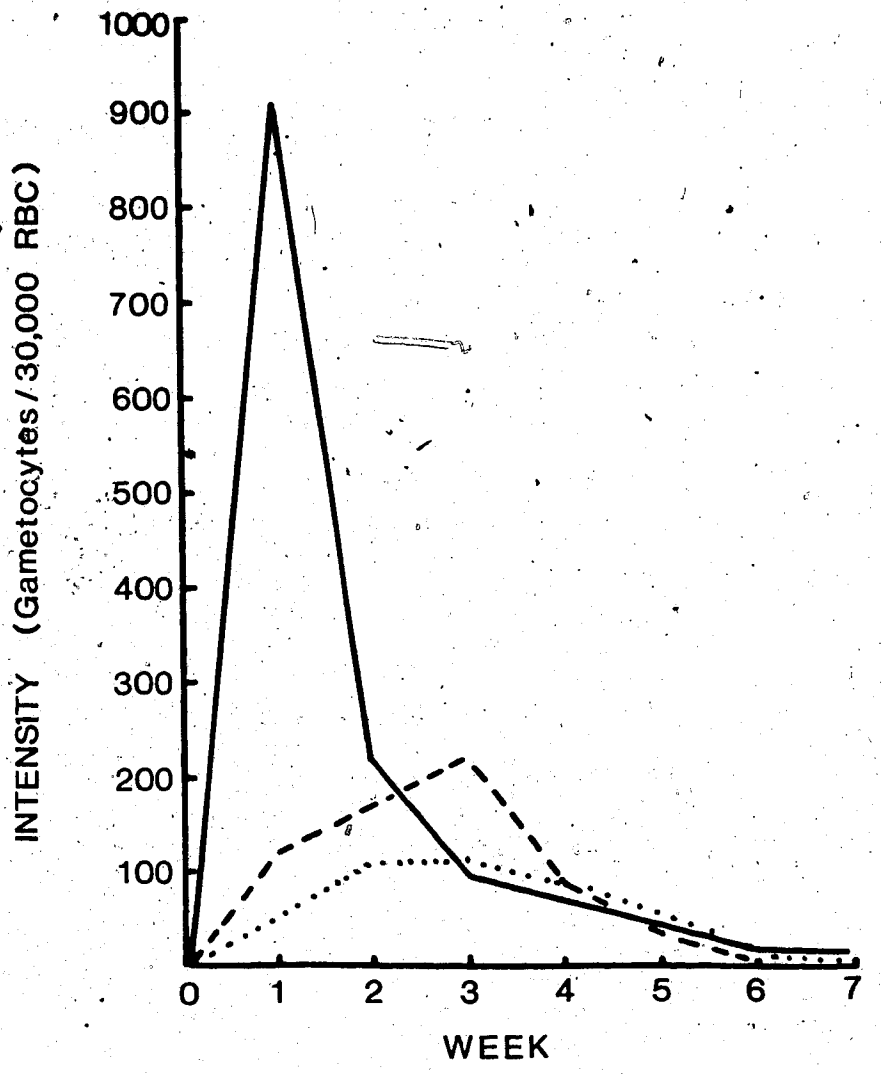

1000
900
800
700
600
500
400
300
200
100
INTENSITY (Gametocytes/30,000 RBC)
0
1
2
3
4
5
6
7
WEEK

TABLE 3. PEAK INTENSITY OF HAEMOPROTEUS MANSONI IN CAPTIVE BLUE GROUSE DURING PRIMARY, RELAPSE AND SECONDARY RELAPSE PERIODS.

BIRD NO.	PEAK INTENSITY+		
	PRIMARY	RELAPSE	SECONDARY RELAPSE
00	22	731	-
38	13	133	72
65	63	629	-
67	10	12	P*
69	699	207	-
75	539	290	-
83	31	184	-
90	127	246	599
92	912	1922	893
95	174	P	-

* gametocytes were present but numbers were less than 1/30,000 RBC

+ peak intensity expressed in gametocytes/30,000 RBC

- bird did not survive.

the previous peak. *Haemoproteus manson*i output obviously varied dramatically from host to host, and from year to year in the same host.

Primary and relapse geometric mean intensities of *H. manson*i in wild blue grouse are presented in Table 4. Primary intensities in chicks were recorded in August of every year. There were no significant differences between years ($F=1.9$, $p>0.05$). Mature grouse returning to the breeding grounds in February and March, 1982, were negative for *H. manson*i, with the exception of one bird which had an intensity of only 1/30,000 RBCs. In all years, the geometric mean intensities were extremely low in April and May, but subsequently rose sharply in June and July (Chapter 2). There was a significant rise in intensity from June to July of 1980 and 1981 (ANOVA, $p<0.01$), but not in 1982 ($F=2.89$, $p>0.05$). There were significant differences in the peak July intensities between years ($F=4.24$, $p<0.05$). Two way analysis of variance revealed significantly higher intensities during relapse than in primary infections ($F=12.57$, $p<0.001$); year did not affect the outcome of the analysis (ANOVA, interaction term $F=1.09$, $p>0.05$).

Comparison of microparasite intensities in yearling and adult blue grouse

Blue grouse which were isolated from reinfection by captivity, produced gametocytes during two consecutive relapse seasons for both *L. bonasae* and *H. manson*i.

TABLE 4. GEOMETRIC MEAN INTENSITY OF HAEMOPROTEUS MANSONI DURING PRIMARY AND RELAPSE PERIODS IN WILD BLUE GROUSE.

PERIOD	MONTH	GEOMETRIC MEAN INTENSITY + (N)		
		1980	1981	1982
Primary (juveniles)	August	816(10)	160(6)	609(35)
Relapse (mature birds)	July	2816(22)	1386(19)	1009(8)

+ = geometric mean intensity expressed as Gametocytes/30,000 RBC.

Gametocytes from *Leucocytozoon* sp. were present during one relapse season. Their presence in a second was not determined as birds died from various causes unrelated to parasite infection before the second relapse period.

Only two chicks (No. 57 and 67) naturally infected in 1981 with *L. bonasae* were held in fly free conditions for two additional summers (1982 and 1983); they produced more gametocytes as yearlings in 1982, than as adults in 1983. From February to July 1982 a total relapse production was 76 gametocytes for bird No. 57, and 62 for bird No. 67. During the same period in 1983, total secondary relapse gametocyte production was 16 for No. 57 and five for No. 67. In all years, wild yearlings had significantly higher relapse intensities of *L. bonasae* than adults. This difference was not observed in the other two parasites (Table 5).

Reexposed yearlings contracted secondary infections of *L. bonasae* (Chapter II). Sudden rises in gametocyte numbers in exposed birds were interpreted as evidence for new infections, as infected control birds did not produce such rises. Bird No. 62 exhibited a mild infection of *Leucocytozoon* sp. (5 gametocytes/150,000 RBC) in 1981. No gametocytes were seen after September, but when reexposed in summer of 1982 a secondary mild infection was detected. Three yearlings, previously infected with *H. manson*i in 1981, were returned to the outdoor aviary in 1982. As all three infrapopulations were intensely productive throughout the reexposure period, it was impossible to determine if

TABLE 5. MEAN GEOMETRIC RELAPSE INTENSITY OF *LEUCOCYTOZOON* SP., *L. BONASAE* AND *HAEMOPROTEUS MANSONI* IN ADULT AND YEARLING WILD BLUE GROUSE.

PARASITE	MEAN GEOMETRIC INTENSITY (N)		F	P
	YEARLING	ADULT		
L. bonasae				
1980	17.6(82)	10.7(140)	11.48	***
1981	17.4(57)	10.9(56)	7.19	**
1982 (April-June)	13.0(34)	7.7(46)	5.95	*
Leucocytozoon sp.				
1980	5.2(64)	5.7(117)	0.26	NSD
1981	3.5(34)	6.2(46)	4.77	*
1982 (April-June)	3.8(29)	4.3(34)	0.28	NSD
H. mansoni				
1980-1982 (July)	2453(18)	1538(30)	2.34	NSD

* significant at 0.05, ** significant at 0.01, *** significant at 0.001, NSD = no significant difference.

reinfection had occurred.

Discussion

Microparasites are transmitted from blue grouse to blue grouse via vectors. In other systems, *Leucocytozoon* species are vectored by simuliids, and *Haemoproteus* species by *Culicoides* (Bennett and Fallis, 1960; Fallis and Bennett, 1961, 1966; Herman and Bennett, 1976). Transmission of a microparasite to an uninfected blue grouse results in a primary infection. Microparasites overwinter in blue grouse, and the reappearance or increase in number of gametocytes in the peripheral blood during the following year is referred to as a relapsing infection. Relapse populations of gametocytes are the result of asexual reproduction in the birds; no further transmission has occurred. Both primary and relapse gametocytes are infective to the appropriate vectors, and thus gametocytes are a vital link in the life cycle of microparasites.

