A QUANTITATIVE ECOLOGICAL STUDY

OF THE HELMINTH PARASITES OF

THE BREAM (ABRAMIS BRAMA (L.))

by

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ABSTRACT

The biology and population size, structure and dynamics
of the bream Abramis brama (L.) have been studied in a small body
of water over a period of one year. The growth of the fish is
compared with other published research carried out in Europe and
the mortality rates and the biomass of the host population are
estimated.

Simultaneously with the examination of the host population,
the biology and population size, structure and dynamics of the helminth
parasites of the bream, Caryophyllaeus laticeps (Pallas, 1781) and
Diplozoon paradoxum (Nordmann 1831) have been studied by designed
sampling schemes.

Particular attention has been paid to the dispersion of the
parasites within the host population and on the analysis of three groups
of variables consisting of host, parasite and environmental parameters.
By the application of a wide variety of statistical methods, including
multivariate procedures, an attempt has been made to isolate key
factors which regulate the size of host and parasite populations.

The application of the use of principal components in multiple
regression has been demonstrated and a new coefficient E, the "coefficient
of Effect" has been proposed to aid in the biological interpretation of
the results.

CONTENTS

Introduction

The interrelationships between host and parasite and the basic concepts of parasitism have been of interest to biologists for many years.

The quantitative description of the dynamics of parasite populations has lagged behind general progress in biology, despite the strong current trend in ecology for the mathematical description of the population dynamics of animal species, particularly in fields of entomology and epidemiology of diseases of medical and economic importance. (Bailey, 1957, 1967; Bartlett, 1960; Watt, 1961, 1962, 1968; Morris, 1963; Holling, 1963, 1964, 1965; Pielou, 1969)

Background information on geographical distribution and qualitative population biology of helminth parasites of fish has been reviewed by Chubb (1970) and Kennedy (1970) respectively. However, generally parasitologists have become exclusively interested in the physiological adaptations of parasitic organisms, including immunological mechanisms, to the exclusion of the study of the quantitative ecological relations between host and parasite and the basic mechanisms which control their population dynamics. The physiological adaptations are solely mechanisms through which individual relationships between host and parasite are regulated and have tended to mask the fact that parasitism is an ecological relationship which can be initially expressed quantitatively in terms of population change.

Some descriptions of the mathematical relations between host and parasite have already been made (Kostitzin, 1939; Lotka, 1934) but have largely been ignored. Crofton (1971) has recently emphasised the need for parasitism and host parasite relations to be expressed in more precise and quantitative terms and favours the use of theoretical

distribution models in the description of these relationships. This, however, is only one method of approach. Initially more quantitative information is required on the mechanisms regulating parasite and host populations. This can best be achieved by the detailed analysis of data aquired from natural populations of hosts and their parasites.

The problem of the analysis of host parasite relations is essentially a multivariate one. Three main groups of variables need to be considered, namely the host, parasite and environmental components. The theory of multivariate analysis has far outpaced the practical biological application of the techniques, with the result that only a few examples are available in the published literature (for example, Jeffers, 1964, 1967; Alcock, Lovett and Machin, 1968; Barkham and Norris, 1970). This lack of practical application in biological fields has partly stemmed from difficulties in computation, but the easy access to digital computers has largely removed this problem. By the use of detailed statistical analysis, including multivariate methods, key factors operating on the host-parasite relationship may be selected for further experimental study, or for trial inclusion in deterministic models to describe the population dynamics of host and parasite. These models may lead to a deeper understanding of the basic mechanisms regulating host-parasite relations.

The problem of studying host-parasite relations can be effectively treated only by simultaneous studies of the population dynamics and biology of both host and parasite in conjunction with measured changes in the macro environment. This method of approach has not previously been applied to the study of helminth parasites.

In this present study the population dynamics and biology of the bream <u>Abramis brama</u> (L.) and two species of its helminth parasites,

an intestinal Cestode, <u>Caryophyllaeus laticeps</u> (Pallas, 1781) and
a gill Monogene, <u>Diplozoon paradoxum</u> (Nordmann, 1832) were investigated
simultaneously. Emphasis is placed on the identification and evaluation
of selected biological factors which were considered of importance in
quantifying the host-parasite relationships. The analyses are based
on data collected from the field during the period February 1969 to
February 1970.

S E C T I O N   1

The Biology and Population dynamics of the fish host

Abramis brama (L.)

Introduction:-

The majority of ecological surveys of fish in Britain have
been concerned almost entirely with salmonid fish and very few
population studies have been made on British freshwater cyprinids.

Hartley (1947a) and (1947b) examined the natural history of
a number of freshwater fish including the bream Abramis brama (L.),
but based his studies on only a few fish. Lemming (1963) carried out
a more detailed biological study of the bream in the river Welland and
Kennedy and Fitzmaurice (1968) examined the distribution of the bream
in Irish freshwaters, studying the biology of the species in a variety
of habitats.

In a number of countries, mainly Finland, Poland, East Germany
and Russia, the bream is a commercial food source and is harvested
annually from enclosed waters and river deltas, and thus most information
concerns the bream in Eastern European waters. This information has
been reviewed by Backiel and Zawisza (1968), their summary containing
information on many aspects of the biology of the bream including its
fishery management.

A few workers have examined the population dynamics and growth
of cyprinid fish from rivers. Williams (1965 and 1967) examined the
population growth, density and mortality of the Roach (Rutilus rutilus
(L.)), Bleak (Alburnus alburnus (L.)) and Dace (Leuciscus leuciscus (L.))
from the river Thames at Reading. A further study of the growth of the
Dace and Roach was carried out in Willow Brook, Northamptonshire, by
Cragg-Hine and Jones (1969). Other extensive studies on the ecology of
non-cyprinid freshwater fish in Britain include those of the Perch (Perca
fluviatilus (L.)) (Le Cren, 1958) and the Pike (Esox lucius (L)) (Frost
and Kipling, 1959).

The Environment

The environment studied was a disused gravel pit which has been stocked with fish since 1948 by the White Hart Dagenham Angling Club.

The gravel pit is situated near Dagenham in Essex and has map reference 512862, ordnance survey sheet 161. The pit is approximately rectangular in shape measuring 330 yards by 240 yards with numerous small bays; the surface area of the water is 15.9 acres. The depth of the water varies from a few inches to over 12 feet deep, but most of the water is about 6-8 feet in depth. During the year of investigation the range of fluctuations in the water level was about 1 foot 6 inches.

The environment is situated in a sheltered hollow with steep banks on all sides. The water is eutrophic in nature; littoral vegetation is abundant in summer; planktonic populations are dense and planktonic "blooms" are characteristic in spring and summer. Because of the high organic content, summer stagnation is apparent in some of the more isolated bays. The turbidity of the water is high due to inorganic and organic material in suspension; planktonic algae form a large part of the material involved. Turbidity decreases productivity in large bodies of water since it decreases light penetration which, in turn, prevents the establishment of photosynthetic plants (Macan and Worthington, 1951). However, at Dagenham this was probably of little importance since most of the water was shallow.

Zoo plankton are very numerous especially during the spring and summer and the major groups present were Cladocera, Ostracoda and Copepoda. The bottom of the pit varied from gravel to deep mud and silt, and supported a large and varied benthic invertebrate fauna. This fauna was dominated by mud dwelling oligochaetes, aquatic crustacea and a variety of molluscs.

Emergent vegetation consists mainly of Junicus species and Typha species, while submerged vegetation is predominantly Elodea canadensis.

The habitat was visited by a wide variety of birds, including several species of gulls (<u>Larus ridibundus</u> (L.), <u>Larus agentatus</u> (Pontoppidan), <u>Larus fuscus</u> (L.)).

Resident species were the great crested grebe (<u>Podiceps cristatus</u> (L.), the coot (<u>Fulica atra</u> (L.)), the moorhen (<u>Gallinula chloropus</u> (L.)) and the mallard (<u>Anas platyrhynchus</u> (L.)).

The water contains a number of species of fish which are listed below:

Cyprinidae	<u>Abramis brama</u> (L.)
	<u>Rutilus rutilus</u> (L.)
	<u>Gobio gobio</u> (L.)
	<u>Cyprinus carpio</u> (L.)
	<u>Tinca tinca</u> (L.)
	<u>Scardinius erythropthalmus</u> (L.)
	<u>Carassius carassius</u> (L.)
Percidae	<u>Perca fluviatilis</u> (L.)
Esocidae	<u>Esox lucius</u> (L.)

A number of physical measurements of the environment were recorded. Water temperature was recorded continuously at a depth of one foot by means of a Cambridge Single Thermograph. Local records of sunshine hours, day length, rainfall inches and air temperature were obtained from Greenwich meteoroligical station and are presented in Fig.(1.1.)

Fig (1.1)   Local meteorological data for Dagenham.

(Monthly mean water temperature $^\circ$C; monthly range of

water temperature $^\circ$C; monthly total hours of sunshine;

monthly mean number of hours of daylight; monthly mean

inches of rainfall per day).

WATER TEMP.

RANGE OF WATER TEMP.

°C.

SUNSHINE

HOURS

DAYLENGTH

MEAN HOURS/DAY

RAINFALL

INCHES

F M A M J J A S O N D J

1969   MONTHS   1970

F M A M J J A S O N D J

1969   MONTHS   1970
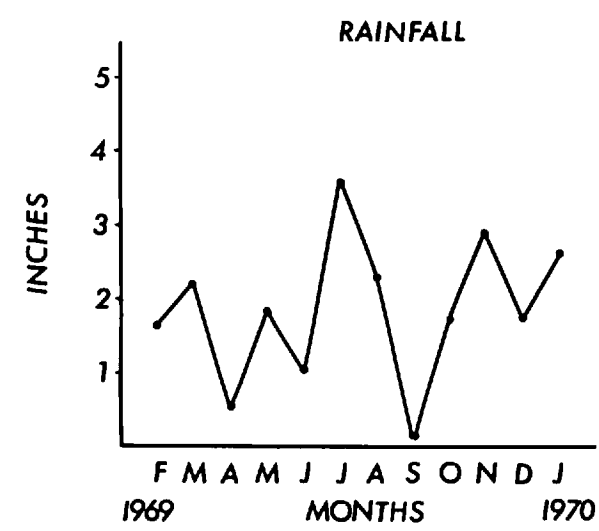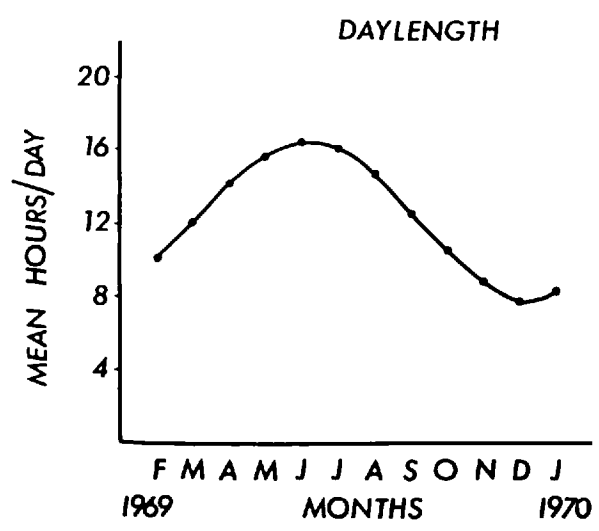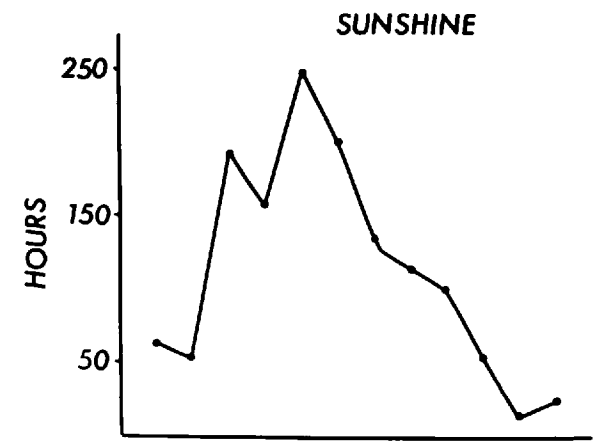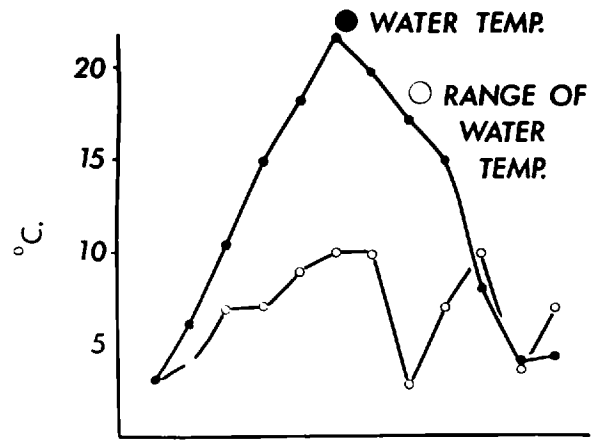
<u>Methods</u>:-

The methods used in this study of the bream population are well documented in the literature concerning fish biology and are reviewed by Ricker (1958) and Gerking (1966). The following terminology has been used throughout this study.

Year class:- All the fish spawned in a particular calendar year are grouped together in a single year class.

Age class:- All the fish of the same age are grouped together in a single age class which is designated a numeral indicating the number of years of life completed. Thus fish in their first year of life belong to the O age class, in the second year to the 1 age class and so on. The numeral is followed by a plus sign; this indicates that the fish concerned have already passed through a portion of the next year of life.

Age group:- For the purpose of later analysis and due to the scarcity of older fish in the environment, the fish were grouped into six age groups. The O, 1, 2, and 3 age groups refer to the same classification as the age classes of fish. The 4th age group consists of 4^+ and 5^+ age classes of fish and the 5th age group of 6^+ and older age classes.

Rings or growth rings:- The rings or growth rings are concentric ridges borne on the outer surface of the scales of the bream and which steadily increase in number as the scale increases in size.

Scale check, or check:- The interruption in the normally regular pattern of growth rings on the scales used in age determination.

(A) <u>Sampling procedure</u>:-

The bream were captured using seine nets. Other methods such as trapping and angling were tried but were found to yield insufficient fish. Two types of seine net were used, primarily to compensate for net selectivity of the fish. A large net, seventy five yards long and eight feet deep, was

used to catch all but the very young fish. This net had a one inch
mesh and measured one half an inch from knot to knot. The second
net, a frynet, was thirty yards long and eight feet deep with a mesh
of one half inch and measuring one quarter of an inch from knot to knot.
Both nets were made of polypropylene synthetic fibre and had polystyrene
floats on the top line and lead weights on the bottom one. The nets
were manufactured by Bridport Gundry Ltd., Dorset.

Three types of sampling were used:-

1) Large numbers of fish were caught at discrete time intervals for the
purpose of mark-release-recapture estimates of the population. The fish
were marked by injecting rubber latex solutions, coloured with various
dyes, into the ventral surface of the fish between the anal fin and tail
(Davis,1955). The solution was injected between the fish scales, just
below the epidermis and formed a coloured mark clearly visible to the
naked eye.

2) To assess the structure of the host population in terms of age and
size large samples of one thousand fish or more were captured at the be-
ginning and end of the year of investigation. From random subsamples
of approximately one hundred fish, the ages of these specimens were
determined using scale readings. Length was measured in the field by
using a measuring board covered with Ethlon (a transparent, plastic
sheeting) on which the anterior extremity of the fish was placed against
a stop at one end of the board. The measured length was then taken as
the distance from the stop to the top of the median rays of the tail fin
of the fish, the position of which was marked on the sheet with a pin prick.
Fork length was the most convenient measurement since bream tend to abrade
or otherwise lose the projecting extremities of the tail fin. The fish
were returned to the habitat after the measurements had been taken, and
scales removed. The ethlon sheet was removed in the laboratory and the
lengths of the sample recorded.

3) Thirty fish or more were removed at the end of each calendar month to give a total of twelve samples for the year. This minimum sample of thirty fish consisted of six random samples of five fish taken from each of the six age groups of fish. In order to stratify the sample, age determination in the field was based on sizes and to allow for misclassification of fish age a larger sample than necessary was taken each month.

(B) Measurement and recording of data:-

The monthly samples of fish were transported live to the laboratory in large water filled bins, killed and deep frozen to a temperature of $-22^{\circ}C$. The following morphometric measurements were recorded:-

(a) Fork length (cms.); (b) Girth, just anterior to the dorsal fin (cms.); (c) Weight (gms.); (d) length of unstretched gut (cms.); (e) Diameter of eye socket (cms.); (f) Diameter of eye lens (in microscope eye piece units); (g) Vertical height of the opercular covering of the gills at the widest point (cms.). Measurements (e), (f) and (g) were recorded as an indication of the size of the micro-environments, the gills and eyes of particular species of parasites.

Other measurements of sex, stage of maturity and condition of the gonads were recorded. The gonads were inspected to determine the sex of a fish. Eggs were readily discernible in the ovaries of adult females, while in adult males the testes were typically smooth, whitish and non-granular in appearance. Sex determination is difficult in immature specimens. However, sexing is sometimes possible by examination of squashed gonad material under a high power microscope. A series of numbered stages were used to record the sex of the fish: 1, immature fish whose sex was indeterminable; 2, male fish; and 3, female fish.

Numerical rankings were used to record the maturity of the fish. Immature fish, rank 1, were young individuals with very small transparent

to grey sexual organs. The eggs, in immature females, were invisible to the naked eye and these fish were judged incapable of spawning in the year of examination. Mature fish, rank 2, were judged capable of spawning in the year of examination. The testes and ovaries of these fish had visible blood capillaries on the surface of the gonads, which were opaque in colour. In mature females eggs were visible to the naked eye.

Mature fish were also examined to assess the condition of their gonads and given a supplementary ranking to the maturity scale. Resting or unripe, rank 1, were fish with gonads of small size occupying less than one half of the length of the body cavity. Developing or ripening, rank 2, were fish with reddish testes, the ovaries being orange red, releasing neither sperms nor eggs when a slight pressure was applied to the body cavity and gonads occupying one half to two thirds of the body cavity. Gravid or ripe, rank 3, were fish with sexual organs filling more than two thirds of the body cavity, and releasing sperms or eggs when a slight pressure was applied to the body cavity.

The scales used in age determinations of the fish were always removed from directly above the anal fin and just below the lateral line. These scales were large and fairly symmetrical and thus convenient for age determination. They were cleaned and examined dry under a binocular microscope. The radius of each scale was measured from the nucleus to the frontal edge. The number of rings outside the last scale check was recorded and gave an indication whether or not the check was recent.

The intestinal contents of the fish examined monthly were surveyed to identify the food organisms present, and thus to assess seasonal and age class patterns of feeding. Due to the efficient grinding pharynx of the bream, assessment of the type of food present proved difficult. Accordingly a qualitative assessment was made of the dominant type of food. The method used was based on a visual judgement of the food organisms numerically and

volumetrically dominant. If two types of food organism were equally
dominant, then both were recorded. Quantitative methods for the
assessment of stomach and intestine contents have been reviewed by
Hynes (1950).

A record sheet was designed to record the measurements made
on individual fish. Data from these record sheets were then transferred
to computer punch cards and then stored on magnetic tape. This
facilitated access to all the data collected for analysis.

The validity of age determination:-

The determination of the age of fish by counting the number of checks on their scales is based on the assumption that check formation occurs annually and is limited to a short and specific period of the year.

The scale consists of a flat bony plate bearing numerous concentric ridges on its outer surface. These ridges, termed rings in this account, were referred to as annuli or sclerites by Hartley (1947a). Possible mechanisms for the production of these rings on the scales of cyprinids have been discussed by Wallin (1957) and Yamada (1962). The surface of the scales also bears a number of radial fissures, some of which extend to the centre of the scale.

The concentric rings are normally laid down in a regular sequence, but periodically there is a break in this regular pattern and a few broken irregular rings are laid down in close proximity to one another. This feature has been called an annual ring by Masterman (1923), a check or ring by Hartley (1947a) and a band of narrow rings by Healy (1956).

Incomplete rings are not necessarily associated with check formation and are occasionally formed at other times. This gives rise to structures which have been called false checks which can be distinguished from true checks since they cannot be traced right round the scale and are present on only a small proportion of the scales of the fish in question. A true check can be traced right round the scale and can be seen on all scales.

To test the assumption that check formation is annual the bream scales were examined throughout the year for the presence of newly laid down checks. These are checks at the very edge of the scale, or with only one or two rings outside them. The mean number of rings outside the last check was also calculated for each age group of fish in each monthly sample. Fig. (1.2) shows the number of fish in monthly samples of five fish for each age group which have recently laid down a scale check. It is clear that the majority of checks are produced from May to July with a

peak in June. It is noticeable, however, that older fish, i.e. in the 4th and 5th age groups, tend to produce checks slightly later in the year than the young fish. For convenience June 1st was taken as the birthday of the bream; thus, for example, fish in the 0^+ age class in May became the 1^+ age class in June. Fish with a large number of rings outside the last check in June were taken to be a year older than the age indicated by the number of checks; also fish with a newly laid down check in May were taken to be a year younger than indicated by the number of checks. A second way to examine the validity of annual check formation is to examine the monthly samples and count the number of rings outside the last check. This must be done separately for each age group since the number of rings laid down in a year decreases with the age of the fish. If check formation is annual, a graph of the mean number of rings outside the last check, plotted against month of the year should show a marked drop during one particular time of the year, which in the bream corresponds with June (Fig. 1.3). This is clear in the younger age groups of the bream, whereas the older fish do not show such a marked pattern, possibly because they lay down fewer rings. Fig (1.3) illustrates the tendency for the older slower growing fish to lay down fewer rings on the scales.

There has been considerable speculation about the nature of the scale check of cyprinids and the reason, or reasons, for its formation. It has been suggested by Hartley (1947a,b) and Williams (1967) that there is a connection between check formation and the breeding cycle. Jones (1949 and 1953) and Wallin (1957) suggest that the check on the scales of roach is formed as a result of the change from very slow winter growth to rapid spring and summer growth. Both explanations could apply to the bream sampled in this present study since the growth rate of most age classes of fish increases around May and June, and also the bream spawn from mid May to mid June.

Much has been written about the nature of body to scale relationship (Bryazgin, 1963). Oliva (1950) found a linear relationship between scale radius and body length with a positive intercept on the body length axis. A similar relationship was found from a sample of 151 bream (Fig. 1.4). The regression

$$y = 5.29x - 14.26$$

removed a significant proportion of the variation $(P(F=1763.0) < 0.001)$

Alternatively this relationship can be represented by

$$L_n - c = \frac{S_n}{S}(L - S) \qquad \text{(Fraser, 1916; Lee, 1920)}$$

where L_n = length of fish when check 'n' was formed

L = length of fish at time scale sample was obtained

S_n = radius of check 'n'

S = total scale radius

c = intercept on the x-axis which is obtained from the regression equation.

By back calculation it is possible to use this formula to estimate the length of any fish in a previous year, from its scale measurements.

Fig (1.2)   Formation of annual scale checks in the bream <u>A.brama</u>

Solid line: the total number of fish with a recently

laid down scale check. Histograms: the number of

fish per age group which have recently laid down a
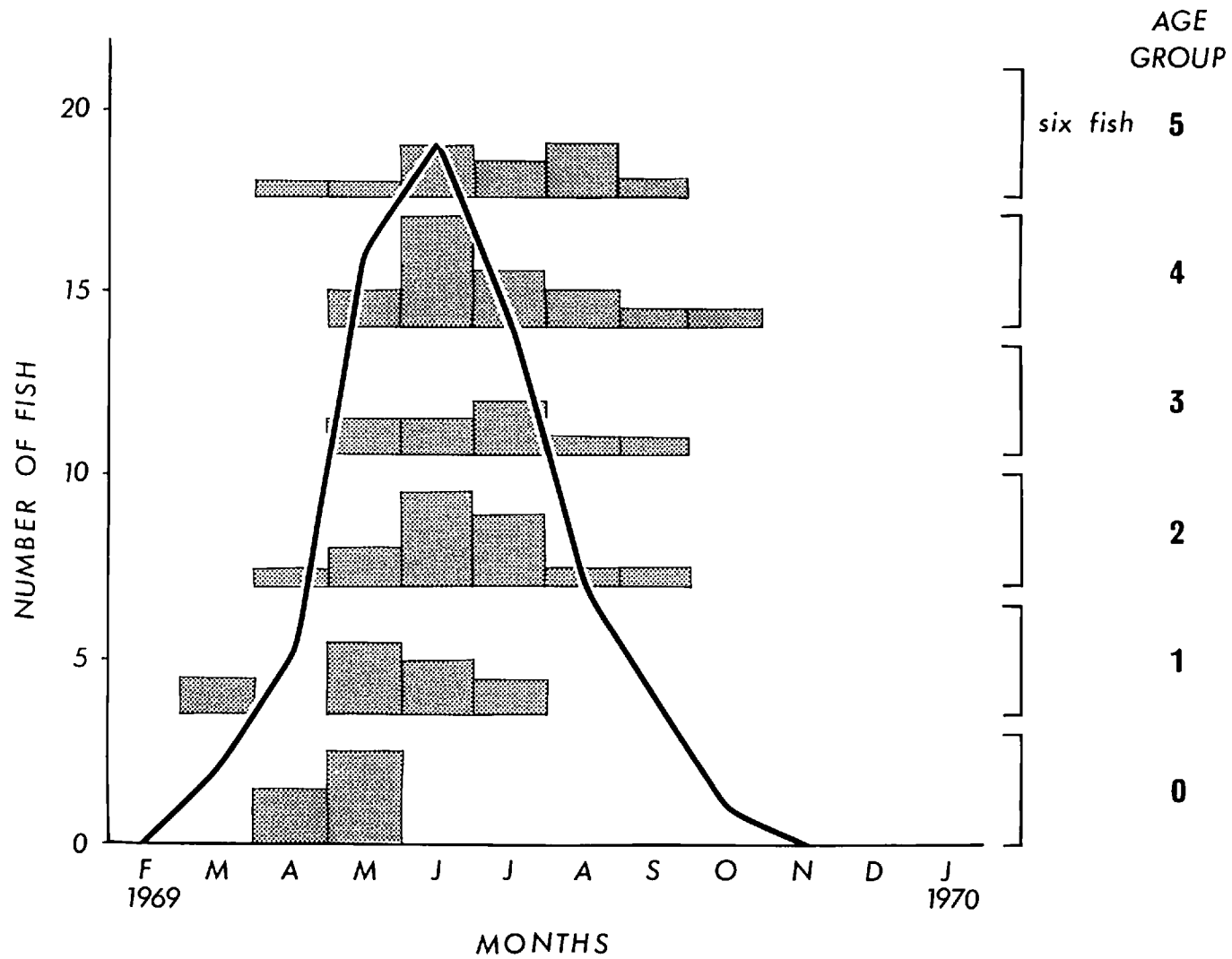
scale check

Fig (1.3)   Number of growth rings on the surface of the scales

of A.brama outside the last scale check in each age

group of fish.
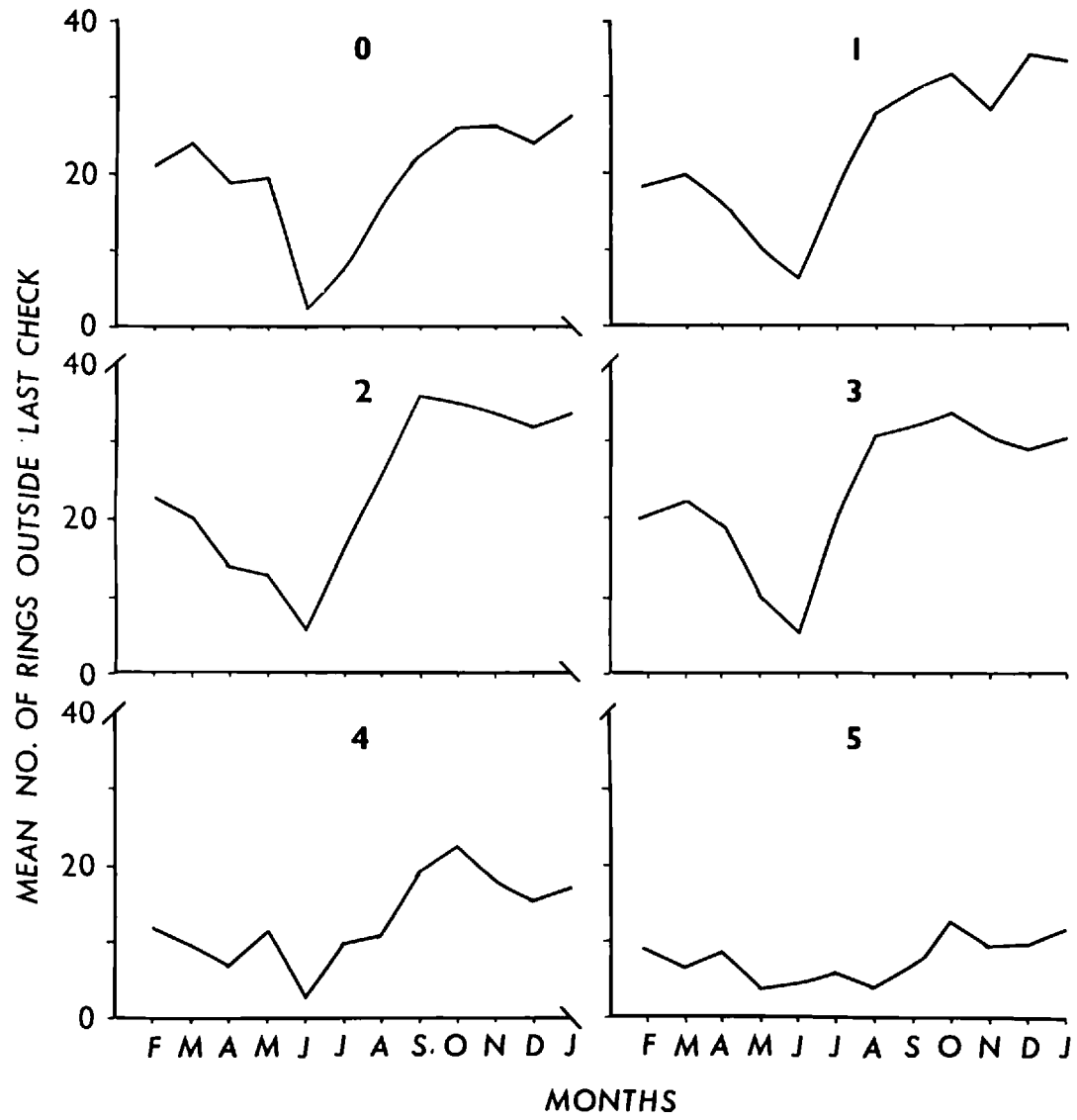
Fig (1.4)   The relationship between body length (cms.) and

scale diameter (microscope eye piece units) of

A.brama.

SCALE RADIUS IN MICROSCOPE EYE PIECE UNITS

LENGTH OF FISH IN CMS.

-25-
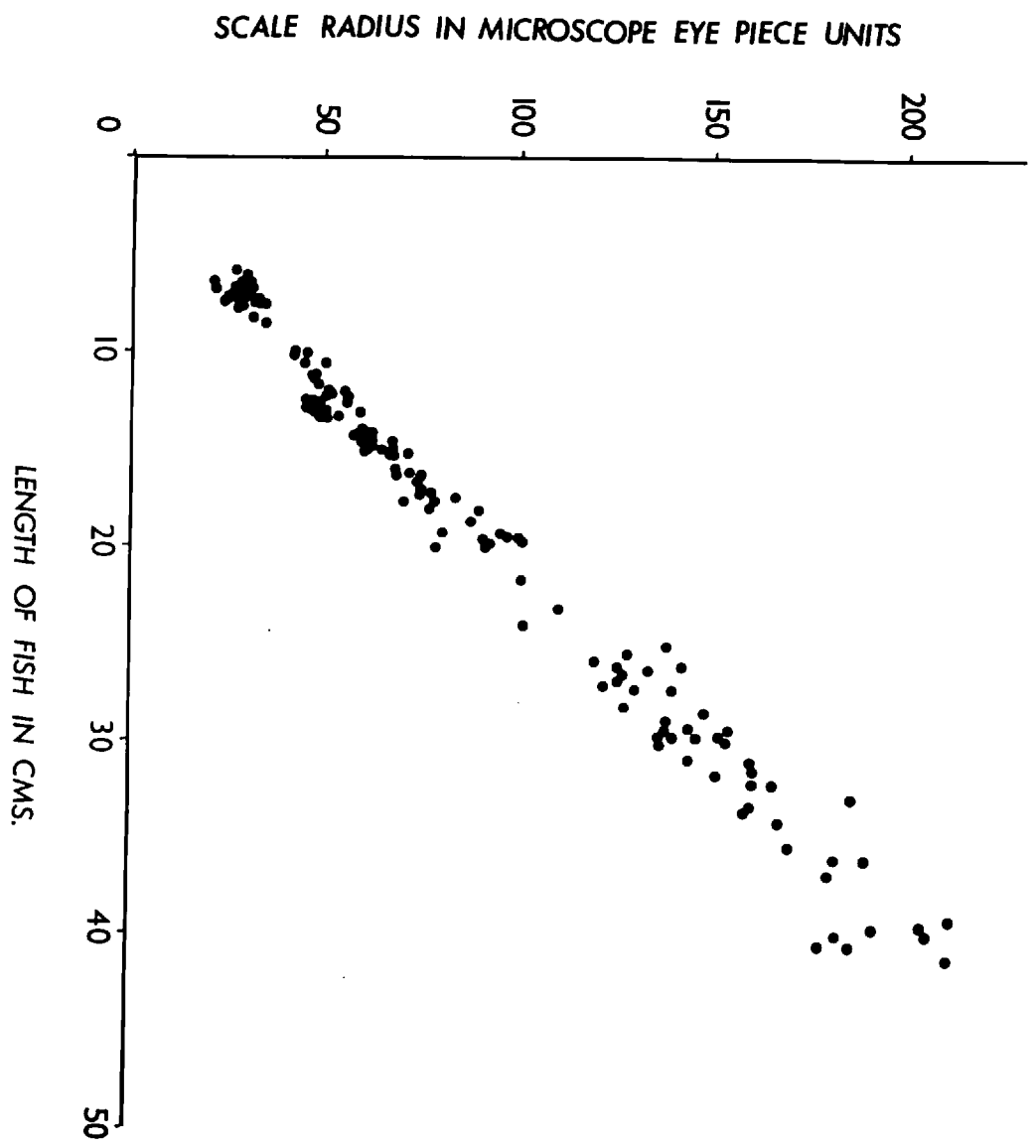
General Biology of the Bream (Abramis brama (L.)):-

The Bream belongs to the Teleost family Cyprinidae which is well represented in European waters. The species occurs in fresh and brackish, preferably slow moving waters of Europe, off the north western part of Asia Minor and in the drainage areas of the Caspian and Aral seas. The natural distribution area has been enlarged eastwards by transplantation. It seems clear that the distribution of the bream is limited by the conditions necessary for reproduction and embryonic development, such as maximum temperatures, oxygen content and salinity of the waters (Alabaster, 1964; Alabaster and Robertson, 1961; Iurovitskii and Reznichenko, 1961).

Most cyprinids produce natural hybrids with many genetically similar species of the same family which spawn at the same period, in the same conditions. The bream population studied in this investigation was no exception; hybrids between bream and roach Rutilus rutilus (L.) and between bream and the rudd Scardinius erythropthalmus (L.) were common in the habitat.

Bream are slow growing fish which often attain a considerable age and size in comparison to other cyprinids. Kennedy and Fitzmaurice (1969) reported a bream of 23 years of age from an Irish lake. At Dagenham the greatest age record was 10 years. The growth of the bream in this habitat will be discussed later.

The bream is heterosexual, but sexual dimorphism of the secondary sexual characters is weak. However, during the spawning season males can be distinguished by spawning tubercles on their scales. There was considerable variation in the age at which fish reached sexual maturity; the majority of fish matured in their fourth year of life (6.7%, 68.2%, 82.1% of the fish examined were mature respectively in their 3rd, 4th and 5th years of life). These figures agree with observations made by Driagin (1952)

and Dementeva (1952,1955). Geyer (1939) pointed out the inter-
dependence of growth rate of bream and time taken to reach sexual
maturity.

The Dagenham bream spawned between mid May and mid June when the
water temperatures were 15°C. or more. The fish usually spawned in
shallow water near the edge of the gravel pit and in places which were
sheltered from wind turbulence and where there was abundant weed growth.
Spawning did not take place in deeper waters. Males and females spawned
repeatedly with different partners. The males delimited territories in
the spawning areas and vigorously pursued females when they entered these
territories; spawning took place in the denser vegetation of the areas.
At the boundaries of the territories displays of aggressiveness were
observed between males, which involved splashing near the surface of the
water. The eggs, which were laid in the dense weed patches and attached
to the vegetation, measured approximately 1.6 - 1.8 mms. Fertilization
of the eggs is external. The quantity of eggs produced by any one female
varies; however, it seems to be related to body size (Bauch, 1963).
The alevin hatched in the laboratory in nine to ten days at a temperature
of 18 - 22°C. and measured between 4 - 5 mm. in length. They first
attached themselves to vegetation, but after a few days the young became
free swimming and began feeding on small planktonic organisms.

Feeding behaviour and nutrition:-

The diet of the bream, like that of other cyprinids, changes as the fish grow, and many authors agree with the general rule that as bream develop they move to deeper feeding grounds and feed on larger organisms. Annual changes in food composition have been observed also in Polish lakes (Pliszka, 1953) and generally reflect changes in the availability of food organisms. The change of feeding habits with age was gradual as shown by the dominant organisms found in the intestine of the bream examined for each group and by month (Table 1.1) The very young bream feed mainly on phytoplankton and thus are characteristic of most other cyprinids. They feed, for the most part, in the marginal shallows of the gravel pit. When the fish move into their second year of life, they feed primarily on small planktonic crustacea such as species belonging to the Cladocera and Ostracoda. These food organisms continue to be important as food sources for older fish. The gravel pit at Dagenham had abundant supplies of these two groups of crustacea and even 6^+ and older fish feed on them to some extent, particularly during the cold winter months. As the bream grow in size and move into the deeper, open waters, their diet changes gradually to include more insects and algal material, and molluscs are eaten by the older fish. Chironimid larvae account for the bulk of the insects eaten. Trichoperta larvae and Gammarus species are also eaten during the summer months by the large fish.

One significant ommission from Table (1.1), namely aquatic oligochaetes, requires comment. Aquatic oligochaetes, especially the tubificid Tubifex tubifex were abundant in the mud and gravel bottom of the pit. These tubificids harbour the larval stages of the cestode Caryophyllaeus laticeps, which was present as the adult parasite in the bream intestine, often in large numbers and thus tubificids as well as other oligochaetes were probably a significant food source for the bream.

Unfortunately, most aquatic oligochaetes are broken down very quickly
by the digestive enzymes and would be detected only if ingested immediate-
ly before dissection and the only evidence of their presence would be the
remains of chaetae.

The observed feeding patterns agree with those recorded for the
bream by Kennedy and Fitzmaurice (1968) in Ireland, Neubaur (1926) at
Stettin and Hartley (1947b) in England. It seems clear from this feeding
data and observations made on bream behaviour in the gravel pit that
each age group of the fish population feeds in a particular niche of the
habitat. The young bream inhabit the marginal shallows and gradually
move into open waters as they become older. Finally the mature bream
become feeders on mud dwelling invertebrates and keep to the deeper parts
of the habitat.

It has been stated by many authors that during the colder months of
the year, bream feed spasmodically since they are mostly inactive (Hartley,
1947a,Nebolsina, 1962). The numbers of immature and mature fish with food
remains in their intestines were recorded. These are presented as percentages
of fish feeding during each month of the year (Fig. 1.5). It is clear from
these histograms that there is a decrease in feeding activity during the
winter months. It is interesting to note that the mature bream did not
begin to feed intensively until after the breeding season from mid May to
mid June. Of the mature bream examined between January and June, with food
organisms present in their intestines, 80% were females and 20% males. The
fact that male fish delimitate spawning territories may inhibit their feed-
ing activity. The cessation of feeding activity during the breeding season
by the bream has also been recorded by Morozova (1952).