Blue grouse can be regarded as a patch in which microparasites may survive and reproduce. The population dynamics of parasites within an individual grouse can be termed within-patch dynamics. Moreover, as each patch has a finite life span, microparasites must transfer from one patch to another. The dynamics of such transfer is referred to as between-patch dynamics. Thus, the population dynamics of microparasites have two components, within-patch and between-patch.

Between-patch dynamics can be measured by the transmission of each microparasite species to the next generation of blue grouse, the chicks. A comparison of the prevalence in chicks from year to year, for each microparasite, should reflect the level of and variation in transmission from patch to patch. Thus, the effectiveness of the transmission system can be compared between species of parasites.

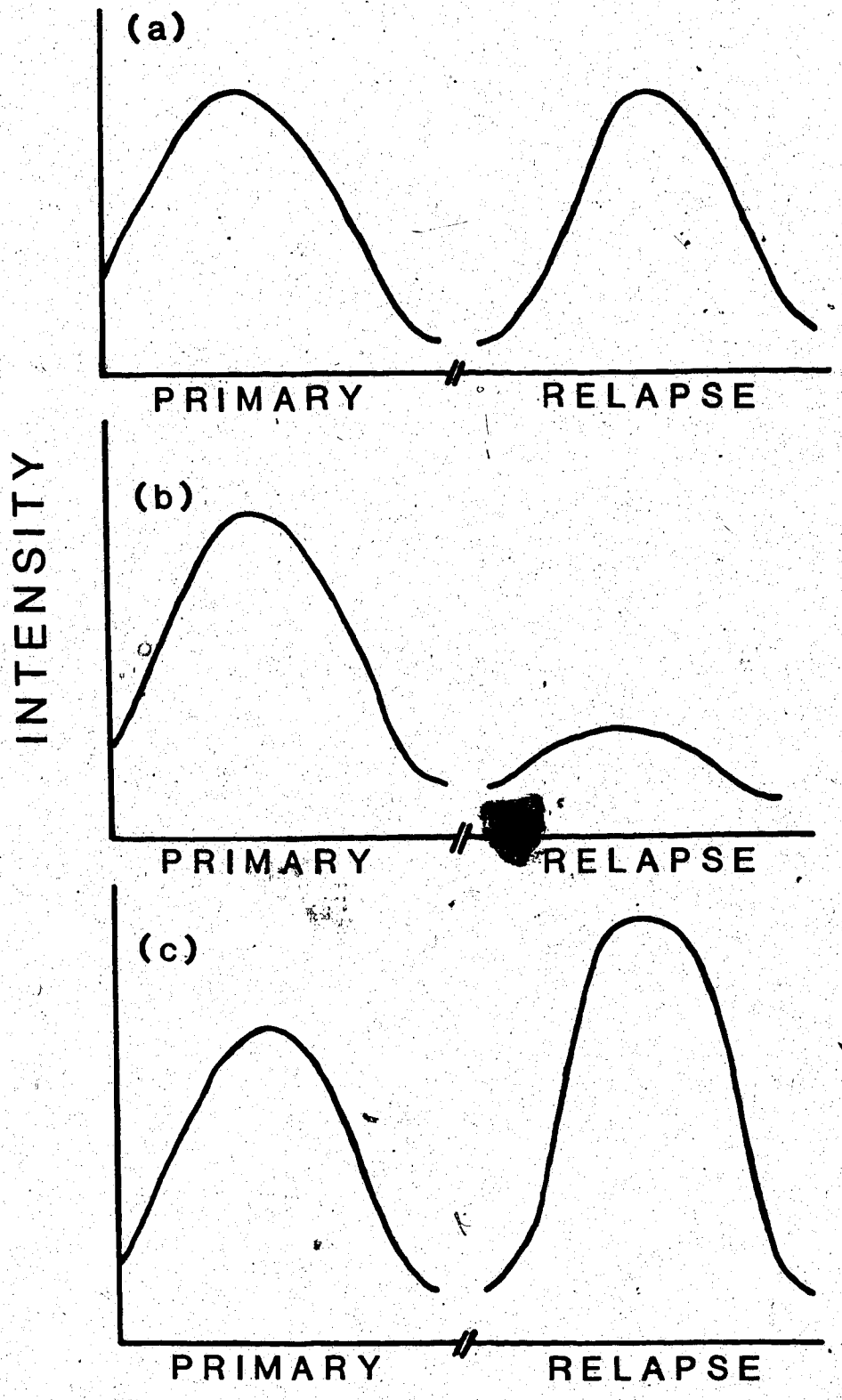
Within-patch dynamics is measured by the survival and reproductive output of each microparasite within the blue grouse. An indicator of survival is the prevalence of a parasite during relapse compared with the prevalence in an earlier primary infection. The reproductive output can be measured by the intensity of gametocytes in the blood during primary and relapse infections. I will use these infrapopulation studies, in both captive and wild grouse, to compare within-patch dynamics in the three microparasites. This assumes that both primary or relapse phases are important for transmission. Reproductive output is important during primary infection, as further transmission involving primary infections in chicks may occur. During relapse, output is critical for maintainance of the parasite population, as transmission occurs via the vector to the new generation of vertebrate hosts, the grouse chicks. Comparison of output during primary infection and subsequent relapse periods can be a measure of the dependability of the vertebrate host for the continuance of the parasite life

cycle.

Infrapopulations of a particular microparasite in individual blue grouse or in populations may follow four possible courses in terms of reproductive output during primary and relapse periods (Figure 9): (a) primary and relapse output are equivalent, (b) primary output is much greater than relapse output, (c) relapse output is greater than primary output, and (d) primary output only, no relapse occurs. Comparison of primary and relapse intensity levels in this way at the infrapopulation level is independent of the intensity of infection attained by each species of parasite, and serves as an interspecies indicator of association with the vertebrate host.

Are there differences in effectiveness of the transmission system from year to year? The prevalence of each microparasite was determined for three years in wild chick populations on Hardwicke Island, allowing an evaluation of the between-patch dynamics of each microparasite. Transmission of *L. bonasae* was highly effective each year, with ≥90% of chicks becoming infected by August. By contrast, prevalence of *Leucocytozoon* sp. was lower and varied widely from year to year. The prevalence of *H. manson*i in wild chicks also varied from year to year. Transmission of this parasite took place late in the summer, and most likely continued after sampling was stopped at the end of August. Therefore data from August may be an inaccurate measurement of effectiveness. However, prevalence

Figure 9. Hypothetical comparison of primary and relapse intensity profiles of microparasites.


(a)
PRIMARY
RELAPSE
INTENSITY
(b)
PRIMARY
RELAPSE
(c)
PRIMARY
RELAPSE

in September of 1981 was 39%; if I assume no transmission occurred in October, then this indicated poor effectiveness of transmission. These features indicate that the effectiveness of the transmission system, which is an indicator of the between-patch dynamics, differed among these microparasites; *Leucocytozoon bonasae* had a highly effective transmission system, whereas *Leucocytozoon* sp. and *H. mansoni* had much less effective transmission systems.

Effectiveness of the transmission system is also variable for other blood microparasites. Beaudoin *et al.* (1971) found varying prevalence from year to year of *Plasmodium* in birds from Pennsylvania. Primary infections in many species of their birds varied from 0-25%; in one year there appeared to be no transmission. Herman and Bennett (1976) used sentinel ducks to estimate prevalence of *L. simondi* and *H. nettionis* in New Brunswick. In three years, the prevalence of *L. simondi* in sentinel ducks was very consistent and high, with over 90% of ducks infected each year. By contrast, the prevalence of *H. nettionis* varied from almost zero (only one bird infected) in 1973, to 40% in 1972. Apparently, *L. simondi* had a effective transmission system, whereas *H. nettionis* did not. This pattern was similar to that in my data for *L. bonasae* and *H. mansoni* in blue grouse. Williams *et al.* (1980) reported variable prevalence of *H. mansoni* in blue grouse chicks on Vancouver Island.

Overwinter survival of each microparasite was measured by the production of gametocyte stages which were present in the peripheral blood during the relapse period. Captive grouse were monitored weekly, and therefore served as a dependable gauge of survivorship for each parasite. All *L. bonasae* and *H. manson*i infected chicks and yearlings had relapse populations the following summer. Fourteen of 15 *Leucocytozoon* sp. infections overwintered and relapsed. Thus, there were no important differences in survivorship between these parasites. Survival to a second relapse period (second summer after primary infection) was also possible to assess in captive grouse (at least for *L. bonasae* and *H. manson*i). All surviving grouse infected with either species had gametocytes during a second relapse period. The survivorship aspect of dependability of the vertebrate host for the reproduction of each parasite does not differ between the three microparasites.

The dependability of blue grouse for the reproduction of each parasite was also assessed by comparing primary and relapse output. *Leucocytozoon bonasae* intensities in wild and captive grouse showed the same patterns. The geometric mean intensities of *L. bonasae* in wild chicks (primary infection) were similar to those found during relapse in mature birds, with the exception of 1982 (Table 1). Likewise, peak primary and relapse intensities in captive grouse were not significantly different. Regression analysis also indicated a relationship between peak primary and

relapse intensities. In general, the reproductive output during relapse was similar to that of primary output in both captive and wild birds. There were differences between wild and captive birds. Relapse intensities in captive grouse peaked in February and March, and declined throughout the summer months. In wild grouse, intensities could not be determined in February and March, but were not significantly different throughout the summer months (Table 1). These differences could be the result of the much lower intensities in captive grouse. Overall, *L. bonasae* produced gametocytes equally well during primary and relapse periods indicating that blue grouse were dependable hosts for this parasite (Figure 9a).

Leucocytozoon sp. had different patterns in primary and relapse intensities. Results were consistent for wild and captive grouse. For all three years, the geometric mean intensities of *Leucocytozoon* sp. in wild chicks (primary infection) were significantly higher than in relapsing mature birds (Table 2). Captive grouse infrapopulation profiles supported these observations. Intensities during relapse were extremely low and there was no relationship between primary peak and relapse peak intensities (Figure 7). This implied a different type of host response than that observed in *L. bonasae*. *Leucocytozoon* sp. may have induced a density-independent immune response, whereas the response to *L. bonasae* appeared more density-dependent. *Leucocytozoon* sp. followed a type (b) (Figure 9b) reproductive output,

with primary output being much greater than relapse output. In addition, primary infections of *Leucocytozoon* sp. gametocytes had poorer survival than *H. manson*i gametocytes or uninfected erythrocytes (Chapter V). Blue grouse constituted an undependable host for this parasite.

*Haemoproteus manson*i in wild blue grouse had higher outputs in relapse than in primary infections. Captive birds provided an accurate assessment of the reproductive output of *H. manson*i, as the peak intensity could be more precisely determined. There were wide variations in the output of *H. manson*i. Infrapopulations followed type (a), (b) and (c) patterns. On average, primary and relapse production was equivalent. Therefore, *H. manson*i can at least sustain reproductive output during relapse, and potential exists for population increase. Grouse constituted a dependable host for *H. manson*i.

These three microparasites can be categorized with respect to the dependability of blue grouse for their reproduction and survival As there was no detectable difference between survival, reproductive output must be the main criterion. Reproductive dependability was high for *H. manson*i and *L. bonasae*, but much less for *Leucocytozoon* sp.

How long is a patch suitable for these parasites? Wild yearlings and adults had infections of all three parasites (Table 5), but intensities of *Leucocytozoon* sp. were low in both age classes which indicated a continued poor association. Wild and captive adult blue grouse appeared to

have increased resistance to *L. bonasae*. Thus, the quality of each patch (bird) was apparently deteriorating with time. This was not the case for *H. mansoni*; the two age classes had equivalent intensities. As *H. mansoni* had a low effectiveness of transmission, continued dependability of adult blue grouse would be advantageous to its survival. On the other hand, *L. bonasae* had a highly effective transmission system, and may not be as dependent on maintainance of infrapopulations in adult blue grouse.

Within-patch dynamics can also be assessed for other Haemosporina. *Leucocytozoon fringillinarum* produced a substantial primary infection, but essentially no relapse (Khan and Fallis, 1970a,b). This species was found in many bird species in the same locality (Khan and Fallis, 1970b). Although there was poor relapse in any one host, the accumulated relapse output from many host species may result in a large total output from the host community. Khan and Fallis (1970b) noted reduced parasitemias in robins and saw-whet owls with relapsing *L. dubreuili* and *L. danilewskyi*, respectively. *Plasmodium relictum* in a population of English sparrows had a much lower mean peak relapse intensity than mean primary intensity (Applegate, 1970). *Leucocytozoon bonasae* and *H. mansoni* in blue grouse were in a more dependable vertebrate host than *L. fringillinarum*, *L. dubreuili*, *L. danilewskyi*, *P. relictum*, and *Leucocytozoon* sp.

This discussion has focused on the effectiveness of the transmission system, and survival and reproductive output of three microparasites. These correspond to an assessment of between-patch and within-patch dynamics. Each parasite fell into different category when considering both effectiveness and dependability parameters. *Leucocytozoon bonasae* had a highly effective transmission system, and a highly dependable association with blue grouse. In contrast, *Leucocytozoon* sp. had a less effective transmission system, and the dependability of the vertebrate host for its reproduction was low. *Haemoproteus manson*i exhibited the lowest effectiveness of transmission, and a dependable association with blue grouse. Such a spectrum of patch dynamics may influence the evolution of microparasites. This idea will be developed in the concluding discussion.

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V. Comparison of decline in *Leucocytozoon* sp. and *Haemoproteus mansoni* primary populations in blue grouse

Abstract

Loss of *Haemoproteus mansoni* and *Leucocytozoon* sp. gametocytes from peripheral blood of blue grouse during the first three weeks of primary infection was compared. Chromium 51 was used to estimate normal loss of uninfected erythrocytes. The loss of *Leucocytozoon* sp. gametocytes, which are intraerythrocytic, was more rapid than the loss of either uninfected erythrocytes or erythrocytes infected with *H. mansoni* gametocytes. This was taken as further evidence for the undependable association between *Leucocytozoon* sp. and blue grouse.

Introduction

The gametocyte stage of *Haemoproteus* spp. and some *Leucocytozoon* spp. develops in erythrocytes (Khan, 1975; Khan and Fallis, 1970; White and Bennett, 1979). In blue grouse (*Dendragapus obscurus*) from Hardwicke Island, British Columbia, *Leucocytozoon* sp. (Chapter III) and *H. mansoni* produced primary populations of gametocytes within erythrocytes. A number of factors may limit the duration of these primary infections: (1) initial peak intensity of infection, (2) immune reaction of host which may reduce or prevent secondary merozoite invasion and/or remove infected erythrocytes from circulation, and (3) the life span of

erythrocytes.

Both parasites had greater gametocyte losses than could be accounted for by the normal removal of erythrocytes, but in *H. manson*i this loss may have been compensated by new merozoite invasion. Limited reproduction of *Leucocytozoon* sp. was implicated as further evidence for an undependable association with blue grouse.

Materials and Methods

Captive grouse were raised and maintained as previously described (Chapter II). Intensity of *H. manson*i and *Leucocytozoon* sp. were monitored weekly as described (Chapter IV). The patent period for *Leucocytozoon* sp. and *H. manson*i was calculated from the week the intensity rose above 1/30,000 RBC, until the intensity dropped below these levels.

Six captive blue grouse (parasite free) were isolated in individual cages and each injected in the brachial vein with 100 ul of ^{51}Cr (Na_2CrO_4). A 0.5 ml blood sample was removed 24 hr after inoculation, and at weekly intervals thereafter. Each sample was mixed with 0.2 ml of sodium citrate. Duplicate 0.2 ml subsamples were removed and washed twice in physiological saline (0.85%) to remove any extracellular radioactivity. The subsample was pipetted into a 10 ml scintillation vial containing 5 ml of saline and counted (counts per minute) on a Beckman Gamma 8000. Packed cell volume (PCV) was also recorded. The loss of

radioactivity was corrected for variation in PCV, and for decay of the isotope. The log decline in ^{51}Cr content of the blood was calculated for the first three weeks from the start of the experiment. This gave an indication of the rate of loss of erythrocytes from the circulation (Rodnan *et al.*, 1957). Similarly, the log decline of the two parasite populations was calculated from the week of peak primary intensity for two succeeding weeks after the peak.

Results

Linear regressions were calculated for number of weeks of patency of *H. mansoni* and *Leucocytozoon* sp. on the peak intensity of primary infection (Figures 1,2) to determine if peak intensity had an influence on patent period. In *H. mansoni*, 39.5% of the variance in primary patent period could be accounted for by the regression ($t=3.527$, $p<0.01$), and in *Leucocytozoon* sp., 33.3% of the variance in patent period could be explained by the regression ($t=3.08$, $p<0.01$). The slopes of *H. mansoni* and *Leucocytozoon* sp. were not significantly different ($F=0.273$, $p>0.05$).