Table (1.1) Dominant Food organisms present in the monthly samples:-

Month	February 1969						March 1969						April 1969					
Age	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
FOOD TYPE																		
Phytoplankton								x										
Algae			x										x					
Cladocera																		
Ostracoda											x		x	x				
Copepoda																		
Mollusca																		
Chirominids																		
Trichoptera																		
Gammarids																		

	May 1969						June 1969						July 1969					
	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
Phytoplankton							x						x					
Algae																		
Cladocera	x			x					x	x	x	x	x			x		
Ostracoda	x	x	x					x	x					x	x	x		
Copepoda		x	x															
Mollusca						x					x							x
Chirominids				x	x	x								x			x	x
Trichoptera																		x
Gammarids																		

(Absence in all columns = no feeding)

Table (1.1) continued.

Month	August 1969						September 1969						October 1969					
Age	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
Food Type																		
Phytoplankton																		
Algae			x															
Cladocera	x	x		x			x	x					x	x	x	x	x	
Ostracoda		x	x	x	x			x	x		x						x	
Copepoda	x																	
Mollusca						x						x						
Chirominids				x	x					x	x	x		x		x	x	x
Trichoptera																		
Gammarids																		

	November 1969						December 1969						January 1970					
Phytoplankton																		
Algae																		
Cladocera	x	x			x			x					x					
Ostracoda			x	x	x		x	x		x				x	x		x	
Copepoda		x																
Mollusca																		
Chirominids						x					x	x						x
Trichoptera																		
Gammarids				x														

(Absence in all columns = no feeding)

Fig (1.5)   The percentages of mature and immature A.brama in

the monthly samples with food in the intestines.
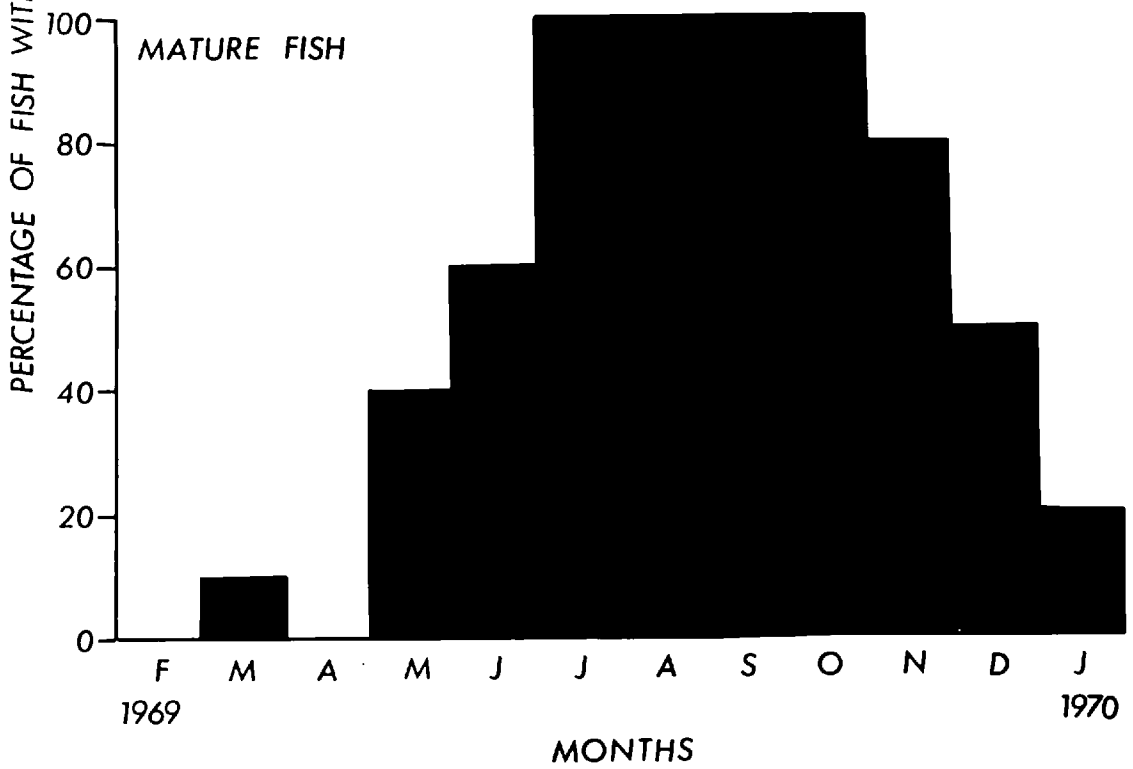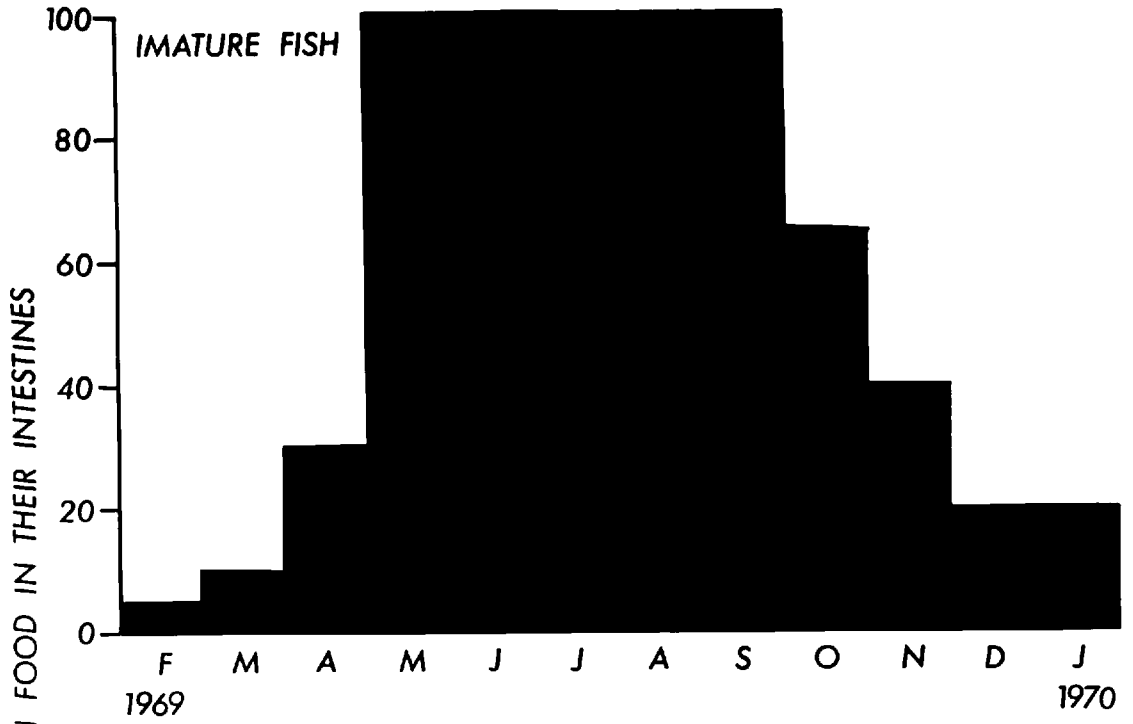
The size and structure of the Bream population:-

(A) The size of the population:

The method of mark-release-recapture to estimate animal population has been widely used by animal ecologists (c.f. reviews by Le Cren,1965; Ricker, 1958; Southwood, 1966). It was originally decided to carry out mark-release-recapture censuses during each month of the sampling year. However, because of the inevitable disruption of the environment and fish population, and also because of the labour involved, it was decided to estimate the population size only at the beginning and end of the year of study. Two different colours of latex were used to differentiate between marks of the two estimates.

Various assumptions underlie all methods of mark and recapture, which include:-

1) Animals are not affected by being marked, and

2) Marks are not lost during the period of survey.

To examine these two assumptions ten bream of a range of sizes were injected with rubber latex solution as previously described and kept under observation in the laboratory. No changes in behaviour were observed and all ten fish survived throughout the year of sampling without loss of marks.

3) Marked animals became completely mixed in the population. To eliminate any inaccuracies caused by inadequate mixing the marked fish were released at a number of points in the pit and not necessarily at their point of capture.

4) The population was sampled randomly with respect to its mark status.

(a) All individuals of the different age strata of the population were sampled in the proportion in which they occurred. By using two nets of different mesh size it was hoped that selectivity for certain strata of the fish was eliminated. This could not be verified.

(b) All individuals whether marked or not are equally available for capture irrespective of their position in the habitat. Since bream are a shoaling fish, netting is a suitable method of sampling. However, older fish lie in hollows in the bed of lakes, ponds etc. and they might be netted less efficiently than other age groups and thus undersampled.

5) Sampling is done in discrete time intervals, and the actual time involved in taking samples is small in relation to the total period of the census. The mark-release-recapture census was carried out on seven days, a set number of samples being taken each day. Sampling time was small in comparison to the week's survey.

6) During the mark and recapture experiments (following Ricker (1958)) no immigration or death in the population should take place. Since the surveys in each case occupied one week only, it was assumed that death and immigration rates were negligible during the period. During the two one week surveys the ratios of the number of fish caught at time 't' multiplied by the number of marked fish at large at time 't' over the number of recaptures at time 't' remained fairly stable. Thus (following the arguments of Ricker, 1958) it was assumed no mortalities occurred during these periods.

7) The probability of capturing an individual is not affected by its capture on a previous occasion. It was not possible to verify this assumption and it introduces a further source of error.

The multiple census technique of Ricker (1958) was used. This type of technique was adopted since insufficient fish could be marked and re-captured on a single occasion due to the labour involved. The precision of the population estimate depends on the proportion of marked fish in the population. Thus, where few fish are marked and caught in each netting, it is advisable to use a multiple census to increase the proportion of marked fish. Fish were marked and added to the population over a period of one week in each survey during which samples were taken and examined for recaptures and replaced. There is some computational advantage in marking

all the fish in a sample, but it is not essential, and not all captured fish were marked in the present study. The total data for each day was the result of six separate seine nettings in different parts of the pond. Daily records were made of the total number of fish netted, the number of fish marked, and the number of fish marked at large. The results for the censuses in February 1969 and in February 1970 are shown in Tables (1.2A) and (1.2B) respectively.

The formula originally described by Schumacher and Eschmeyer (1943) and more recently by De Lury (1958) was used for the population estimates.

$$\frac{1}{\hat{N}} = \frac{\sum (R_t M_t)}{\sum (C_t M_t)^2} \qquad (1.1)$$

where $\hat{N}$ = estimate of population size

C_t = number of fish caught on day t

M_t = number of marked fish at large on day t

R_t = number of recaptures on day t.

The variance of $\frac{1}{\hat{N}}$ was calculated from

$$s^2 = \frac{\sum (R_t^2 / C_t) - (\sum R_t M_t)^2 / \sum (C_t M_t^2)}{m - 1}$$

where m = the number of days on which samples were taken. (1.2) (Schumacher and Eschmeyer, 1943)

The method of De Lury (1958) was used to estimate the standard error of the more symmetrically distributed $\frac{1}{\hat{N}}$ (when compared with $\hat{N}$ from

$$\frac{s}{\sqrt{\sum C_t M_t^2}} \qquad (1.3)$$

and the confidence limits for $\hat{N}$ follow from $\hat{N} \pm t \frac{1}{s_{\frac{1}{\hat{N}}}}$ where t is

students t corresponding to P = 0.05 and m-1 degrees of freedom.

The Schumacher and Eschmeyer population estimates using equation
were calculated as:-

$$\hat{N} \text{ for February 1969 } = 6344 \text{ fish}$$

$$\hat{N} \text{ for February 1970 } = 5173 \text{ fish}$$

Using equation 1.2 and 1.3 the De Lury 95% confidence limits
were calculated as:-

For February 1969 lower limit = 5862, upper limit = 6916

"   "   1970   "   "   = 3948,   "   "   = 7501

A change in the population size has occurred during the year of
investigation. This is indicated by the corresponding estimates,
ignoring the 95% confidence limits. However, these limits when applied
to the February 1970 estimate encompass the estimate for 1969, although
the reverse is untrue. It is difficult to account for this decrease in
precision of the second estimate since the same procedure was employed on
each occasion, and approximately the same number of fish were marked.
However, fewer fish were caught in total during the 1970 survey. This
might have been due to the fact that the fish became "net shy" as a
result of intensive netting during the year. Although lower captures
might be accepted as a plausible reason for the wide confidence limits
of the second estimate, it seems fairly certain that mortalities occurred
throughout the year causing the drop in population size. In which age
classes these mortalities occurred and possible explanations for them
will be discussed later.

Table (1.2) Computations for Schnabel and Schumacher estimates of population size for Bream, from net recaptures:-

A. February 1969

Date	Number caught	Recaptures	Number marked (less removals)	Marked fish at large
	C_t	R_t	-	M_t
1st	557	0	248	0
2nd	1191	48	121	248
3rd	1853	102	50	369
4th	1304	91	4	419
5th	720	42	3	423
6th	478	30	0	426
7th	842	64	0	426
Totals Σ	6945	377	426	2311

B. February 1970

Date	C_t	R_t	-	M_t
3rd	296	0	296	0
4th	128	11	40	296
5th	43	2	30	336
6th	6	0	6	366
7th	105	4	10	372
8th	249	19	10	382
9th	78	7	0	392
Totals Σ	905	43	392	2144

(B) The Structure of the population:-

Fish size and age compositions of the population were examined in
conjunction with the population size estimates at the beginning and end
of the one year sampling period.

The lengths of the fish captured were divided into half centimetre
classes, and plotted as a length/frequency polygon. This polygon is
shown for 1665 fish captured and measured in February 1969 (Fig.1.6).
and for 1328 fish captured and measured in February 1970 (Fig. 1.7).
Scales were removed from a sub sample of 211 fish in 1969 and 110 fish
in 1970 for age estimates.   This data is represented as a length/age
histogram and is superimposed on the length/frequency polygons in
Figs.

In order to calculate the mean length of successive age classes the
two groups of data were combined, assuming that the various year classes
of the bream were distributed in the whole sample as in the subsample in
which the ages of each fish were estimated. Thus, if 50% of the fish
aged in a particular length class were 2^+ and 50% 3^+, it was assumed that
these year classes were represented in the same proportions in the total
sample of fish in that length class. Knowing the estimated size of the
total population on each time occasion,and, assuming the length/frequency
polygons to be representative of the whole population, it was possible to
calculate the number of fish within each age class in the total population.
These estimates are shown for 1969 and 1970 in column one of Table   .
The percentage contributions from each of the year classes to the total
population size are shown in column two. It can be seen that the vast
majority of fish in the population are of age three years or less, i.e.
91.6% in 1969 and 93.9% in 1970. Since these fish are immature they make
no immediate contribution to the reproductive capacity of the total
population. Heavy mortalities must occur during these early years of

life since the older mature fish form only a small proportion of the fish population. This observed age composition of the bream population is not unusual for fresh water cyprinids, nor bream populations studied in eastern Europe (Dementeva, 1952).

Figs. 1.6 and 1.7 illustrate the variation in the contribution of the 0^+ year classes from year to year. The 0^+ year class formed 8.1 % of the total population in February 1969, while in February 1970 the 0^+ year class formed 40.05 %. These fluctuations may be due to the influence of temperature and other climatic factors during the spawning period. These weather factors could influence the availability of food organisms, such as phytoplankton, to the young fry. The different mortality and survival rates of each year class will be discussed later.

The monthly samples indicated that the sex ratio among the young immature bream was approximately 1:1, but that the number of males present in the population declines as the fish age. Females predominate in the older year classes and the ratio of females to males was calculated as 1:0.66.

Fig (1.6)   The length-frequency curve for a sample of 1665

fish and the length-frequency histograms for

each age class in a subsample of 211 fish for

A.brama in February 1969.
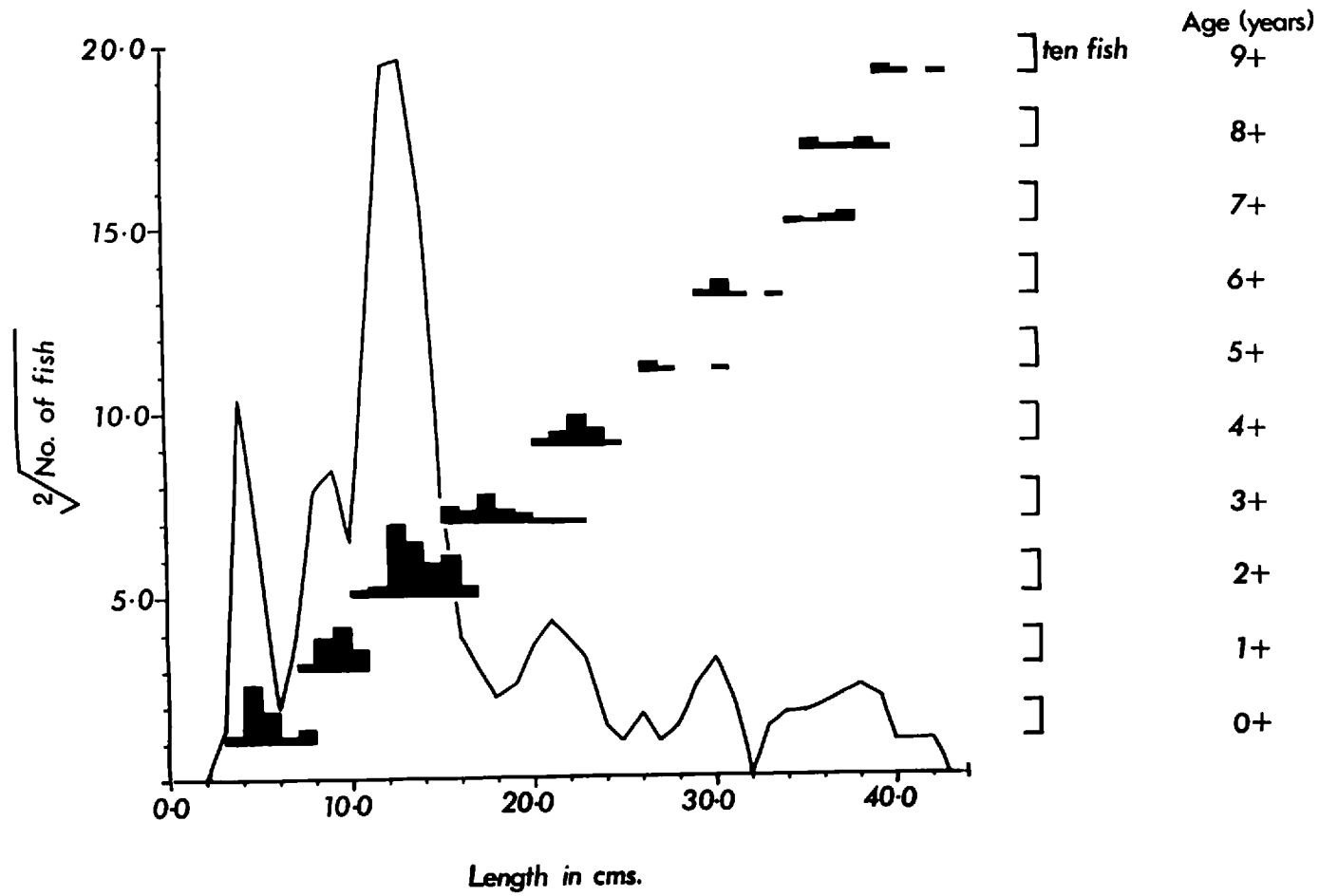
Fig (1.7)   The length-frequency curve for a sample of 1328

fish and the length-frequency histograms for each

age class in a subsample of 110 fish for A.brama

in February 1970.
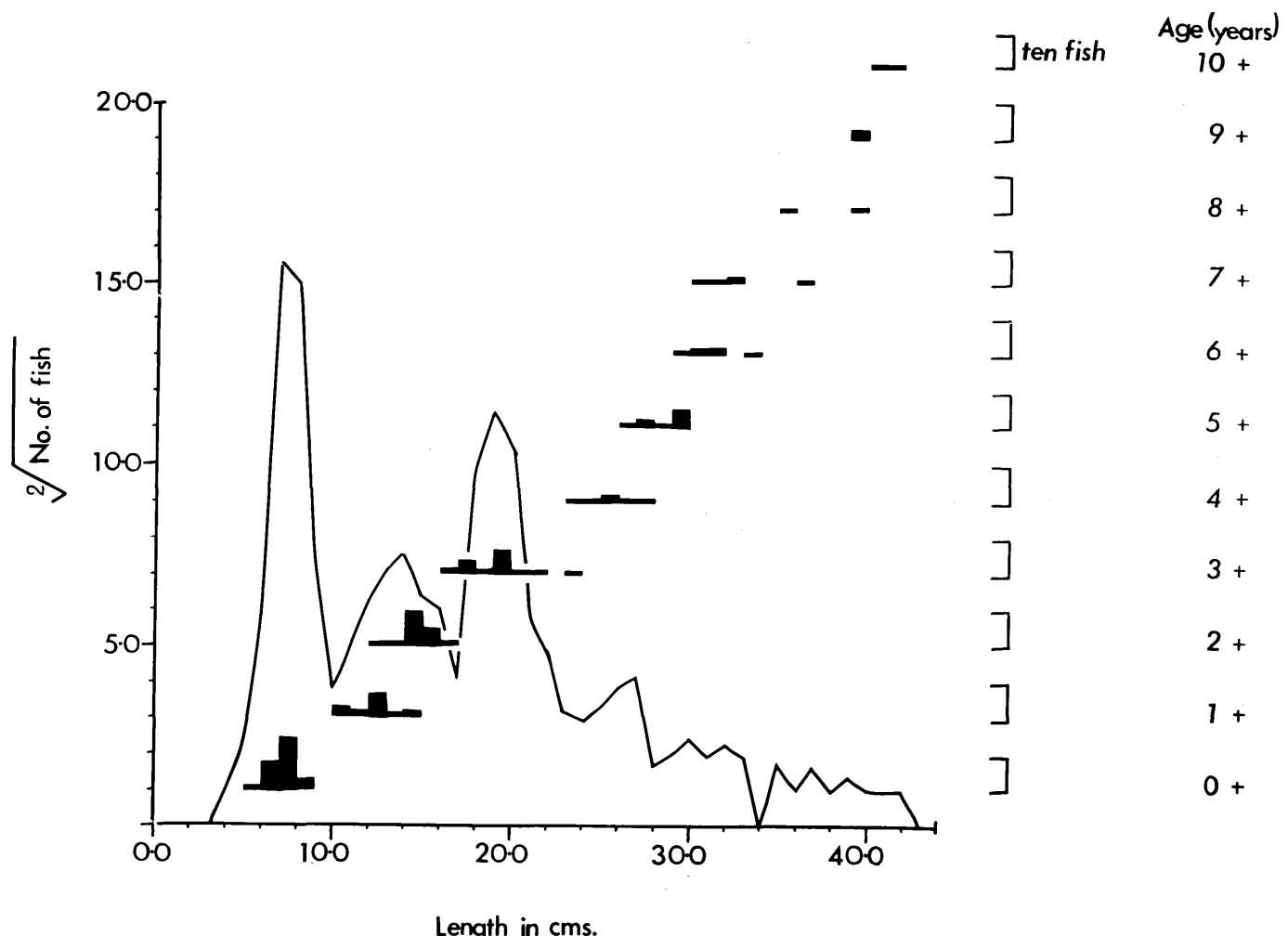
Length in cms.

Growth:-

Growth can be expressed in many ways. From the point of view of fish production increase in weight is a more important measure of growth than increase in length, but has usually been derived indirectly from length. In this investigation the growth rates of each year class of fish during the sampling year from February 1969 to February 1970 were of particular interest in relation to parasite burdens. Therefore, both growth in length and in weight were examined. From the length/frequency and length/age data, and from the lengths and ages of the fish sampled monthly, the growth of each year class was determined.

For the purpose of this investigation growth was expressed by:-

1) Growth curves of each age class of fish during the year of study, produced by plotting the mean lengths of successive age classes against time.

2) Overall growth curves for the bream population were produced by plotting the mean lengths and mean weights calculated from the length/ frequency and length/age data against age. A growth curve based on lengths of all members of each age class in the combined monthly samples was computed for comparison with other published data. The resultant means were therefore the lengths of the age classes at the mid point of the year of investigation. The sexes were combined as the means of the males and females were not significantly different.

3) Instantaneous growth rates were determined as the natural logarithm of the ratios of final weights to initial weights for a unit of time (one year) (Ricker, 1958). These values were then plotted against age.

In fishes, weight varies as some power of length (Ricker, 1958), for example

$$w = al^b \qquad (1.4)$$

where w = weight, l = length and a and b are constants. Thus lengths can be transformed into terms of weight. The length/weight relationship was

determined for (a) each age group of bream separately and (b) all the age groups combined. A linear regression analysis was carried out for each group. By taking logarithms equation (1.4) becomes

$$\log_{10} w = \log_{10} a + b \log_{10} l$$

Thus the slope of the regression of $\log_{10} w$ against $\log_{10} l$ is an estimate of the exponent b and the antilog of the $(\log_{10} a)$ intercept is an estimate of a in (1.4)

Fig.(1.8) shows the plot of log l against log w.

The value b = 3 would describe "isometric" growth, which would characterize a fish having an unchanging body form and unchanging specific gravity (Ricker, 1958). Bream, like many species of fish, approach this ideal, and the calculated equation for all age groups combined is

$$\log w = 3.134 \log l - \log 2.001$$

The b's within an age group are slightly different due to the smaller range of sizes included at each age grouping, but still approach "isometric" growth. The above b value has been used to calculate the mean weights of each age class.

Growth curves:-

The monthly changes in length of each age class are shown in Fig.(1.9) A clear seasonal growth pattern is seen in the younger fish. Little growth occurs during the early and late part of the year, while a marked period of growth occurs from May to the beginning of October. The seasonal check on the scales, as indicated earlier, is laid down in the early period of this growth period. The seasonality of growth is less marked in the older mature fish, which may be due to the low numbers of 8^+, 9^+ and 10^+ fish caught and examined. However, in the 4^+, 5^+ and 6^+ age classes, where adequate samples were collected, growth seems to be less rapid during the summer months, a slow, steady growth rate occurring throughout the year, with a slight decrease in the colder months. Very little growth seems to occur in the very old fish.

It is interesting to note that, particularly in the young fish, growth

seems to have been good during the year of study in comparison with the

previous year, with the young fish in February 1970 being larger on

average than fish of similar age in February 1969. This is particularly

marked in the 0^+ age class. The late spring and early summer of 1970

were periods of warm temperatures and long sunshine hours in the London

area, supposedly ideal weather conditions for the growth of young fish.

Similar correlations have been observed by Le Cren (1958) and Williams

(1965). Both authors suggest that fish grow faster during years which

are warmer than average. The increased scale growth during the year of

investigation indicated in Fig.(1.3) is further proof of increased growth

by the fish.

The choice of growth curves based on the average lengths of

successive age classes of fish in the length frequency data collected in

February 1969 and 1970 had two advantages. First, large numbers of fish

were measured and aged, and second, the two groups of data combined were

from the same month of the year, a month when little growth occurred

throughout all the age classes. Using the length weight relationship

from the mean lengths of each age class, the mean weights were calculated

(Fig.(1.10, graphs 3 and 4). The patterns illustrated are fairly

characteristic of cyprinid fish, with a marked decrease in growth rate

occurring in the older fish.

Several authors have published growth curves for bream in various

habitats and some are illustrated in Fig.(1.11). The growth curve 4

represents the bream from Dagenham calculated from the lengths of all

members of each age class in the combined monthly samples. However, care

must be taken in absolute comparisons of these curves since other authors

have based their curves on samples collected at varying times of the year.

However, all the data presented in the graphs was reduced to fork lengths

rather than total lengths; thus trends of growth are comparable. It is
interesting to note that curves 5, 6 and 7 exhibit a continual steady
increase in length with age, while 1 and 2 show a decrease of growth rate
with age. The Dagenham bream fall between these two categories, showing
a steady increase in length with a slight fall off in the rate of growth
as the fish reach the observed maximum age for the study area. It is also
interesting that Wundsch (1939) stated, when comparing the growth rates of
different populations of European bream, that it was impossible to find any
dependence on the geographic position or climatic conditions. The prime
influence on growth rate is probably food abundance and inter and intra-
specific competition for food and space.

Von Bertalanffy (1934, 1938) developed a useful relationship
which has been widely used to describe fish growth.

$$l_t = l_\infty \left(1-e^{-K(t-t_o)}\right) \tag{1.5}$$

where l_t = length of fish at time t

l_∞ = the value at which l assumes, as age increases indefinitely

and is called the average maximum or asymptotic length of

the fish.

t_o = a constant defining the time scale, with origin $t = t_o$

K = a constant determining the rate of change in length

increment. Each year's growth increment is less than the

previous year's by (l-k) of the latter where $k = e^{-K}$

Ricker (1958) presents the above expression in the form

$$l_{t+1} = l_\infty (1-k) + kl_t$$

which has been developed empirically by Walford (1946). A graphical plot
of the relationship of l_{t+1} with l_t is convenient for interpretation since
the slope of the line is equal to k and the Y axis intercept is $l_\infty (1-k)$
from which l_∞ can be calculated. The asymptotic length l_∞ is the point
at which the line cuts the $45°$ diagonal from the origin.

The growth curve constructed from the length/frequency data, was tested for goodness of fit to equation (1.5) by first using a 'Walford plot' (Fig. (1.12). The points fitted a straight line with an l_∞ value of 62.0 cms. The validity of this value of l_∞ was tested by plotting the natural logarithms of $(l_\infty - l_t)$ against time as demonstrated by Ricker (1958); the slope of this line is K. For a better fit the l_∞ obtained from a free hand Walford line can be used as a trial value in an expression (Beverton, 1954) derived from equation (1.5) by taking logarithms

$$\log_e(l_\infty - l_t) = \log_e l_\infty + Kt_0 - Kt \qquad (1.6)$$

Thus a graph of $\log_e(l_\infty - l_t)$ against t should be a straight line; this straightness is sensitive to changes in l_∞. The l_∞ value 62.0 cms. was found to yield a straight line. For this value, the slope of the natural logarithm line is $K = 0.105$ (hence $k = e^{-0.105} = 0.904$) and the Y axis intercept is 6.43. Equating the latter to $\log_e l_\infty + K$ in equation (1.6) with $\log_e l_\infty = \log 620 = 6.43$

$$to = \frac{Y \text{ axis intercept} - \log_e \infty}{K} = 0.002$$

which is negligible and thus $t - 0.002$ becomes t

Equation (1.5) becomes

$$l_t = 62.0(1 - e^{-0.904t})$$

This equation represents the observed growth pattern constructed for the population, by using the observed intervals between the mean lengths of age classes. However, the growth pattern for the year during which the population was investigated can be more accurately assessed by using the length/frequency data of the mean lengths of the age classes calculated at time t = February 1969 and time t + 1 = February 1970. The new Walford plot (Fig. 1.13) shows that although the young 0^+, 1^+, 2^+, 3^+ and 4^+ fish indicate an asymptotic value, l_∞, of 62.0 cms. the older age classes make little growth and tend to an l_∞ of 45.0 cms. K was calculated as

0.40, k as 0.67, the Y intercept being 6.36 thus to = 0.627. The

Bertalonffy growth equation for the older fish ($> 4^+$) during the year

of investigation was thus

$$L_t = 45.0 \ (1-e^{-0.40(t-0.627)})$$

These calculations clearly illustrate the poor growth of the 5^+

and older fish in comparison with the young fish, and to the observed

growth of the old age classes in previous years. They also illustrate

the danger of using the mean lengths of age classes of fish sampled at

one instant in time to calculate growth equations. The older bream have

markedly different feeding habits from the young fish and thus this poor

growth pattern may be due to a relatively poorer supply of their major

food organisms. Competition is an unlikely explanation since few old

fish are present in the environment and under ideal conditions bream can

live to a considerable age even at higher density. These aspects will be

discussed later in connection with parasitic infections.

Growth rates:-

The instantaneous growth rates (G) were also calculated from the

weight increments of each age class during the year of investigation

(Table 1.3 ; Fig. 1.10, graph 2). These data emphasise the decline in the

rate of weight increase as the bream enter the later age groups.

The average weight of the fish in any age class and the relative

abundance of each class may be used to estimate the change in total bulk

or biomass during the period. The estimated biomass for each age class

in February 1969 are represented in Table (1.4) and for February 1970 in

Table (1.5). It is interesting to note that, although the size of the

population was reduced during the year, the biomass of the population

increased. This was primarily due to the growth in weight of the numer-

ically large and dominant 2^+ age class which, although suffering heavy

mortalities during the year, increased in biomass on recruitment to the

3^+ age class. A marked increase in biomass occurred in all the young age classes of fish, again indicating that 1969-70 was a good year for the growth of individual young fish.

Fig (1.8)   The relationsbip between body length (cms.) and body

weight (gms.) of Dagenham bream (406 fish).

LOG.₁₀ LENGTH (in gms.)
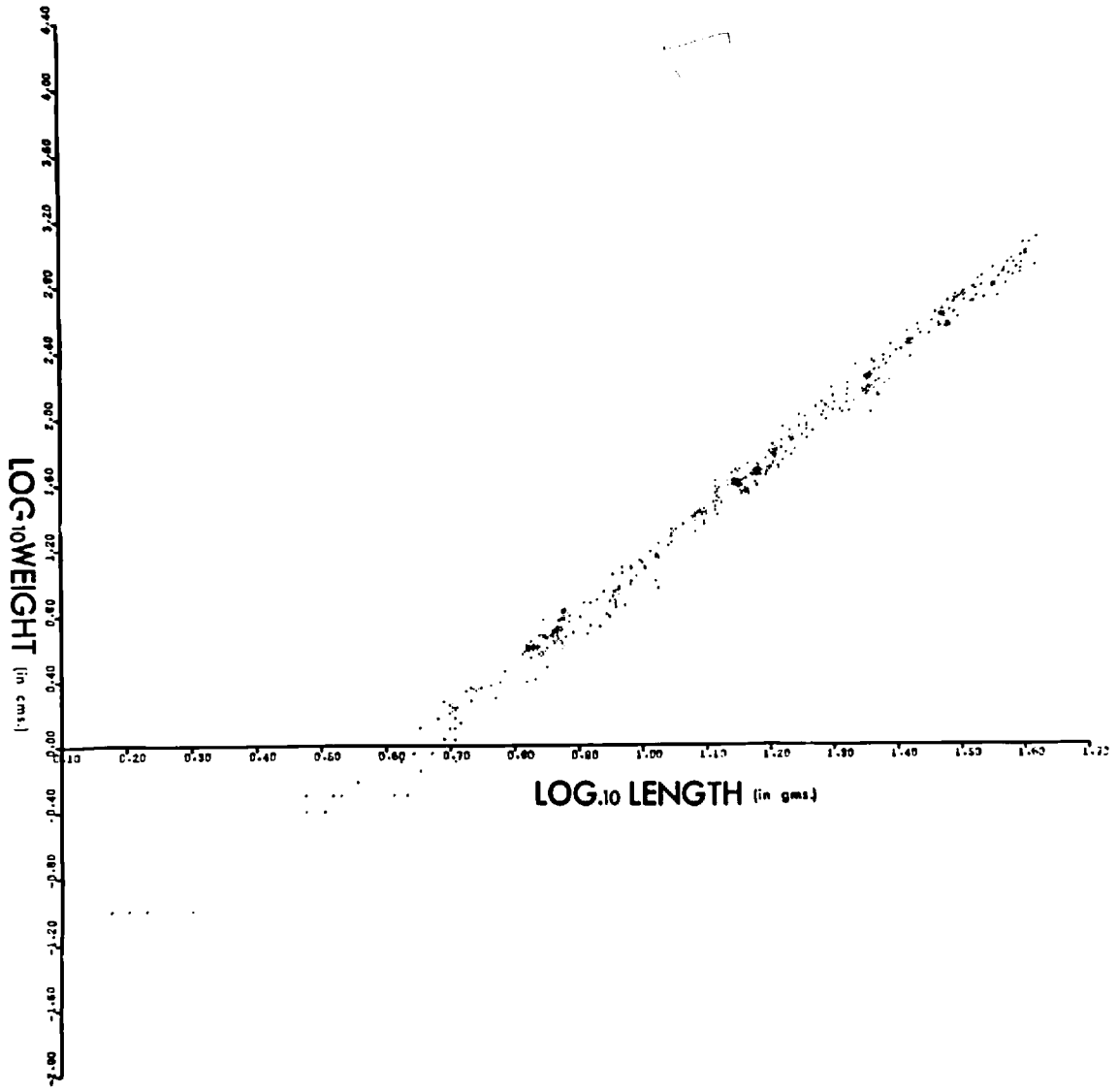
Fig (1.9)   Growth in length of each age class of bream from

February 1969 - February 1970.