The log decline of uninfected ^{51}Cr labelled erythrocytes had a slope of -0.195. The decline in *Leucocytozoon* sp. was greater than that of uninfected erythrocytes (Table 1) (Signs test, $p<0.01$). However, the log decline of *H. mansoni* intensity was not significantly different from the decline of uninfected erythrocytes (Table 1). Chromium 51 label could not be detected in erythrocytes

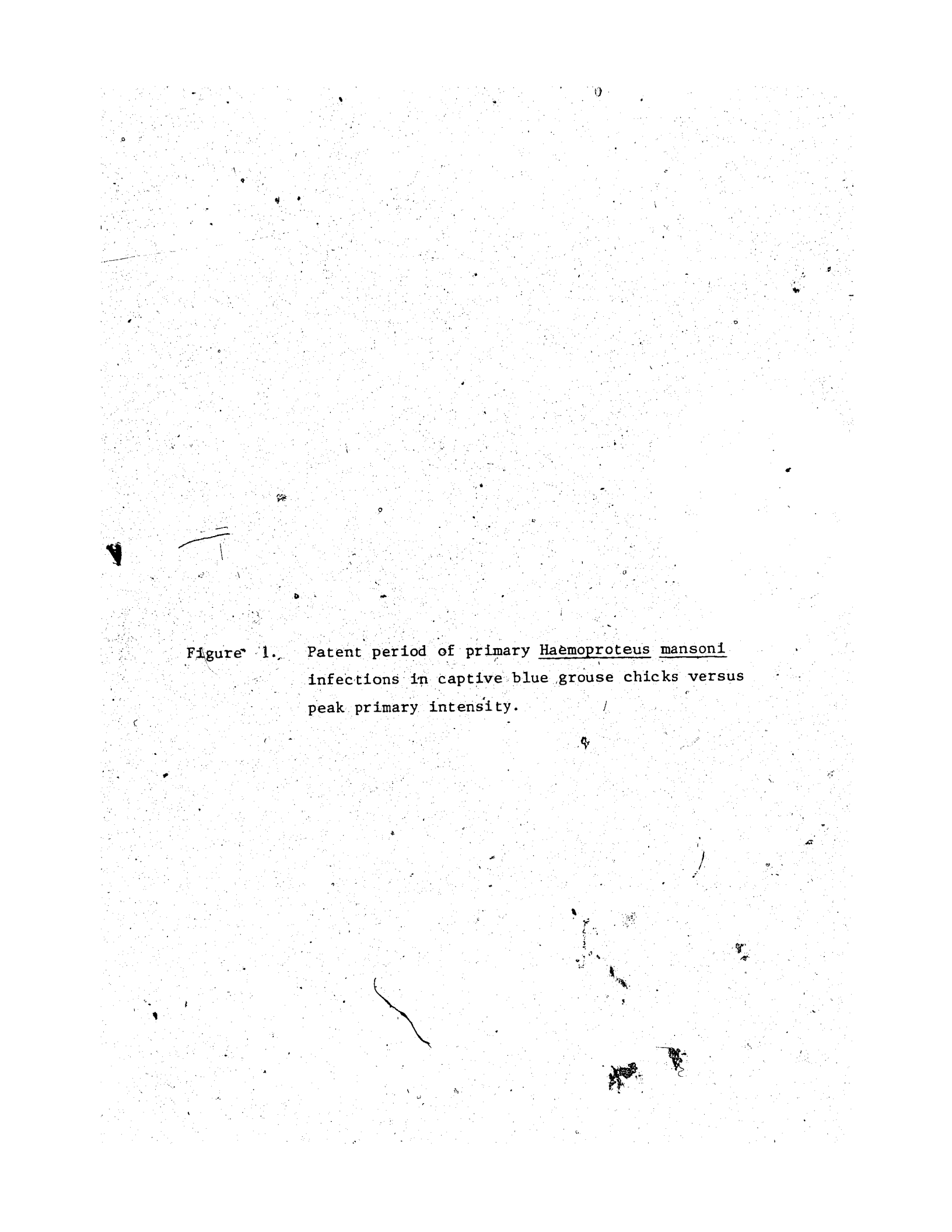
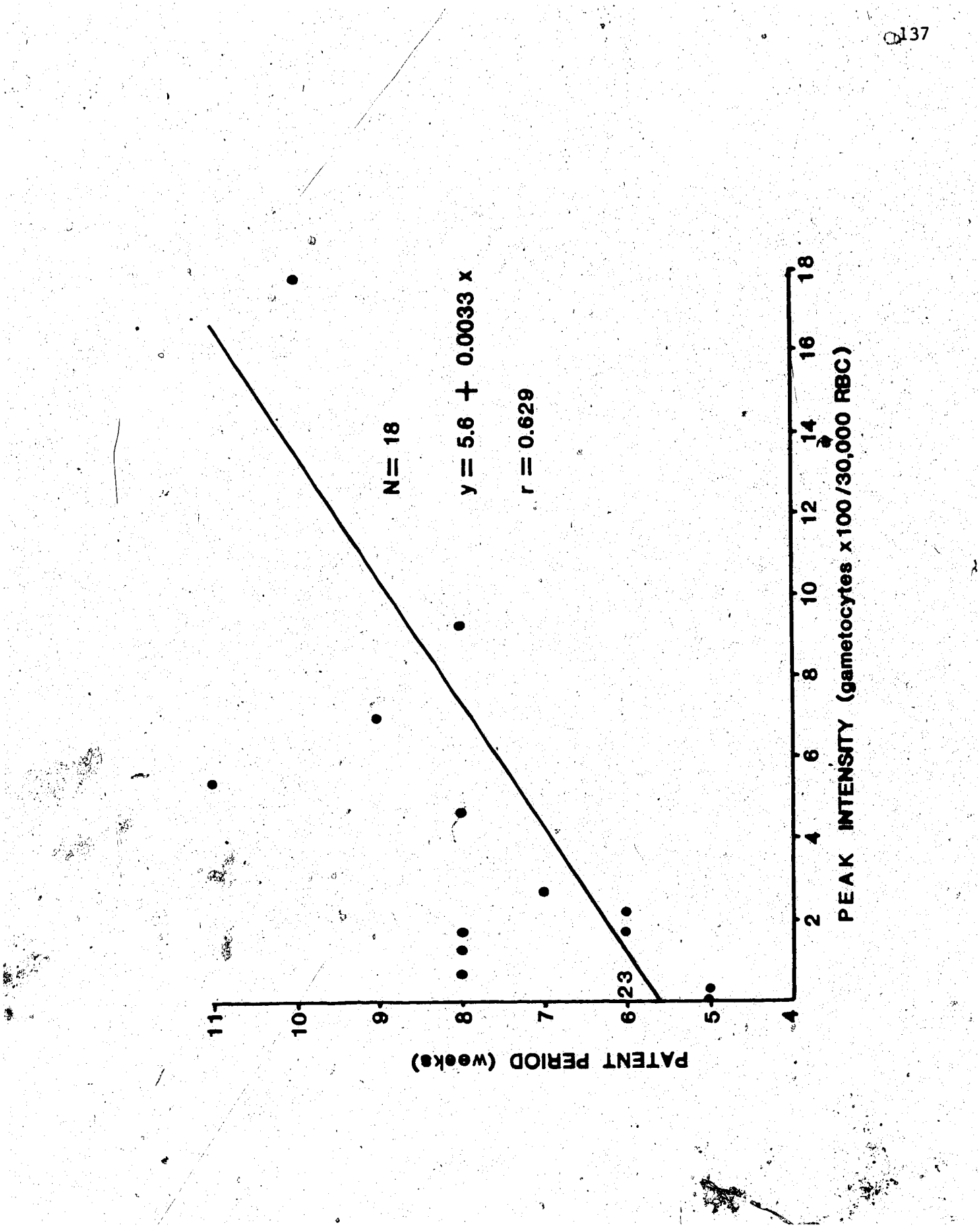


Figure 1. Patent period of primary Haemoproteus mansoni infections in captive blue grouse chicks versus peak primary intensity.