-50-
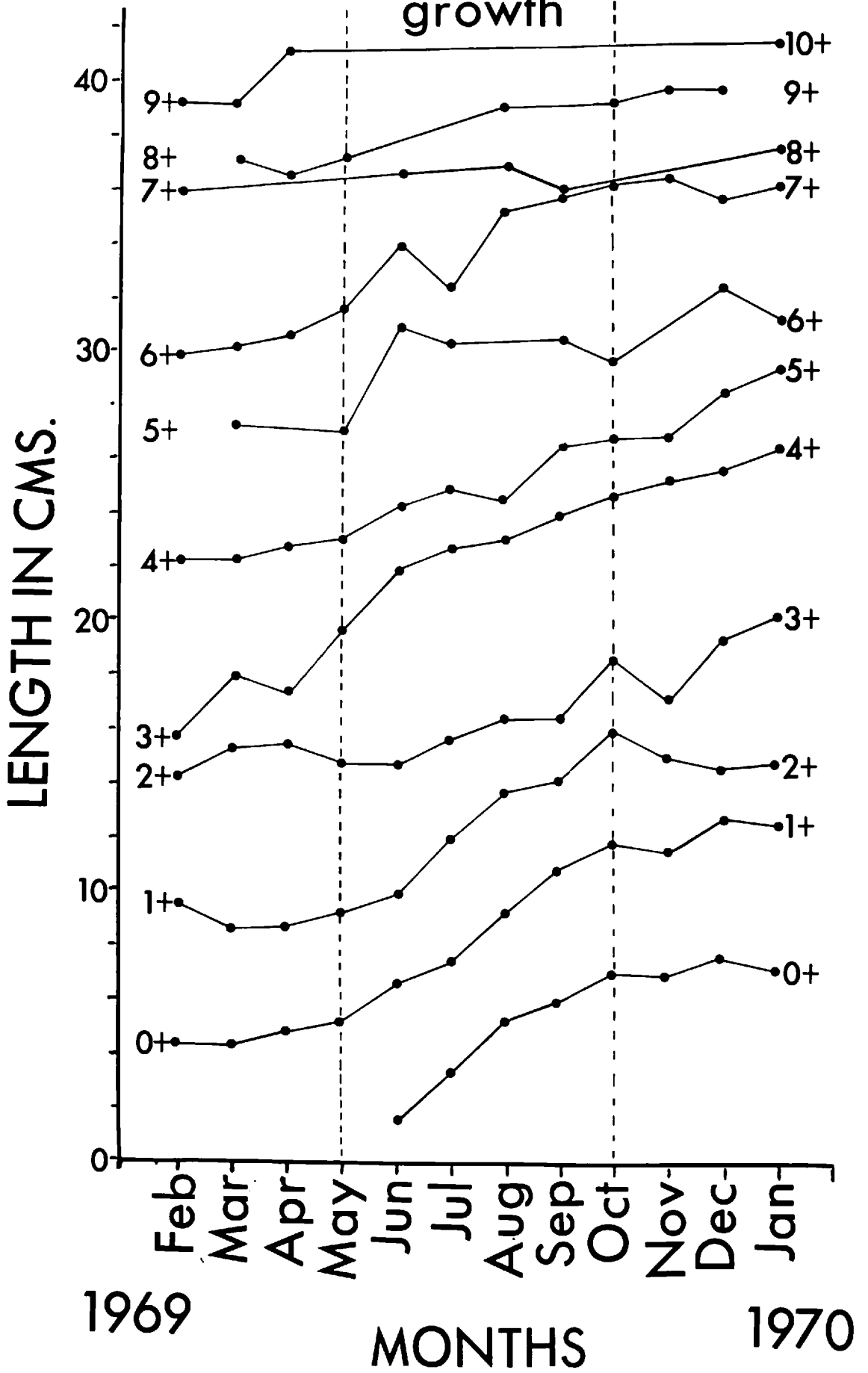
Fig (1.10)   1: Catch curve for bream of successive ages

(logarithm of frequencies). 2: Instantaneous rate

of weight increase of successive ages. 3: Mean

weight (kilo grams) of successive ages. 4: Mean

length (centimetres) of successive ages. (Means

of the grouped data from large samples collected

in February 1969 and 1970).
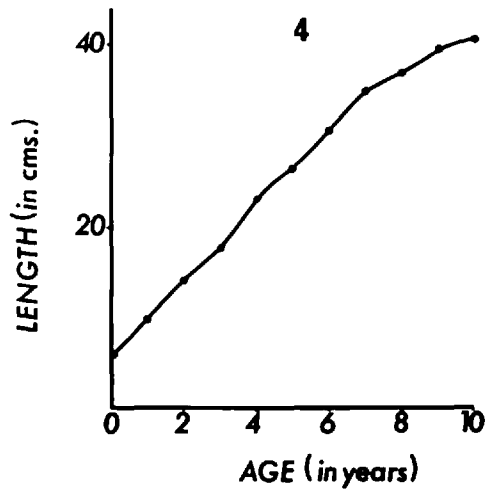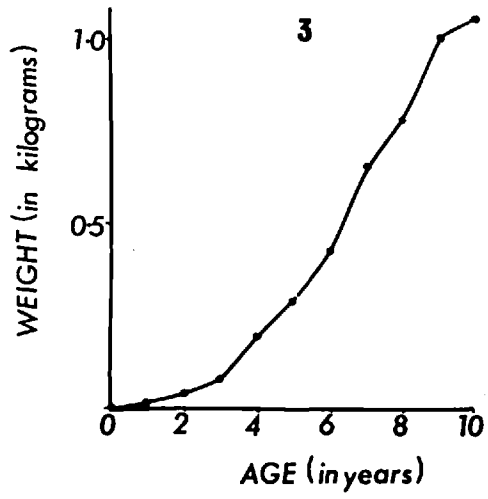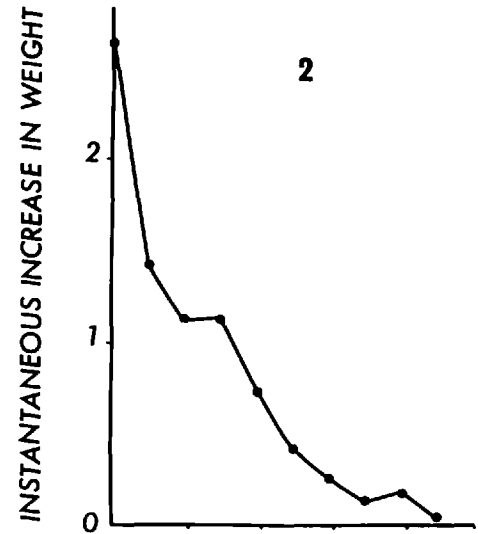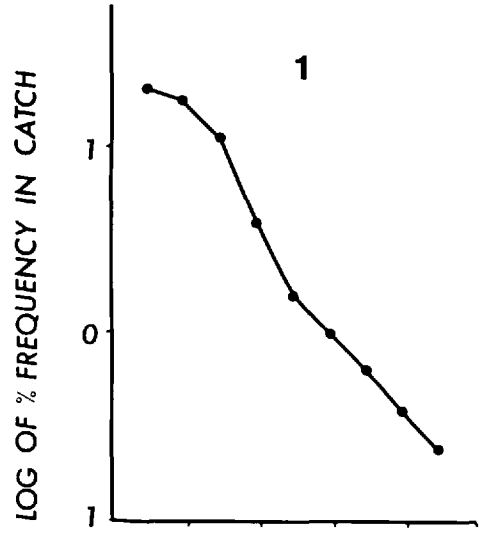
Fig (1.11)   Growth of bream in Dagenham compared with growth in

some European waters. 1: Ural delta - Berg (1949).

2: Caspian Sea - Dementeva in Shorygin (1952).

3: Madingley Lake, England - Hartley (1947b)

4: Dagenham, England. 5: Average from 36 North

German Lakes - Bauch (1963). 6: Hjalmaren, Sweden -

Alm (1947). 7: Tuusula, Finland - Järnefelt (1921).
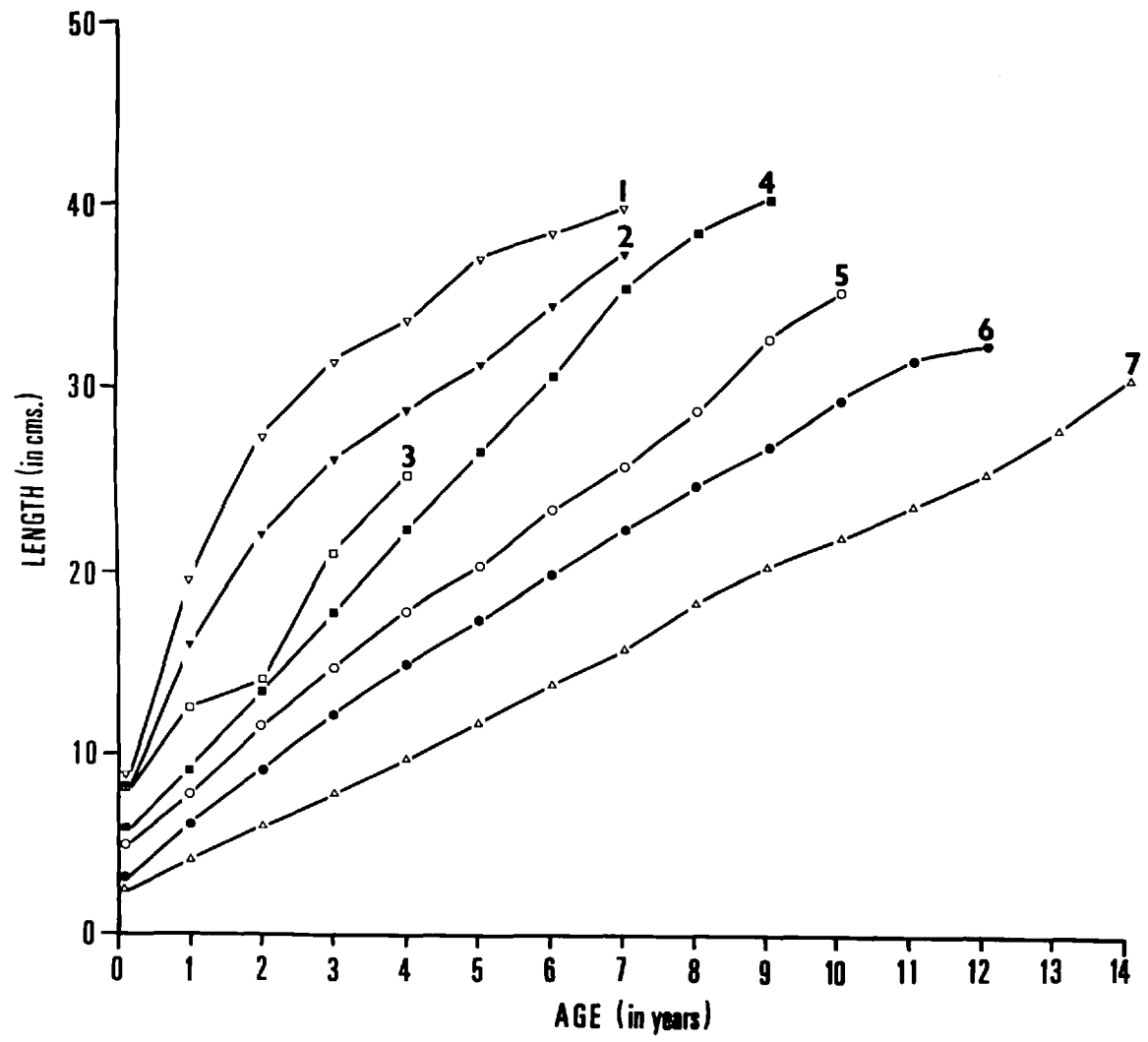
Fig (1.12)   Walford plot of mean length at age (t+1) against

the mean length at age (t) calculated from the

observed intervals between the mean length of

age classes. Log_e $(l_\infty - l_t)$ plotted against age

for l_∞ = 62.0cms.
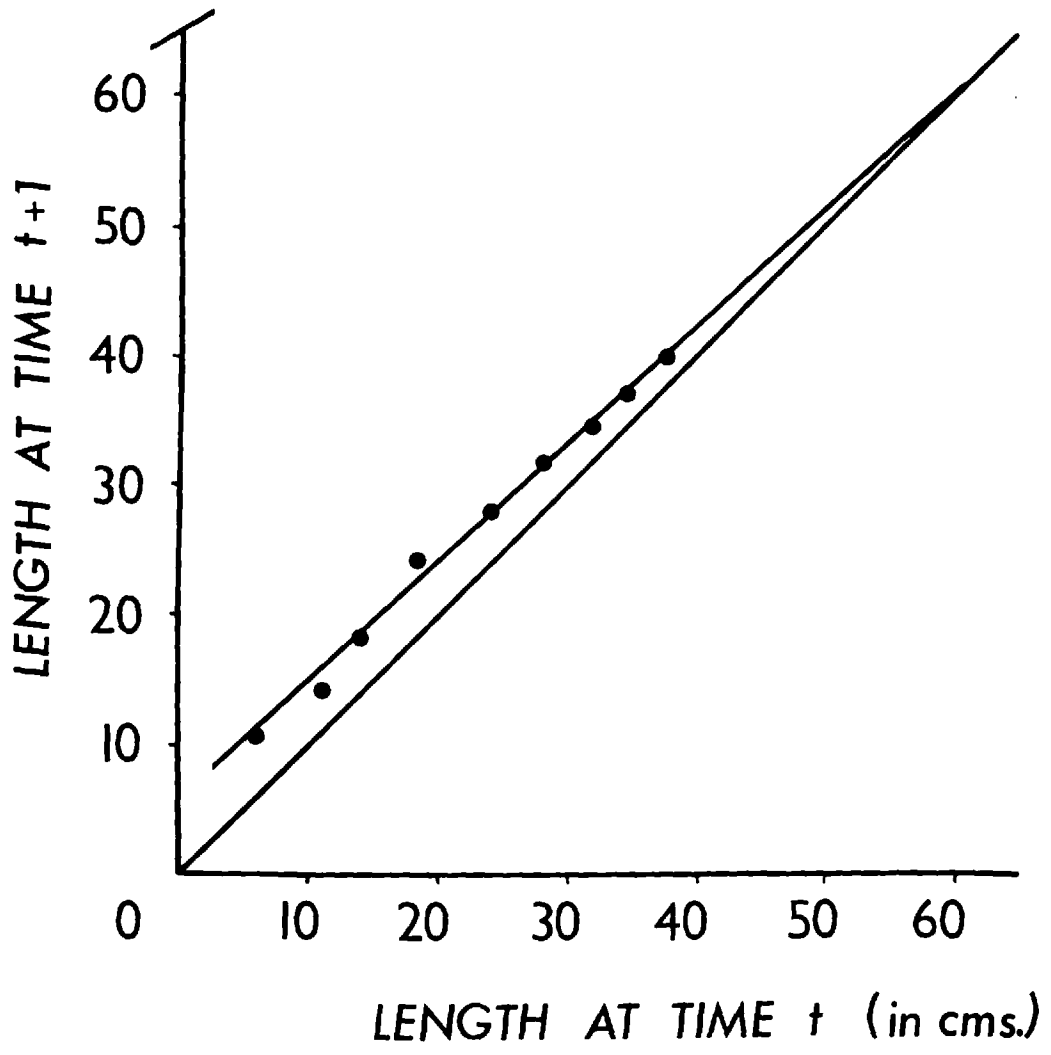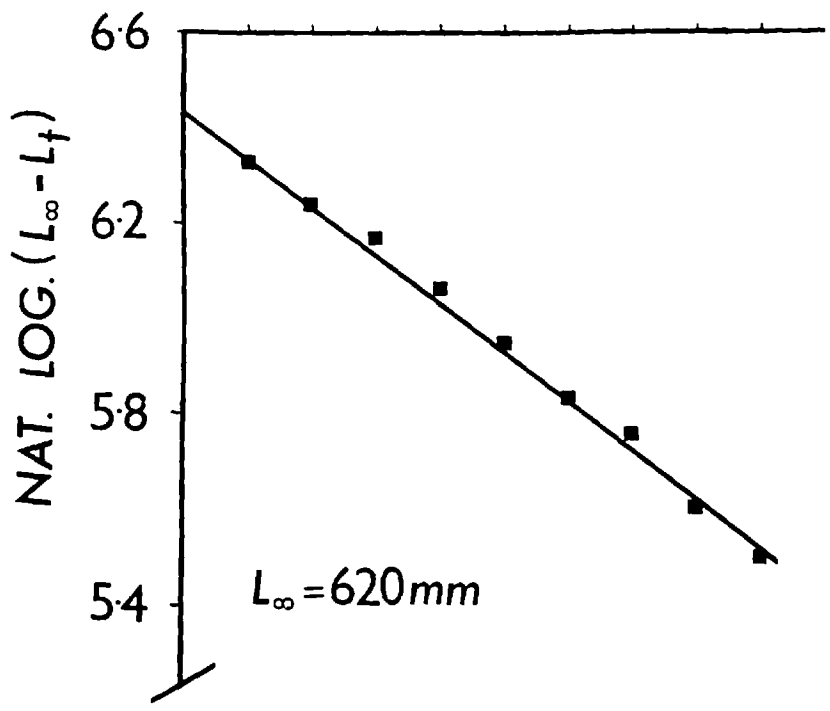
Fig (1.13)   Walford plot of mean length at age t+1(= February

1970) against mean length at age t (= February 1969);

$\text{Log}_e(l_\infty - l_t)$ plotted against age for l_∞ = 45.0 cms.
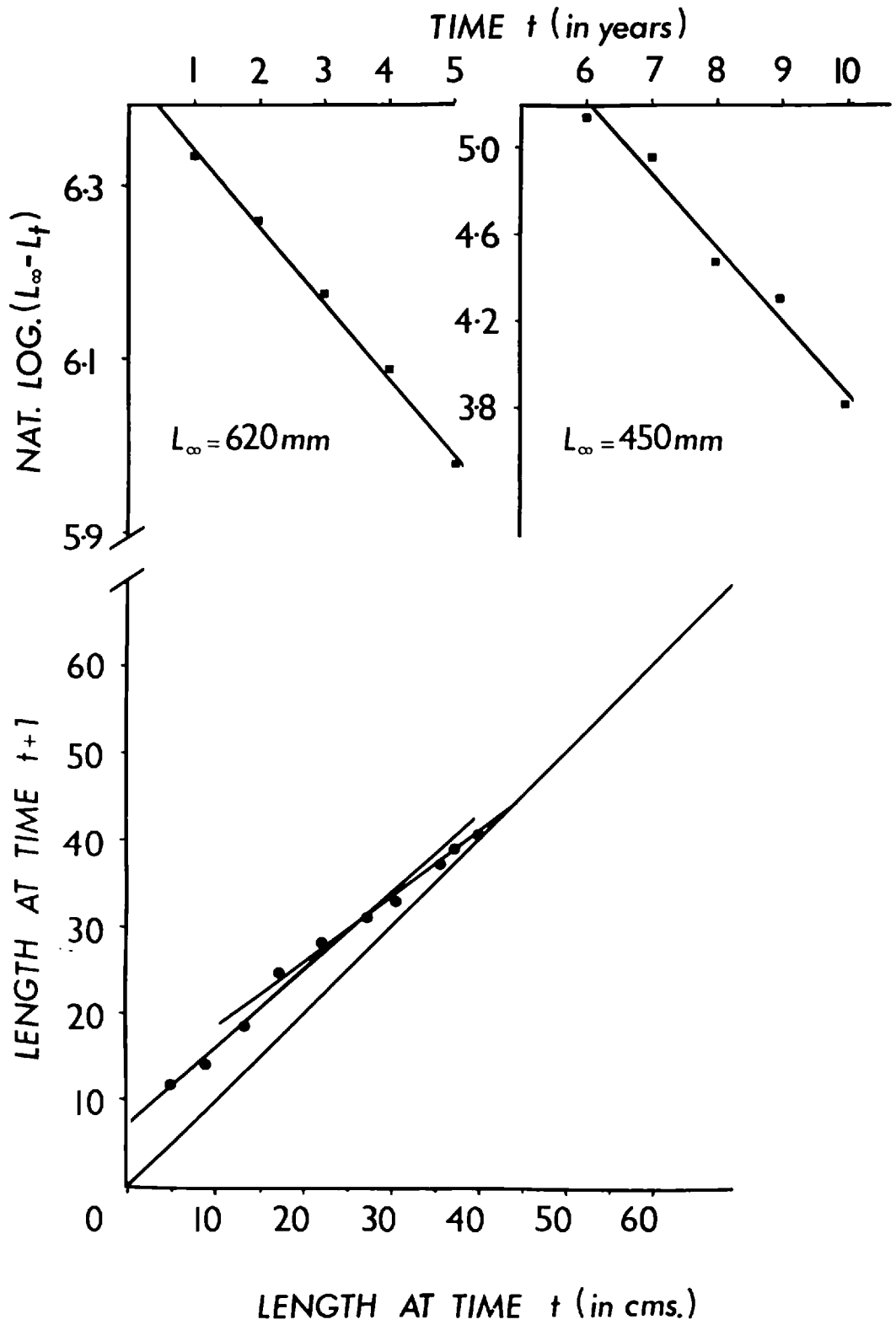
Table (1.3) Mortality and survival parameters for successive age
classes of bream during the year February 1969 to
February 1970.

February 1969   February 1970
  (time t)   (time t + 1)

Age class	Number of fish	Age class	Number of fish	Instantaneous growth rates G.	Natural survival rates S.	Mortality Rates i.
0^+	514	1^+	619	2.653	1.204	-0.204
1^+	655	2^+	448	1.404	0.683	0.316
2^+	4644	3^+	1671	1.102	0.359	0.640
3^+	127	4^+	97	1.135	0.763	0.236
4^+	182	5^+	148	1.732	0.813	0.187
5^+	55	6^+	43	0.399	0.781	0.218
6^+	84	7^+	39	0.247	0.464	0.536
7^+	38	8^+	16	0.104	0.421	0.579
8^+	30	9^+	12	0.174	0.400	0.600
9^+	15	10^+	8	0.010	0.533	0.467

Table (1.4) Growth parameters for successive age classes of bream in
February 1969

Age class	Estimated number of fish	% frequency	$\log_{10}N$	$\log_{10}F$	Mean length (cms.)	Mean weight (gms.)	Biomass (kilograms)
	N	F			l.	w.	B.
0^+	514	8.10	2.7110	0.9084	5.196	1.75	0.897
1^+	655	10.33	2.8162	1.0128	9.258	10.67	6.989
2^+	4644	73.20	3.6670	1.8645	13.620	33.20	154.181
3^+	127	2.00	2.1038	0.3016	17.840	83.30	10.579
4^+	182	2.87	2.2601	0.4579	22.450	171.16	30.958
5^+	55	0.87	1.7404	-0.0648	27.700	331.20	18.216
6^+	84	1.32	1.9243	0.1206	30.720	458.20	38.488
7^+	38	0.60	1.5798	-0.2219	36.200	765.70	29.096
8^+	30	0.48	1.4771	-0.3188	37.500	855.60	25.668
9^+	15	0.23	1.1761	-0.6383	40.510	1090.00	16.350

Estimated population size = 6344 fish

Total Biomass = 331.422 kilograms

Table (1.5)   Growth parameters for successive age classes of bream in
                      February 1970

Age class	Estimated number of fish	% frequency	$\log_{10}N$	$\log_{10}F$	Mean length (cms.)	Mean weight (gms.)	Biomass (kilograms)
	N	F			l.	w.	B.
0^+	2072	40.05	3.3159	1.6021	7.2	4.8	9.862
1^+	619	11.96	2.7916	1.0792	12.1	24.9	15.413
2^+	448	8.66	2.6512	0.9395	14.5	43.6	19.514
3^+	1671	32.30	3.2230	1.5092	18.9	100.1	167.267
4^+	97	1.87	1.9868	0.2718	25.6	258.6	25.084
5^+	148	2.86	2.1702	0.4564	28.3	354.0	52.392
6^+	43	0.83	1.6334	-0.0869	31.5	494.5	21.260
7^+	39	0.75	1.5910	-0.1249	33.3	589.7	22.991
8^+	16	0.30	1.2041	-0.5228	37.4	847.5	13.564
9^+	12	0.23	1.0762	-0.6383	39.7	1020.6	12.246
10^+	8	0.15	0.9031	-0.8239	40.8	1100.8	8.806

Estimated population size = 5173 fish

Total Biomass = 368.394 kilograms

Mortality and survival:-

A catch curve (Ricker 1958) was developed by plotting (Table 1.6)
the logarithm of the average percentage frequency of each age class for
February 1969 and 1970 against age (Fig.1.10 , Graph 1). The curve was
smoothed using running averages over three points. If it can be assumed
that fishing mortalities are absent or negligible, the descending limb
of the catch curve represents sampling and natural mortality which will
be discussed later.

The survival rates (S) of the age classes during the year were
calculated as the number of fish alive at the end of the year, divided
by the initial number and the instantaneous mortality rates (i) as (1-S)
(Table 1.3). The survival and mortality rates of the 0^+ age class were
artifically high and low respectively which could be due to sampling
errors in the length/frequency data and might be caused by net selectivity.
Additions to the stock did not occur in the study year. The decrease in
abundance of the bream over the year is primarily due to the high mortality
rate in the 2^+ age class which in February 1969 represented 78.2% of the
total population. This is most probably due to competition for available
food materials upon which the class feeds, notably planktonic crustacea.
In general, for the remaining age classes mortality rates increase with
age, thus survival rates decrease. This is typical for natural fish
populations, and represents the cumulative effects of 1) intra and inter
specific competition for food 2) predation 3) parasites and other diseases
and lastly 4) spawning deaths.

Table (1.6) Population parameters for each successive age class of
bream calculated from the combined data of February 1969
and February 1970.

Age class	Mean number of fish	% frequency	$\log_{10}$ of % frequency	running average of 3 points	Mean Length (cms.)	Mean Weight (gms.)
0^+	1293	24.05	1.3802		6.2	3.2
1^+	637	11.14	1.0450	1.34	10.7	17.8
2^+	2546	40.95	1.6117	1.28	14.0	38.4
3^+	899	17.15	1.2330	1.07	18.4	91.7
4^+	139	2.37	0.3747	0.63	24.0	214.3
5^+	102	1.86	0.2707	0.22	28.0	342.6
6^+	63	1.07	0.0294	0.42	31.1	476.3
7^+	39	0.67	-0.1739	-0.18	34.8	677.7
8^+	23	0.39	-0.4089	-0.41	37.4	851.6
9^+	13	0.23	-0.6383	-0.62	40.1	1055.2
10^+	4	0.15	-0.8239		40.8	1100.8

Discussion

The spawning behaviour, early development, growth and food of the bream population at Dagenham were fairly typical for the species in European waters (Kennedy and Fitzmaurice, 1968; Backiel and Zawisza, 1968). The spawning period between mid May and mid June was somewhat late, however, thus inhibiting the length of the growing season, since early spawning implies a longer growing season in the first years of life. High summer temperatures offset this late spawning to a certain extent, causing good growth and survival of the fry during the year investigated. The structure of the population was typical for cyprinid fish with certain age classes, notably 2^+, dominating the population. The reasons for a particularly high survival rate of fry in one particular year are complex. There is some evidence (Smyly, 1952) that the predominance of one particular year class in perch fry may depend on the survival rates of the larvae soon after hatching, in fact in the first few hours of life; the weather and presence of certain planktonic organisms during this period may be crucial. It is likely that the survival of bream fry is equally determined by these factors, which must have been ideal in 1966 to produce the large 2^+ year class observed. In February 1969 the 2^+ age class formed 73.2 % of the total population. It is interesting to note that the summer of 1969 was also good for fry survival and in February 1970 the 0^+ year class formed 40.1 % of the population. With cumulative mortalities building up over the years the older mature bream form only a small percentage of the total population.

The feeding pattern of the age classes of bream undergoes a gradual change with age, planktonic crustacea being replaced by bottom dwelling invertebrates as a diet for the fish. A slightly unusual fact in relation to diet was the lack of variety of food organisms detected in the diet of bream. These narrow preferences including Cladocera, Ostracoda, Chirominids

and Molluscs could reflect the abundance of these groups of invertebrates in the environment. The intensity of feeding decreases during the cold winter months and also during the spawning season of the mature fish. The change in diet with age of the fish is also correlated with a change in ecological niches occupied by the different age classes. The very young bream inhabit the marginal shallows of the gravel pit, slowly moving into open water, but remaining in the middle and surface layers of the water. Finally, as mature fish, they move into the deeper layers of the habitat. All ages of bream exhibit shoaling behaviour, with the exception of the mature fish during the breeding season. The changes in feeding habits correlated with the change in ecological niche are important in relation to parasitic infections which will be discussed more fully later.

Scale check formation was found to be annual, with the check laid down in June. The number of rings outside the check gives a measure of growth made in a single year. Growth mainly occurs in the young immature fish and mainly in the period May to the beginning of October; a marked acceleration in growth is evident during this period. The mature fish grow steadily throughout the year with a slight pause during the cold winter months. The year during which samples were collected was of particular interest because of the variation in growth rates and patterns of the different age classes. The young bream up to the 3^+ age class grew well in comparison with previous years, these good growth rates most probably reflecting the abundance of small crustacea upon which these fish feed and also the long warm summer of 1969. The older bream from the 4^+ age class upwards grew less well, however. Taking into account an expected decrease in growth with age, which is usually observable in cyprinid fish, the year was still poor in comparison with previous years. This may reflect a lack of large invertebrates, such as molluscs and chirominids, in the habitat, since old bream were sometimes found to have eaten small crustacea.

These differences in growth rates of young and old bream are well
illustrated by the Walford plots of growth in length (Figs. 1.12 and
1.13).

Mortality and survival of the fish during the year were of
particular interest due to possible relationships with parasitic infections.
As might be expected the highest mortalities occurred in the largest 2^+ age
class, which dropped from forming 73.2% to 32.3% of the total population
during the year. It is, however, very interesting to note that this fall
in numbers did not prevent an increase in biomass of the age class,
associated with an improved growth rate for individuals. In general,
mortalities increased with age. Mortality can be ascribed to two main
factors 1)Fishing mortalities 2) Natural mortalities. Fishing mortalities
were significant due to the demands of the investigation, approximately
500 fish being removed during the year. This still leaves approximately
600 fish to be accounted for by natural mortalities. Natural mortalities
may be attributed to four main causes (1) intra and inter specific
competition for food (2) predation (3) spawning deaths (4) disease, includ-
ing parasitic infections. In the Dagenham gravel pit intra and inter
specific competition for food must have been considerable, since other
large cyprinids populations, such as the roach, were competing for similar
food types. This factor mainly reduces growth rate, but it may also have
caused mortalities. Predators of the mature bream were absent. The young
fish, however, were preyed upon by the Great Crested Grebe (Podiceps
cristatus (L.)) and the Pike (Esox lucius (L.)). Both were present only
in small numbers, the former for only a small part of the year, but they
must have contributed to overall mortalities of fish. Spawning losses of
mature fish were observable during the breeding season, but were not
visibly large. Mortalities due to disease and parasitic infections are
the most likely causes of mortality but augmented by the combined effects

of the previous factors. The role of parasitic helminth infections
in causing mortalities will be discussed later.

S E C T I O N 2

The Biology and Population Dynamics of the

helminth parasites Caryophyllaeus laticeps (Pallas, 1781)

and Diplozoon paradoxum (Nordmann, 1831)

SUBSECTION I The Biology of the parasites:-

Introduction:-

The majority of studies on the parasites of freshwater and marine

fish have been concerned with problems of life histories and distributions

(Bayliss, 1928, 1939; Nicoll, 1924; Brown 1927; Meggit, 1914). More

recently extensive reviews of the general ecology of the parasites of

freshwater fish in Eastern Europe have been carried out by Dogiel et al

(1961), Dogiel (1964) and Baur (1962).

A review of the current information available on the distribution

and occurrence of freshwater fish parasites has been carried out by

Chubb (1970) and a reference list of host-parasite records has been

compiled by Chappel and Owen (1969). A number of workers have made

general surveys of the parasite fauna of the fish species within particular

environments in the British Isles; these include Chubb (1964),Rizvi (1964),

Mishra (1966) and Davies (1967).

The ecology of single species of parasites in specific fish

populations has been investigated by Arme and Owen (1968), Awachie

(1965, 1966, 1968), Chappel (1969a, 1969b), Chubb (1963), Hopkins (1959,

Kennedy (1968, 1969), Kennedy and Hine (1969), Paling (1965), Thomas (1964)

and Walkey (1967). A review of the population biology of fish helminth

population is given by Kennedy (1970).

The majority of these studies, however, lack quantitative precision

due to the difficulties encountered in sampling the fish host populations.

Also both host and parasite population dynamics have not been studied

simultaneously by designed sampling schemes.

This section deals with the methods of collection and the general

biology of the parasites selected for study. The method and results of

the detailed analyses of the data are dealt with later.

Although ten species of parasites were found, consisting of 1 Protozoa, 1 Annelid, 1 Crustacea and 7 helminths (Table 2.1), only two parasites <u>Caryophyllaeus laticeps</u> (Pallas, 1781) and <u>Diplozoon paradoxum</u> (Nordmann, 1832) have been singled out for a detailed examination of their population dynamics and distributions, both within their microenvironments and in the host population. This is due mainly to the quantity and nature of the data collected concerning these two parasites. Both these species occur in the fish host throughout the year and in sufficient numbers for analysis.

Table (2 . 1)   Parasite list for the bream <u>Abramis brama</u> in
Dagenham gravel pit.

Protozoa

       Myxobolus mulleri   (Butschlii,1882)

Monogenea

       Diplozoon paradoxum   (Nordmann,1832)

       Dactylogyrus crucifer (Wagner,1857)

       Gyrodactylus elegans   (Nordmann,1832)

Cestoda

       Caryophyllaeus laticeps (Pallas,1781)

       Ligula intestinalis   (Linnaeus,1758)

Digenea

       Diplostomum spathaceum (Rudolphii,1819) *

       Diplostomum clavatum   (Nordmann,1832) *

Annelidia

       Psicola geometra   (Linnaeus,1758)

Crustacea

       Argulus foliaceus   (Linnaeus,1758)

(* New host record for English waters)

Methods:-

Live fish caught in the seine nets were sampled as previously
described and transported back to the laboratory in 16 gallon dust bins
filled with water which was oxygenated during the journey. On arrival
at the laboratory the fish were killed by pithing and deep frozen within
an hour to a temperature of $-22^{o}C$.

Fish were thawed when required for examination and a record was
made of any external symptoms of damage or disease. Each fish examined
was regarded as one sample unit, and all were examined in the same manner.
The data recorded for each individual fish was placed on record sheets
before transfer to punch cards and then onto magnetic tape.

All the fish tissues were examined and records made of the
numbers and positions of all the parasite species found. The tissues
were first examined macroscopically and then under a binocular dissecting
microscope.

Examination of the fish:-

External surfaces of the fish were examined for ectoparasites;
when present their numbers and positioning on the host were recorded.
During the process of freezing and thawing most ectoparasites are lost;
however records of those remaining were kept. The eyes of each fish were
removed and dissected and examined dry on a dark background by reflected
light. The lenses were removed and examined separately. The numbers of
parasites present were recorded. The buccal cavity and the walls of the
branchial cavity, including the internal surfaces of the operculum were
also examined. Each gill arch was removed separately from the fish and
examined under water by a reflected light on a light background. Care
was taken to search between the primary filaments of each gill arch. The
exact position of each parasite found was recorded. To aid in future
analysis of the data each gill arch was divided lengthwise approximately
into four sections of equal area and the numbers of parasites in each

section were recorded. The body cavity was opened by a longitudinal mid ventral slit from cloaca to a position anterior to the pectoral girdle. The viscera and mesenteries were examined for parasites, free in the body or encysted on the viscera. The swim bladder, kidneys, gonads and heart were also examined for parasites. The oesophagus and intestine were removed in one piece from the body cavity and the overall length of the intestine measured. The gut was divided into 24 sections of equal length, and the number of parasites present in each section was recorded. In the case of tapeworms whose length might occupy numerous sections, position was determined by the location of the scolex.

Preservation_of specimens:-

All platyhelminth parasites were removed, fixed and stored for assessment of their state of maturity. Fixed permanent preparations of the parasites were made to aid in their identification. The Monogenes and Cestodes were fixed in cold neutral formal saline and washed and stored in 70% ethanol. A number of methods were used to examine the parasites microscopically. Some species could be identified from un-stained squashed preparations. However the majority were stained and mounted. Specimens were stained in Gowers carmine or alcoholic carmine hydrochloride, dehydrated in ethyl alcohol, cleared in methyl benzoate and mounted in canada balsam. Measurements of length were made on fixed specimens using a calibrated ocular micrometer.

Maturation_determination:-

The state of maturity of the parasites was determined from stained preparations. Three stages of maturity were recorded for both <u>Caryophyllaeus laticeps</u> and <u>Diplozoon paradoxum</u>:-

1) Immature worms - Primordial germinal cell mass present, but gonads plus associate structures not distinct.

2) Mature worms - Gonads distinct. Vitalleria fully developed.

3) Mature gravid worms - Eggs present in uterus.

All specimens of C.laticeps found were examined and, as they were

fixed in a uniform manner, variations in the length of the worms were used

as an indicator of growth changes in the population. Large numbers of

D.paradoxum were encountered; thus samples were taken from each age group

of hosts for examination. The length of one of the worms in a couple was

recorded. The length measured was the distance from the tip of the haptor

to the junction of the couple. This region is not so susceptible to

variations due to contraction and contortion caused by fixing.

Sampling_and_examination_of_intermediate_hosts:-

Caryophyllaeus laticeps, one of the two helminths examined in

detail, has a life cycle involving tubificid intermediate hosts which

will be discussed later. Tubificids were sampled bimonthly throughout the

year at four stations in the gravel pit. Samples of the mud at these

points were taken using an Ekman dredge, emptied into plastic containers

and then transported to tanks in the laboratory. Ten subsamples of 50 ccs.

of mud were taken from each of the four samples and examined on each

occasion with the exception of May 1969 when approximately 100 subsamples

were examined to obtain information on the extent and form of the tubificids

distribution. The subsamples were examined within 72 hours of being

collected. A combination of three sieves, mesh sizes 1100 microns, 850

microns and 300 microns, was found to be the most efficient in extracting

the worms from each subsample. The numbers of tubificids in each subsample

was recorded and each was then examined individually for the presence of

C.laticeps in the body cavity. The infected worms were counted and then

dissected. The parasites were removed and allowed to relax before fixing

and staining as described above.

Caryophyllaeus laticeps (Pallas) 1781):-

Cestodes of the family Caryophyllaeidae are unsegmented tapeworms occurring in freshwater Teleosts of Africa, Asia, Australia, Europe and North America. Larval stages of the worm develop in the body cavity of aquatic oligochaetes of the family Tubificidae. Reviews of the family Caryophyllaeidae have been presented by Hunter (1930), Hymen (1951) and Yamaguti (1958).

C.laticeps is a common parasite of cyprinid fish and has been reported from bream by Schäperclaus (1954), Kane (1956), Barysheva and Baur (1957), Agapova (1960) and Pacak (1962). Adult worms occur in the intestine of the fish host, attached to the intestinal wall by the large scolex. Mature eggs are laid in the lumen of the gut and pass to the exterior with the faeces of the hosts. The eggs are ingested by Tubificid oligochaetes, the hexacanth larvae hatching and burrowing via the intestinal wall into the body cavity. In the Dagenham gravel pit, the species Tubifex tubifex was the main intermediate host of C.laticeps. Development of the larvae up to and including the formation of genitalia takes place in the body cavity of the tubificid. However, eggs are only produced in the definitive fish host. Kulakowskaja (1962 and 1964) reported that the larvae become infective to fish after four months and can remain infective in the intermediate host for up to two years, although the survival of the adult worm in the fish is not longer than two months. Both the adult and larval parasite are not ostensibly host specific, the former infecting a number of cyprinid species and the latter developing in various species of tubificids.

<u>Diplozoon paradoxum</u> (Nordmann, 1832):-

    <u>Diplozoon paradoxum</u>, a Monogenetic trematode, is a common parasite of the gills of freshwater cyprinid fish. Before 1959 the majority of parasites in the genus Diplozoon recovered from cyprinid fish were given the specific name <u>D.paradoxum</u>. Bychowsky and Nagibina (1959) pointed out that morphological differences existed between parasites recovered from different hosts, and divided the genus into several species according to host. Since then the genus Diplozoon has been reviewed by Reichenbach-Klinke (1961) and Gläser and Gläser (1964). The species of Diplozoon parasitising the bream in Dagenham was identified as <u>D.paradoxum</u>. This species has been recorded infesting bream by a variety of workers. (Bychowsky and Nagibina (1959), Bychowsky (1962), Lucky and Dyk (1964), Bovet (1967), Wiles (1968)). Work on the host specificity of <u>D.paradoxum</u> has been carried out by Halvorsen (1969) and Bovet (1967), both concluding that <u>D.paradoxum</u> is fairly host specific to the bream, but will also parasitise the roach (<u>Rutilus rutilus</u>) and the hybrids of the two species of cyprinid.

    The parasite has a direct life cycle. The eggs have a long spiral filament serving as an anchoring device either to the gills of the host or to the substratum or vegetation of the environment. A small free swimming ciliated larva, the oncomiracidium, hatches from the egg and is passively swept into the branchial chamber of the host (Bovet, 1967) and attaches to the gills. The larva once attached loses the ciliated coat and majority of sense organs and develops into a small larva called a diaporpa. The larva which is motile in a leech like fashion continues to grow on the gills of the host, but development is arrested when the anchoring apparatus reaches a certain stage. Further development is possible only when two larvae meet and fuse together. The fusion and development of the larvae have been described by Bovet (1967). The attached parasite is thus

composed of two fused individuals (Nordmann, 1832) which are united

in the middle region of their bodies. Each individual is attached by

four pairs of clamps to secondary lamellae of the host gills; the

functional adult is thus secured by eight pairs of clamps. The morphology

and functioning of the attachment apparatus has been described by Owen (1963a,

1963b). Wiles (1968) has described the occurrence of D.paradoxum in northern

England, and its distribution on the gills of various cyprinids including

the bream. The life span of the parasite seems to vary depending on the

host and habitat. However, Bovet (1967) reports a two year life span at

least for D.paradoxum on the bream in Switzerland.

Occurrence of other species of parasites:-

Seven other parasitic species which were also found during the examination of the monthly samples are listed below.

	Parasite	Number of fish infected (out of 360)	Site of infection	Time of occurrence
Protozoa	Myxobolus mulleri (Bulschli, 1882)	284	gills	All the year round
Monogenea	Dactyologyrus crucifer (Wagner, 1857)	8	gills	May, June
	Gyrodactylus elegans (Nordmann, 1832)	3	gills	May
Digenea	Diplostomum spathaceum (Rudolphii, 1819)	311	eye lens	All the year round
	Diplostomum clavatum (Nordmann, 1832)	318	vitreous humour of the eye	All the year round
Cestoda	Ligula intestinalis (Linnaeus, 1758)	2	body cavity	September
Annelidia	Psicola geometra (Linnaeus, 1758)	6	skin	June, August, September, December
Crustacea	Argulus foliaceus (Linnaeus, 1758)	19	skin	All the year round

<u>Discussion</u>:-

Although the parasite fauna of the bream from an enclosed aquatic environment has not been previously studied in England, the ten species of parasites found seemed fairly characteristic for the host, in type and number of species, when compared with other published literature from Eastern Europe. (Kosheva, 1957; Kozicka, 1951; Titova, 1957). Bream seem to harbour a variety of parasites in particular intestinal helminths due to the fishes' varied invertebrate diet, through which infective stages of the parasites are picked up. It is unusual, in comparison to other studies, that the bream examined did not have more than one species of intestinal helminth. However, this may have been due to the lack of suitable intermediate hosts for other parasite species within the environment. The very low incidence of <u>Ligula</u> <u>intestinalis</u> is also interesting since the roach (<u>Rutilus rutilus</u> (L.)) population in the same habitat was heavily infected. In Eastern Europe, bream have been reported to be the most common host of <u>L.intestinalis</u> (Kosheva, 1957; Schäperclaus, 1954), the parasite often causing considerable damage to young fish. The situation in Dagenham may have arisen because bream do not feed on the species of copepod harbouring the infective stages of <u>L.intestinalis</u>, while roach do.

<u>Caryophyllaeus laticeps</u>, <u>Diplozoon paradoxum</u> and <u>Myxobolus muelleri</u> seem fairly specific to the bream population and are not very common to other fish species in the habitat examined. This is unusual since it is difficult to justify reasons why these comparatively non host specific parasites (Dogiel, 1964; Baur, 1962; Chubb, 1970) are very host specific in this habitat. It seems likely that further work is required on the taxonomy of the above genera. <u>Diplostomum spathaceum</u>, <u>Diplostomum clavatum</u>, <u>Psicola</u> <u>geometra</u> and <u>Argulus foliaceus</u>, however, parasitised all the fish species present in the habitat.

SUBSECTION II   METHODS OF ANALYSIS

A number of parametric and non-parametric statistical tests were used in the analysis of the data. Most of the analyses were calculated using the London University C.D.C. 6600 Digital computer. Standard statistical programmes were obtained either from the I.B.M. System /360 Scientific Sub-routine Package (360A-CM-03X) Version III Programmer's Manual (referred to as S.S.P. programmes), or the Biomedical Computer Programmes (Dixon, 1967) (referred to as B.M.D. programmes). Statistical programmes not available from these sources were written in FORTRAN IV (McCracken, 1965; Veldman, 1967).

Transformation of raw data:-

Where there was a statistical relationship between the mean $(\bar{x})$ and the variance (s^2) of raw counts (x_i) a transformation of the raw data was carried out before using parametric statistical analysis. The method devised by Taylor (1961) was employed throughout this study to determine the relationship between the mean and variance and to calculate a suitable transformation. Taylor (1961) showed that the variance is proportional to a fractional power of the mean.

$$s^2 = a\bar{x}^{-b} \quad \text{where} \quad s^2 = \text{variance}, \ \bar{x} = \text{mean}$$

and a and b are constants.

By calculating the linear regression of the $\log_{10}$ of $\bar{x}$ and $\log_{10}$ of s^2 the value of b can be obtained from the regression equation

$$\log_{10}s^2 = \log_{10}a + b \log_{10}\bar{x}$$

The apparent variance stabilizing transformation function $\int (\bar{x})$ is of the form:-

$$\int (x) = Q \int \bar{x}^{-b/2} d\bar{x}$$

and the transformed value $z_i = x_i^{(1-\frac{1}{2}b)}$

A random sampling procedure was simulated within the computer (programme TAYP) to calculate sample means and variances. For the raw counts of the number of C.laticeps per host and of the number of D.paradoxum per host, from the total sample of 406 fish, 50 samples of 100 fish each were obtained by sampling with replacement. The means and the variances of these samples were calculated. For C.laticeps $Z = x^{-0.549}$ and since zero values of x were present the transformation used was $\dfrac{1}{\sqrt{x+1}}$.

For D.paradoxum $Z = x^{0.495}$ and the transformation used was $\sqrt{(x+1)}$.

The transformation for the number of C.laticeps per gut section and the numbers of D.paradoxum per gill section was $\dfrac{1}{(x+1)}$ and $\sqrt{(x+1)}$ respectively. All regression equations calculated in the above analyses were significant at the 1% level.

To test the efficiency of the calculated transformations the distributions of the residuals $r_i = Z - \bar{Z}_i$ were examined (programme RESID) by plotting the residuals calculated against their frequency. In all cases the residuals were not skewed and were evenly distributed around the zero value.

The fitting of theoretical distribution models:-

A programme TOPFIT (Reyna Robles, 1969) was used to fit theoretical distribution models to observed frequency distribution of the number of parasites per sampling unit. The methods used in fitting a wide range of theoretical distribution models are described in detail by the author.

Up to five theoretical distribution models were tested for goodness of fit to each observed frequency table depending on the variance/mean ratio. For ratios less than 1.0 the goodness of fit to the Binomial and Poisson distributions were tested. For ratios greater than 1.0 the Negative Binomial

(Bliss and Fisher, 1953), the Neyman A (Neyman, 1939) and the logarithmic series (Fisher, 1941; Fisher, Corbet and Williams, 1943) were tested for goodness of fit.

The χ^2 test was used to test the goodness of fit of the observed data to the theoretical distribution models and the criterion of grouping all expected frequencies of less than 5 was followed. However, in many cases frequencies were below 5 and an alternative χ^2 test was used employing these small frequencies (Cochran, 1954). Cochran has demonstrated that when the number of frequency classes is five or more one unexpected frequency as low as unity has negligible effect on the significance level of the test and may be safely omitted. This point is worth emphasising since it is often the case that the main difference between an observed and an un-expected distribution lies in the tails of the distribution and might be overlooked or blurred if categories were combined unnecessarily (Maxwell, 1961).

Such comparisons between actual and expected frequencies by χ^2 tests may be distorted by chance irregularities. Two alternative tests have been described (Anscombe, 1950). These tests are based on the differences between the actual and expected moments compared with their standard errors (Bliss and Fisher, 1953). In the case of fits to the Negative binomial distribution tests of T and U based on the second and third moments respectively have been used. The values of T and U are divided by their standard errors and tested as normal deviates.

The Analysis of Variance:-

Analysis of variance (S.S.P. programme Analysis of Variance)
the distribution of D.paradoxum and C.laticeps within their respective
micro environments. The programme performs a factorial analysis using
three special operators (Hartley, 1962). The analysis uses replicates as
a factor but since the replicates were always random samples from within
a category, e.g. age group, the sums of squares for replicate main effect
and replicate interaction with other factors were pooled to form the
error sums of squares. Interpretation of only the main effects and
second order interactions were attempted. The appropriate models used in
each analysis are dealt with in the relevant parasite sections.

Regression Analysis:-

a) Polynomial_regression:-   For the purpose of the empirical
description of trends in seasonal change of the parasite population size,
polynomial regression analysis has been applied to a number of sets of data.
The fourth degree polynomial was the maximum form used.

The polynomial function is of the general form
$$\hat{y} = a + bx + cx^2 + dx^3 + ex^4$$
where y = dependent variable, x = independent variable and a, b, c, d and
e are the regression coefficients calculated in the analysis. The S.S.P.
programme POLYNOMIAL REGRESSION was used in the analysis of the data. The
polynomial regressions calculated are empirical fits and generally no
structural meaning can be ascribed to the terms x^2, x^3 and x^4 nor to their
associated coefficients. The curve fitting procedure was stepwise and at
each step of increasing degree a significance best (F-ratios) was used to
test the improvement in fit. (Steel and Torrie, 1960)

b) Multiple_regression techniques:-   One of the major problems
in the analysis of the collected data, was that of defining the relation-
ships between the size of the parasite populations and two or more sets of

what were thought to be possible controlling factors. In statistical

terms this is the problem of defining the relations between a dependent

or criterion variable and a set of independent or predictor variables.

It is convenient to think of cause and effect, or predictor and criterion,

in terms of temporal succession in a given order, but it is not necessary

for the criterion variable to succeed predictor variables in time.

The method specifies a linear relationship between the observations

on each of p independent variables $x_1 x_2, \ldots x_p$ and the observations p + 1

the dependent variable Y

The general model is

$$Y_i = B_0 + B_1 x_1 + B_2 x_2 \ldots B_p x_p + \epsilon_i$$

where ϵ_i is a random error variate, and $B_0 \ldots B_p$ are partial regression

coefficients. The assumptions on which this model is based are well known

as is the method of estimating the B coefficients (Kendall, 1948; Steel

and Torrie, 1960; Draper and Smith, 1966). The solution is the best squares

fit of the coefficients to the observations and the regression surface.

Due to differences in the scales of the measurements used in this

study the variables, dependent and independent, were standardized to zero

mean and unit variance. The regression equation then becomes

$$\frac{Y_i - \bar{Y}}{S_y} = B_1 \frac{(x_1 - \bar{x}_1)}{S_1} + B_2 \frac{(x_2 - \bar{x}_2)}{S_2}$$

$$\ldots \quad B_p \frac{(x_p - \bar{x}_p)}{S_p} + \epsilon_i$$

where $\bar{x}_p$ = mean of variable p, S_p = standard deviation of variable p,

$B_1 \ldots B_p$ are the standardized partial regression coefficients.

The important assumptions in the multiple regression model are

often abused in biological analysis. Multiple regression is in essence

a univariate model, since only the dependent variable and ϵ are treated as

subject to errors. In biological situations often some or all the X's

are themselves random variables subject to errors of observation. It is

thus important to minimize error when measuring the X's. One of the

basic assumptions in the model is that the predictor variables are in-

dependent of one another (i.e. they are uncorrelated). This is rarely

true in biological analysis, due to the inherent complexities and inter-

actions of multivariate biological systems. If intercorrelations exist

one or more of the latent roots (λ) of the correlation matrix may be zero

or nearly so. Thus the determinant of the matrix approaches zero, introduc-

ing an element of instability which can be seriously misleading. For

example,in the case of three independent variables, if two latent roots

are nearly zero it means that two variables can be approximately expressed

in terms of the third. A correlation matrix with one or more very small

latent roots can be made to yield several solutions. The regression line

in this case is unreliable in the sense that a different sample or set of

observations might give entirely different coefficients. However, although

the coefficients may be different, the equations are nevertheless equally

efficient in predictive power. (Kendall, 1957).

Of importance in biological research is the "relative importance"

of the variables incorporated in the regression equation, in their

contribution to accounting for the variance of the criterion variable.

When significant intercorrelations exist between the supposedly independent

variables, misleading conclusions can be drawn about the influence of the

predictor variables. (Kendall,1957) Kendall goes as far as to say "no

reliance whatever can be put on individual coefficients in regression

equations embodying all the predictor variables."

Stepwise regression among other selection techniques has been

proposed by Efroymson (1962) and Draper and Smith (1966) as an improvement

on standard multiple regression techniques. The stepwise procedure uses

the simple correlation matrix and enters into regression the x variable

most highly correlated with the criterion (call this variable X_1). The

partial correlation coefficients of all variables not in the regression
are then calculated and the variable with the highest partial correlation
coefficient is then selected to enter regression (call this variable X_2)
The method then examines the contribution X_1 would have made if X_2 had been
entered first and X_1 second. A partial F-ratio test is calculated and if
it is significant X_1 is retained. The stepwise procedure then selects as
the next variable to enter, the one most highly partially correlated with
the criterion (Call this variable X_3, given that X_1 and X_2 have already
been entered into the regression). At this point partial F tests for X_1
and X_2 are made to determine if they should remain in the regression equation
and so on, until no more variables are removed or included in the regression.
The partial F criterion for each variable in the regression at any one stage
is compared with F of preselected probability. This method provides a
judgement on the contribution made by each variable as though it had been
the most recent variable entered, irrespective of its actual point of entry.
This selction procedure although an improvement over standard multiple
regression does not eliminate the problem of intercorrelated predictors
variables. Also from the point of producing purely predictive equations
the more variables included in an equation the better the predictive power,
assuming none have zero correlation with the criterion variable.

Cooley and Lohnes (1962) have further reservations concerning step-
wise regression. They point out that as the model of multiple regression
is essentially univariate, then any effort to generalize from sample to
population is open to serious danger of capitalization on chance variation,
particularly if it involves selecting some predictors and discarding others.
Johnson and Jackson (1959) have also expressed concern specifically about
the dangers of interpreting tests of significance of partial regression
coefficients, since the significance test values only apply in terms of
the fixed values of the predictor variables for which they were calculated,

in other words, from samples in which only a given range of X values occur.

Because of the criticisms of multiple and stepwise regression procedures mentioned above, another approach was adopted in the analysis of this problem. Kendall (1957) suggests that the use of principal components in multiple regression analysis might "throw some new light on certain old but unsolved problems; particularly (a) how many variables do we take? (b) how do we discard the unimportant ones? and (c) how do we get rid of multicollineorities in them?" This promising approach to the problem was applied and is described in detail below. It has been termed Orthogonalised Multiple Regression Analysis.

c) Orthogonalised Multiple Regression Analysis:- Kendall's method has been applied on few occasions (Jeffers, 1967; Alcock, Lovett and Machin, 1968) and the interpretation of the regression equations, in particular the regression coefficients, has not been developed.

Ths principle of this method of analysis is basically to replace the original correlated predictor variables with new orthogonal (uncorrelated) components. This is achieved by Principal component analysis (P.C.A.) The basic technique of P.C.A. is well described by Kendall (1957), Quenouille (1962), Seal (1964), Morrison (1967)and many others.

Component analysis seeks linear transformations of the type

$$Z_k = \sum_{j=1}^{p} a_{kj} x_{ij} \qquad i = 1, 2 \ldots n$$

$$j = 1, 2 \ldots p, \quad k = 1, 2 \ldots m \ (m \leqslant p)$$

where p = number of original variables, x_{ij} = the ith observation on the jth variate. The coefficients $\underline{a}$ are chosen so that the first new variate Z_1 has as large a variance as possible and the second variate Z_2 is chosen so as to be uncorrelated with Z_1 and to have as large a variance as possible and so on. In this way transformation to new uncorrelated variates which account for as much of the variation as possible in descending order is achieved;

The first one or two components may account for nearly the whole of the variation, perhaps as much as 85 or 90%. Thus it can be said that the variation is represented approximately by the first one or two components. These new axes are completely orthogonal to one another and thus may be used to replace the original predictor variables in the regression equation. A computer programme (ORTHREG) was developed to perform the analysis. The raw data was standardized as described previously and the latent roots of the correlation matrix were calculated by iteration, the computational details of which are given by Lawley and Maxwell (1963). Normalized latent vectors were calculated so that the sums of squares of the elements of a vector equal unity. These vectors were used to calculate the principal components, and to aid in the interpretation of these components in terms of the original variables; each combination of two was plotted by means of the CALCOMP plotting facilities at Imperial College computer centre. Examples of the interprestation of these components are given in the relevant parasite sections.

The mathematical procedures for the calculation of the regression equation incorporating the components are given by Kendall (1957). Principal component analysis is performed on the variables $x_1 \ldots x_p$ and new components $Z_1 \ldots Z_m$ are derived and used in the regression equation

$$y = \sum_{j=0}^{m} \alpha_j Z_j + \epsilon$$

where the α_js are linear functions of the standard partial regression coefficients (β). The Gauss-Markoff theorem then applies (Kendall, 1957) to give estimates of the α_js which are the same linear function of the estimates of the βs

Since all Z_js are orthogonal

$$\alpha_j = \frac{\sum y Z_j}{\sum Z_j^2}$$

Further, the reduction in variance due to the fitting of Z_j is $\alpha_j^2 \lambda_j$ where λ_j is the latent root corresponding to the principal component Z_j

Thus the value $\alpha^2_j \lambda_j$ is the square of the correlation coefficient between the dependent variable and the component Z_j, which measures the proportion of the variance of the dependent variable accounted for by principal component Z_j. Student's 't-test' can be used to test whether the α_j s are significantly different from zero; a simpler method is to test the significance of the correlation coefficient $\sqrt{(\alpha^2_j \lambda_j)}$ between the dependent variable and the component Z_j. This technique provides a method whereby significant components can be selected and incorporated into the regression. The products of the normalised latent vector V_i (where $i = 1.2 \ldots p$) and the value α_j for component Z_j are the orthogonalised standard partial regression coefficients b_i. The summation of these coefficients over the j components yields the standard partial regression coefficients B, which are identical in value with those produced by multiple regression analysis on the original variables. Thus the structure of these B coefficients can be studied.

To gain information about the "relative importance" of each predictor variable, only the significant components are incorporated in the orthogonal regression equation. The orthogonal standard regression coefficients are summed over the significant components and provide the regression weight for the new equation. From these coefficients, coefficients of separate determination d (Hope, 1968) can be calculated as follows

$$d_j = r_j \, b_j \qquad \text{where}$$

r_j = the correlation coefficient between the dependent variable and the jth independent variable. These coefficients d are thus the product of the b coefficient with the corresponding predictor- criterion correlation coefficient and are the predictor's contribution to the magnitude of R^2, the square of the multiple correlation coefficient for

that equation. They are thus a measure of the proportion of the
criterion variance which is predictable by that variable. It follows
that the sum of these coefficients of separate determination over the
j variables is equal to R^2. Hope (1968) pointed out that it is often
convenient to express a variable's coefficient of separate determination
as a proportion or percentage of R^2. However, when these d s are negative
absurd results are obtained.

To solve the problem of negative d. coefficients and to calculate
a quantitative estimate of the _effect_ of each independent variable in its
association with the criterion variable a new coefficient E is proposed,
"the coefficient of Effect". E is calculated as follows

$$E_j = \frac{\left| d_j \right|}{\sum\limits_{j=1}^{p} \left| d_j \right|} \qquad (j = 1, 2 \ldots p)$$

It must be stressed, however, that this coefficient is _not_ a
measure of the "relative importance" of each predictor variable in account-
ing for the variance of the dependent variable as in the coefficient of
separate determination.

When considering a particular multiple regression whether
orthogonal or not, care must be taken in developing conclusions about the
effect of the predictor variables in their prediction of the criterion
variable. It must be remembered that multiple regression maximizes the
value of R, the multiple correlation coefficient, for the particular set
of data to which it is applied. In applying the deduced regression
equation to a further set of data, the value of the correlation may well
drop considerably. Secondly and most important, correlation does not
necessarily support causation which is often implied in the biological
application of multiple regression analysis.

Thus the "coefficients of Effect" measure the <u>direct</u> or <u>indirect</u> absolute association between the predictor variable and the criterion variable. The direction of this association is not indicated; whether it is positive or negative is given by the sign of the correlation coefficient. Often in biological analysis the direction is not of primary importance, the main point of interest being whether a change in an independent variable is associated with a change in the response, or not.

To illustrate the misleading conclusions that can be drawn from multiple regression analysis, when compared with orthogonal regression, two examples are mentioned briefly in this subsection.

The first example concerns the regression of five weather factors on the mean number of parasites in a specific age group of hosts. Twelve observations on the total of six variables were made during one year and five components were extracted from the independent variable data matrix which accounted for 60.51%, 28.45%, 5.51%, 4.89% and 0.64% of the total variation respectively.

The fourth component was found to have a significant correlation with the dependent variable and accounted for 33.96% of the variation of this variable. Having calculated the coefficient of effect, the percentage effects of each original independent variable were calculated using the contributions from the significant component four (Fig. 2.1,D). The percentage effect in this histogram is labelled the percentage contribution to the dependent variable. Alternatively all the original variables were used in a separate analysis and the coefficients of effect and percentage effect of each variable calculated (Fig. 2.1C). A markedly different pattern of effects is produced in this histogram, primarily due to the inter correlations of the original but supposedly independent variables.

The second example concerns the regression of six host measurements on the number of parasites per host. Six components were extracted from data for the independent variables and accounted for 85.71%, 10.1%, 3.07%, 0.82%, 0.26% and 0.04% of the total variation respectively. The first principal component had a significant correlation with the dependent variable and accounted for 34.91% of its variation. The percentage effects of each of the original variables are shown in Fig.(2.1 A) and the percentage effects calculated from the regression of all the original independent variables are shown in Fig. (2.1 B).

The difference between the values of each variable in the two methods are again marked and illustrate the danger of interpreting the effects of inter correlated predictor variables when all the variables are used in a regression equation.

It is interesting to note that in the first example, the predictive power of the regression equation is considerably less when applying orthogonal regression, the R^2 value is 0.3396 compared with a value of 0.7330 for multiple regression. In the second example however, little reduction in predictive power occurs; the R^2 for orthogonal regression is 0.3491 compared to 0.3911 for multiple regression.

In conclusion the advantages and disadvantages of orthogonalised multiple regression are summarized.

1) The method provides flexibility in approach, different levels of significance can be selected for the entry of principal components into the regression.

2) The principal component analysis provides additional useful information on the basic structure and major sources of variation in the data.

3) The components used in the regression analysis are completely orthogonal.

4) The basic dimensions of the problem can be reduced by selection of the first few components which may account for the majority of the variation. This however must be done with caution since components which account for little of the total variation in the system of independent variables may account for the bulk of the variation of the dependent variable.

5) The proposed coefficient of effect provides a method of interpreting the absolute effects of the independent variables in their association with the dependent variable.

6) The coefficient of effect is <u>not</u> a measure of the relative importance of each predictor variable in accounting for the variance of the criterion variable.

7) Care must be taken in interpretation since correlation between predictor and criterion variables may often be due to another unmeasured factor operating in both.

8) For predictive purposes the selection of significant components, by reducing the quantity of information in the predictive equation, can reduce the predictive power of the equation. It is advisable for predictive purposes to incorporate as much information as possible.

This method of analysis would seem to have a wide application in many fields of ecological research.

Fig (2.1)   The interrelationships between the number of C.laticeps

per host and six host factors in January 1970.

A. The percentage "effects" of the independent variables

in their associations with the dependent variable.

(analysis incorporating all six components)

B. The percentage "effects" of the independent variables

in their associations with the dependent variable

(analysis incorporating the significant component 1)

The interrelationships between the mean number of

D.paradoxum per host for the fifth age group and the

weather factors.

C. The percentage "effects" of the independent variables

in their associations with the dependent variable

(analysis incorporating all five components)

D. The percentage "effects" of the independent variables

in their associations with the dependent variable

(analysis incorporating the significant component 4)

Diag. A

%
CONTRIBUTION
TO DEPENDENT
VARIABLE

100

50

0

1 2 3 4 5 6

INDEPENDENT
VARIABLES

Diag. B

100

50

0

1 2 3 4 5 6

1 - WEIGHT
2 - LENGTH
3 - GIRTH
4 - SEX
5 - MATURITY
6 - AGE

Diag. C

%
CONTRIBUTION
TO DEPENDENT
VARIABLE

100

50

0

1 2 3 4 5

INDEPENDENT
VARIABLES

Diag. D

100

50

0

1 2 3 4 5
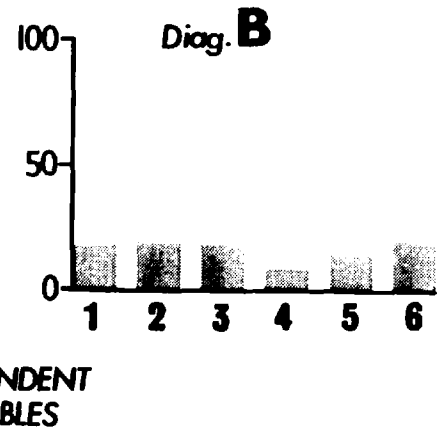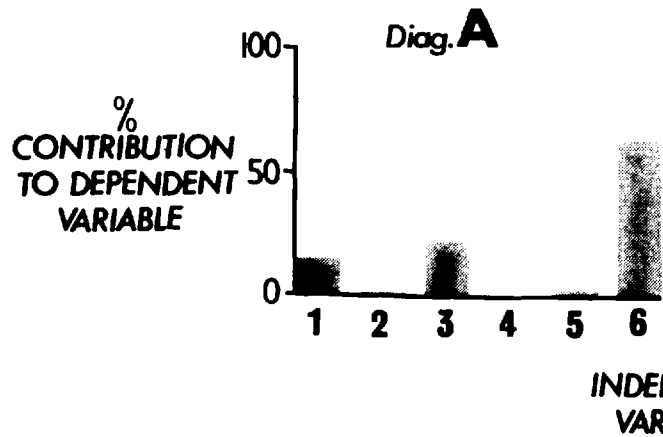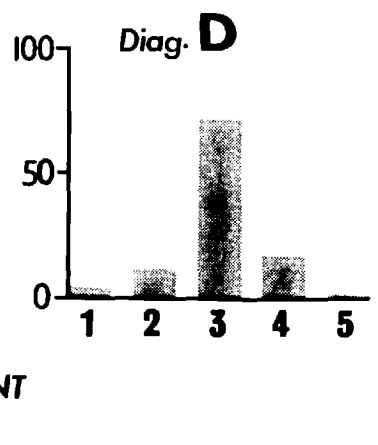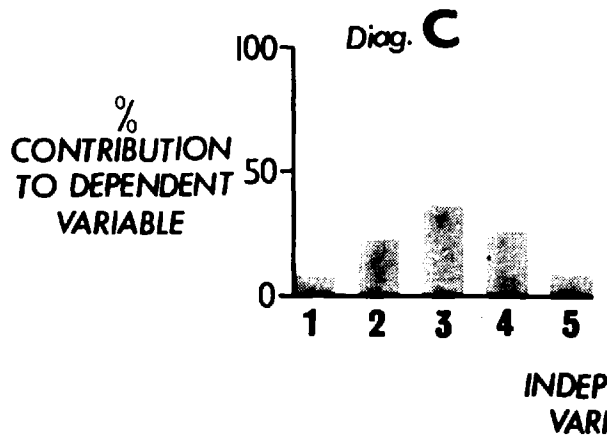
1 - RAINFALL
2 - SUNSHINE
3 - WATER TEMP.
4 - DAYLENGTH
5 - RANGE OF WATER
    TEMP.

SUBSECTION III   Analysis of the Ecology of Caryophyllaeus laticeps:-

The distribution of Caryophyllaeus laticeps, as an adult and larvae in its respective host population:-

In describing the distribution and estimating the abundance of parasites in their hosts, it is becoming increasingly evident that parasites of any one species are rarely randomly disposed within their host population. Most commonly the distribution of the number of parasites per host is such that the variance is in excess of its random expectation, the mean. Parasites are thus over dispersed or aggregated within the host population (Fisher,1941); Milne, 1943; Donald and Leslie, 1969; Kennedy, 1968; Crofton, 1971). This overdispersion within the host population reduces the precision of estimates of abundance and yields large standard errors. However, an observed over-dispersed pattern may provide information about the behaviour of the hosts and parasites concerned, and suitable mathematical models which describe this pattern may help in understanding the ecological principles involved.

Frequency distribution models provide a method of expressing the quantitative relationships between hosts and their parasites. Empirically frequency distributions provide a method of condensing the sample data so that any given population may be described by a few parameters which are readily comparable with the corresponding parameters of another population. Fundamentally, however, these distribution models are based on theoretical considerations of the biological processes involved. Cassie (1962) notes the following danger in applying fundamental models. "The fundamental model on the other hand is based on some hypothesis of real biological significance. If it fits the data better than other possible models, it provides some justification for the hypothesis concerned. This principle must however be used with caution, since the same distribution may often be generated by different and even contradictory sets of postulations. Ideally the empirical and fundamental models are the same." This problem is also noted by Crofton (1971).

The frequency distribution of C.laticeps in the bream:-

The sampling frequency data (Fig 2.2) of the numbers of C.laticeps per host for twelve months each consisted of random samples of five fish from each of six age groups. The observed distributions for each month were tested by chi-square for goodness of fit to seven discrete theoretical distribution models, namely the Poisson, Negative binomial, Neyman type A, Truncated poisson, Truncated negative binomial, Truncated Neyman type A and Fisher's logarithmic series; truncation implies the censoring of zero counts. Grouping of frequency classes was done when expectations were less than 5 but, when there were too few degrees of freedom for the standard chi-squared test, an alternative chi-squared test was used (Cochran, 1954).

In each month the negative binomial distribution provided the best fits to the data (Table 2.2). The comparison of observed and expected frequencies by chi-squared may be distorted by chance irregularities in individual counts. To guard against this danger two moment statistics, T and U were computed (Anscombe, 1950). The two moment statistics divided by their respective standard errors can be treated as normal deviates. They have the advantage that they take into account the few large values which are hidden by grouping the lower frequencies in the tails of the distributions. The deviates were not significant and thus confirmed the applicability of the negative binomial model.

The negative binomial distribution has been shown to be widely applicable to insect dispersion (Evans, 1953; Bliss and Fisher, 1953; Southwood, 1966). Fisher (1941) suggested that it may have importance in a wide variety of parasitological problems. Recently Crofton (1971) stressed the importance of this distribution, not only for the empirical description of data, but also for defining the ecological nature of parasitism.

The probability generating function of the distribution is

$$G(z) = p^k/(1-qz)^b \quad o < p < 1, \quad q = 1-p$$
$$o < k < \infty$$

The two parameters of the distribution, are k, a constant defining

the degree of aggregation (Waters, 1959) and μ the mean. As $k \to \infty$

the distribution tends to the poisson form and as $k \to o$ it tends to Fisher's

logarithmic series. Since k varies inversely with the degree of aggregation

it can be seen that C.laticeps is distinctly aggregated within the host

population, with contagion varying from month to month (Table 2.2). Methods

have been devised to calculate a common k for several samples (Bliss and

Owen, 1958) which can be used to apply a transformation to the raw sampling

data. However, a common k can only be applied when $\bar{x}$ and k do not vary

widely and could not be estimated for these sample data.

Although the negative binomial is a unimodal distribution (Anscombe,

1950) an idea of its versatility is indicated by the number of ways in which

it can arise, or be approximated (Anscombe, 1950); Southwood, 1966; Reyna

Robles, 1969). Possible models with reference to parasites in general and

to C.laticeps in particular will be discussed later.

Because samples were small (5 fish) it was not possible to test the

goodness of fit of the negative binomial distribution within each age group

for each month. However, for a sample of thirty 3[+] fish collected in March

1969, the negative binomial distribution provided the best fit (Table 2.3).

Thus, it seems improbable that the observed negative binomial distribution of

the monthly samples arises from the compounding of poisson distributions

with varying means for each age group of hosts.

The frequency distribution of the intermediate host and larval stages of

C.laticeps:-

To develop an understanding of the input of parasites into the fish

host and in order to derive a fundamental model for the negative binomial

distribution of C.laticeps the spatial pattern of the tubificid intermediate host was examined.

A frequency distribution was constructed from the number of tubificids in each of 123 samples of mud collected from the bottom of the gravel pit in April 1969. The negative binomial distribution provided the best fit (Table 2.4). The negative binomial distribution also provided the best model of the number of infected tubificids per sample (Table 2.4).

Analyses of aggregation may be affected by quadrant or sample size (Reyna Robles, 1969). Thus the sample date of infected tubificids was combined in groups of 2, 4, 6 and 8 samples. The negative binomial distribution fitted the 2 and 4 groups (Table 2.4). However, distributions could not be fitted to the larger groups possibly because of the low number of observations. The variance / mean ratios for the 6 and 8 groups were greater than 1.0 indicating contagion

To examine the distribution of infected samples groups of six samples were selected at random and a frequency distribution of the number of infected samples in each group of six was constructed. The group size of six samples was selected to give a possible total of seven stages of infection and sufficient degrees of freedom for the testing of distribution models. Since all samples contained tubificids it was not possible to use this approach to test the distribution of aggregates of tubificids (infected and non-infected). Thus a basic aggregate size of ten tubificids was selected based on the mean number of worms per samples of 15.38 (Table 2.4) and the distribution of aggregates greater than ten was tested.

Both frequency distributions of the number of samples with infected worms present and the number of samples with more than ten tubificids were tested for goodness of fit to a variety of theoretical distributions. In both cases the poisson distribution provided the best fits (Table 2.5).

Since over 90% of the tubificids had single infections of <u>C.laticeps</u>, the mean number of larvae per tubificid being 1.033, the distribution of larval tapeworms in the host population was not tested.

From the evidence presented above, it is possible to suggest a model for the spatial distribution of the intermediate host in the habitat and of infected tubificids within the population.

1) The tubificids are aggregated in the mud on the bottom of the gravel pit.

2) The aggregates of ten or more worms are distributed at random.

3) The infected tubificids are aggregated.

4) These aggregates of infected tubificids are distributed at random.

This is represented diagrammatically in Fig (2.3).

The observed clumping of infected tubificids could arise from aggregates of parasite eggs in the fish faeces falling to the bottom of the pond and thus tubificids immediately in the vicinity of the faeces, which can be regarded as a focus of infection, would be at a greater risk of infection than those some distance away. An alternative hypothesis of infected tubificids actively aggregating can be discounted.

Fundamental models of the negative binomial distribution

The negative binomial distribution of parasites in an individual host over a period of time, and in a host population observed at one instant in time or over a period of time can arise in a number of ways (Anscombe, 1950; Bliss and Fisher, 1953).

First, consider the ways in which a contagious distribution can arise in one individual host over a period of time.

1) Random exposures to infective stages over periods of time, with random input of numbers at one instant in time:-

When the mean, λ , of a poisson distribution varies with time, the combined distribution will be of the hetrogenous poisson type. A negative

binomial distribution with exponent k will result if λ has a Pearson

type III distribution. (Greenwood and Yule, 1920; Fisher 1941;

Feller, 1943; Reyna Bbles, 1969). In terms of parasitism this implies

that, if the exposure to infection is random in time and the number of

infective stages at each exposure has a poisson distribution, then a

compounded poisson distribution results. Thus, if one host was examined

at different time intervals, the distribution of counts of the number of

parasites per host would be contagious.

2) Contagious_exposure to_infective_stages over_periods_of time, with

random_input_of numbers at_one_instant_in time:-

When the number of exposures to infective stages is contagious in

time, and the number of infective stages at each exposure has a poisson

distribution, then an aggregated distribution of parasite numbers in a

host will result. A contagious input with time could result from a number

of biological situations. The feeding behaviour of a host, or the avail-

ability of infective stages may vary throughout the seasons of the year.

The exposure of the host to a parasite in a previous time interval may

affect the susceptibility of the host to exposure, or, for example, the

immune response of the host may vary with temperature which will also vary

with time.

3) Contagious_or random_exposure to_infective_stages over_a_period of

time_with contagious_input_of numbers at_one_instant_in time:-

Contagious input at one instant in time is by itself sufficient

to generate a negative binomial distribution. Thus whether input at one

instant in time was random or contagious would not effect the basic over-

dispersed nature of the distribution. (Tallis and Leyton, 1969) Contagious

input of infective stages could arise in two ways:

a) where an intermediate host contains a large number of infective

stages and is consumed by the definitive host, or

b) where the intermediate hosts are contagiously distributed, or the free living infective stages or eggs are aggregated.

4) Birth,_death_and_immigration_processes:-

Stochastic models of the population growth of parasites in an individual host during an interval of time could lead to a negative binomial distribution of parasite numbers within hosts. Simple models incorporating infection, loss and reinfection have been described by Kendall, 1948; Bartlett, 1960 and Bailey, 1957.

Discussion:-

It is relevant at this stage to consider the ways in which a negative binomial distribution of the number of parasites per host could be generated in a sample from the fish population.

Samples of thirty fish, five from each of the six age groups, were examined each month for twelve months. The observed numbers of parasites per host will have resulted from combinations of events in previous time intervals. The susceptibility of each host is probably varied by genotype, age and sex and also by previous exposures to infection. The numbers of parasites available for infection will vary with feeding site and behaviour of the host, and also month by month. Any of the conditions considered in (1) to (4) could lead to the observed negative binomial distribution. The distribution could also arise from the logarithmic distribution. If the infected hosts containing the parasite colonies are distributed at random, the parasites being distributed logarithmically, then the observed distribution of the number of parasites per host in the total host population would be negative binomial due to the generalization of a poisson distribution by a logarithmic series. (Quenouille, 1949; Skellam, 1952)

The distribution evidence for C.laticeps in the intermediate and definitive hosts, and the spatial distribution of the intermediate host suggests a fundamental model for the contagious distribution of parasites

in the fish host. It seems probable that contagious input of the
infective stages occurs because of the feeding behaviour of the fish and the
clumped nature of the infective stages in the mud. Once an infected tubificid
has been ingested the probability of ingesting other infected worms is in-
creased because of their aggregated distribution. As pointed out in (3)
above a negative binomial distribution could be generated purely from a
contagious input of larval stages. Although it has been shown that the
aggregates of infected tubificids are distributed at random and therefore
will be encountered randomly by a feeding fish, this would not affect the
outcome.

Thus aggregation of C.laticeps within the bream population could
be caused by the summation of a number of events which are summarized below:-

1) Contagious input of larval parasites.

2) Differing susceptibility to both parasite ingestion
and establishment in differing strata of the host population.

3) Differing susceptibility of the hosts to infection and
establishment in different months of the year.

4) Availability of infective stages varying with time.

The resultant observed distributions of C.laticeps over all the age
groups of hosts in monthly samples are the result of combinations of
hetrogeneous negative binomial distributions.

Variations in susceptibility between age groups, due to previous
infections and feeding behaviour, will be discussed later.

Table (2.2) Parameters and test statistics of the fits of the monthly counts of the number of C.laticeps per host to the Negative binomial distribution.

Months	1969 Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	1970 Jan
Total number of parasites/ 30 fish	49	77	77	92	58	70	31	21	32	11	22	44
mean number of parasites/ host	1.63	2.56	2.56	3.06	1.93	2.33	1.03	0.70	1.06	0.36	0.73	1.46
variance	5.55	31.75	22.52	22.40	13.09	13.40	6.24	2.01	7.37	0.58	2.27	8.33
variance/ mean	3.39	12.40	8.77	7.31	6.77	5.74	6.04	2.87	6.91	1.59	3.09	5.67
k_3	0.435	0.292	0.365	0.478	0.408	0.301	0.185	0.296	0.115	0.477	0.215	0.230
Alternative χ^2	3.84	5.81	8.59	2.32	3.65	4.82	0.64	0.24	0.25	0.27	1.01	2.99
d.f	5	6	5	7	5	5	4	3	4	1	3	5
Probability	.5	.3	.1	.9	.5	.3	.95	.95	.99	.1	.7	.7
T	-13.19	-157.76	-147.44	-100.56	-42.53	-70.42	-20.70	-3.06	-38.56	-.39	-5.29	-33.12
Normal deviate of T	-0.265	-0.385	-0.585	-0.460	-0.472	-0.241	-0.243	-0.274	-0.143	-0.372	-0.22	-0.234
U	-2.21	6.57	1.92	-0.33	2.00	-7.02	-0.56	-0.34	-3.54	-0.06	-0.96	-2.51
Normal deviate of U	-1.55	1.369	0.490	-0.908	0.889	-1.654	-0.258	-1.526	-0.832	0.344	-1.255	0.830

Fig (2.2) Histograms of the frequency distributions of the number

of C.laticeps per host.
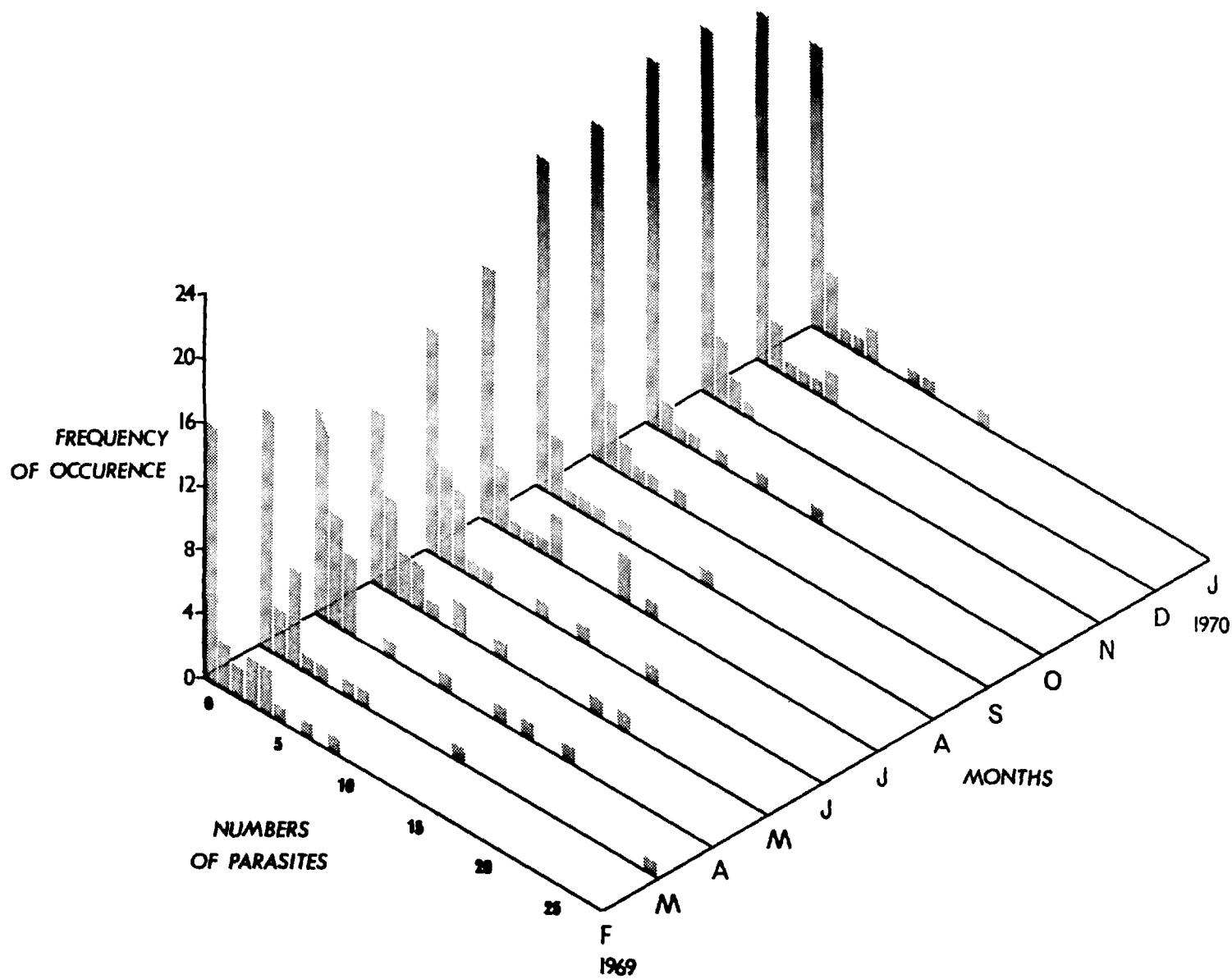
Table (2.3) The Negative binomial distribution applied to counts of
C.laticeps per host of the 3rd age group (March, 1969)

Total number of hosts	30
Total number of parasites	102
Mean number of parasites/host	3.4
Variance	17.48
Variance/mean	5.14
k_3	1.186
Alternative χ^2	8.83
d.f	8
Probability	.30

Table (2.4) Parameters and test statistics of the fit of the counts
of infected and non infected tubificids to the Negative
binomial distribution.