N= 18
y = 5.6 + 0.0033 x
r = 0.629
PATENT PERIOD (weeks)
PEAK INTENSITY (gametocytes x100/30,000 RBC)
23

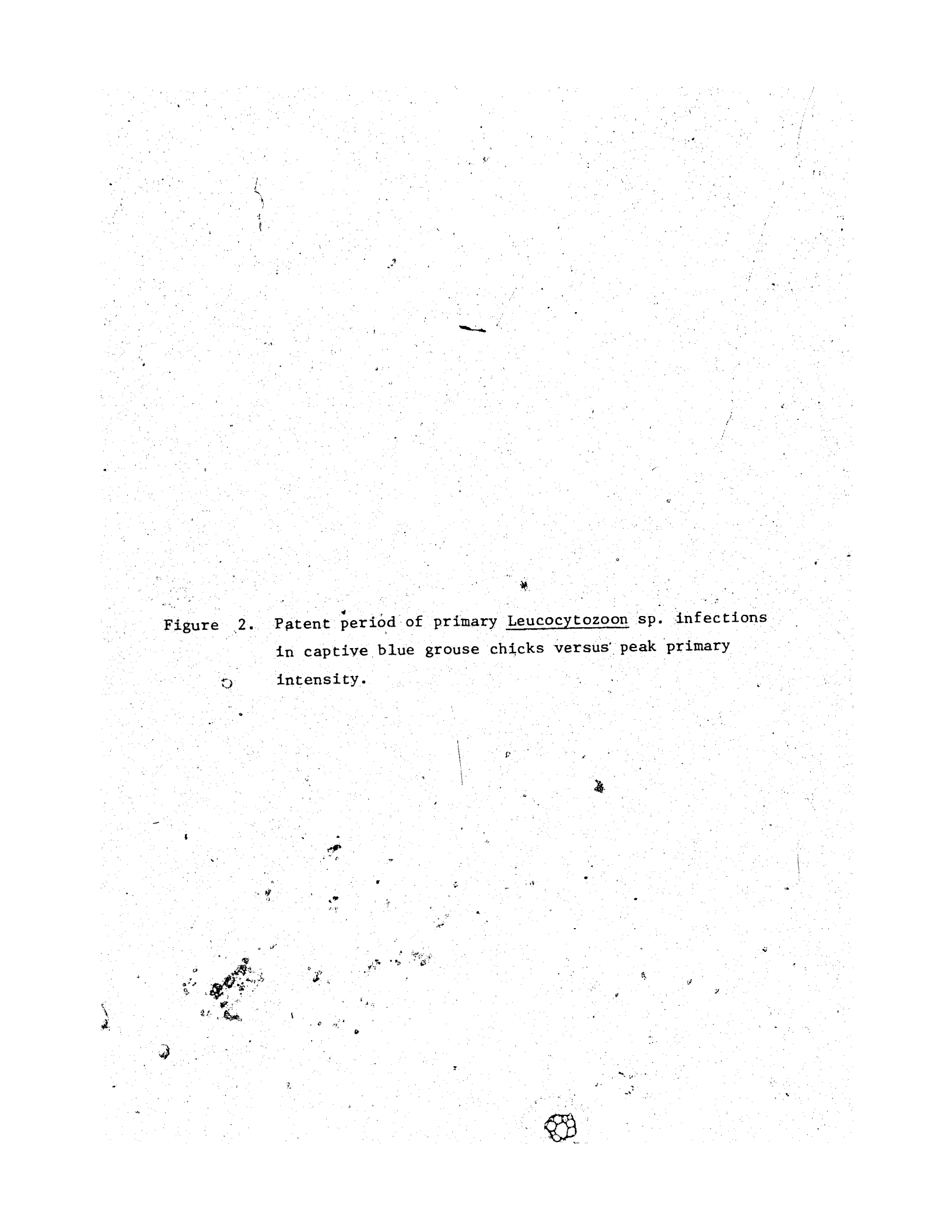


Figure 2. Patent period of primary Leucocytozoon sp. infections in captive blue grouse chicks versus peak primary intensity.

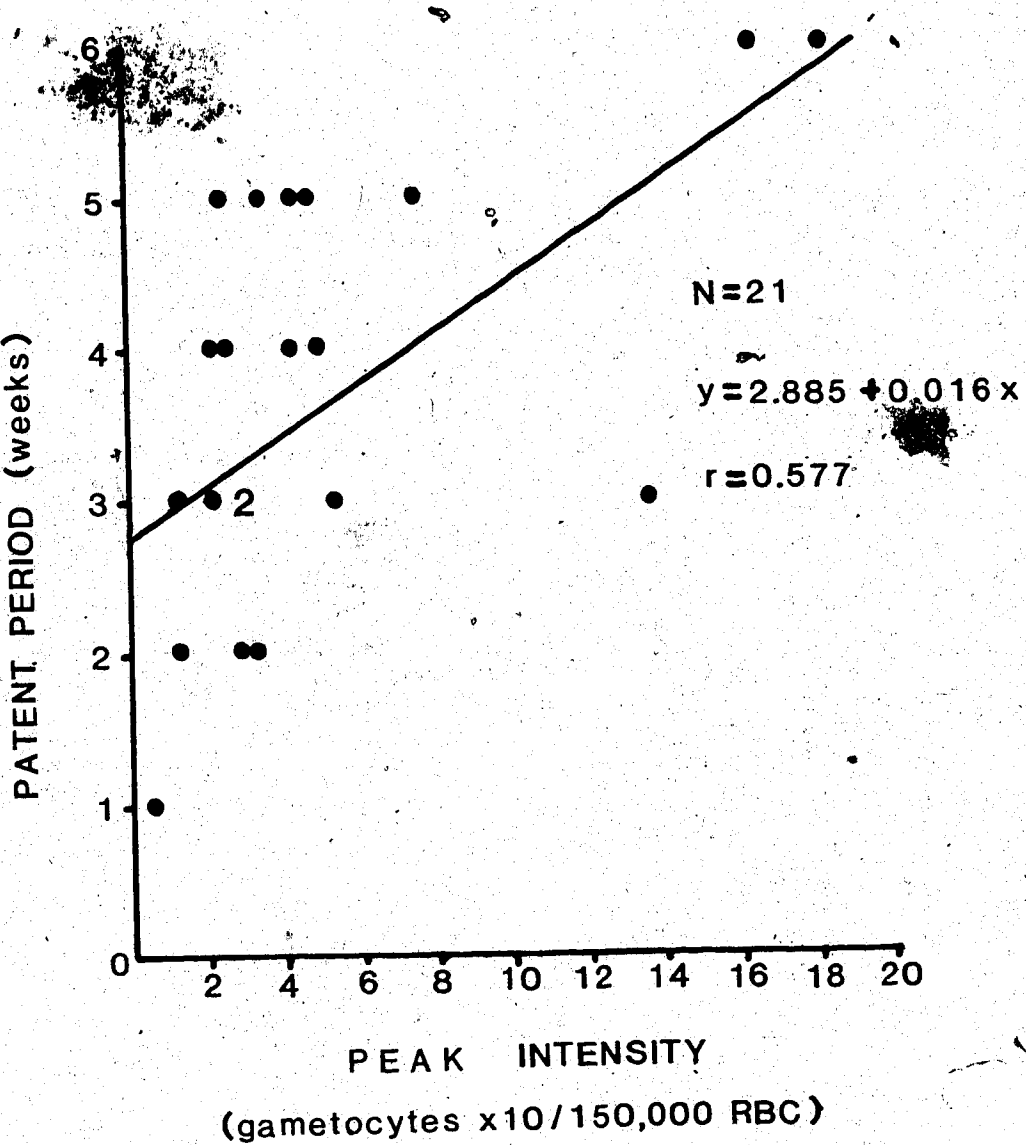

PATENT PERIOD (weeks)
0
1
2
3
4
5
6
2
N=21
y=2.885+0.016x
r=0.577
0
2
4
6
8
10
12
14
16
18
20
PEAK INTENSITY
(gametocytes x10/150,000 RBC)

TABLE 1. COMPARISON OF LOG SLOPE OF DECLINE IN PRIMARY *HAEMOPROTEUS MANSONI* AND *LEUCOCYTOZOON* SP. GAMETOCYTE POPULATIONS WITH LOG SLOPE OF LOSS OF ^{51}Cr LABELLED ERYTHROCYTES.

Case	$\bar{X}$ slope	N	No. with slope > RBC	Significance
Erythrocytes	-0.195	-	-	-
H. mansoni	-0.25	19	11	NSD
Leucocytozoon sp.	-0.49	19	17	0.01*

* $p = 0.01$ by Signs test (Siegel, 1956).

beyond the fifth week after injection. Therefore, the maximum life span of erythrocytes was five weeks. Almost all *Leucocytozoon* sp. infections had patent periods shorter than five weeks, whereas *H. mansoni* infections lasted longer (5-11 weeks) than the longest lived uninfected erythrocyte.

Discussion

Regression of peak intensity on weeks indicated that in both parasites the higher the peak intensity the longer the infection lasted. It was curious that in both organisms approximately one third of the variance was explained by this relationship; however, this may be a coincidence, or a function of the measurement technique. A large part of the variance in patency was not correlated with peak intensity. Wide variation in patency may be the result of variable resistance of the host.