	No.of tubificids /sample	No. of infected tubificids /sample	No. of infected tubificids /2 samples	No. of infected tubificids /4 samples	No. of infected tubificids /6 samples	No. of infected tubificids /8 samples
Total number of samples	123	123	61	43	28	21
Total number of tubificids	1892	105	103	151	132	148
Mean number of tubificids /sample	15.38	0.85	1.69	3.51	4.71	7.05
Variance	155.37	1.29	2.35	11.92	7.54	25.85
Variance/ mean	10.10	1.51	1.39	3.40	1.60	3.67
k_3	1.96	1.41	4.92	1.43	6.14	3.05
χ^2	15.16	1.18	0.82	1.55	-	-
d.f	16	1	2	2	-	-
Probability	.50	.20	.50	.30	-	-
T	0.23	-0.63	0.14	16.86	-	-
Normal deviate of T	0.0003	-0.978	0.094	0.487	-	-

Table (2.5)   Parameters and test statistics of the fits of tubificid
               aggregates to the Poisson distribution. (two replicates
               A and B)

	Number of samples with infected tubificids present in total of 6 original samples.		Number of samples with more than 10 tubificids in total of 6 original samples.	
	A	B	A	B
Total number of samples	29	29	29	29
Total number of aggregates	85	85	92	103
Mean number of aggregates	2.93	2.93	3.59	3.55
Variance	2.99	2.71	3.04	2.90
Variance/mean	1.02	0.92	0.84	0.82
χ^2	1.13	0.62	2.04	2.30
d.f	2	2	2	2
Probability	.50	.70	.10	.10

Fig (2.3)   Diagrammatic representation of the spatial distribution

of tubificids infected with the larval stages of
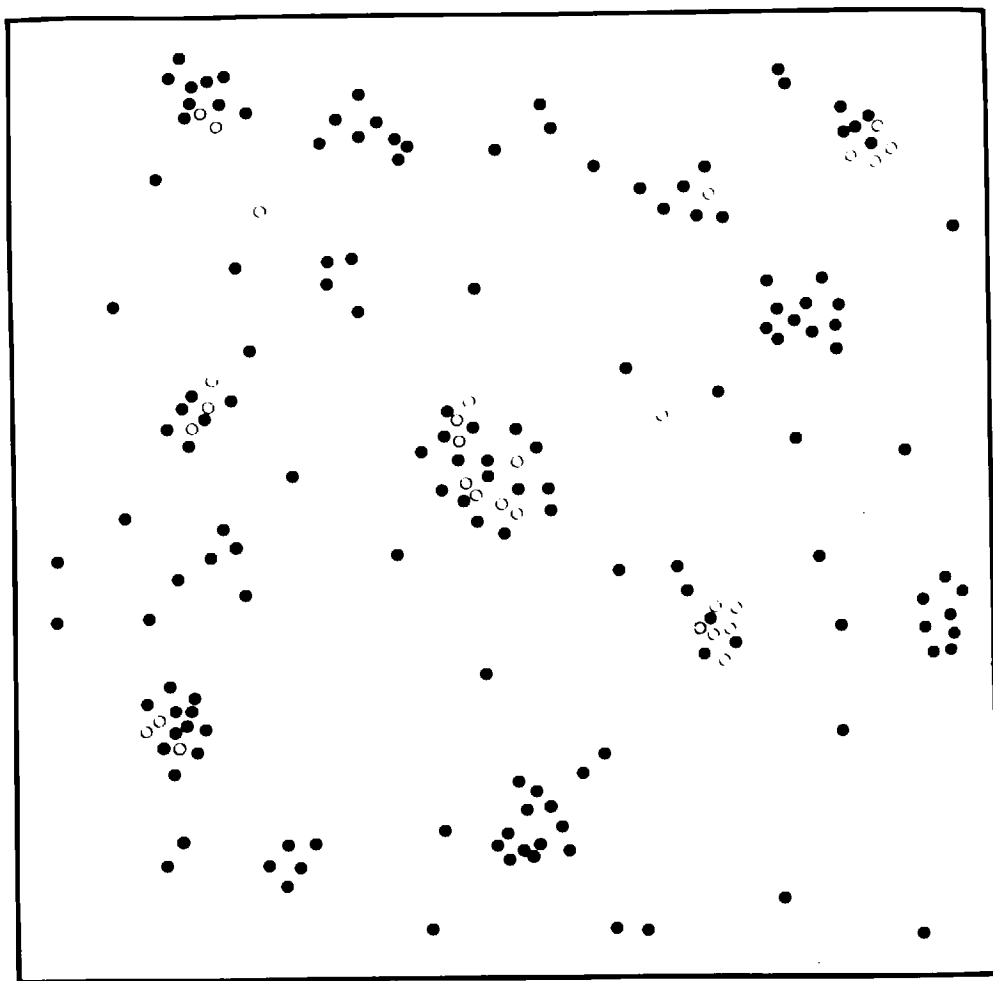
C.laticeps.

● TUBIFICIDS
○ INFECTED TUBIFICIDS

Table (2.6)   The monthly derived mean number of _C.laticeps_ per host, in each age group.

Age group	0	1	2	3	4	5	Age class 1	2
Month 1 Feb 1969	0.0	0.304	0.539	1.817	1.930	0.515	0.304	0.539
2 Mar	0.0	0.111	1.488	2.922	0.579	0.485	0.111	1.488
3 Ap	0.0	0.154	0.429	1.727	0.875	0.219	0.154	0.429
4 May	0.0	0.250	1.127	4.86	0.622	2.139	0.250	1.127
5 June	0.0	0.0	0.111	1.113	1.609	1.069	0.0	0.111
6 July	0.0	0.0	0.111	0.500	3.000	8.097	0.111	0.50
7 Aug	0.0	0.0	0.0	0.111	1.019	1.180	0.0	0.111
8 Sept	0.0	0.0	0.0	0.0	0.415	0.590	0.0	0.0
9 Oct	0.0	0.0	0.0	0.154	0.200	0.769	0.0	0.154
10 Nov	0.0	0.0	0.0	0.154	0.250	0.974	0.0	0.154
11 Dec	0.0	0.0	0.111	0.350	0.714	0.979	0.111	0.355
12 Jan 1970	0.0	0.111	0.395	0.350	0.818	1.95	0.395	0.350

Table (2.7)   The percentage of each age group of hosts infected with _C.laticeps_.

Age group	0	1	2	3	4	5
Month 1 Feb 1969	0	40	60	100	80	40
2 Mar	0	20	80	100	60	40
3 Ap	0	20	60	100	80	20
4 May	0	40	80	100	60	100
5 June	0	0	20	80	100	80
6 July	0	0	20	60	100	100
7 Aug	0	0	0	20	80	60
8 Sept	0	0	0	0	40	60
9 Oct	0	0	0	0	20	60
10 Nov	0	0	0	20	40	80
11 Dec	0	0	20	40	60	60
12 Jan 1970	0	20	40	40	80	80

The Population size structure and dynamics of C.laticeps:-

The population structure and dynamics of C.laticeps in the different age strata of the host population are examined: particular attention is paid to changes in the variations in intensity and incidence of infection between months. The intensity of infection is measured by the mean number of parasites per host and the incidence of infection by the percentages of infected hosts in the population. The structure of the parasite population in each month of the year is also examined in relation to density dependent and independent factors (Watt, 1968). Estimates of the population size and structure are also discussed. In this section emphasis is placed on trends of population change rather than on an explanation of minor observed fluctuations.

Intensity and Incidence of infection:-

Intensity and incidence of infection vary with age of the host and with time within age groups (Tables 2.6, 2.7). The mean number of parasites per host were derived from the back transformed mean of $\dfrac{1}{\sqrt{(x + 1)}}$, where x is the individual count. Since intensity and incidence of infection vary with age of the host and with time within age groups, the derived means were plotted against age group and month on a three dimensional scheme (Fig 2.4). Polynomial curves for each age group were used to smooth the data in order to aid interpretation (Table 2.8).

Polynomials were satisfactorily fitted to the younger age groups, but not to the 4th and 5th age groups. The fourth degree polynomial was considered adequate for the purpose of smoothing and higher degrees were not attempted. Curves for percentage infection (Fig.2.5) were smoothed by taking the running means of three points: the first and last points represent the averages of two data points. The original data is shown in Table

The trends of the curves of intensity and incidence of infection are very similar and illustrate a number of points:

1) The intensity of infection varies with the age of the host and the time of the year.

2) The 0 age group (equivalent to the 0^+ age class of fish are uninfected throughout the year.

3) Older fish harbour large numbers of parasites except in the early part of the year.

4) The peak of intensity of infection in the young mature bream occurs in the period February to April, where in the older mature bream the peak occurs in the period May to August.

5) The parasite is lost by the young immature fish in the summer months but is present in the fish of other age groups throughout the year.

The similarity between the curves of incidence and intensity requires further consideration. Since the negative binomial distribution is a good empirical model of the data the proportion of uninfected hosts, P_0, is

$$= \frac{1}{(1 + \frac{x}{k})^k} \qquad \text{(Bliss and Fisher, 1953)}$$

where $\bar{x}$ is the mean number of parasites per host and k is a parameter of the negative binomial distribution. Thus for a given k, Po decreases as the mean increases. Therefore an increase in the mean number of parasites per host increases the percentage infection in the host population. However, in

terms of incidence the peaks are less marked than for intensity, possibly indicating that although intensity decreases in some months, there is a proportionate decrease in the proportion of infected hosts but each retains fewer parasites. This difference is probably related to k, which is a measure of aggregation of the parasites. This and the possible biological explanations for the trends in incidence and intensity of infection will be discussed later.

It must be remembered that in the discussion above the patterns of infection in each age group of host and not in each age class have been considered. In June each age class passes into the next age category with the formation of the annual checks on the scales. It is not possible to examine the patterns of infection of C.laticeps in the older age classes which are grouped into the 4th and 5th age groups and are based on few individuals per age class. However, curves can be fitted to the fish born in 1966 and 1967. In 1969-70 the 1966 fish were the 2^+ age class before June 1969 and the 3^+ age class thereafter, while the 1967 fish were the 1^+ age class and 2^+ age class respectively in the same period. A fourth degree polynomial has been fitted to the derived means for each of the year classes (Table 2.6 and Fig. 2.6). These curves further indicate the drop in intensity of infection in young fish during the summer months, with a peak in the period February to April.

Table (2.8) Polynomial regression equations for the mean intensity of

infection of the bream with C.laticeps.

Age group of hosts		degrees of freedom	F.ratio
1	$y = 0.364 - 0.089x - 0.005x^2$	2/9	9.979**
2	$y = 0.149 + 1.191x - 1.250x^2 + 0.041x^3 - 0.001x^4$	4/7	3.616*
3	$y = 1.530 + 4.285x - 1.250x^2 + 0.125x^3 - 0.004x^4$	4/7	3.570*
4	$y = 4.205 - 3.431x + 1.120x^2 - 0.134x^3 + 0.005x^4$	4/7	1.881
5	$y = 2.860 - 3.810x + 1.626x^2 - 0.216x^3 + 0.009x^4$	4/7	0.802

Year class of hosts			
1966	$y = 0.460 - 0.247x + 0.072x^2 - 0.009x^3 + 0.001x^4$	4/7	7.834***
1967	$y = -0.161 + 1.185x + 0.376x^2 + 0.039x^3 + 0.001x^4$	4/7	2.970**

$$*** \quad P < .01 \qquad ** \quad P < .05 \qquad * \quad P < .10$$

where y = mean number of parasites/fish, x = month of the year:-

February 1969 = 1 ...... January 1970 = 12.

These dummy variates have been used throughout this study for all analysis

involving months.

Fig (2.4)   Mean intensity of infection of each age group of

bream with C.laticeps during the year February 1969 -
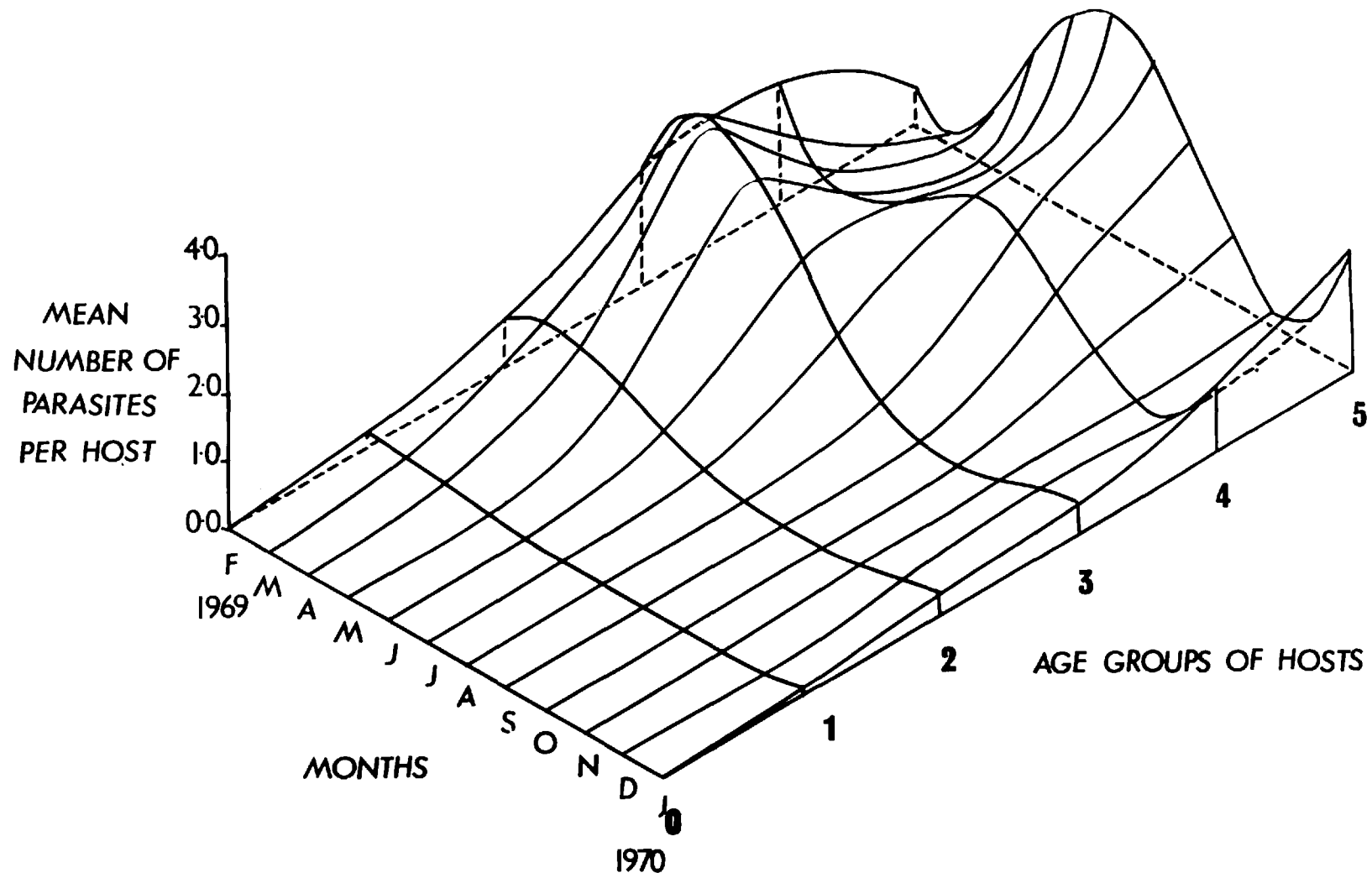
January 1970.

Fig (2.5)   Percentage of each age group of bream infected with

C.laticeps during the year February 1969 - January

1970.

PERCENTAGE INFECTION

100

50

0

F
1969 M
A
M
J
J
A
S
O
N
D
J 0
1970

MONTHS

1

2

3

4

5

AGE GROUPS OF HOSTS
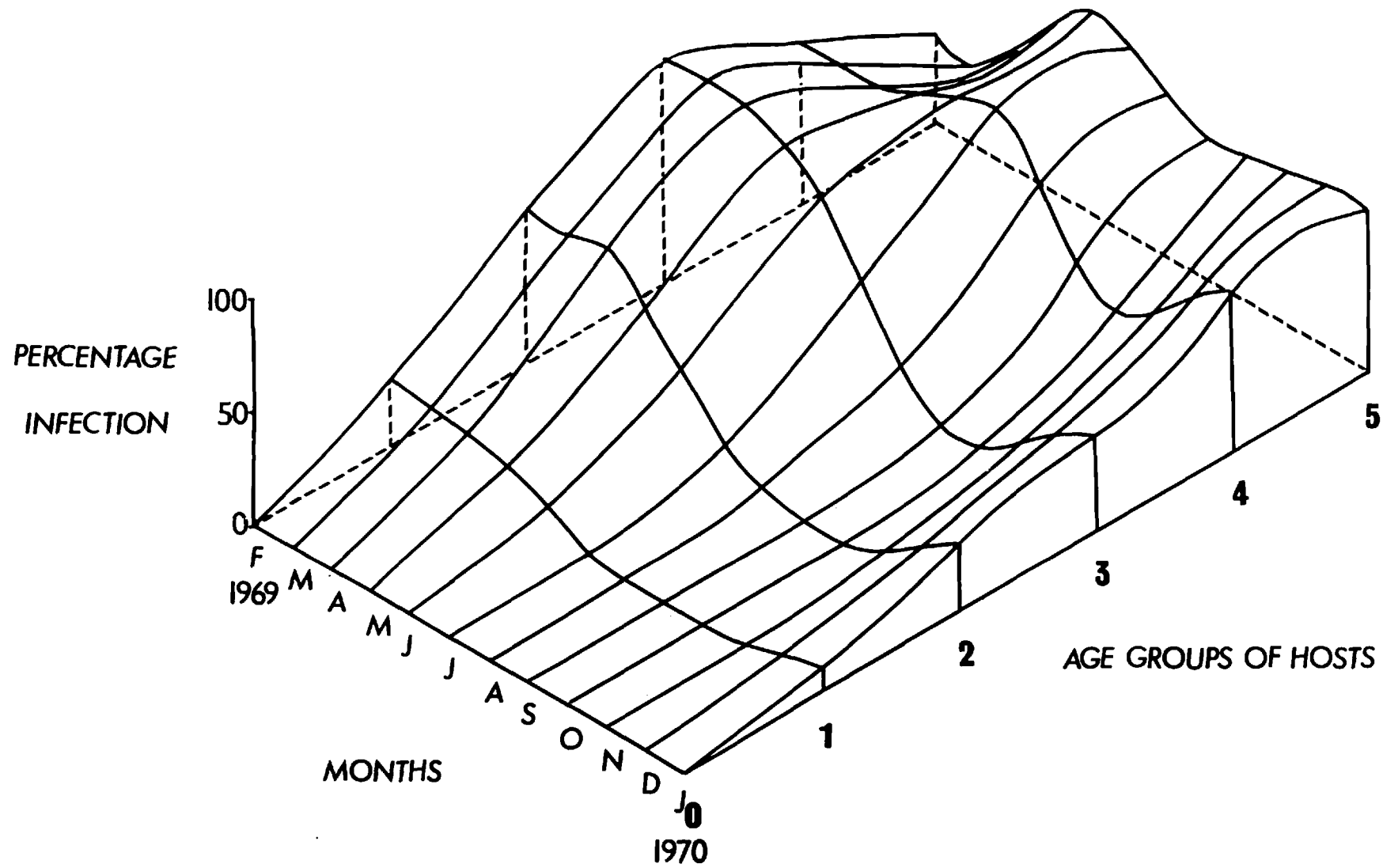
Fig (2.6)   Mean intensity of infection of the bream with C.laticeps

in the 1966 and 1967 year classes of fish during the

year February 1969 - January 1970.
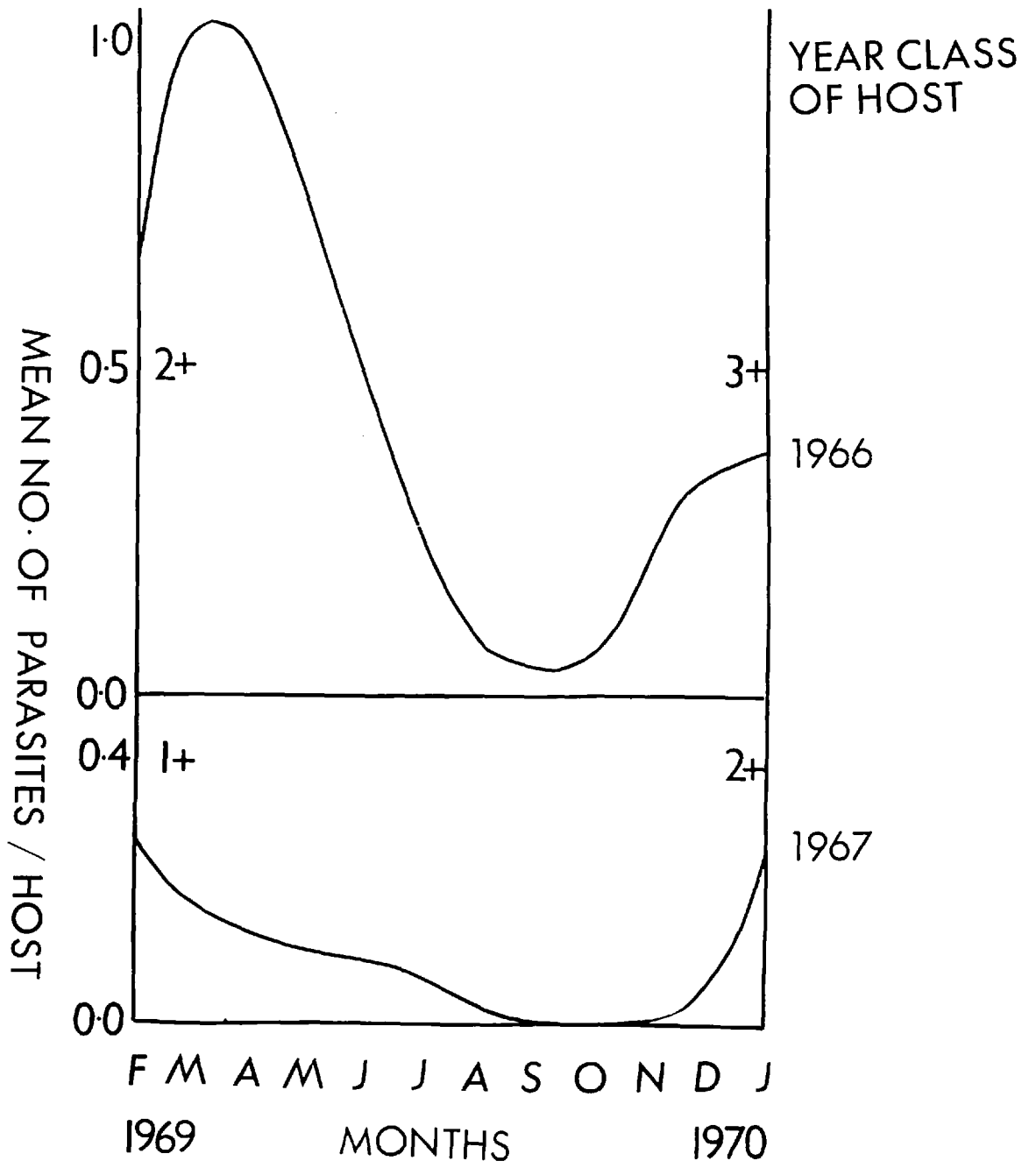
The population structure of C.laticeps:-

Because of the low numbers of parasites usually found in each fish, the total numbers of parasites in the unit sample of 30 fish examined each month were used in order to analyse the population composition of C.laticeps in the host. As described previously (p. 69) the cestodes encountered were counted and grouped into maturity classifications (Table 2.9 , Fig 2.7A). Fourth degree polynomials were fitted to the total number of worms (solid line)

$$y = 3.87 + 57.42x - 12.60x^2 + 0.8x^3 - 0.01x^4$$

$$(df = 4/7. \quad P(F = 13.257) < .001)$$

and to the total number of immature worms (broken line)

$$y = -5.74 + 32.78x - 6.52x^2 + 0.38x^3 - 0.004x^4$$

$$(df = 4/7. \quad P(F = 13.126) < .001).$$

The largest numbers of worms in the unit sample occurred from March to May 1969. The percentage of immature worms in the unit sample is markedly increased (Table 2.9) in the summer months. The significance of these data will be discussed later.

There is a clear indication that the number of mature worms increases linearly with the total (Fig 2.8D). However, the percentages of mature C.laticeps which become gravid are not related to the population size (Fig 2.7C). and the largest proportion of gravid mature worms occurred in the warm summer months. When these percentages are plotted against mean monthly water temperature (Fig.2.7D), the relationship between the proportion of egg producers and temperature is clearly seen.

Maturation of C.laticeps:-

Experimental infections of the fish were carried out in the laboratory to assess the time taken to reach maturity.

Methods:-

Twenty bream from a small lake near Chelmsford, Essex, were found to be free of C.laticeps. Twelve fish of the 4[+] age class from the same lake were assumed to be free of infection and used in the experiment. The fish were starved for two days, anaesthetized using M.S.222 and force fed by means of a syringe with eight tubificids, each infected with the late larval stages of C.laticeps and kept in tanks at $12^\circ C$. Three fish were dissected and examined for parasites every eight days until 32 days after infection. All parasites found were relaxed in cold water, fixed, stained and examined to assess their state of development.

Results:-

Days after infection	Number of parasites recovered	Number of mature parasites	Number of gravid parasites
8	14	0	0
16	16	0	0
24	9	1	0
32	5	5	2

It can be concluded that at $12^\circ C$, C.laticeps takes approximately four weeks to reach maturity which compares with maturation of C.laticeps in dace (Leuciscus leuciscus) (Kennedy and Walker, 1969), although the development time is slightly longer. Kulakowskaja (1962) also reported a maximum of 1.5 months for C.laticeps to develop to maturity in the fish host.

It is interesting to note that only 20.8% of the parasites entering the fish survived to reach maturity. Unfortunately time and labour did not allow repetition of these experiments under different temperature conditions. It is probable, however, that the time to develop to maturity is dependent on water temperature. Further work is required on this aspect since information concerning the developmental rate of adult helminth parasites in fish hosts is not available in the literature. However, there is in-vitro

evidence that temperature plays an important part in maturation of tape-worms (Smyth, 1952; Meyer and Vik, 1963; Hilliard, 1959). It has also been reported that cestode eggs have an increased rate of development at higher temperatures (Guttowa, 1958).

The difference between the total number of immature worms in the unit samples at time t, i.e. N_t, and the number of mature worms at time t + 1, N_{t+1}, where the unit of time is one month, is an estimate of the number of parasites lost from the population during one time interval. Thus $\frac{N_{t+1}}{N_t}$ is an estimate of survival rate. (Table 2.9). These estimates will be subject to sampling error and to error due to differences in development rate to maturity in each month. There is no clear relationship between N_{t+1} and N_t (Fig 2.8A). However, two groupings of monthly estimates occur. First for the cold winter months (February to April and November to December) and second for the warm summer months (May to October). The members of these groups appear to fall on two separate lines with survival rate constant for each, since N_{t+1}/N_t estimates the slope of the line when the intercept is zero, with temperature varying the slope of the line. The relationship between survival rates and temperature is clarified when N_{t+1}/N_t is plotted against water temperature (Fig. 2.8B) and against month (Fig. 2.7B).

The fourth degree polynomial fitted to the N_{t+1} N_t by month data, is

$$y = 0.255 + 0.393x - 0.186x^2 + 0.02x^3 - 0.001x^4$$

$$(df = 4/6; \quad P(F = 8.57) < .01)$$

and the linear relationship between N_{t+1}/N_t and mean water temperature is

$$y = 0.649 - 0.02x \quad (df = 1/9; \quad P(F = 30.32) < .001)$$

The critical temperature affecting the survival rate seems to be between 10 and 15°C which coincides with a separation between the two groups (Fig. 2.8B). Although the linear regression accounts for a significant proportion of the variation in y, the ratio N_{t+1}/N_t, a curve of the form shown in Fig (2.8B), might be expected. Fig (2.8C) is a plot of the input of

parasites at time t against the loss or output in the interval t to t+1.
For loss rates (input versus output, line (a) Fig. 2.8C) the linear
regression for warm months is

$$y = 0.92x - 1.96 \ (df = 1/4; \ P(F=791.5) < .001)$$

and for the cold months (line (b), Fig. 2.8C) is

$$y = 0.59x - 1.60 \ (df+ 1/3; \ P(F=2343.9) < .001)$$

The two regression coefficients are significantly different
$(df=1/7; \ P(F=73.26) < .01.$ Thus the relationship between input and output
of C.laticeps is linear, but two separate relationships exist for the warm
and cold months of the year. The evidence presented so far indicates the
importance of the density independent factor temperature in controlling
the size of the parasite population in the bream.

A constant development rate has been assumed in this discussion but
it is reasonable to assume that the time taken to reach maturity will be
less than one month during warmer periods. Thus more than one generation
of parasites would pass through the host between sampling periods, which
would lead to a loss of synchronization between the estimates of N_t and
N_{t+1} in the monthly unit samples. However, the input of immature parasites
during the summer months is still relatively large in relation to the total
number of parasites. This is further illustrated by the percentages of
immature parasites in the total samples, which are largest during the
period June to September (Table 2.9). Thus, even though the time taken
to mature is likely to be less than one month, the loss rate is still very
high at this time of the year. Therefore the survival rates, although not
precisely determined, do drop during the warmer summer months of the year.
(Fig. 2.7B). Thus it seems probable that both developmental and loss
rates of the parasite are affected by temperature. From the data collected
it is not possible to deduce the precise relationship between water temperature
and survival of the parasites to maturity. Possible biological mechanisms
causing a temperature dependent loss rate will be discussed later.

Table (2.9) The structure and size of the population of <u>C.laticeps</u>
in unit samples of 30 fish per month.

Month	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan
Total number of parasites	49	77	77	92	58	70	31	21	32	11	22	44
Number of immature parasites	22	36	38	53	35	39	18	15	15	6	14	20
Number of maturing parasites	23	30	23	22	16	26	9	4	13	1	4	17
Number of mature parasites	4	11	16	17	7	5	4	2	4	4	4	7
Number of gravid parasites	1	4	7	6	5	4	4	2	2	1	2	2
Percentage of mature worms gravid	25.0	36.3	43.7	35.2	71.2	80.0	100	100	50.0	25.0	50.0	28.6
Ratio N_{t+1}/N_t	0.50	0.44	0.45	0.13	0.14	0.10	0.11	0.27	0.27	0.66	0.50	-
Percentage of immature worms in total	44.8	46.7	49.3	57.6	60.3	55.7	58.1	71.4	46.8	54.5	63.6	45.4
Percentage of mature worms in total	8.2	14.3	20.8	18.5	12.1	7.1	12.9	9.5	12.5	36.1	18.2	15.9

The pattern of infection with C.laticeps in the intermediate host
population:-

The mean numbers of tubificids per sample of mud in the bimonthly
collections shows a marked drop in the mid summer period (Fig.2.9C).
This drop corresponds with the annual breeding period of the tubificid
Tubifex tubifex, following which large numbers of young worms were found
in the months of July and September. The adult tubificids seem to die
after one year and on completing reproduction. Similar annual cycles in
tubificid populations have been noted by Kennedy (1969) and Brinkhurst and
Kennedy (1965). Throughout the year little variation in the percentages
of infected tubificids is observed (Fig. 2.9D). The vast majority of
hosts harboured single infections ($\bar{x}$ = 1.033). It is possible that
multiple infections are lethal to either C.laticeps or to the tubificid.
It is interesting to note that a decline in the population of the inter-
mediate host, measured by the mean number of worms per sample, is not
associated with a decreased incidence of infection of the tubificids
with larval C.laticeps.

The size of the parasite population

It is possible to estimate the total numbers of parasites in each age group of the host population from the population estimates of each age group of hosts in February 1969 and 1970, and the mean number of parasites per age group in February 1969 and January 1970. (Fig 2.9A and B).

By interpolating between the two host estimates and using the intensity figures for August 1969 it is possible to estimate the parasite population for that month (Fig. 2.9A and B). This is only a rough guide since the fish mortalities may have been spread non uniformly over the year and no independent observations of this effect have been made.

These estimates illustrate a marked drop in the parasite population size (63.7%), corresponding with the drop in the host population (46.8%) during the year. From these figures it is possible to suggest that the parasite population size is dependent on host population size, but a number of years data are required to determine the density dependent relationship between parasite and host.

Fig (2.7)   A: Solid line - Seasonal trend of the total numbers of

C.laticeps present in each unit monthly sample of 30

fish. Broken line - Seasonal trend of the numbers of

immature C.laticeps present in each unit monthly sample

of 30 fish.

B: Survival rate of C.laticeps in each month of the

year (N_{t+1}/ N_{t}); unit time interval = 1 month.

C: The percentages of mature C.laticeps gravid in the

unit monthly samples.

D: The relationship between percentage of mature

C.laticeps gravid and water temperature. (1 = February

1969 ..... 12 = January 1970).
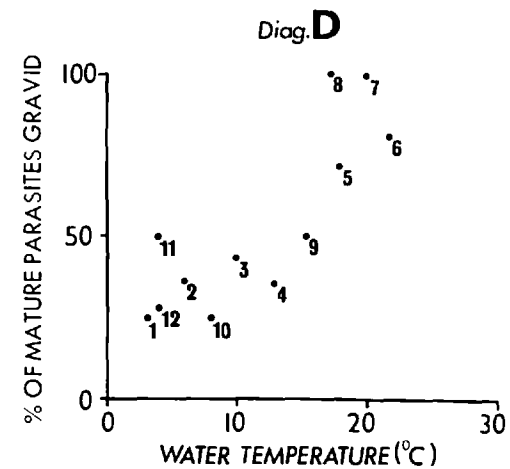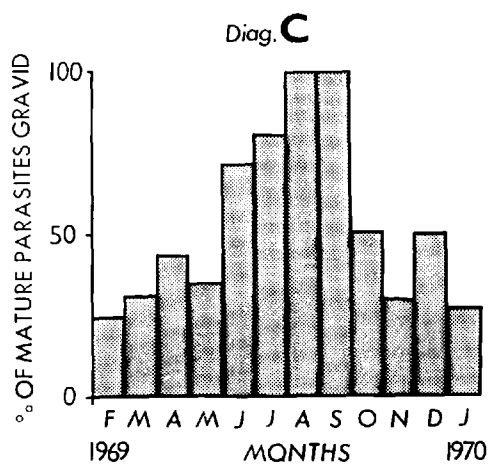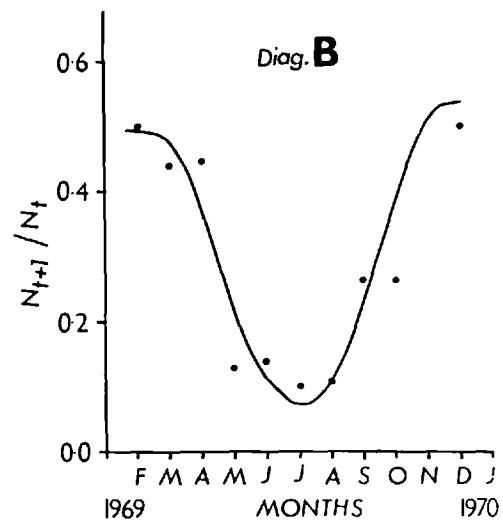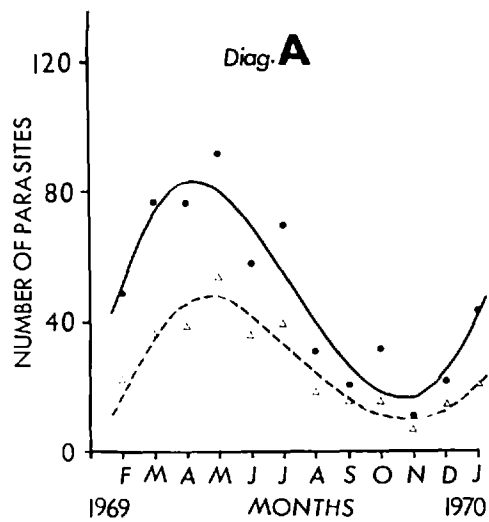
Fig (2.8)   <u>A</u>: The relationship between number of <u>C.laticeps</u> at time

t+1(N_{t+1}) and the number at time t(N_t) where a unit time

interval = 1 month.

<u>B</u>: The relationship between the survival rate of

<u>C.laticeps</u> (N_{t+1} / N_t) and water temperature.

<u>C</u>: The relationship between number of immature <u>C.laticeps</u>

at time t (INPUT) and number of mature parasites at time

t+1 (OUTPUT).

<u>D</u>: The relationship between the number of mature

parasites and the total number of <u>C.laticeps</u> in the unit

monthly samples.

(1 = February 1969 ...... 11 = December 1969)

Diag. A

Diag. B

Diag. C

Diag. D
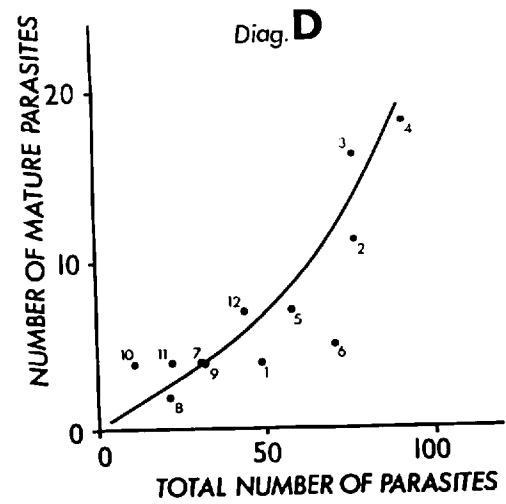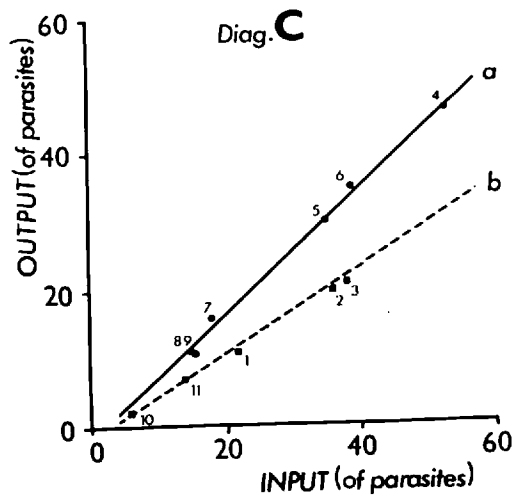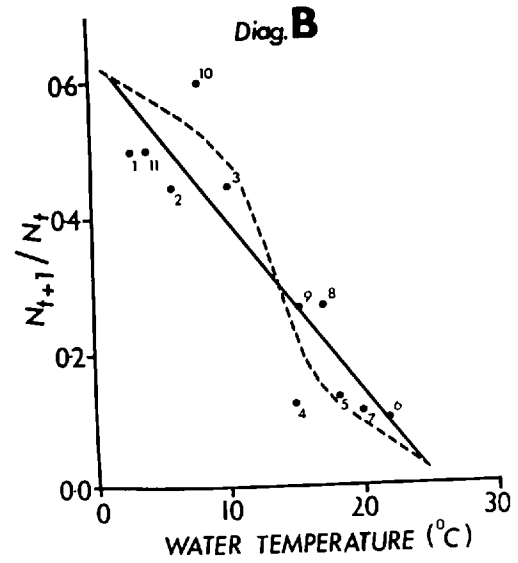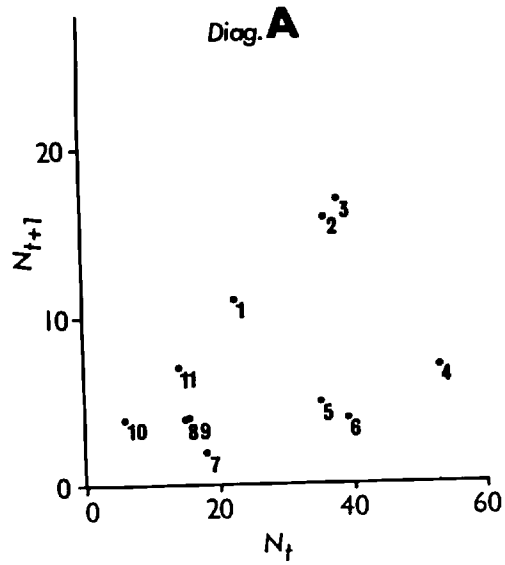
Fig (2.9)   A: Percentage of estimated population of C.laticeps

in each age group of fish.

B: Population estimates of C.laticeps in each age

group of hosts.

C: The abundance of tubificids during the year

February 1969 - January 1970.

D: The percentage of the tubificids infected with

larval stages of C.laticeps.

Diag. A

% OF PARASITES IN AGE GROUPS OF HOST POPULATION

100

50

0

AGE GROUPS OF HOSTS

1 2 3 4 5

Diag. B

NUMBER OF PARASITES

2000

1000

0

AGE GROUPS OF HOSTS

1 2 3 4 5

Feb. 1969

Aug.

Feb. 1970

MONTHS

Diag. C

MEAN NO OF TUBIFICIDS SAMPLE

16

12

8

4

0

Diag. D

% OF TUBIFICIDS INFECTED

20

15

10

5

0

Feb Apr Jun Aug Oct Dec

1969 1970

MONTHS
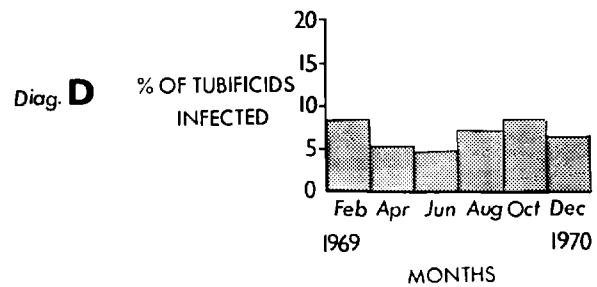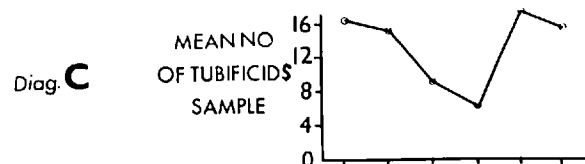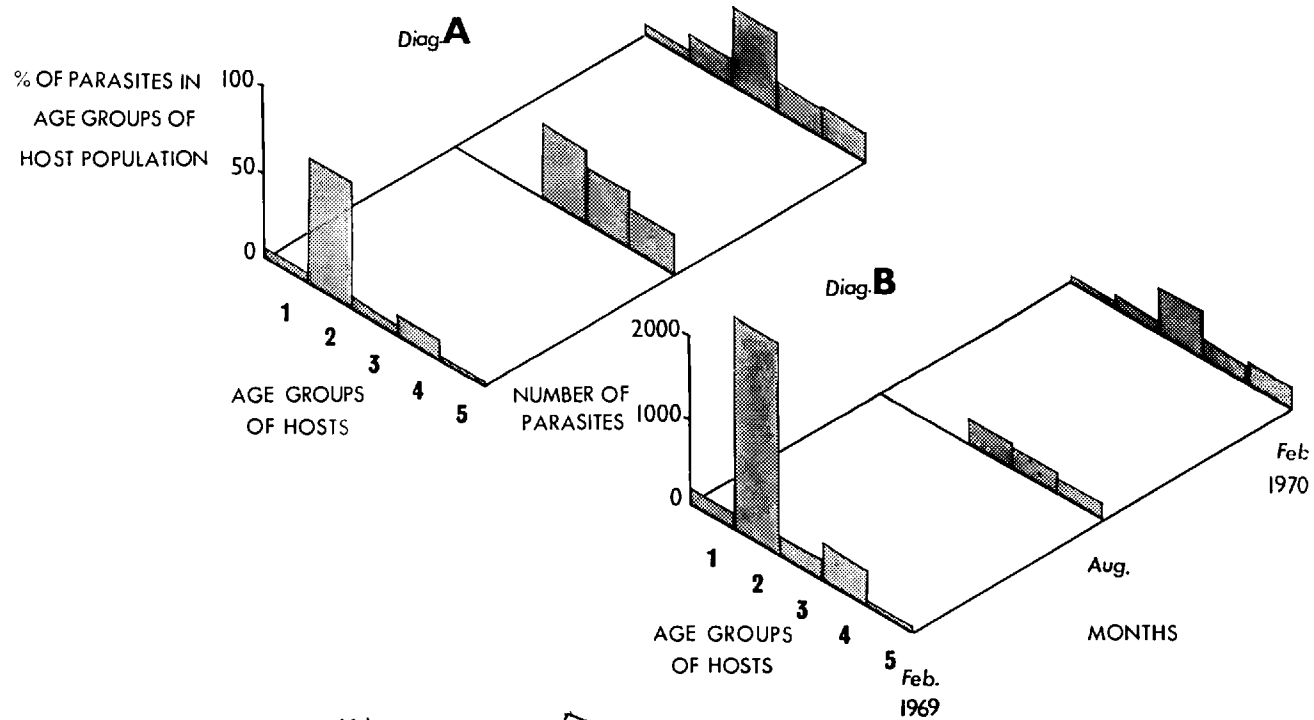
The ecological niche of the parasite within the host:-

Bream have no stomach, the food being crushed by a grinding pharynx before being passed into the long intestine. C.laticeps attaches to the villi on the walls of the intestine by the scolex and the body of the tapeworm hangs free in the lumen of the gut. The position of each parasite scolex was recorded as occurring in one of the 24 sections of the gut (see p 69). For the purpose of representation, the number of sections was reduced to a total of six and the mean number of parasites per section for each age group of fish in each month of the year was plotted (Fig. 2.10).

The majority of parasites were attached in the first two sections at the anterior end of the intestine but these were mainly immature stages: 72.8% of the immature parasites were found in sections one and two. The majority of mature parasites (64.3%) were found in sections three and four. It is in section three that the bile ducts secrete their products into the lumen of the intestine.

The data were analysed as a factorial design. The model for the analysis, to represent the data X_{ijkl}, is as follows where X_{ijkl} = the transformed number of parasites per gut section. $\sqrt{}$Sections = S, Replicates = R, Age groups = A, Months = M, where they have a, b, c and d levels respectively. Thus i = 1...a, j = 1...b, k = 1...c, l = 1...d$\underline{7}$ μ = overall mean.

$$X_{ijkl} = \mu + S_i + R_j + A_k + M_l + (SR)_{ij} + (SA)_{ik}$$
$$+ (SM)_{il} + (RA)_{jk} + (RM)_{jl} + (AM)_{kl}$$
$$+ (SRA)_{ijk} + (SRM)_{ijl} + (SAM)_{ikl}$$
$$+ (RAM)_{jkl} + (SRAM)_{ijkl} + \epsilon_{ijkl}$$

ϵ_{ijkl} is the error variation within a particular factor level. In the analysis summary the error mean square is calculated from the sum of the replicate interactions plus the replicate main effect $+ \epsilon_{ijkl}$

Thus error mean square E

$$= R_j + (SR)_{ij} + (AR)_{jk} + (MR)_{jl}$$

$$+ (SRM)_{ijl} + (SRA)_{ijk} + (RAM)_{jkl}$$

$$+ (SRAM)_{ijkl} + \epsilon_{ijkl}$$

The raw data were transformed using $\dfrac{1}{\sqrt{(x+1)}}$

Table   <u>Factorial analysis of the number of C.laticeps in the fish gut</u>

Factor A = Sections of the gut   levels = 24

Factor B = Age groups of hosts   levels = 6

Factor C = Months of the year   levels = 12

Factor	Degrees of freedom	Mean square	F-ratio	
A	23	.18310	9.7497	***
B	5	1.10370	58.7699	***
AB	115	.03766	2.0053	***
C	11	.21209	11.2934	***
AC	253	.03072	1.6357	**
BC	55	.15835	8.4318	***
ABC	1265	.01486	0.7013	
Error Term	6912	.01878		
Total	8639			

*** = P $<$ .001

** = P $<$ .01

It can be seen from this analysis that there are significant differences between the levels in each factor. This was to be expected for months, since large numbers of parasites are present in the mature fish in the summer, while the converse is true for the immature fish. Similarly a significant difference between sections is also to be expected, due to the active selection by the parasites for the optimum position in the micro-environment; this will be closely related to the physio chemical

conditions in each section of the gut. The mature worms selecting the mid section is perhaps correlated with the entrance of the bile ducts in this region of the intestine. The significant difference between age groups is also to be expected since older fish feed more extensively on the benthic invertebrates, thus acquiring larger numbers of parasites, and have larger guts thus providing a large area for attachment for C.laticeps.

The interaction terms are however more complex. The interaction term between sections and age of host is significant. This result is surprising since it was not expected that the selection for an optimum site would alter with age of the host. This may be related to the change in feeding habits with the age of the host. The interaction between months and sections is also significant perhaps due to the seasonal variation in the activity of digestive enzymes in the gut as demonstrated by Ananichev (1959) in the bream.

It is illustrated by the analysis and Fig. (2.10) that C.laticeps has a preference for particular regions of the gut. The backward migration of the parasite, as indicated by the percentage of immature and mature worms in the first four sections of the gut, is unusual. Hopkins (1959) demonstrated that Proteocephalus filicollis in Gasterosteus aculeatus (L.) migrate forward in the intestine, immature worms favouring the anterior part of the fish gut. Archer and Hopkins (1958) showed the same phenomenon in Diphyllobothrium latum in rats. The position of the adult C.laticeps must be regulated by specific preferences for intestinal regions controlled by the physiochemical conditions of the gut (Read, 1950).

Fig (2.10)   The spatial distribution of C.laticeps in the bream

intestine (mean number of parasites per gut section)

in each age group of hosts for each month of the year.

MONTHS
COMBINED

MEAN
NO. OF
PARASITES

SECTIONS
OF THE GUT

AGE GROUPS

AGE GROUPS

AGE
GROUPS
COMBINED

MONTHS

1969

1970
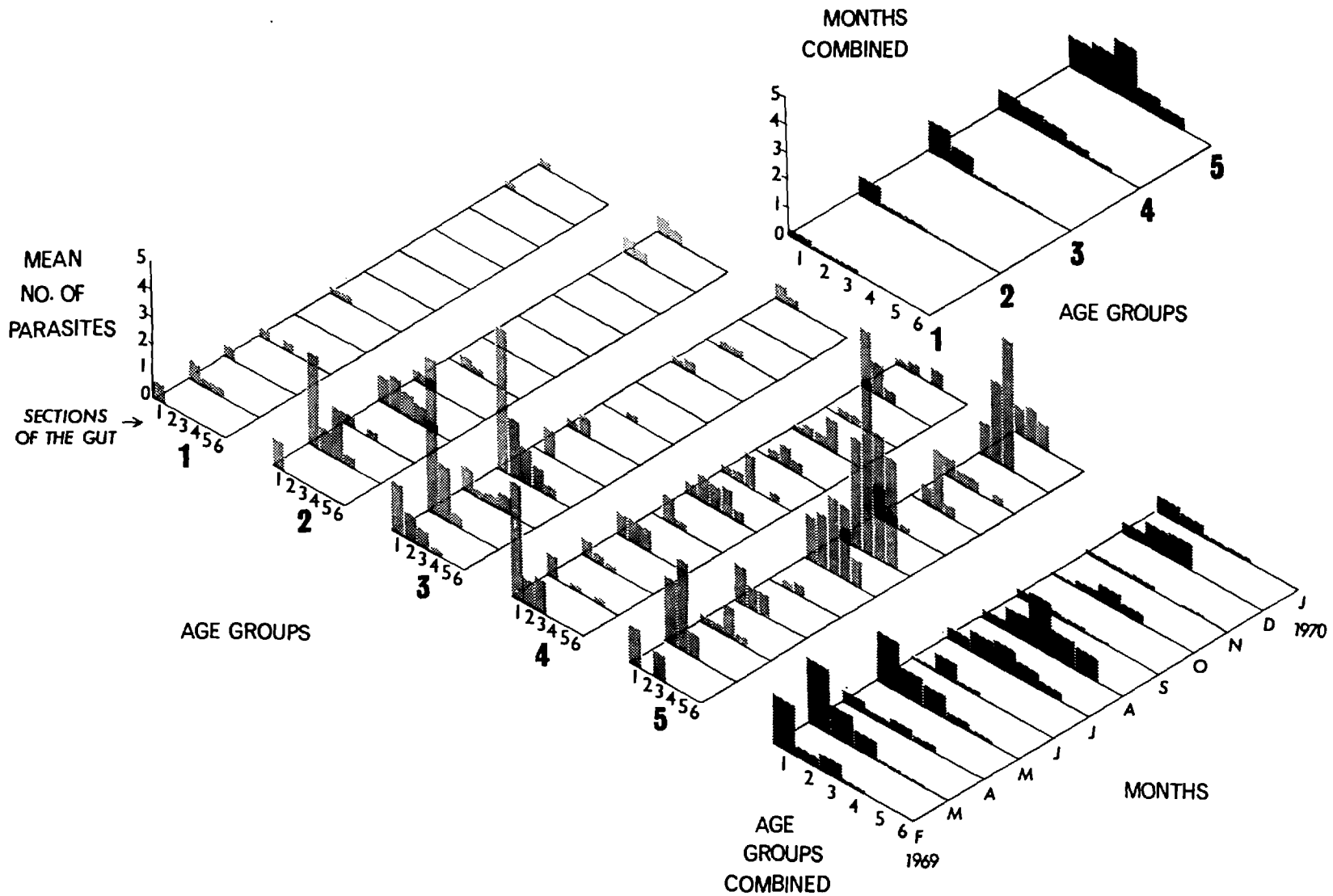
The inter relationships between the number of C.laticeps per fish and host and weather parameters

It has been shown in this study that the intensity of infection with C.laticeps varies with the age of the host. This relationship is now examined in more detail by the use of orthogonalised multiple regression. The computational details, methods of interpretation and advantage of this method over the more standard techniques of multiple and stepwise regression are discussed earlier (p 79 to 82). This method was also used to investigate the relationships between a number of local meteorological factors and the mean number of parasites per fish in each age group of hosts. Throughout this account of the results of orthogonalised regression analysis the number of parasites per host or the mean number of parasites per age group of hosts will be referred to as the dependent or criterion variable, whilst the groups of host and weather measurements will be referred to as the independent or predictor variables.

The aims in these analyses are threefold:-

1) To determine whether a significant amount of the variance of the dependent variable could be accounted for by the measured independent variables.

2) If a significant amount of variation had been accounted for, to produce predictive linear equations in terms of the criterion variable y and the n predictive variables $x_1 \ldots \ldots x_n$.

3) To arrive at tentative conclusions concerning the effects of each independent variable in its association with the dependent variable.

Care must be taken in the interpretation of results due to the inherent difficulties of using correlation and regression techniques. These are discussed on page

<u>Host features and parasite numbers.</u>

The host features used in this analysis were as follows:-

Variable number 1) Weight (gms)

2) Length (cms)

3) Girth (cms)

4) Sex (dummy variable)

5) Maturity (dummy variable)

6) Age (years)

Since the number of parasites per host was used as the dependent variable, the values were transformed using the function $x_2 = \dfrac{1}{x_1 + 1}$ where x_1 = raw count and x_2 = transformed count, as calculated by applying Taylor's Power law. Each month's data was analysed separately. Principal component analysis of the above independent variables produced six components. The interpretation of these components in terms of the original variables was achieved by the examination of the normalised latent vectors. The structure of the components was very similar in each month of the year; thus the vectors are only shown for one month, January 1970, (Fig 2.11). These are typical of the other months. The six components can be interpreted as follows:-

<u>Component one.</u>

This component measures a general trend in size of the fish, having approximately equal contributions from the variables, weight, length, girth, maturity and age. Large fish have large component scores.

<u>Component two.</u>

This component measures the sex of the fish, negative values being assigned to immature fish, small positive values to male fish and large positive values to female fish.

<u>Component three.</u>

Component three measures the maturity of the fish, large component

values being assigned to mature fish.

Component four

This component contrasts weight with length and girth, for example, separating heavy fish which are small in size for their weight and vice versa.

Component five

This component contrasts age with weight and girth. Thus high component scores are given to old fish which are unusually small in girth and weight and vice versa.

Component six

Component six contrasts length with girth, thus is a measure of shape. High component scores are assigned to fish with small fat bodies and low scores to long slim fish.

The correlations between the transformed dependent variable and the independent variables were large within each monthly analysis (Table 2.10) thus the above components were used in the multiple regression analysis. The new correlation coefficients between the dependent variable and the components are shown in Table (2.11). The percentage variances of the dependent variable accounted for by each of these components (Table (2.12), Fig (2.12)) when summed give the total percentage variance accounted for, which is equivalent to the total variation accounted for by the original independent variables. These totals when divided by one hundred equal the square of the multiple correlation coefficients (R).

Within each month a significant amount of variation (Table 2.12) in the transformed dependent variable has been accounted for by the measured independent variables. As described previously (p 85), the components with significant correlations with the dependent variable (Table 2.11) were used in the subsequent multiple regression analysis. The significance levels of the r or R^2 values, depending on whether one

or more components were used in the analysis, are shown in Table (2.13).

The calculated standardized partial regression coefficients for each independent variable (a) using all the components and (b) using only the significant components are shown in Tables (2.14) and (2.15) respectively. Using the coefficients in Table (2.14) and the means and standard deviations of the variables (Table2.16) predictive equations can be constructed. The form of these equations is described on page Using the standardized partial regression coefficients calculated from the analysis using only the significant components, coefficients of separate determination were calculated. From these coefficients of separate determination, "coefficients of effect" were calculated and thus the absolute percentage effects of each independent variable in its association with the dependent variable were derived (Fig 2.13).

From Fig (2.13) it can be seen that the relative effect of each independent variable varies in each month of the year. However two phases are observable. In February, March and April the variable sex is important, whereas the variables weight, length, girth, maturity and age are equally important in the remaining months. Thus, in the latter months of the year, a dependence on size correlated with age and maturity is shown.

Since C.laticeps enters the fish in the food a possible explanation of these trends may be sought in the feeding behaviour of the fish. Female fish have the highest weighting in the dummy variable sex. Thus the positive association of the numbers of parasites per host with the sex variable implies that female fish in the months February, March and April harbour a large parasite population when compared with male and immature (unsexable) fish. To confirm this association two further statistical tests were carried out on the data. A X^2 test was performed on the numbers of un-infected and infected male and female fish. The Null Hypothesis was that there is no difference between the number of male and female fish infected.

This test was carried out on the date (a) from months February to June 1969 and (b) the months July to January 197). The x^2 value for (a) was 10.49 which is significant at the 5% level. For (b) the x^2 value was 0.28 which is non significant. Thus during the early part of the year females are more prone to infection than males. A second test, the Mann Whitney U test, was carried out on the numbers of parasites in infected female and male fish in the months February to June 2969. The Null Hypothesis was that female and male fish harbour the same numbers of parasites. The test criterion, the normal deviate Z was equal to 0.13; has a two tailed probability p = 0.857 and thus the null hypothesis is accepted.

From the above two tests it is possible to conclude that during the early part of the sampling year, while the mature bream were involved in preliminary spawning behaviour in preparation for the spawning season, female fish have a higher probability of being infected with C.laticeps. However, those male fish which are infected with C.laticeps do not harbour significantly more or less parasites than infected females. A possible explanation for this observed pattern of infection of the hosts lies in the spawning behaviour of the female and male fish. As described earlier (p 27) mature males have spawning territories which they guard by continually circling the perimeter, fighting off other males. It seems therefore that male fish have little time for feeding during the early spawning period which is supported by the lack of food present in mature males during this period. Female fish, on the other hand, wander throughout the male territories looking for potential spawning sites; it seems likely that they do feed during this period, although not extensively as indicated by the feeding data.

Those male fish which do feed however, pick up as many parasites as the females. These males may not take part in spawning due to shortage of and competition for territories. It seems unlikely that there is a

hormonal influence on the parasites since infected males carry similar worm burdens to the females and thus a difference in feeding behaviour seems the most likely explanation.

During the remaining months of the year, size and age are the factors determining parasite burdens. There is no evidence of age immunity since older and larger fish harbour more parasites than younger fish. This is again most probably due to feeding behaviour; young fish during the summer months and later in the year are surface feeders on planktonic crustacea, the older fish, however, feed primarily at the bottom of the pit on benthic invertebrates and thus are more likely to pick up the infected tubificids. It is also likely that within the groups of mature fish the large individuals consume more food and thus have a higher probability of eating infective stages of C.laticeps.

Table 2.10 The correlation coefficients (r) between the transformed number of C.laticeps per host and the host factors.

Independent variables	Weight	Length	Girth	Sex	Maturity	Age
	1	2	3	4	5	6
Months						
1969 Feb	-.0247	-.2720	-.2816	-.5887	-.0127	-.2655
March	-.1613	-.2926	-.3130	-.5393	-.0181	-.2704
April	-.0104	-.2301	-.2208	-.5437	-.0840	-.2133
May	-.3929	-.5519	-.5623	-.4930	-.2674	-.5240
June	-.4601	-.6767	-.6969	-.5328	-.5270	-.6974
July	-.7959	-.8903	-.8913	-.7145	-.8683	-.8799
Aug	-.8333	-.8346	-.8369	-.4261	-.8555	-.8167
Sept	-.5117	-.5696	-.5473	-.3376	-.4913	-.5792
Oct	-.6163	-.5596	-.5454	-.2718	-.5334	-.5441
Nov	-.4765	-.5375	-.5121	-.2961	-.4452	-.5516
Dec	-.6003	-.6285	-.6227	-.4516	-.5262	-.5940
1970 Jan	-.5603	-.5833	-.5744	-.4138	-.5179	-.6105

Table 2.11 The absolute values of the correlation coefficients (r) between the number of C.laticeps per host and the host components.

Components	1	2	3	4	5	6
Month						
1969 Feb	.2445	.5796****	.1414	.2047	.1897	-
Mar	.2769	.4498***	.4814***	.1411	.0964	.0424
Apr	.2131	.5189***	.1304	.2545	.1192	.1091
May	.5095***	.1363	.3532**	.3328*	.2315	-
June	.6363****	.0374	.0346	.4239**	.0374	.2271
July	.9079****	.0245	.1292	.0640	.0223	.0728
Aug	.8563****	.1594	.1192	.2382	.0447	.0010
Sept	.5568****	.0683	.0671	.1783	.2487	.2496
Oct	.5613****	.2005	.0500	.1628	.1104	.0768
Nov	.5217***	.0565	.0894	.1763	.1852	.2827
Dec	.6297****	.0316	.0825	.0436	.2946	.1789
1970 Jan	.5908****	.0010	.0632	.0458	.1895	.0000

(**** $P < .001$; *** $P < .01$; ** $P < .05$; * $P < .10$)

Table 2.12 **C.laticeps** The Percentage variance of the dependent
variable accounted for by each host component.

Components Month	1	2	3	4	5	6	Total (100xR²)%
1969 Feb	5.98	33.59	0.02	4.19	3.60	–	47.39*
Mar	7.67	20.24	23.18	1.99	0.93	0.18	54.19**
April	4.54	26.93	1.70	6.48	1.42	1.19	42.24*
May	25.96	1.86	12.48	11.08	5.36	–	56.74**
June	40.49	0.14	0.12	17.97	0.14	5.16	64.33***
July	82.44	0.06	1.67	0.41	0.05	0.53	85.16***
Aug	73.34	2.54	1.42	5.68	0.20	0.00	83.18***
Sept	31.01	0.37	0.45	3.18	2.21	6.23	43.45*
Oct	31.50	4.02	0.25	2.65	1.22	0.59	40.27*
Nov	27.22	0.32	0.80	3.11	3.43	7.99	42.88*
Dec	38.53	0.10	0.68	0.19	8.68	3.20	51.39**
1970 Jan	34.91	0.00	0.40	0.21	3.59	0.00	39.11*

(*** $P<.001$; ** $P<.01$; * $P<.05$)

Table 2.13 **C.laticeps** Significance of r or R^2 calculated in the
multiple regression analysis using only the
significant components.

Months	r	R^2	F-ratio	d.f	Probability
Feb	.5796	–	–	28	.001
Mar	–	.434	10.351	2/27	.001
April	.5189	–	–	28	.01
May	–	.495	8.495	3/26	.001
June	–	.585	19.030	2/27	.001
July	.9079	–	–	28	.001
Aug	.8563	–	–	34	.001
Sept	.5568	–	–	38	.001
Oct	.5613	–	–	38	.001
Nov	.5217	–	–	33	.01
Dec	.6207	–	–	40	.001
Jan	.5908	–	–	42	.001

Table 2.14   **C.laticeps**   Standardized partial regression coefficients calculated from the multiple regression analysis using all the components.

Independent Variable Month	1 Weight	2 Length	3 Girth	4 Sex	5 Maturity	6 Age
1969 Feb	.687	-.866	-.964	-.405	.285	.938
March	.139	.412	-1.084	-.568	.865	-.088
April	.744	-2.262	2.150	-.402	.413	-.941
May	.569	-1.262	-1.222	-.154	.786	.875
June	.516	3.284	-3.038	.121	.009	-1.513
July	-.074	1.573	-1.665	-.177	-.506	-.162
Aug	-.851	.175	-.183	-.341	-.697	.767
Sept	.293	-3.737	3.540	.257	-.125	.722
Oct	-.737	-1.620	.977	-.121	-.171	1.011
Nov	.201	-3.969	4.018	.307	.005	-.983
Dec	-.979	-4.167	1.661	-.357	.484	2.635
1970 Jan	.362	.011	.515	.014	.052	-1.434

Table 2.15   **C.laticeps**   Standardized partial regression coefficients calculated from the multiple regression analysis using only significant components.

Independent Variable Month	1 Weight	2 Length	3 Girth	4 Sex	5 Maturity	6 Age
Feb	.181	-.067	-.008	-.563	.239	.028
March	-.077	-.061	-.061	-.666	.807	-.042
April	.185	.022	.022	-.537	.027	.040
May	.546	-.575	-.516	.005	.721	-.567
June	.652	-.428	-.463	.225	-.065	-.514
July	-.160	-.175	-.175	-.139	-.158	-.170
Aug	-.159	-.171	-.172	-.106	-.151	-.171
Sept	-.105	-.108	-.108	-.079	-.095	-.108
Oct	-.102	-.109	-.109	-.078	-.099	-.108
Nov	-.099	-.103	-.102	-.072	-.088	-.103
Dec	-.114	-.120	-.120	-.083	-.111	-.119
Jan	-.107	-.114	-.114	-.081	-.105	-.113

Table 2.16 The mean standard deviations of the dependent and independent variables, used in the ortho-gonalised regression analysis of parasite numbers and host features, for <u>C.laticeps</u> and D.paradoxum:-

/Variable Number   1 = Weight   2 = Length   3 = Girth   4 = Operculum Size   5 = Sex   6 = Maturity

7 = Age   8 = <u>C.laticeps</u>   9 = <u>D.paradoxums</u> /

Month	February (1969)		March		April		May		June		July	
Variable	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
1	150.12	253.29	146.45	231.84	162.90	267.35	144.59	209.15	142.20	247.90	136.30	199.60
2	16.77	10.01	17.02	9.83	17.45	10.49	17.53	9.71	14.59	10.79	15.30	9.57
3	12.97	8.21	13.24	8.61	12.89	8.32	12.98	7.75	11.25	8.87	12.20	7.96
4	2.69	1.59	2.40	1.48	2.49	1.60	2.62	1.57	2.29	1.89	2.45	1.64
5	1.87	0.86	1.77	0.82	1.77	0.82	1.93	0.87	1.53	0.71	2.00	0.91
6	1.23	0.43	1.33	0.48	1.33	0.48	1.40	0.50	1.30	0.47	1.33	0.48
7	2.83	2.35	2.87	2.39	2.97	2.59	2.83	2.30	2.97	2.46	2.83	2.21
8	0.63	0.39	0.64	0.38	0.71	0.32	0.56	0.36	0.71	0.33	0.65	0.39
9	3.43	1.46	3.57	1.79	3.43	1.58	3.46	1.56	2.68	1.68	2.29	1.80

	August		September		October		November		December		January (1970)	
Variable												
1	131.23	231.28	163.82	242.72	157.00	229.71	153.00	237.31	196.10	263.76	247.65	328.40
2	14.90	9.88	16.19	9.72	16.97	9.14	15.74	9.47	18.43	9.46	19.19	10.30
3	11.77	7.71	12.99	8.48	13.31	7.69	12.58	8.23	14.60	8.10	15.56	8.82
4	2.51	1.86	2.60	1.84	2.80	1.55	2.57	1.81	3.03	1.66	3.16	1.81
5	1.78	0.80	1.86	0.83	1.90	0.90	1.83	0.82	1.87	0.83	1.86	0.82
6	1.25	0.44	1.19	0.40	1.27	0.45	1.17	0.38	1.31	0.47	1.34	0.48
7	2.42	2.43	2.55	2.42	2.45	2.37	2.46	2.38	2.75	2.45	2.98	2.82
8	0.83	0.30	0.89	0.26	2.45	2.37	0.89	0.26	0.80	0.33	0.76	0.35
9	2.21	1.92	2.79	2.45	2.82	1.61	2.66	2.35	3.20	1.72	3.10	1.61

Fig (2.11)   The interrelationships between the number of C.laticeps

per host and the host parameters.

The Normalised latent vectors of each component for

January 1969 (Figures in brackets represent the

percentage of the total variation accounted for by each

component)

NORMALISED LATENT VECTOR

Component 1 (85·7%)

Component 2 (10·1%)

Component 3 (3·1%)

Component 4 (0·8%)

Component 5 (0·3%)

Component 6 (0·1%)

INDEPENDENT VARIABLES
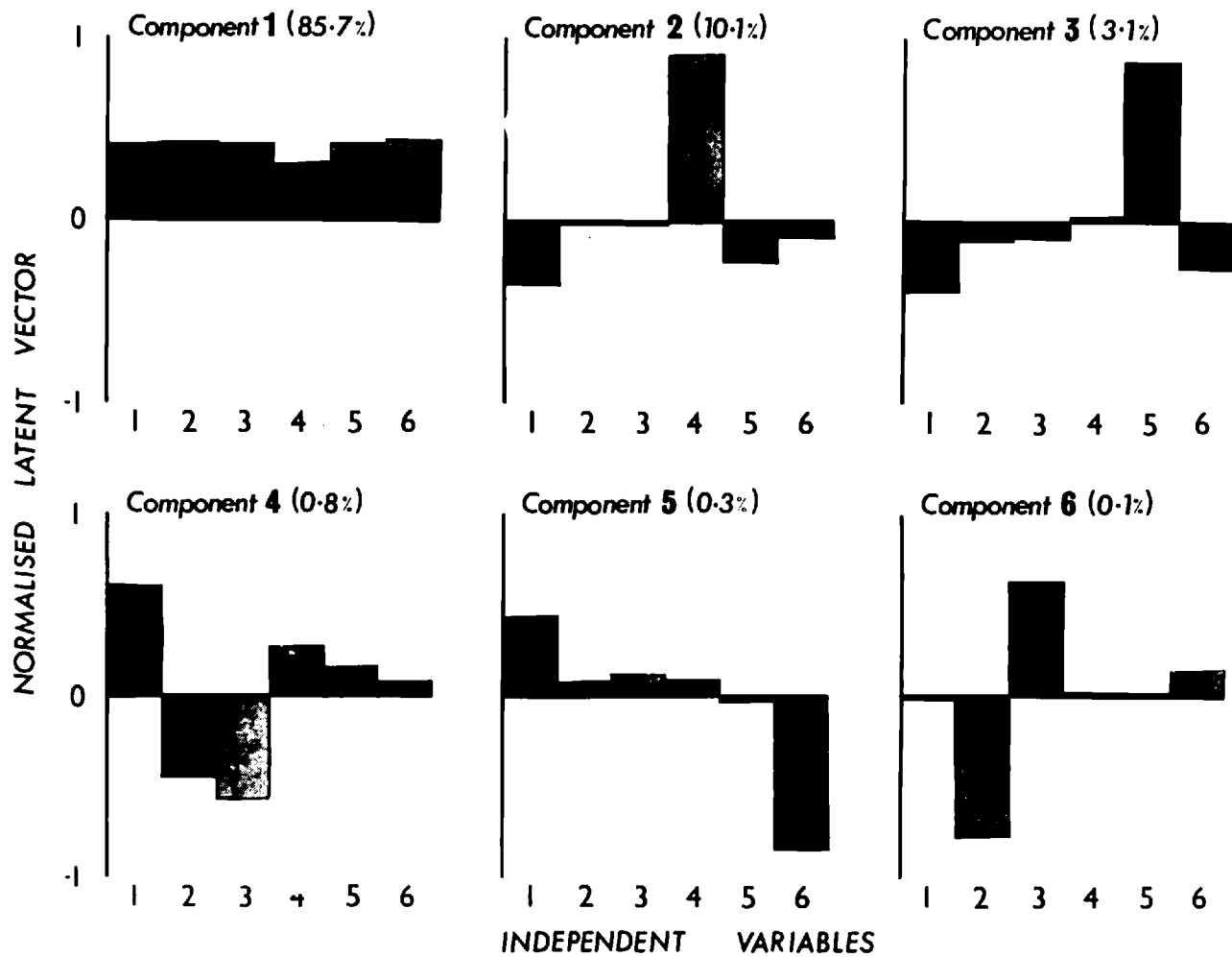
1 · WEIGHT 4 · SEX
2 · LENGTH 5 · MATURITY
3 · GIRTH 6 · AGE

Fig (2.12)   The interrelationships between the number of

C.laticeps per host and the host parameters.

The percentage variance of the dependent variable

and the total variation accounted for by the
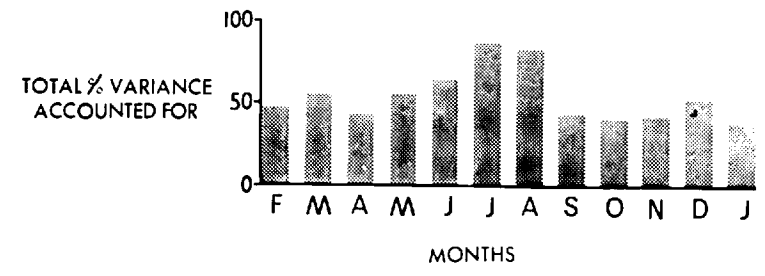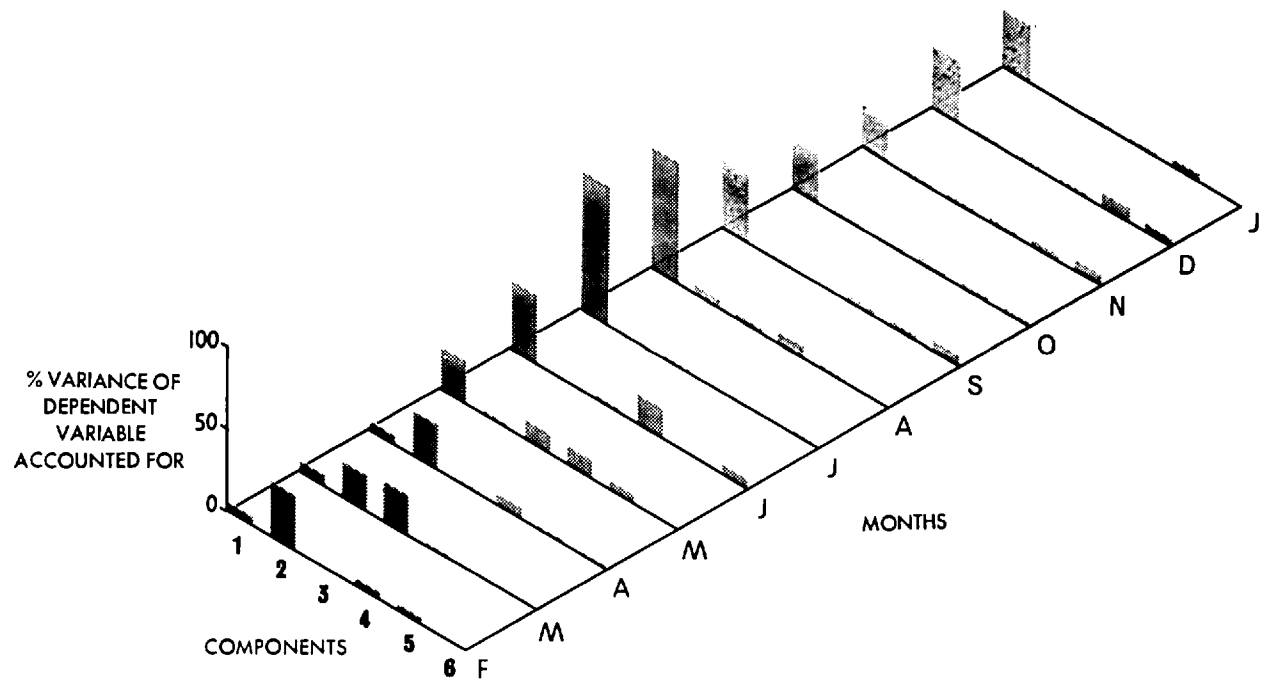
components.

Fig (2.13)   The interrelationships between the number of C.laticeps

per host and the host parameters.