A comparison of log slope of decline in erythrocytes with log decline in *Leucocytozoon* sp. and *H. mansoni* indicated that gametocytes of *Leucocytozoon* sp. declined more rapidly than normal erythrocytes or gametocytes of *H. mansoni*. During the second and third week after the peak in *H. mansoni* intensity a few young gametocytes were present in the population (Chapter III). Thus, the equivalent slope of RBC infected with gametocytes of *H. mansoni* and uninfected normal erythrocytes may have resulted from a combination of the removal of mature gametocytes and the addition of young developing gametocytes stemming from continued invasion by

merozoites.

As evident from the log slope of decline of *Leucocytozoon* sp. the loss of gametocytes was much more rapid, and there were no indications of continued invasion of erythrocytes; the loss was not compensated. In both parasites, removal of the infected erythrocyte was presumably through an immunological response, as in *Plasmodium* infections (Cohen, 1978; Cohen *et al.*, 1977; Poels *et al.*, 1978). Additional invasion of erythrocytes by merozoites of *H. mansoni* would also account for the longer patent period in this parasite.

Khan and Fallis (1970) followed primary infections of *L. dubreuili* in robins and *L. fringillinarum* in grackles. I calculated the slope of decline of gametocytes to be approximately -0.225 for *L. dubreuili* and -0.21 for *L. fringillinarum*. This was very similar to the decline of *H. mansoni*, or erythrocytes in blue grouse. However, similar slopes could be the result of removal of old gametocytes and new gametocyte development as in *H. mansoni*, or just normal rate of loss of uninfected erythrocytes.

The poor dependability of blue grouse for the reproduction of *Leucocytozoon* sp. was reflected in a short primary patency, whereas *H. mansoni* had a longer patency. In addition, although *Leucocytozoon* sp. had a longer transmission window than *H. mansoni* (Chapter III), the former had a shorter patency which would reduce between-patch transfer from primary infected blue grouse to

uninfected chicks. Reproduction of *Leucocytozoon* sp. appeared to be severely curtailed.

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VI. CONCLUDING DISCUSSION

Attempts to understand the selective pressures which lead to different life history strategies have been the focus of research in evolutionary ecology, see reviews by Stearns (1976, 1977). MacArthur and Wilson (1967) first introduced the terms r and K selection, in the context of environmental correlates which select for different suites of characteristics within species. These ideas have stimulated much controversy, which has resulted in the expansion of the meanings for r and K selection (see review by Parry, 1981). The model presented here will invoke habitat stability as the sculptor of life history strategies for microparasites with two hosts (vector and vertebrate). K-selected species are found in habitats that remain available for long periods of time, whereas r-selected species occur in ephemeral habitats. I attempt to relate evolutionary processes in microparasites to general ecological theory, and make no evaluation of these theories any further than is necessary to understand microparasite systems.

Parasitologists have attempted to place parasite life history strategies in the context of r- and K-selection (Esch *et al.*, 1977; Jennings and Calow, 1975; Mitchell, 1974; Seidenberg *et al.*, 1974); Jennings and Calow (1975) concluded that endoparasitic Platyhelminthes followed both r- and K-strategies. Seidenberg *et al.* (1974) interpreted loss of adult male enteric nematodes, *Longistriata adunca*,

as an r-strategy. Esch *et al.* (1977) also concluded that most parasites exhibited r-selected characteristics. However, a third selective process, adversity selection ("A-selection") may alter the interpretation of some parasitic life history strategies. Greenslade (1972 a,b) recognized this process, which has been applied to both plants and animals (Greenslade, 1983; Grimes, 1979; Southwood, 1977; Whittaker, 1975). A-selection occurs in consistently adverse environments. Greenslade (1983) cites cool wet montane forests as an adverse habitat for log dwelling Coleoptera. As temperatures are constant year round, this environment is predictably unfavourable for these organisms. Similarly, arctic habitats are adverse for insects, as many critical factors are limiting. However seasonal changes are predictable (summer follows winter).

All three selective processes have been incorporated into a life history strategy model for microparasites. In the model, I have focussed on within-patch and between-patch dynamics, as suggested by Price (1980).

The Life History Strategy Model

Southwood (1977) interpreted habitat characteristics in terms of heterogeneity in time and space. The time a habitat is suitable for the survival and reproduction of an organism is critical; heterogeneity in this feature is described by habitat predictability. Relatively permanent habitats are predictable, whereas ephemeral habitats are unpredictable.

These extremes will create vastly different selective pressures. Heterogeneity in space also varies, with some patches more favourable than others. Southwood (1977) noted that this feature will influence migration from one patch to another. Habitat favourableness refers to this characteristic. Greenslade (1983) incorporated these ideas into his habitat templet (Figure 1). The two habitat parameters, favourableness and predictability, interact to produce three selection processes: r-selection in unpredictable habitats, K-selection in predictable and favourable habitats and A-selection in predictable but unfavourable habitats (Greenslade, 1972b, 1983). Greenslade (1983) did not use the terms within or between-patch when referring to his system. Although his interpretation of the habitat templet inferred patch dynamics, no clear line was drawn between within and between-patch features. Both his axes appeared to focus on within-patch phenomenon. I adopted the format of his model because of its simplicity, but used Price's (1980) ideas on patch dynamics for my model.

In the microparasite's habitat there are two environments (vector and vertebrate). This complication requires a judgement on which of the time and space parameters will be critical in determining life history strategies in parasites. This model is intended to describe a particular type of microparasite system, in which the vector is seasonal (present in summer months), so that the parasite must overwinter in the vertebrate host, and relapse

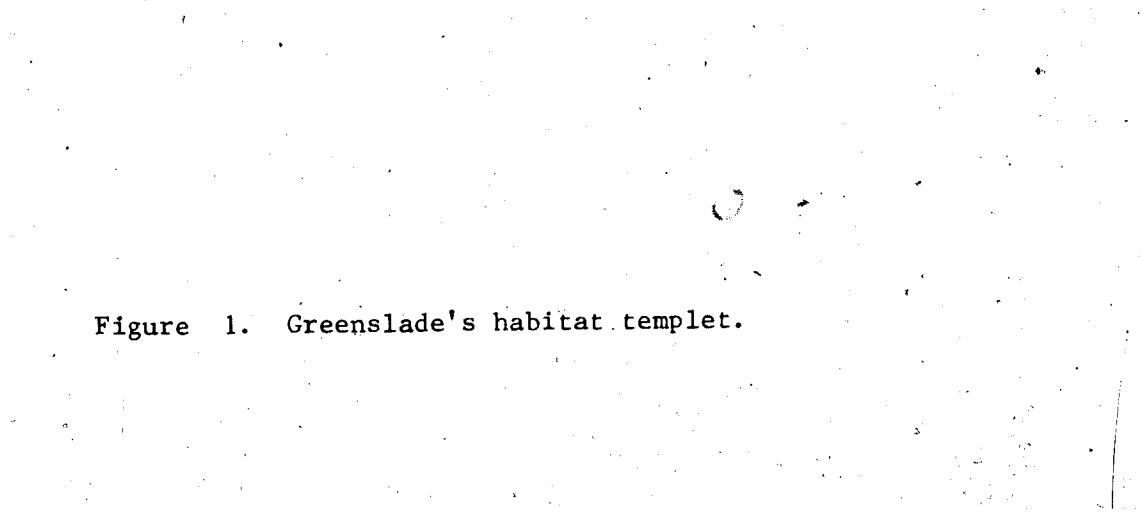
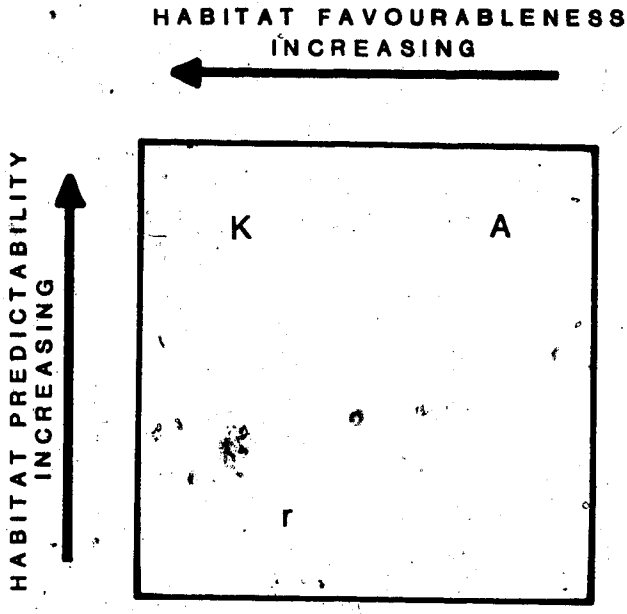


Figure 1. Greenslade's habitat templet.


HABITAT FAVOURABLENESS
INCREASING
HABITAT PREDICTABILITY
INCREASING
K
A
r

in the succeeding transmission season. Thus, the habitat templet must be defined differently from Greenslade's. As the critical overwintering stage of the parasite occurs in the vertebrate host, heterogeneity in time will translate into time available in the vertebrate host (within-patch dynamics), and is measured by the dependability of the vertebrate host for the reproduction (primary and relapse) of the parasite. Heterogeneity in space (habitat favourableness) will refer to variation in the transfer of parasites from vertebrate to vertebrate, or between-patch dynamics as measured by transmission effectiveness. As discussed in Chapter III the transmission window width appeared important in the blue grouse - microparasite system. Different population behaviours were exhibited during primary and relapse periods; these behaviours were presumably the result of strong selective pressures on the parasites to coordinate reproductive activity with availability of the vectors. In addition, transmission window width affected transmission effectiveness. *H. manson*i had the narrowest transmission window and the least effective transmission, while *L. bonasae* had the widest transmission window and most effective transmission (Chapter III,IV). Parasite systems where vectors are available throughout the year will not be influenced by transmission window constraints, and may not require a relapse period, therefore their life history strategies will not necessarily be measured by the parameters in this model.

The population dynamics of microparasites can be viewed from the perspective of the parasite, as in this model, or from the direction of the host, as in a model of population regulation by Bradley (1972). Both approaches are based on patch dynamics; thus if they describe real phenomenon, they should be compatible. Bradley (1972) developed a general theory for regulation of parasite populations, in which he proposed three regulatory mechanisms: "(1) by transmission, (2) at the host population level by immunological and pathological processes, and (3) at the individual host level by premunition and other partial immune processes." Type I involves density-independent determination of parasite numbers through environmental factors extrinsic to the parasite (weather influences on vector populations which results in variable transmission of the parasite). Type II regulation requires more effective transmission than in Type I. Parasite populations are regulated by death of heavily infected hosts, or a successful immune response destroys the parasite population in that host, and leaves the host immune. Type III regulation requires highly effective transmission. Individual hosts regulate parasite populations; the parasite persists, but through an immune response its numbers are prevented from rising.

In the following discussion of my model, for the sake of brevity, I will be concerned only with extremes of each selection type. For each strategy, I will describe the habitat parameters and the corresponding

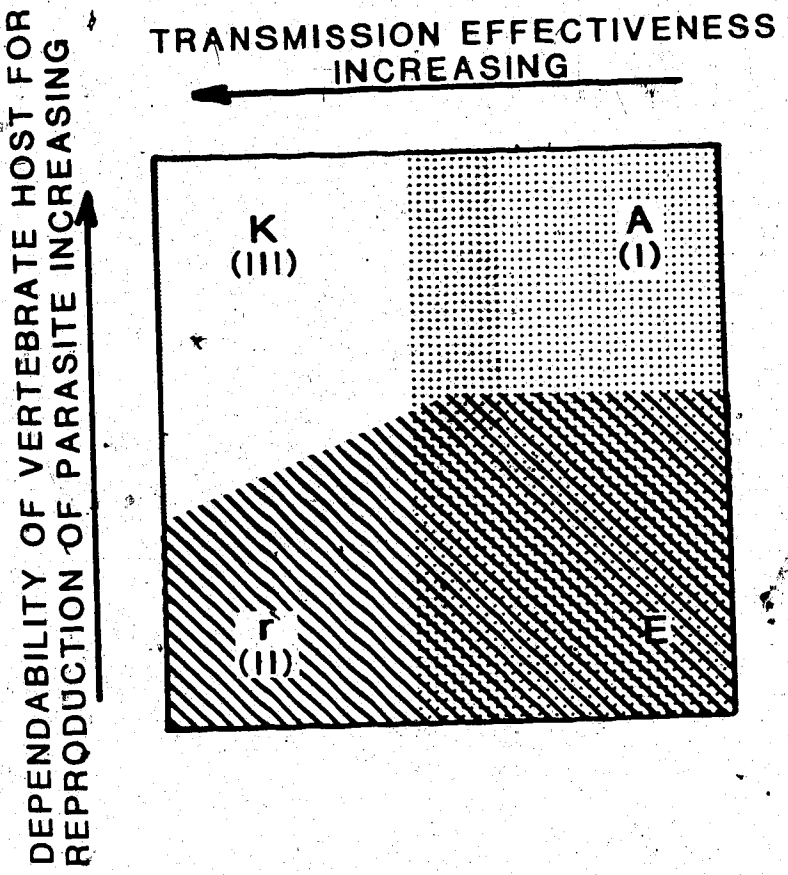
population-determining mechanism from Bradley's theory.

K-selected microparasites occur in reproductively highly dependable vertebrate hosts, and the vector system will be extremely effective from season to season. Within and between-patch dynamics are highly favourable for these microparasites. Population regulation of K-selected species corresponds to Bradley's type III (Bradley, 1972); density-dependent regulation by individual hosts (Figure 2).

Extreme A-selected microparasites also occur in reproductively highly dependable vertebrate hosts, but the between-patch dynamics are unfavourable; the transmission system is very ineffective. Populations are determined through density-independent events related to the variability of the transmission system. This is Bradley's type I mechanism. Between the A and K extremes lies an A-K continuum. Parasite populations are determined within the continuum by a mixture of density-dependent and density-independent mechanisms (represented by overlap in shaded areas in Figure 2).

A reproductively undependable host, coupled to an effective transmission system will result in extreme r-selection. Reproduction in the vertebrate occurs only once; therefore, any ineffectiveness in transmission will increase the probability of extinction of a local population. As the transmission parameter approaches its lowest value, probability of extinction reaches 100% (denoted by E in Figure 2). Population regulation of

Figure 2. General life history strategy model for microparasites. The parameters are: Dependability of the vertebrate host for the reproduction of the parasite and effectiveness of transmission. K = K-strategy, r = r-strategy, A = A-strategy, E = extinction, I, II and III are Bradley's regulation mechanisms. Shaded areas indicate parts of the templet under the influence of each selection/regulation type.


TRANSMISSION EFFECTIVENESS
INCREASING
DEPENDABILITY OF VERTEBRATE HOST FOR
REPRODUCTION OF PARASITE INCREASING
K
(III)
A
(I)
r
(II)
E

r-selected microparasites is through Bradley's type II (Figure 2). This is regulation at the vertebrate host population level. Death of the host or complete immunity removes that infrapopulation.

The general life history strategy model presents two environmental parameters that shape the life history strategies of microparasites. Selection processes result in A-, K- and r-strategies where populations are determined by Bradley's type I, III and II, respectively. Between the extremes are transitional areas where selection processes combine (overlapping areas in Figure 2). Reduction of the reproductive dependability of the vertebrate host, or of the transmission effectiveness, will increase the probability of extinction for a microparasite population.

Position of blue grouse microparasites in model

For each parasite, I will determine its position on the life history strategy model using my parameters and Bradley's; this should determine if they are compatible approaches to modeling parasite populations.

Leucocytozoon bonasae had a highly effective transmission system, coupled with dependable reproduction in the vertebrate host; it was classed as an extreme K-selected species. In blue grouse, *L. bonasae* did not cause clinical signs of illness or death. Type II regulation did not appear to be operating. Density-independent effects through

transmission (type I) was probably not a factor in population determination of *L. bonasae*, as prevalence was always >90%, in both chicks and mature birds. This indicated that transmission was high enough in all years to saturate the host population. Intensity levels of *L. bonasae* gametocytes may be affected by accumulated sporozoite load (sporozoites are the parasite stage passed on by the vector). Captive chicks had lower primary intensities of *L. bonasae* than wild chicks; the former were restricted in their movements by captivity, and therefore were likely to encounter fewer bites from vectors. The additive effect of more frequent encounters with vectors may have resulted in higher primary intensities in wild chicks. Therefore, transmission may have an accumulated affect on *L. bonasae* populations, but it was impossible to assess. I will assume the effect to be slight.

Type III regulation did appear to be operating in *L. bonasae*. Second relapse gametocyte production in two captive adult grouse was much reduced from the first relapse season (yearling grouse). A similar pattern was observed in the field, where every year, adult grouse had significantly lower relapse intensities than yearlings (Chapter IV). As wild adults were exposed in at least two transmission seasons, and yearlings only one season, this result was opposite to that expected. Adults should have had higher intensities than yearlings, as additional transmissions to adults must have occurred. A type III immune mecha-

appeared to be operating.

Leucocytozoon sp. had a less effective vector system, and undependable reproduction in the vertebrate host. It was not an extreme r-selected species as it did not induce complete immunity or death of the host, nor did it have a highly effective transmission system. Therefore, it was classed as a relatively r-selected species, as it had characteristics which placed it towards K- and A-selected species. In *Leucocytozoon* sp., populations were determined partly through transmission (type I) as prevalence levels in chicks changed from year to year. Severe reduction in reproductive capacity during relapse, in part, may be the result of strong resistance by the host. This suggested a type III immune mechanism with very different characteristics from that observed for *L. bonasae*. *Leucocytozoon* sp. did not cause signs of illness or death. However, the strong immune response could be intermediate to type II, as little reproduction occurred in any age class of blue grouse, after primary infection. *Leucocytozoon* sp. population was determined by a combination of type I and III/II.