The percentage 'effects' of the independent variables

in their associations with the dependent variable

(analysis incorporating the significant components)

% CONTRIBUTION TO DEPENDENT VARIABLE

100

50

0

1 2 3 4 5 6

INDEPENDENT VARIABLES

MONTHS

F M A M J J A S O N D J
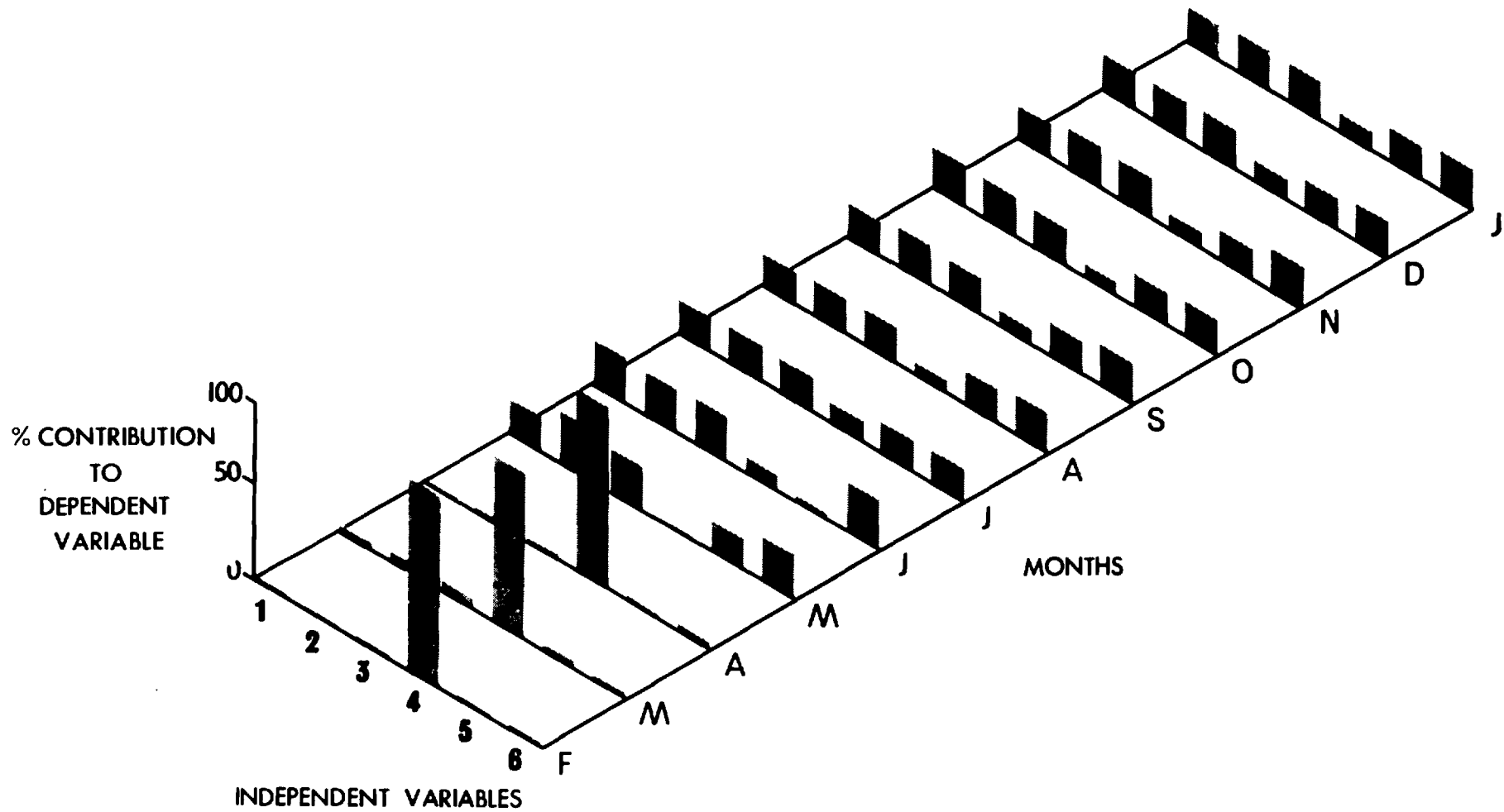
Weather factors and parasite numbers:-

The weather factors used in this analysis were as follows:-

Variable number
1) monthly mean of the inches of rainfall/day

2) monthly total hours of sunshine

3) monthly mean water temperature $^{\circ}C$

4) monthly mean number of hours of daylight/day

5) monthly range of water temperature $^{\circ}C$.

The variables were selected due to their possible influence on the behaviour or physiology of the host and parasite. Five separate analyses were carried out over the twelve months using the mean number of parasites per host in each age group of fish. Since means were used no transformation of the dependent variable is required. The O group of hosts are uninfected throughout the year and thus were not included in the analyses.

Principal component analysis of the above independent variables produced five components accounting for 60.5%, 28.4%, 5.5%, 4.9% and 6.4% of the total variation respectively. The size of the normalised latent vectors in each component (Fig 2.14) allowed the following interpretations in terms of the original variables.

Component one

This component measures a general seasonal trend of the variables day length, sunshine, water temperature and to a lesser extent range of water temperature; rising to a maximum in summer and falling to a minimum in winter.

Component two

This component measures the irregular seasonal trend of rainfall and range of water temperature, giving high weighting to months with a high rainfall and large range of water temperature.

Component three

This component contrasts rainfall and range of water temperature. Those months with a high rainfall but low range of water temperature have a high component score and vice versa.

Component four

Component four contrasts water temperature with day length and sunshine, illustrating the slight lag in increase and decrease of water temperature when compared with sunshine hours and day length. The main contribution however is from water temperature; thus months with a high average water temperature have high component scores.

Component five

This component contrasts sunshine hours with day length. Thus months with a large number of sunshine hours in the early or late part of the year have high component scores.

The percentage variances of the dependent variables and the total variation, accounted for by the components in multiple regression analysis, are shown in Fig (2.15) and Table (2.22) for each age group of hosts. Only in the case of the second age group of hosts is a significant amount of the variation in the dependent variable, R^2, accounted for by the components (Table 2.19). Of the correlation coefficients between each component and the dependent variable (Table 2.18) only three coefficients are significant, two in the second age group and one in the first age group.

Since so little variation is accounted for and so few significant correlations are present, the coefficients of effects for each weather variable are not shown. The standard partial orthogonalised regression coefficients for all the components combined, and for the selected components, however, are shown in Tables (2.20) and (2.21) respectively. The means and standard deviations of the dependent and

independent variables are shown in Table (2.23). The predictive

equations produced from these coefficients seem of little value,

except perhaps in the case of the young age groups of fish, the first,

second and third groups. Here it can be seen that, by using selected

components (Table2.22) which account for a comparatively large amount

of variation in the dependent variable, a negative correlation exists

with water temperature. This is most probably due to the change in

feeding habits of the younger fish during the summer months indicated

by examination of the food of these age groups throughout the year.

It can be concluded that the seasonal cycles of intensity

of infection in the different age groups of hosts cannot be satisfactorily

accounted for by the seasonal change of the measured weather factors.

This indicated the probable dependence of the intensity of infection on

factors such as feeding behaviour of the hosts, which although seasonal,

is not closely correlated with water temperature, day length and sunshine

hours. It has been shown earlier that temperature affects the loss rate

of the parasites from the host. However, this must not be confused with

the observed intensity of infection for, even if loss rate is higher in

summer months, but input rate is proportionally large, an observed in-

crease in intensity of infection will result.

Table 2.17   The correlation coefficients (r) between the selected
              weather factors and the mean number of C.laticeps per host.

Independent Variable	Rainfall	Sunshine	Water Temperature	Day length	Range at Water Temperature
Age group	1	2	3	4	5
1	-.2302	-.0207	-.4453	.0357	-.4107
2	.0662	-.1216	-.3208	.1225	-.3197
3	-.0413	.1033	-.1518	.2925	-.2206
4	.1096	.0261	-.0977	.0135	-.2441
5	.4703	.0163	.3090	.1867	.3068

Table 2.18   The absolute values of the correlation coefficients (r)
              between the mean number of C.laticeps per host and the
              weather components.

Component	1	2	3	4	5
Age group					
1	.224	.323	.184	.677**	.010
2	.167	.085	.505*	.528*	.419
3	.082	.185	.409	.536	.320
4	.070	.037	.401	.199	.550
5	.232	.409	.334	.229	.331

(** $P < .05$;   * $P < .10$)

Table 2.19   C.laticeps   The square of the multiple correlation coefficient
                            R and its significance for each age group of hosts.

Age group	R^2	F-ratio	d.f.	Probability
1	0.647	2.199	5/7	-
2	0.745	3.640	5/7	$< .10$
3	0.591	1.734	5/7	-
4	0.515	1.274	5/7	-
5	0.493	1.168	5/7	-

Table(2.20)   __C.laticeps__   Standardized partial regression coefficients
calculated from the multiple regression analysis using all
the components.

Independent Variable	1 Rainfall	2 Sunshine	3 Water Temperature	4 Day length	5 Range at Water Temperature
Age group					
1	0.112	0.435	-1.125	0.682	-0.369
2	0.047	-1.408	-0.920	2.178	-0.028
3	0.013	-0.912	-0.866	1.852	-0.059
4	1.139	2.245	-0.002	-1.367	-1.434
5	0.150	-1.563	0.427	1.198	0.303

Table(2.21)   __C.laticeps__   Standardized partial regression coefficients
calculated from the multiple regression analysis using only
the significant components.

Independent Variable	1 Rainfall	2 Sunshine	3 Water Temperature	4 Day length	5 Range at Water Temperature
Age group					
1	0.152	0.523	-1.131	0.526	0.056
2	0.640	0.299	-0.712	0.820	-0.623
3	0.121	0.416	-0.900	0.418	0.045
4	0.701	2.194	0.211	-1.835	-0.881
5	0.274	-0.067	-0.015	-0.065	0.182

Table (2.22)   __C.laticeps__   The Percentage variance of the dependent
variable accounted for by each weather component.

Component Age Group	1	2	3	4	5	Total $(100 \times R^2)\%$
1	5.05	10.46	3.39	45.84	0.01	64.74
2	2.80	0.73	25.51	27.90	17.58	74.53*
3	0.67	3.38	16.74	28.69	10.27	59.14
4	0.49	0.14	16.55	3.96	30.37	51.52
5	5.32	16.72	11.16	5.23	10.95	49.30

(* $p < .05$)

Table 2.23   The means and standard deviations of the dependent
             and independent variables used in the orthogonalised
             regression analysis of the mean number of parasites
             per host and environmental factors, for C.laticeps
             and D.paradoxum.

		$\bar{x}$	S
1	Rainfall	1.88	0.98
2	Sunshine	114.31	74.99
3	Water temperature	12.00	6.78
4	Day length	12.32	3.13
5	Range of water temperature	6.75	2.70

Age group of hosts	Parasite	C.laticeps		D.paradoxum	
		$\bar{x}$	S	$\bar{x}$	S
1		0.14	0.21	2.57	1.66
2		0.73	1.03	7.85	5.88
3		2.22	2.92	12.68	7.62
4		2.06	1.77	19.55	3.79
5		5.05	4.24	27.50	10.23

Fig (2.14)   The interrelationsbips of the mean number of C.laticeps

and the mean number of D.paradoxum per age group of

hosts with the weather parameters.

The Normalised latent vectors of each component.

(Figures in brackets represent the percentage of the

total variation accounted for by each component.
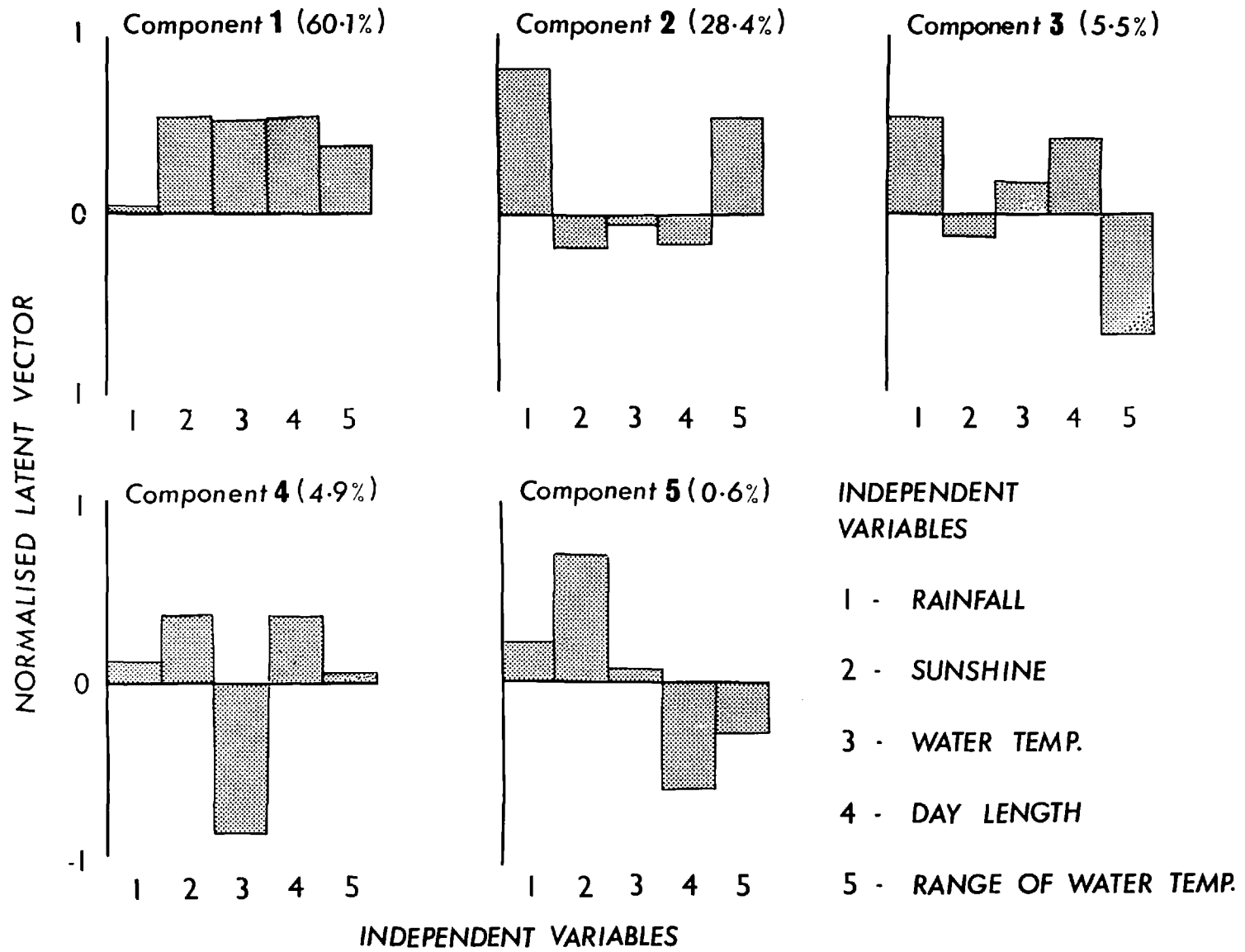
Fig (2.15)   The interrelationships between the mean number of

C.laticeps per age group of hosts and the weather

parameters.

The percentage variance of the dependent variable and

the total variation, accounted for by the components.

% VARIANCE
OF DEPENDENT
VARIABLE
ACCOUNTED
FOR

100

50

0

1

2

3

4

5 1

COMPONENTS

AGE GROUP
OF HOSTS

5

4

3

2

TOTAL %
VARIANCE
ACCOUNTED
FOR

100

50

0

1   2   3   4   5

AGE GROUP
OF HOSTS
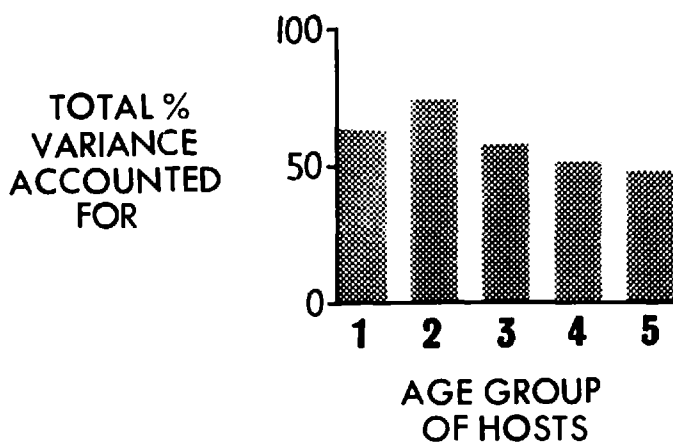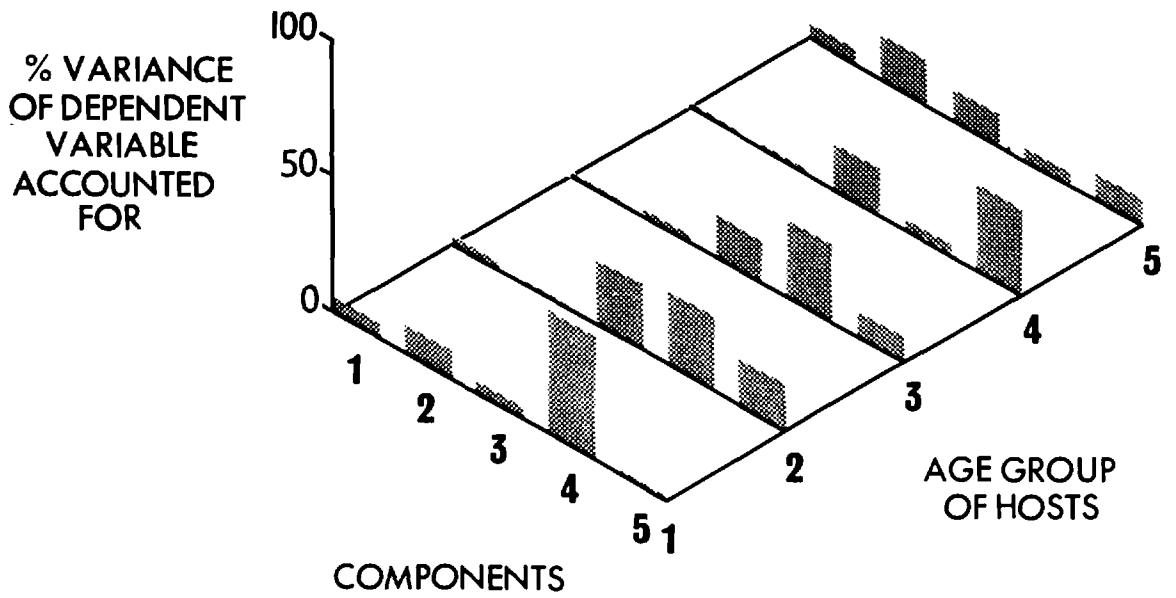
Discussion

From the evidence presented in this section it is possible to make a number of deductions concerning the population biology and relations of C.laticeps with the host and environment.

1) The intensity and incidence of infection of the host varies with the age of the fish and the time of the year.

2) The variation in intensity and incidence of infection of the host are dissimilar in immature and mature fish.

3) The parasite is present in the host population throughout the year, although absent from the young immature fish during the warm summer months.

4) A large proportion of the parasites gaining entry to the host fail to reach maturity.

5) The survival rate of the parasite to maturity in the host seems to be controlled by the density independent factor temperature.

6) Gravid parasites are present throughout the year, but a large percentage of the mature parasites become gravid in the warm summer months.

7) A decrease in the parasite population size in the environment occurred during the sampling year which may be caused by a decrease in the host population.

8) Although the percentages of infected intermediate hosts of C.laticeps did not alter markedly throughout the year, the density of the intermediate host population falls during the mid-summer months.

9) Variations in intensity of infection of the host cannot be closely correlated with measured weather factors.

10) During the early part of the year, more females than males are infected with C.laticeps. However, infected male bream do not harbour significantly different parasite burdens to the females.

11) There is a significant positive correlation between the number of parasites per host and fish size and age throughout most of the year. This is most probably explained by differences in host feeding behaviour.

A few of these points require further clarification. It is not surprising that feeding behaviour is an important aspect affecting parasite burdens since the helminth parasite gains entry to the definitive host via an intermediate host which is a food organism.

In the present study this was indicated in three ways:

1) The variation in incidence of infection between female and male fish during the early part of the year seems to have been due to variation in feeding behaviour of the hosts, particularly at the time of spawning.

2) The delay in increase of intensity of infection at the beginning of the year in mature fish, when compared with immature fish, was again most likely due to lack of intensive feeding activity in mature fish during the spawning season.

3) The loss of the parasites in the young immature fish during the warm summer months seems to have been related to the change in diet to planktonic crustacea, feeding occurring mainly in the mid and surface layers of water.

Another cause for the decrease in parasite population size in mature fish during the warm summer months was most probably related to a decrease in the survival rate of the parasite to maturity with an increase in water temperature. Kennedy and Walker (1969), experimenting with C.laticeps in the Dace (Leuciscus leuciscus (L.)), noted a temperature dependent loss rate. This the authors attributed to a temperature dependent immune response by the fish host. A number of workers have demonstrated a temperature dependent production of antibodies in fish and amphibia (Allen and McDaniel,1937; Bisset, 1948; Finsted and Good, 1964).

In general, increased environmental temperature tends to shorten the latent period and to enhance the intensity of the immune response. It is possible that the observed decrease in survival rate of C.laticeps in bream during the summer months is due to a temperature dependent immune response. The threshfold for increase in this response seems to be a water temperature of between 10-15° centigrade.

C.laticeps does not have a seasonal cycle of egg production, gravid worms occurring throughout the year. However an increased number of mature worms do become gravid during the warm summer months. Kennedy (1968) stated that for a population of C.laticeps in dace, the number of parasites reaching maturity and becoming gravid was not related to water temperature. This is not confirmed in this present study, the reverse being indicated. From the nature of biological reactions, an increased egg production with increased temperature seems likely since the processes involved in egg maturation must be mediated by an enzyme or enzymes. An increase in temperature is thus likely to increase the speed of the reaction and thus possibly the production of eggs.

The decrease in the parasite population size is interesting since it is connected with a corresponding decrease in the host population during the sampling year. Host density has often been stated as a factor of prime importance in controlling the size of parasite populations (Dogiel, 1964). However, no detailed population studies of parasite in different sized host populations have been carried out. The results in this study must be interpreted with caution since more years of study are required to confirm and quantitatively define a density dependent relationship between host and parasite.

SUBSECTION IV   Analysis of the Ecology of Diplozoon paradoxum

The distribution of D.paradoxum in the host population

The observed frequency distributions of the counts of D.paradoxum per host within a monthly sample are difficult to analyse due to the large differences between the mean number of parasites per host in each age group of fish (Table 2.25). When age groups are combined in the monthly analysis of the data over dispersion results, because of the combination of several heterogenous distributions with differing means (Table 2.27). A trend exists for the variance/mean ratios to increase during the summer months.

It is more informative to examine the variance/mean ratios within the age groups of fish in each month of the year (Table 2.26). It can be seen that a range of patterns exist in the samples from under dispersion through randomness to over dispersion. Under dispersion is more marked in the immature, 1, 2 and 3 age groups whilst over dispersion is more apparent in the older fish. The observed pattern in the older fish may be due to combining a wider range of different ages into the two age groups (4 and 5). The sample sizes from which the ratios are calculated are small (5 fish/ sample); thus under dispersion or over dispersion may be due to sampling error in certain age groups of fish.

To gain more information about the distribution of D.paradoxum thirty 3^+ fish were examined in March 1969, and the counts of the number of parasites per fish were tested for goodness of fit to a variety of discrete theoretical distribution models.

The positive binomial distribution provided the best fit (P (x^2 = 18.107), $>$ 0.05, 10 d.f). The mean was 19.103 and variance 11.810 with the ratio 0.618.

The binomial distribution describes random events with a given probability, p, for occurring and q = (1-p) for not occurring. When used in ecological distributions it tends to describe plants or animals with regular patterns of dispersion.

The individual terms of the binomial expansion are given by

$$P(x) = \frac{k.^1}{x.^1 \, (k-x).^1} \, q^{k-x} \, p^x$$

where P(x) is the probability of x events occurring and k is the absolute maximum number of individuals (parasites) which can occur in one quadrant (one host) (Turner and Eadie, 1957; Greig-Smith, 1964). Thus k is probably determined not only by the physical size of D.paradoxum on the gills, but also by the size of the sampling unit, the size or age of the host. The binomial distribution can be described by a general model discussed by Feller (1943) and implying "negative contagion" in which the probability of finding a parasite in any host is decreased by the presence of other parasites (Student, 1919; Nef, 1967).

This model would seem applicable in the case of D.paradoxum. If an area on the gill is already occupied by a parasite, a new infective stage has less chance of establishing on the gills. This probability will vary with the size of the host (available space for attachment) and also with the number of parasites already attached. An alternative model is suggested by Bailey (1964) where the number of survivors from a finite population will follow the binomial distribution if the individuals are independently subjected for a given period to a constant mortality rate. Application of this model to dispersion of parasites within hosts would additionally require a constant number of individuals in all hosts at the beginning of the process

and the absence of migration during the relevant time interval.
Skellam (1952) noted that these conditions could conceivably be met, if
only approximately, in some plant populations. This seems an unlikely
model in the case of D.paradoxum.

Randomness or regularity of the distribution of D.paradoxum
in the host population is further indicated by the transformation of the
raw counts calculated by applying Taylor's power law. The transformation
was calculated as $\sqrt{x}$ which is a standard transformation for the Poisson
and Binomial distributions.

From the evidence presented above in relation to the age
group variance/mean ratios, and the fit of a single set of data to the
binomial distribution, it would appear dangerous to propose a fundamental
model to describe the distribution of D.paradoxum. However, the following
ideas are presented with the reservation that they are based on small
amounts of data.

It seems probable that the binomial distribution would
adequately describe the dispersion of D.paradoxum in the host population
(assuming that the large variance/mean ratios in the old age groups of
fish are due to combinations of age classes) if large samples had been
collected within each age group of fish. Regularity of the dispersion is
most probably due to competition for space on the gills of the host; thus
the presence of one parasite decreases the chance of another attaching to
the gills. This decreased probability would be more marked when little
space was available for attachment (in young fish) and also when large
numbers of parasites were already present on the host. The initial invasion
of the host is most likely random, due to the mechanism of infection, where
the infective oncomiracidium are sucked into the gill chamber via the
respiratory water currents; no active attraction to the host's presence

apparently occurring (Bovet, 1967). Aggregation of these infective stages in the water seems unlikely because water currents would tend to disperse them and the eggs from which they hatch. Thus, if competition for space did not occur due to few infective stages in the environment, a Poisson distribution would probably result, the mean of which would vary with the age of the host due to an increase in the volume of water circulated round the gills and the larger area of the gills in older fish. It is possible that the final distribution could arise from a series of waves of random exposures to infections at different periods of time. A compounded Poisson distribution of varying means could thus occur, resulting in over dispersion described by either the negative binomial or neyman A models. This may operate in older fish where competition for space is less acute than in the younger hosts.

Table(2.24) The Percentage infection of each age group of fish with
D.paradoxum in each month of the year

Month Age group	0	1	2	3	4	5
1	0.0	100	100	100	100	100
2	0.0	100	100	100	100	100
3	0.0	100	100	100	100	100
4	0.0	80	100	100	100	100
5	0.0	0.0	100	100	100	100
6	0.0	0.0	40	60	100	100
7	0.0	20	20	100	100	100
8	0.0	40	60	60	100	100
9	0.0	100	60	100	100	100
10	0.0	80	100	100	100	100
11	0.0	100	100	100	100	100
12	0.0	80	100	100	100	100

Table(2.25) The Derived mean numbers of D.paradoxum/host, in each age
group of hosts in each month of the year

Month	0	1	2	3	4	5	0	1	2
				Age group				Age class	
1	0	4.395	14.834	14.628	19.328	22.340	0.0	4.395	14.834
2	0	2.910	15.288	17.106	20.238	26.310	0.0	2.910	15.288
3	0	2.260	13.406	15.336	18.367	20.721	0.0	2.260	13.406
4	0	2.107	13.444	16.828	21.355	23.053	0.0	2.107	13.444
5	0	0.0	2.480	6.099	15.490	28.924	0.0	2.481	6.099
6	0	0.0	0.511	0.892	18.177	21.647	0.0	0.511	0.892
7	0	0.172	1.172	1.376	20.372	53.991	0.172	0.172	1.376
8	0	0.511	1.540	2.283	26.660	43.090	0.511	1.540	2.283
9	0	3.764	3.388	14.370	20.302	23.904	3.764	3.388	14.370
10	0	3.051	5.736	12.106	15.969	21.246	3.051	5.736	12.106
11	0	2.526	7.448	16.421	21.540	27.782	2.526	7.448	16.421
12	0	3.386	12.831	18.424	20.821	23.214	3.386	12.831	18.424

Table(2.26) <u>Monthly variance/mean ratios for counts of D.paradoxum</u>
<u>per host divided into age groups</u>.

Age group Month	0	1	2	3	4	5
Feb 1969	0.0	1.26	0.93	1.67	4.60	8.30
March	0.0	0.50	0.57	2.10	6.92	9.67
April	0.0	0.41	1.12	0.17	10.02	2.27
May	0.0	2.62	1.93	0.25	2.02	3.10
June	0.0	0.00	0.88	0.60	0.53	0.43
July	0.0	0.00	1.33	1.00	2.65	6.75
Aug	0.0	1.00	1.00	0.20	1.15	6.53
Sept	0.0	1.30	1.78	4.67	3.01	12.79
Oct	0.0	1.62	3.62	1.18	1.52	5.14
Nov	0.0	1.71	0.38	3.89	9.80	6.04
Dec	0.0	2.21	1.88	0.89	3.00	1.11
Jan 1970	0.0	2.16	0.88	2.82	5.42	2.22

Table (2.27) <u>Monthly means and variances for the number of D.paradoxum</u>
<u>per fish host</u>

Month	mean	variance	variance/mean ratio
Feb 1969	12.87	86.81	6.74
March	14.90	172.57	11.58
April	13.17	106.83	8.11
May	13.33	98.44	7.38
June	8.90	116.51	13.91
July	7.37	132.45	17.97
Aug	12.93	478.96	37.04
Sept	13.07	409.31	31.32
Oct	11.40	116.45	10.21
Nov	10.40	119.49	11.49
Dec	12.90	125.75	9.75
Jan 1970	12.40	94.18	7.59

The population size structure and dynamics of D.paradoxum

The population structure and dynamics of D.paradoxum in the different strata of the host population were examined throughout the year. Particular attention is given to changes in the intensity and incidence of infection. As in the previous section on C.laticeps emphasis is placed on trends of population change rather than an explanation of every observed fluctuation.

Intensity and Incidence of infection

Polynomials were fitted to the derived mean number of parasites in each age group throughout the year (Fig 2.16). The calculated polynomial regression equations are given in Table (2.28).

Good fits for the fourth degree polynomial were obtained for the 1, 2 and 3 age groups. As for C.laticeps the fits to the older age groups were unsatisfactory. The isometric plots of the percentage infection of each age group of fish throughout the year (Fig 2.17) were produced using running averages over three percentages with the first and last point in each age group of hosts being the average of the first and last two percentages respectively.

The seasonal trends in incidence and intensity of infection of the bream with D.paradoxum are very similar and illustrate a number of points (Figs. 2.16, 2.17).

1) The mean intensity of infection varies with the age of the host and with the month of the year.

2) The parasite is present in the second, third, fourth and fifth age groups throughout the year, but is lost from the first age group in the mid summer. The O age group at any time of the year is uninfected.

3) The mature fish of the fourth and fifth age groups harbour more parasites than the young immature fish.

4) An increase in intensity of infection occurs in the mature fish during the summer months, whilst a decrease occurs in the immature fish in the same period.

The relations between incidence and intensity of infection require further clarification and are discussed in detail later.

Following the method developed for C.laticeps the intensities of infection within age classes were calculated from the age group information (Fig.2.18) using June as the birthday of the year class. The 0^+, 1^+ and 2^+ age classes were born in the years 1968, 1967 and 1966 respectively and in June 1969 they entered the 1^+, 2^+ and 3^+ age classes. Fourth degree polynomials were fitted to the derived means of the number of D.paradoxum per fish for each of the year classes (Table 2.28).

These curves further illustrate the drop in intensity of infection in the young immature fish during the warm summer months, with a build up in the late autumn and early winter periods. Possible reasons for the seasonality of the intensity and incidence of D.paradoxum in the age groups and classes of bream will be discussed later.

Table(2.28) <u>Fitted fourth degree polynomials to the derived mean number</u>
<u>of D.paradoxum per age group and age class of bream</u>

Age group
of hosts   d.f.   F-ratio

1      $y = 4.28 + 0.54x - 0.69x^2 + 0.11x^3 - 0.01x^4$ 4/7   6.64 *

2      $y = 6.98 + 11.68x - 4.50x^2 + 0.51x^3 - 0.02x^4$ 4/7   19.89 ***

3      $y = -2.19 + 23.32x - 8.19x^2 + 0.95x^3 - 0.03x^4$ 4/7   9.24 **

4      $y = 23.29 - 4.10x + 1.14x^2 - 0.11x^3 + 0.01x^4$ 4/7   0.14

5      $y = 45.53 - 27.80x + 9.24x^2 - 1.00x^3 + 0.04x^4$ 4/7   1.03

Year class
of hosts

1966 $y = 2.53 + 17.30x - 6.51x^2 + 0.78x^3 - 0.03x^4$ 4/7   10.57 **

1967 $y = 4.87 - 0.70x - 0.04x^2 + 0.01x^3 - 0.001x^4$ 4/7   55.19 ***

1968 $y = -0.94 + 1.38x - 0.57x^2 + 0.08x^3 - 0.003x^4$ 4/7   9.34 **

$\sqrt{\ }$where y = mean number of parasites per fish, x = month of the year

   Feb. 1969 = 1 .... Jan. 1970 = 12,   *** P $<$.001,   ** P$<$.01,

   * P$<$.05 $\underline{\ }$/

Fig (2.16)   Mean intensity of infection of each age group of bream

with D.paradoxum during the year February 1969 -
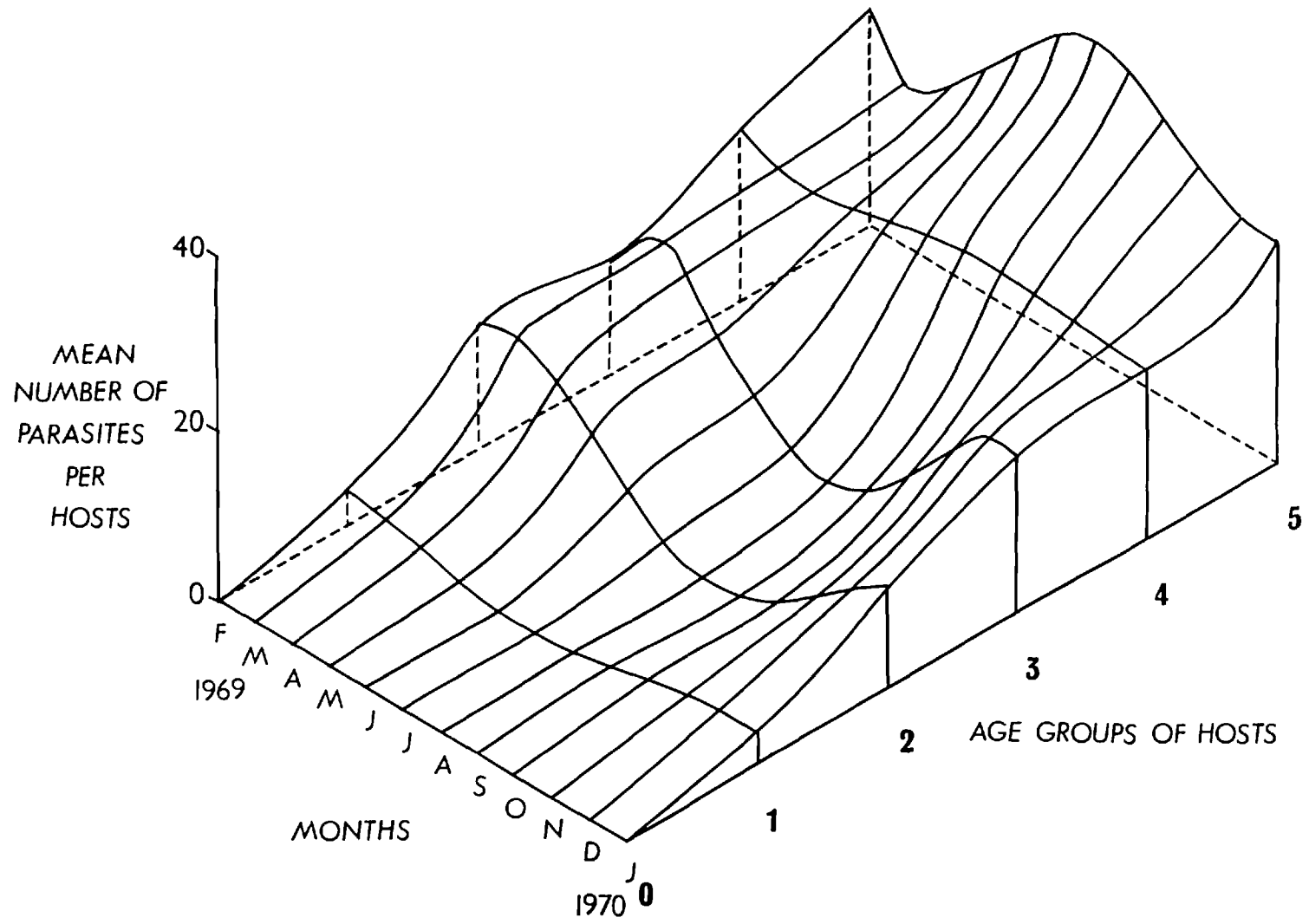
January 1970.