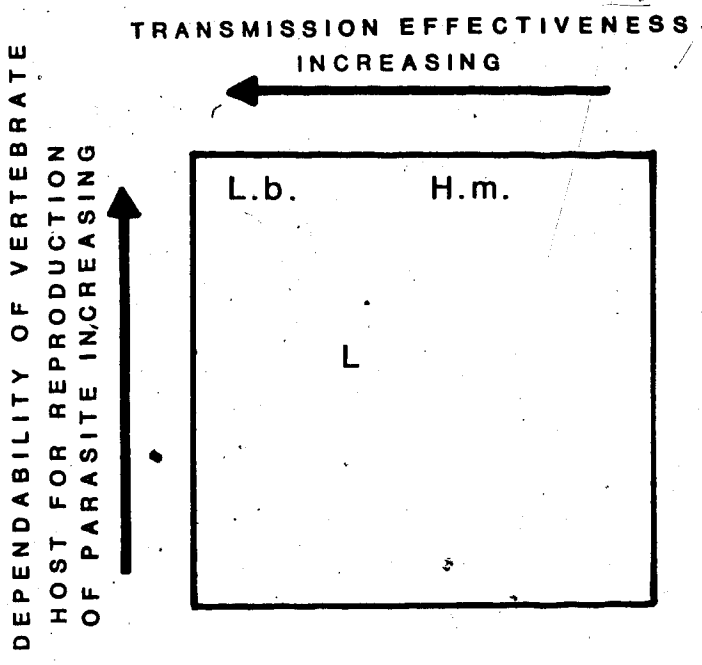
*Haemoproteus manson*i was in a relatively dependable vertebrate host, but the transmission system was the least effective of the three parasites. Transmission was very effective in one of the three years of this study. Therefore, *H. manson*i was not an extreme A-selected microparasite, but it did lie on the A side of the A/K

continuum. Although no reinfection by *H. manson*i was detected, 100% of primary infections relapsed, and there were no clinical signs of illness or death. Therefore Bradley's type II regulation does not appear to be operating. *Haemoproteus manson*i populations were determined predominately by type I, with a possible influence of type III. Transmission of *H. manson*i to chicks varied from year to year. Such variable transmission suggested that populations were determined through type I. Fluctuations in peak intensity from primary to relapse periods was very much dependent on the individual host. Regulation at the individual host level may be contributing to population regulation of *H. manson*i, but it was operating in a very imperfect manner.

The life history strategy model and Bradley's model agree in the classification of these microparasites. In this study, *L. bonasae* was regulated largely by type III regulation and was K-selected, while *H. manson*i populations were determined by type I with a possible influence of type III, and were relatively A-selected. *Leucocytozoon* sp. populations were determined by a combination of type I and a type III/II mechanism, and were relatively r-selected. In Figure 3, I have placed each parasite in its appropriate position on the habitat templet.

Other avian Haemosporina exhibit Bradley's regulation mechanisms. Lee *et al.* (1969) presented evidence of type II regulation of *L. caulleryi* through mortality in domestic

Figure 3. Position of blue grouse-microparasites in the life history strategy model. L = *Leucocytozoon* sp., L.b. = *L. bonasae* and H.m. = *Haemoproteus mansoni*.


TRANSMISSION EFFECTIVENESS
INCREASING
DEPENDABILITY OF VERTEBRATE
HOST FOR REPRODUCTION
OF PARASITE INCREASING
L.b.
H.m.
L

chickens. *Leucocytozoon dubreuili*, *L. danelewskyi* and *L. fringillinarum* had lower relapse intensities than primary intensities (Khan and Fallis, 1970b), as did *L. smithi* in turkeys (Alverson and Noblet, 1977) and *P. relictum* in English sparrows (Applegate, 1971). Fallis *et al.* (1951) noted increased resistance to secondary *L. simondi* infections in ducks. These can be interpreted as possible examples of type III regulation, but lack of information on response to reinfection (with the exception of *L. simondi*) prevents any definite conclusions.

Predictions of Model

For a model to have value, it must make some predictions about other life history characteristics. Table 1 gives properties and predictions for each selection type.

A-selected species may have a brief prepatent period to compensate for a narrow transmission window. This would increase the probability of between-patch transfer. But if ineffective transmission is due to vector population fluctuations there would be no direct selection affecting prepatent period; it could be short or long. A short prepatent period is predicted for r-selected species. Reproduction occurs only once in the vertebrate host (time restricted), therefore rapid transfer to new hosts would be advantageous; this would result in selection for a short prepatent period. Thus both r- and A-selected species are predicted to have short prepatent periods in response to

TABLE 1. ENVIRONMENTAL PROPERTIES AND PREDICTIONS ABOUT LIFE HISTORY CHARACTERISTICS FOR EXTREME r-, K- AND A-SELECTED MICROPARASITES.

	A	K	r
PROPERTIES			
Dependability of the vertebrate host	High	High	Low
Effectiveness of transmission	Low	High	High
Mechanism of population determination (Bradley's)	I	III	II
PREDICTIONS			
Specificity for vertebrate host	Specialist	Specialist	Generalist/ Specialist
Pathogenicity	Low	Low	Low/High
Prepatent period	Short/Long	Long	Short

time restrictions that are from two different directions; in some A-selected species time may be limited by the transmission window, whereas in r-selected species time is restricted in the vertebrate host. K-selected species have little time restrictions in either situation, and thus could have longer prepatent periods.

For r-selected parasites time is restricted in the vertebrate host. Restrictions may be the result of two processes: (1) the host becomes immune and the parasite is expelled (host induced time restriction), or (2) the host dies from the infection (parasite induced time restriction).

Ewald (1983) recognized disease severity as an evolutionary advantage in vectored parasites, which facilitated vectoring. He restricted his discussion to parasites that I would class as r-selected. They included such pathogens as *Borrelia*, *Plasmodium*, *Trypanosoma* and yellow fever virus. In this case, time restrictions result from death of the host. He proposed that increased severity in these vectored parasites was a coevolved phenomenon that resulted in a reduced ability of the host to swat or move away from vectors; thus, the parasite increased the probability of being transmitted to a new host, as in the example below. Day and Edman (1983) experimented with mice infected with various pathogenic species of *Plasmodium*, and observed reduced antimosquito behaviour in infected mice. The authors concluded that reduced antimosquito behaviour would increase susceptibility to vector feeding. Such

coevolution occurred with the major host, but did not occur in parasite/host systems which were less specialized. Ewald (1983) cited data to show that nonspecific parasites, that used humans less, produced lower disease severity in humans. His adaptive severity hypothesis is applicable to specialist parasites.

If the mechanism for reduced time in the host is a consequence of the host's acquired sterile immunity, then the parasite may be forced to use different strategies to maintain a population. The parasite could reproduce in many host species (expanding choice of patches). Such a generalist would likely not be as pathogenic (low pathogenicity), as evolution of severity requires maximum contact between host and parasite. (Pathogenic interactions between parasite and host do occur in nonevolved situations where a parasite infects an unusual host, see Garnham, 1977.) In addition, increased pathogenicity may result in reduced time in a host, without the benefit of increased transmission (cost outweighs benefit). Thus, limited time in a patch may result in r-selected pathogenic/specialists or, r-selected less pathogenic/generalists.

A- and K-selected species would be selected for low pathogenicity, as survival in the vertebrate host for long periods of time is advantageous. In addition, they are predicted to be vertebrate host specialists, as tracking host population genetics is essential to counteract any changes in the host which may be detrimental to a dependable

relationship (Table 1).

I suggest that the r-selected species in blue grouse, *Leucocytozoon* sp., is a generalist and can complete its life cycle in a number of avian hosts, including blue grouse (Chapter IV). *Leucocytozoon fringillarum* was also a generalist (Khan and Fallis, 1970a). Neither species appeared pathogenic. In addition, neither *L. bonasae* and *H. manson*i appeared pathogenic in blue grouse.

A life history strategy model for microparasites may be useful in directing research into the environmental parameters that impinge on the evolution of microparasites. A number of predictions were made regarding regulation of parasite populations which comply with previous ideas. Additional predictions were suggested for life history characteristics that differ between r-, K- and A-selected microparasites. Application of this approach to modeling microparasites and other two host-parasite systems may help in understanding pathogenic organisms and their similarities and differences with other less pathogenic species.

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