Fig (2.17)   Percentage of each age group of bream infected with

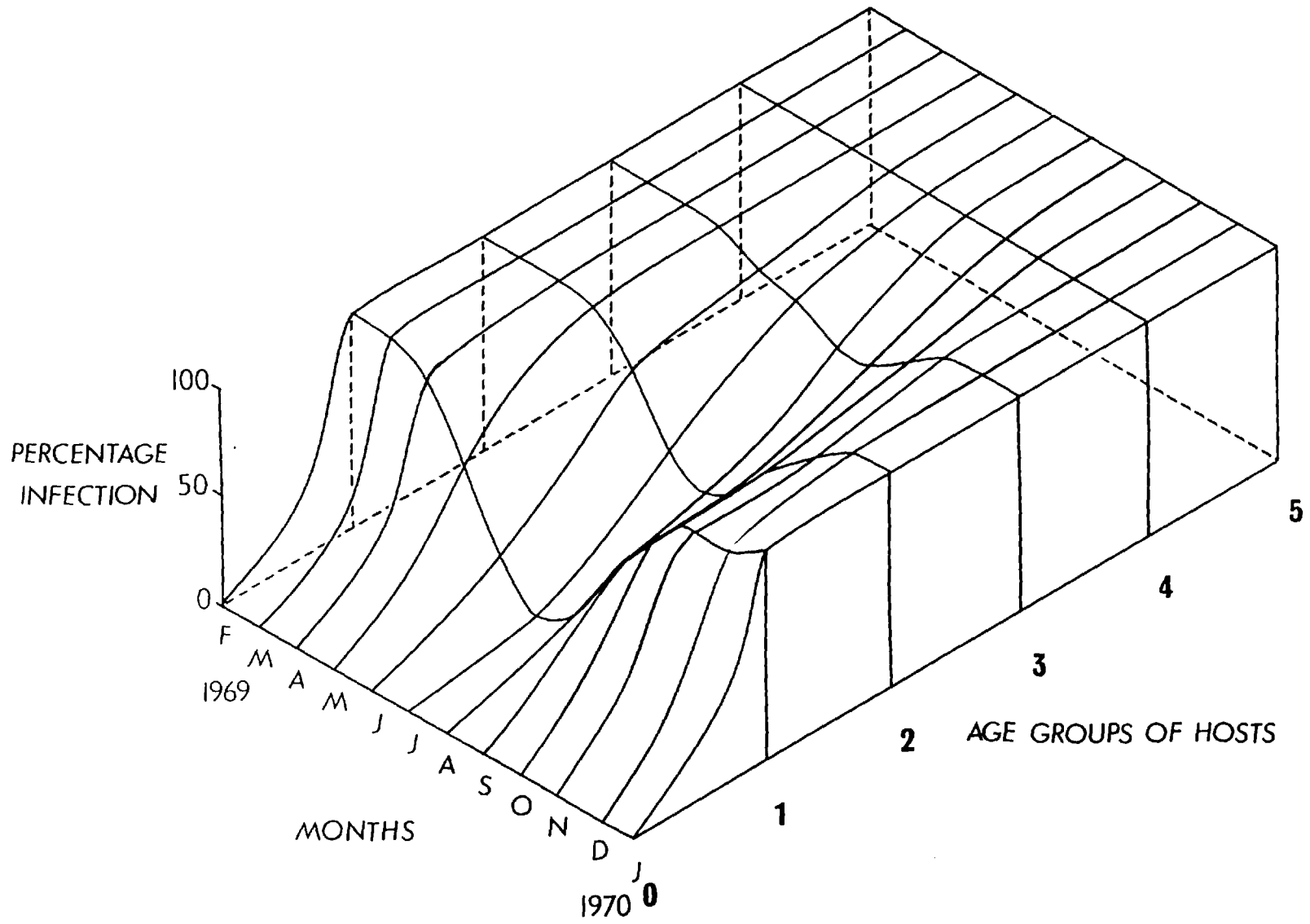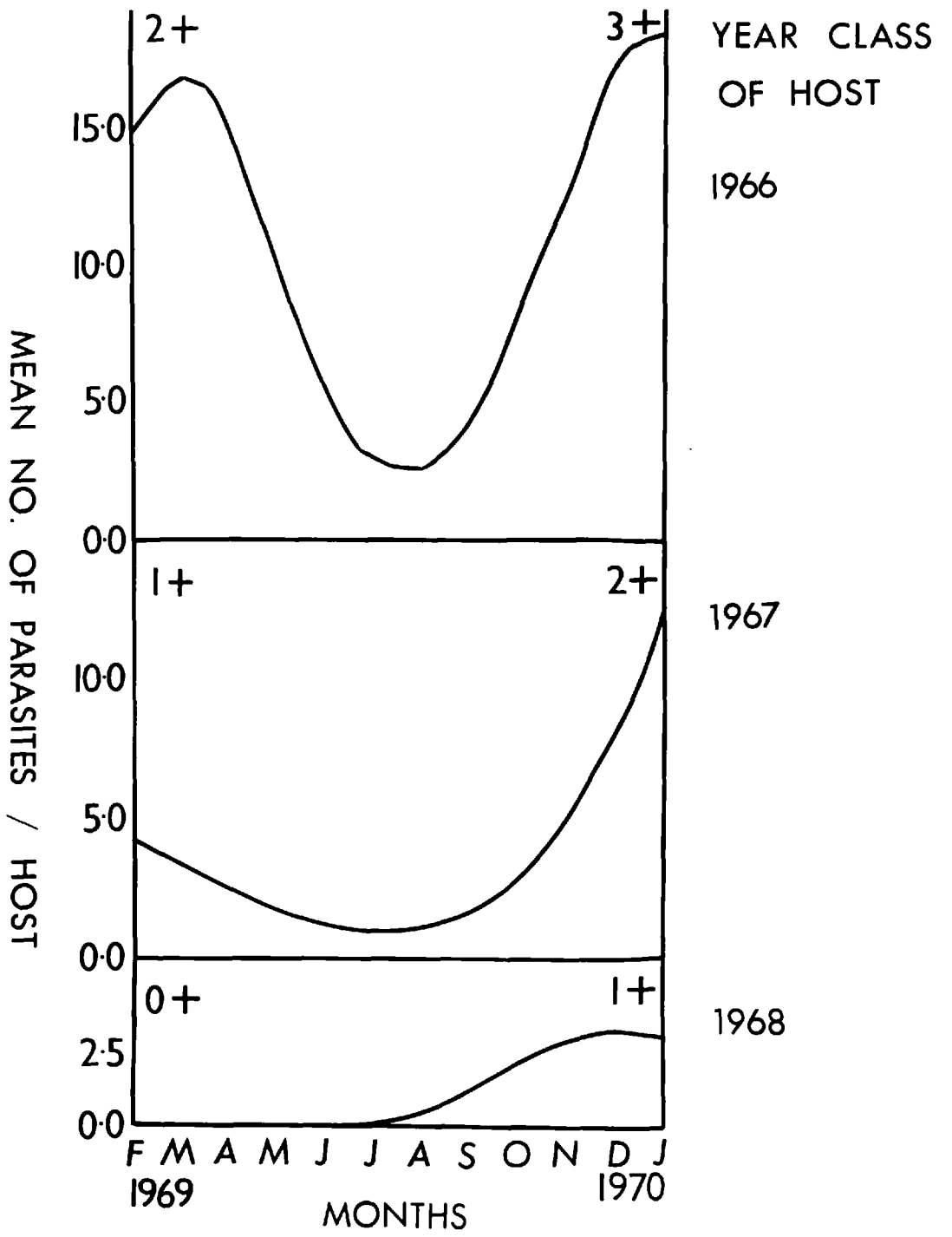D.paradoxum during the year February 1969 - January

1970.

Fig (2.18)   Mean intensity of infection of the bream with $\underline{D}$.

paradoxum in the 1966, 1967 and 1968 year classes

of fish during the year February 1969 – January 1970.

Population structure of D.paradoxum:-

D.paradoxum is reported to have a life span of between two to three years (Zeller, 1872, Bychowsky, 1961; Bovet, 1967; Davies, 1967). A closely related monogene Discocotyle sagittata is thought to have a life span of four years (Paling, 1965). The longevity of the parasite hinders the accuracy of ageing the worms by assessment of their state of maturity since sexual maturity is reached within one year (Wiles, 1965). Length was used to assess their age by making the assumption that length increases with age (Paling, 1965). This assumption is supported by studies on two other genera of monogenes which show an increase in length with age (Paling, 1965). As stated earlier (p 70) one worm in each couple was measured, from the tip of the haptor to the junction of the couple. This region was chosen because it seemed less susceptible to variation caused by contraction and distortion during fixing.

The period during which the build up of the parasite population in each age group of host takes place, occupies only three to four months. Thus, the number of peaks in a length frequency graph of the parasites sampled at one time should indicate the number of age classes of parasites in the population (sample). To eliminate overlap in lengths of different age classes of parasites due to the combination of several months' samples, a large sample of 406 parasites was measured in October 1969 and the percentage frequencies of each size (length) class $\underline{/}$ size class = 0.1 microscope eye piece units = 0.1 mm $\underline{/}$ were calculated (Fig. 2.20). For each of the 1, 2, 3, 4 and 5 age groups of hosts, 21, 30, 94, 149 and 112 parasites were measured respectively.

It is clear that the young bream harbour only small parasites, while the large bream are hosts to both small and large specimens. This leads to two alternative explanations. Either the size of the parasite is physically limited in some way by the size of the host, or the parasite

lives for more than one year and continues to increase in size over a period of years. The latter seems to be more plausible since there is no record of the size of the host affecting the size of monogene parasites.

If we accept that D.paradoxum lives for more than one year, the explanation for the varied structure of the parasite population in the different age groups of hosts follows. In addition the probable life span of the parasite can be deduced. The 0 age group (0^+ age class) of fish are uninfected; thus parasites on the 1 age group or 1^+ age class must be parasites in their first year of life. The population of parasites of the 2 age group or 2^+ age class of fish, therefore, will be composed of some parasites in their first year of life and others in their second year. Likewise the 3 age group or 3^+ age class will harbour parasites in their 1st, 2nd and 3rd years of life, and similarly the 4 age group, the 4^+ and 5^+ age class will harbour parasites in their 1st, 2nd, 3rd and 4th years of life and possibly 5th year, depending on whether their hosts are 4^+ or 5^+. No further increase in length is indicated in the 5th age group of fish. The parasites may die after this time or alternatively they may survive without undergoing further increase in size. However, if the latter point was correct it would be expected that there would be a marked increase in the percentage of parasites in the larger size ranges in the 5th age group of hosts. It can be seen from Fig (2.20) that this is not so and thus it appears that the parasites die after four or five years. It is not possible to determine which year exactly because of the grouping of the 4^+ and 5^+ fish into the 4th age group.

In view of the regularity of the parasites' dispersion between hosts of any one age group $\underline{/}$discussed on p 152$\underline{/}$ which is most probably due to competition for space, then the mortality rate of an age class of parasites within a year and the size of the host will determine the available space for attachment for the next year's parasites. Thus, if

mortalities are few in any age group of host in one year, and if the

gills are saturated with <u>D.paradoxum</u>, then few parasites will be able

to establish in the following year. This may be a possible explanation

for the observed length frequency data (Fig 2.20) for the 4th and 5th age

groups of hosts where few small parasites were found. An alternative

explanation is that the hosts once they pass a certain age, do not come

into contact with the infective oncomiracidia in their particular

ecological niche. This however does not seem plausible since it can be

shown, particularly in the 5th age group of hosts, that an increase in

the intensity of infection occurs in the summer months (Fig 2.16). How-

ever, a loss of parasites follows in September, October and November. The

length frequency data (Fig 2.20) was collected from the October sample;

thus it seems that this loss is not due to old parasites dying, but to the

failure of the young invading <u>D.paradoxum</u> to become established on the gills.

Table 2.29 records the percentage frequencies of the length

classes of <u>D.paradoxum</u> in six samples taken in February, April, June,

August, October and December: the age groups of hosts are combined. The

total percentage frequencies calculated from the combination of all the

monthly samples are also shown. Four frequency peaks are present, providing

further confirmation of the plausability of a life span of four years. The

young small parasites are only found in certain months (Table 2.29) and

recent infections are not apparent in the February and April samples.

This may be due to low temperature inhibition of egg production or hatching

and release of the oncomiracidia. Care must be taken however in the inter-

pretation of the results from the October and December samples, since,

although young forms are present, they may have been picked up earlier

but failed to grow due to low water temperatures.

Fig (2.21) represents the percentages of the total number of

parasites found which were gravid in each month of the year. Similar

observations have been recorded for D.paradoxum by Davies (1967) in the

Dace Leuciscus leuciscus. The solid black line in Fig (2.21) represents

the period during which diaporpa were found on the gills of the bream.

From these results it seems clear that the bream are susceptible to

infection by D.paradoxum throughout most of the year; however larger

numbers of infective stages are present in the environment during the

warm summer months. Temperature may thus play a part in determining the

rate of egg production by the parasite (Davies, 1967). Temperature also

influences the rate of hatching of the eggs and inversely the period of

time during which the oncomiracidia are active (Bychowsky, 1962; Sterba,

1957; Bovet, 1967). Although the infective stages are active for a

shorter period in warm water, the degree of activity is increased (Wiles,

1965) presumably enhancing the chances of infection of a host.

Table 2.29   The percentage frequency of each length class of
D.paradoxum in the six bimonthly samples.
$\angle$ 0.1 units =.1 mm$\angle$

Months	February 1969	April 1969	June 1969	August 1969	October 1969	December 1969	Total
Size class							
0.4	-	-	1.3	-	-	-	0.1
0.5	-	-	6.3	4.1	3.5	0.5	2.7
0.6	-	-	6.3	5.1	3.0	1.1	2.7
0.7	3.2	-	1.3	3.1	3.2	4.3	3.1
0.8	6.3	2.0	-	1.0	2.5	3.2	2.5
0.9	4.8	2.0	-	3.1	4.9	7.5	4.7
1.0	11.1	6.1	3.8	3.1	7.7	10.7	7.6
1.1	6.3	16.3	5.1	8.2	9.6	11.3	9.6
1.2	4.8	12.2	7.6	3.1	8.2	7.5	7.4
1.3	4.8	6.1	7.6	3.1	5.9	6.4	5.8
1.4	11.1	4.1	8.9	5.1	6.2	8.1	6.9
1.5	10.5	12.2	1.3	6.1	7.4	8.1	7.3
1.6	7.9	10.2	8.9	8.2	8.2	10.7	8.9
1.7	4.8	6.1	8.9	14.3	6.2	5.4	7.0
1.8	3.2	4.1	3.8	13.3	4.7	4.3	5.3
1.9	3.2	2.0	7.6	5.1	4.9	2.7	4.4
2.0	6.3	6.1	7.6	6.1	4.2	2.1	4.5
2.1	1.6	4.1	6.3	6.1	2.7	2.1	3.3
2.2	4.8	2.0	3.8	1.0	3.7	2.1	3.1
2.3	3.2	2.0	3.8	-	2.0	0.5	1.7
2.4	1.6	-	-	-	0.5	0.5	0.4
2.5	1.6	2.0	-	1.0	0.5	-	0.6
2.6	-	-	-	-	0.25	0.5	0.3

The size of the parasite population:-

Three approximate estimates of the total number of parasites in each age group of hosts can be calculated using estimates of the number of fish in each age class and the mean number of D.paradoxum in each age group of hosts (p 118).

Estimates of the percentage of the parasite population in each age group of fish (Fig 2.19A) and the numbers of parasites in these age groups (Fig 2.19B) illustrate a decrease in the parasite population during the sampling year, mirrored by the drop in the host population. A decrease in host numbers of 46.8% is parallelled by a 43.3% decrease in the parasite population. The reduced size of the parasite population is most probably due to high mortalities in the host population. However, a number of years data are required to demonstrate a density dependent relationship between host and parasite. It is again interesting to note that although more parasites are harboured by older thus larger fish, the bulk of the parasite population is present in the large February 1969 2^+ age class of fish. These parasites can only be in their first or second years of life; thus the older parasites form a small proportion of the total population. Due to the absence of these parasites in the young fish in the mid summer months, the parasite population is markedly reduced in this period of the year. The parasites present however are responsible for producing the large number of infective stages required for the build up of the population in the young fish in the later part of the year.

Fig (2.19)   A: Percentage of estimated population of D.paradoxum

in each age group of fish.

B: Population estimates of D.paradoxum in each age

group of hosts.

Diag. **A**

% OF PARASITES IN
AGE GROUPS OF
HOST POPULATION

100

50

0

1
2
3
4
5

AGE GROUPS
OF HOSTS

Feb.
1969

Aug.

MONTHS

Feb.
1970

Diag. **B**

TOTAL
NUMBER OF
PARASITES

100000

50000

0

1
2
3
4
5

AGE GROUPS
OF HOSTS
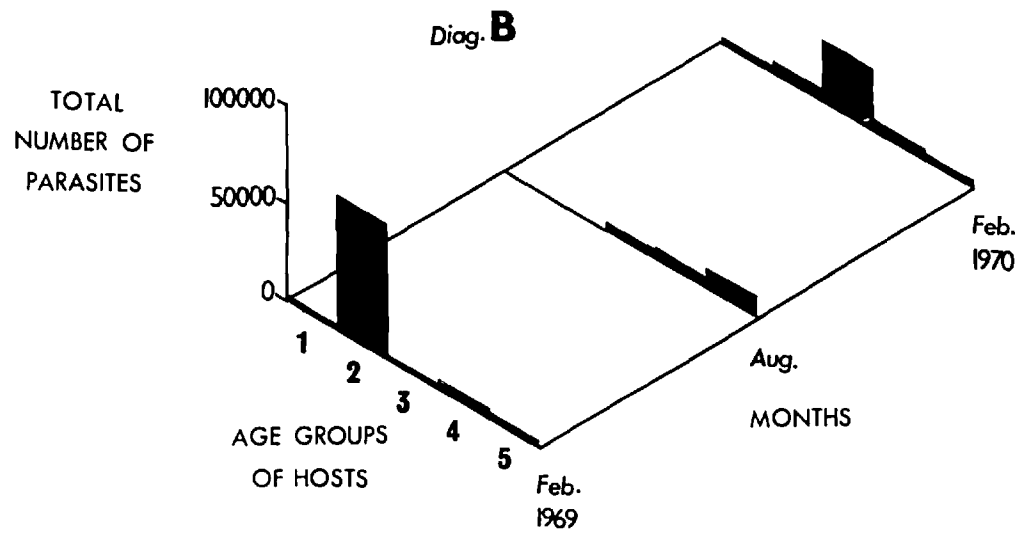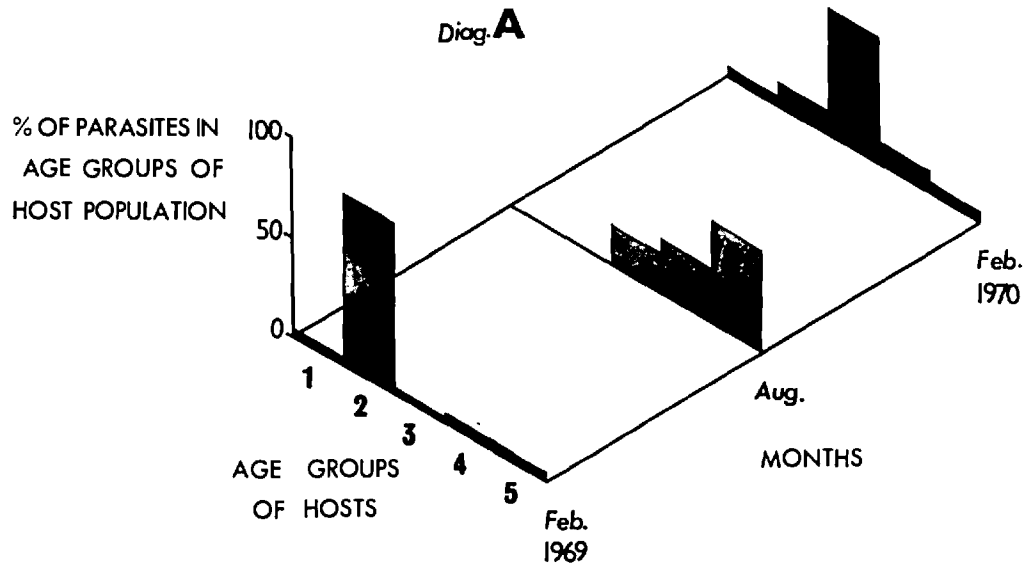
Feb.
1969

Aug.

MONTHS

Feb.
1970

Fig (2.20)   Size frequency histograms of <u>D.paradoxum</u> in each age

group of bream (Sample of 406 parasites from October

1969)

PERCENTAGE
COMPOSITION

SIZE CLASSES
OF PARASITES

(*in microscope eye piece units*)

AGE GROUPS OF HOSTS
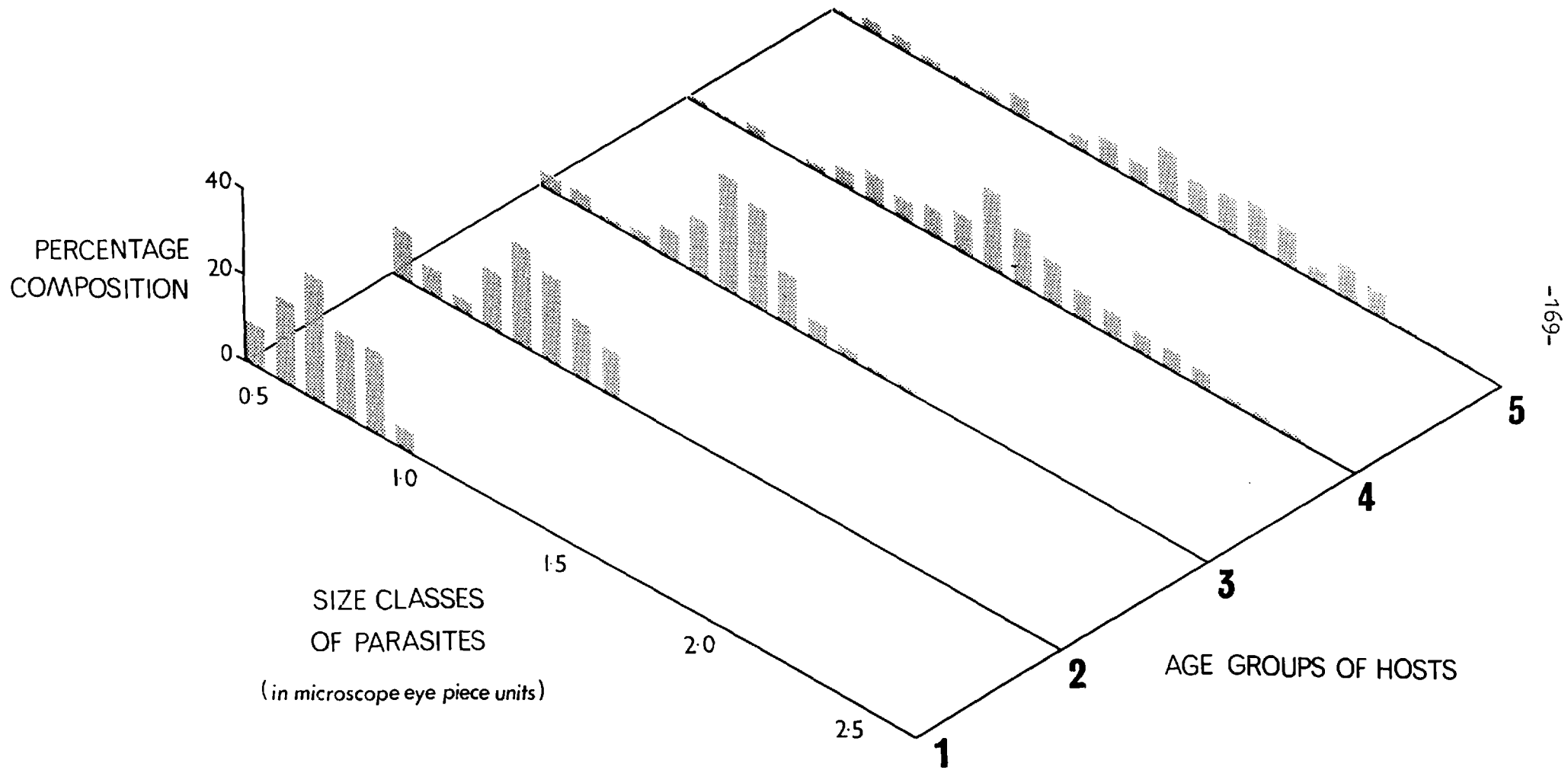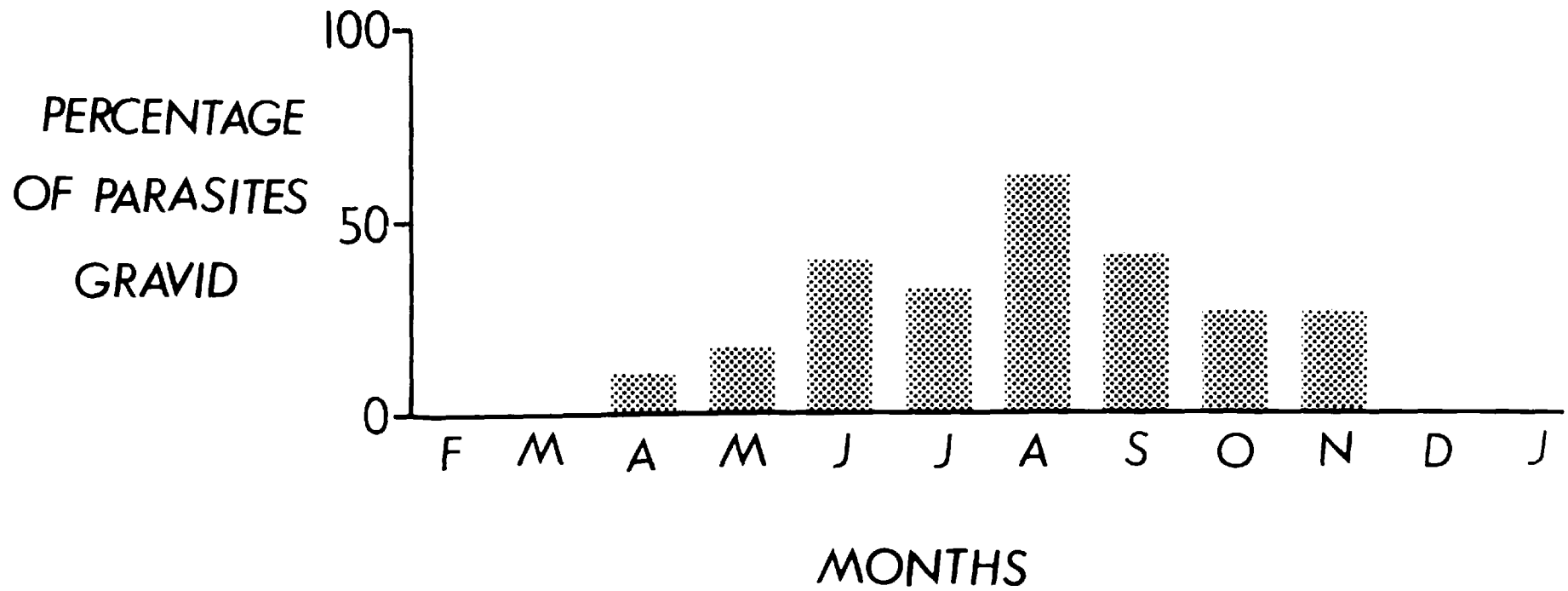
Fig (2.21)   The percentage of D.paradoxum gravid in each monthly

sample.

The Ecological niche of the parasite within the fish host

A detailed analysis of the parasites positioning on the gills was carried out to gain information on the dispersion of D.paradoxum throughout the different gill sections, filaments (hemibranchs) and arches of each age group of hosts throughout the year. The oncomiracidia of D.paradoxum are recorded (Bovet, 1967) as showing no attraction to their host's epithelium. Instead they are swept, by chance, passively with the respiratory current and attach to the gills as the water flows across them. The mechanisms of attachment of D.paradoxum and its position- ing on the gill primary and secondary lamellae are described by Owen (1963a, b) and Bovet (1967).

The distribution data of the parasite on the different gill areas was analysed by a factorial analysis of variance. As described earlier (p 68) each gill filament was divided approximately into four sections along its length, section one representing the dorsal section and section four the ventral section.

Two types of analysis, A and B were used.

Analysis A

The four factors used in analysis A were as follows:-

1) Gill sections at 4 levels; sections 1, 2, 3 and 4.

2) Gill filaments (hemibranchs) at 2 levels; inner and outer.

3) Gill arches at 4 levels; arches 1, 2, 3 and 4.

4) Replicates at 10 levels; 5 fish were examined in each age

group. However, since in a preliminary analysis of

variance using left and right gill chambers as a separate

factor at 2 levels, no significant differences occurred

between the two gill chambers, they were regarded as

further replication making 10 replicates in total in

five fish.

The replicates were incorporated as a factor in the factorial design of the analysis. However, the main effect of the replicates and their interaction terms were pooled in the analysis and combined as the error mean square. In this analysis only the interpretation and significance of the main effects of the factors is attempted.

Seventy-two analyses, 12 months x 6 age groups, were completed. The model for analysis A, to represent the data X_{ijkl} is as follows where X_{ijkl} = the transformed number of parasites per gill section. (Sections = S, Filaments = F, Arches = A and Replicates = R where they have a, b, c and d levels respectively. Thus i = 1...a, j = 1...b, k = 1...c, l = 1...d). μ = overall mean.

$$X_{ijkl} = \mu + S_i + F_j + A_k + R_l + (SF)_{ij}$$
$$+ (SA)_{ik} + (SR)_{il} + (FA)_{jk} + (FR)_{jl}$$
$$+ (AR)_{kl} + (SFA)_{ijk} + (SFR)_{ijl} + (SAR)_{ikl}$$
$$+ (FAR)_{jkl} + (SFAR)_{ijkl} + \epsilon_{ijkl}$$

ϵ_{ijkl} is the error variation within a particular factor level and takes into account all those factors which have not been controlled. It is assumed to be normally distributed about zero mean.

In the analysis the error mean square is calculated from the sum of the replicate interactions and the term ϵ_{ijkl} plus the replicate main effect.

Thus the error term E

$$= R_l + (SR)_{il} + (FR)_{jl} + (AR)_{kl} + (SFR)_{ijl}$$
$$+ (SAR)_{ikl} + (FAR)_{jkl} + (SFAR)_{ijkl} + \epsilon_{ijkl}$$

Therefore in summary the model becomes in essence a 3 factor design

$$X_{ijkl} = \mu + S_i + F_j + A_k + (SF)_{ij} + (SA)_{ik}$$
$$+ (FA)_{jk} + (SFA)_{ijk} + E.$$

The 3 factors in analysis A, sections, filaments and arches are regarded as having 'systematic' effects in contrast to the random effects of the replicates. This term 'systematic' effect implies that all possible levels in each factor are incorporated in the analysis. This type of analysis is referred to as Model I by Huitson (1966). In a Model I type of analysis, to test the main effect of a specific factor, the mean square of this factor is divided by the calculated error mean square, as described above, to yield the F-ratio. This procedure has been carried out in this analysis.

Analysis B

The analysis followed A except for the addition of age effect by combining all age groups within each month. The model for analysis B is as follows:- Age groups = G; (m = 1.... e)

$$
\begin{aligned}
X_{ijklm} = \mu & + S_i + F_j + A_k + R_l + G_m + (SF)_{ij} \\
& + (SA)_{ik} + (SR)_{il} + (SG)_{im} + (FA)_{jk} + (FR)_{jl} \\
& + (FG)_{jm} + (AR)_{kl} + (AG)_{km} + (RG)_{lm} + (SFA)_{ijk} \\
& + (SFR)_{ijl} + (SFG)_{ijm} + (FAR)_{jkl} + (FAG)_{jkm} \\
& + (ARG)_{jkm} + (FRG)_{jlm} + (SAG)_{ikm} + (SRG)_{ilm} \\
& + (FAR)_{jkl} + (SFAR)_{ijkl} + (SFAG)_{ijkm} \\
& + (FARG)_{jklm} + (SARG)_{iklm} + (SFRG)_{ijlm} \\
& + (SFARG)_{ijklm} + \epsilon_{ijklm}
\end{aligned}
$$

This analysis B is similar to A, each factor excepting replicates is systematic and the calculation of the error mean square is the same in principle.

Results of analysis A:-

The significant F-ratio for the main effects of each factor (Sections, Filaments and Arches) in each age group of hosts for each

month are shown in Fig (2.22A). It can be seen that the factors which
are consistently significant during the twelve months are identical in
each age group of hosts.

Factor 1: sections of the gills:-

This factor is consistently the most highly significant in all
age groups of hosts, but predominately in the 4th and 5th groups. A
significant difference between the mean number of parasites per section
occurs less frequently throughout the year in the young groups of hosts.
Fig (2.23) represents diagrammatically the derived mean numbers of
parasites on each gill section within each gill filament and arch, for
the combined monthly samples in each age group of hosts. It can be seen
that the distribution across the sections becomes progressively less
uniform with the age of the host. A "preference" for the dorsal gill
sections is recorded. The derived means for age groups combined in each
monthly sample are shown in Fig (2.24).

No significant differences between the number of parasites on
each gill section in the 1st, 2nd and 3rd age groups of hosts occur in
the mid summer months (Fig 2.22A), which is the period when heavy
mortalities occur in the parasite population in those age groups. These
losses seem to make the parasites' distribution more regular over the gill
sections.

Factor 2: filaments (hemibranch) of the gills:-

The inner and outer hemibranch of the gills are referred to as
inner and outer gill filaments. Significant differences occur in 2 months
in the 1st age group of hosts, 4 months in the 2nd, 5 months in the 3rd,
6 months in the 4th and 10 months in the 5th age group of hosts,
significantly larger numbers of parasites occurring on the inner gill
filaments for these months and the "preference" increases with the age
of the host. This trend may occur due to competition for space being more

acute in young fish with the parasites distributed on the majority of available space. In older fish however, there is more gill surface available per parasite, affording each parasite a "selection" of sites for attachment. These factors will be discussed more fully later.

Factor_3: arches of_the_gills:-

Only two significant F-ratio (at P = 0.05) occur in the total of 60 possible occurrences and these are assumed to be due to chance. It would appear therefore that the parasites are distributed uniformly over the four gill arches.
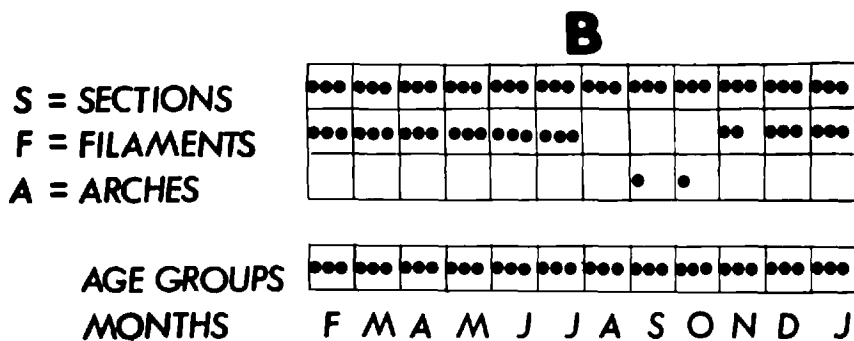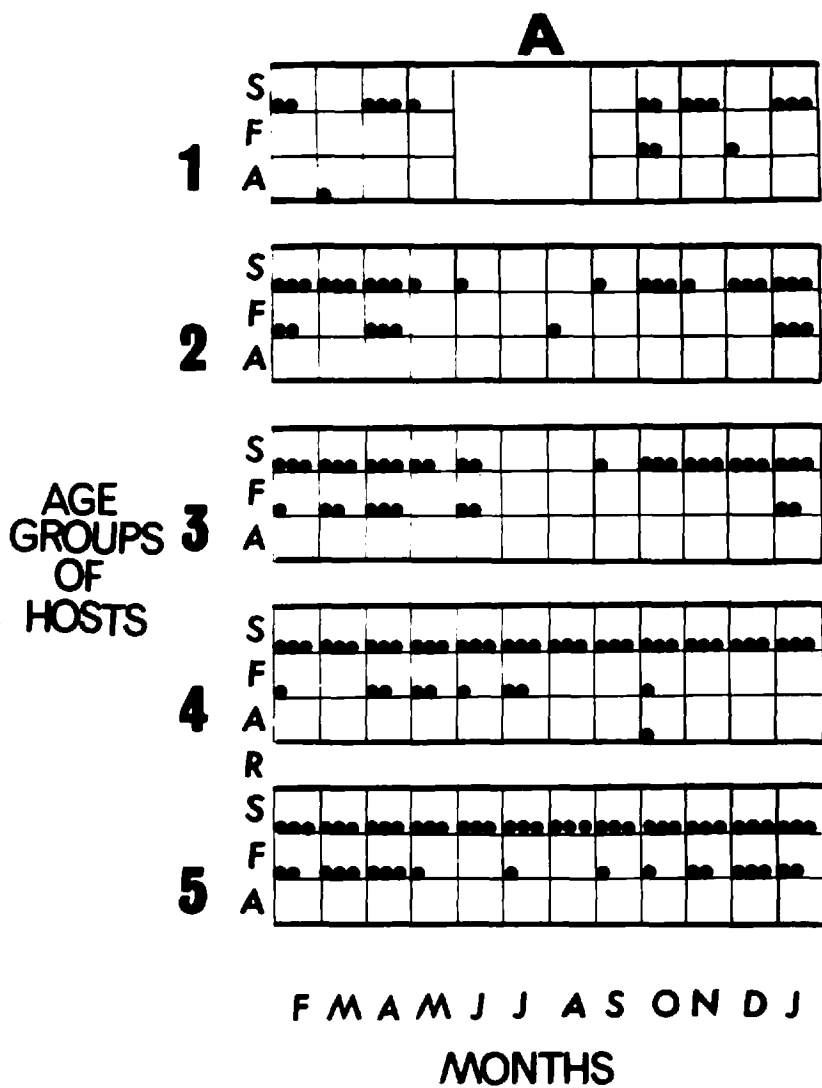
Results of_analysis B:-

The significant F-ratios for each of the four factors in the twelve monthly samples are represented in Fig (2.22B). As expected the age factor is significant in each month, large numbers of parasites occurring in older hosts (Fig 2.23). As in analysis A sections are significant, and arches non significant. Probably because of pooling, filaments are more consistently significant in each month in this analysis. As expected this analysis generally demonstrates the same trends indicated by analysis A.

When months of the year are included in the analysis then, as indicated previously (Fig 2.16), differences between months are significant $\angle$P (F = 16.0509)$<$0.001, d.f. (11,20736)$_\angle$. These differences in the number of parasites per host, per month, vary with the age of the fish.

No advantage is gained by including the fifth and sixth factors, age and month, in this type of analysis, for with the large number of degrees of freedom there is a tendency for all interactions and main effects to be significant. It is more informative to carry out small separate analyses for each age group of hosts in each monthly sample as in analysis A.

Fig (2.22)   Analysis of the spatial distribution of <u>D.paradoxum</u> on

the gills of the bream.

<u>A</u>: Significance of F-ratios for analysis A.

<u>B</u>: Significance of F-ratios for analysis B.

A

AGE
GROUPS
OF
HOSTS

1

2

3

4

5

F M A M J J A S O N D J

MONTHS

B

S = SECTIONS
F = FILAMENTS
A = ARCHES

AGE GROUPS

MONTHS   F M A M J J A S O N D J

• P ≤ ·05
•• P ≤ ·01
••• P ≤ ·001

Fig (2.23)   The spatial distribution of <u>D.paradoxum</u> on the gills

of the bream (derived mean number of parasites per

gill section) in each age group of hosts with monthly

samples combined.

MEAN NUMBER
OF PARASITES

1·0

0·5

0·0

GILL SECTIONS
GILL ARCHES 1

2

3

4

OUTER GILL FILAMENT

1 2 3 4

1

2

3

4

INNER GILL FILAMENT
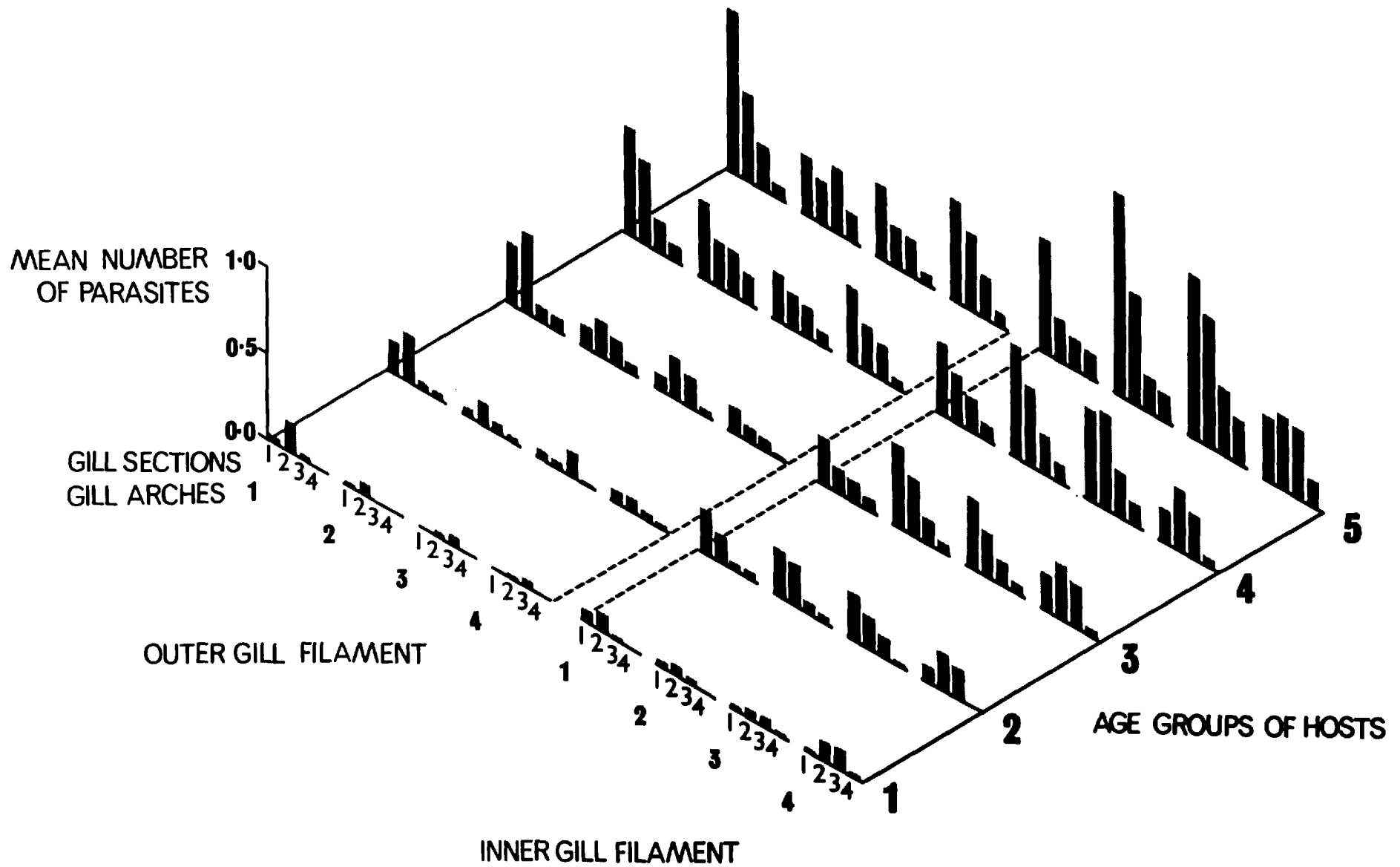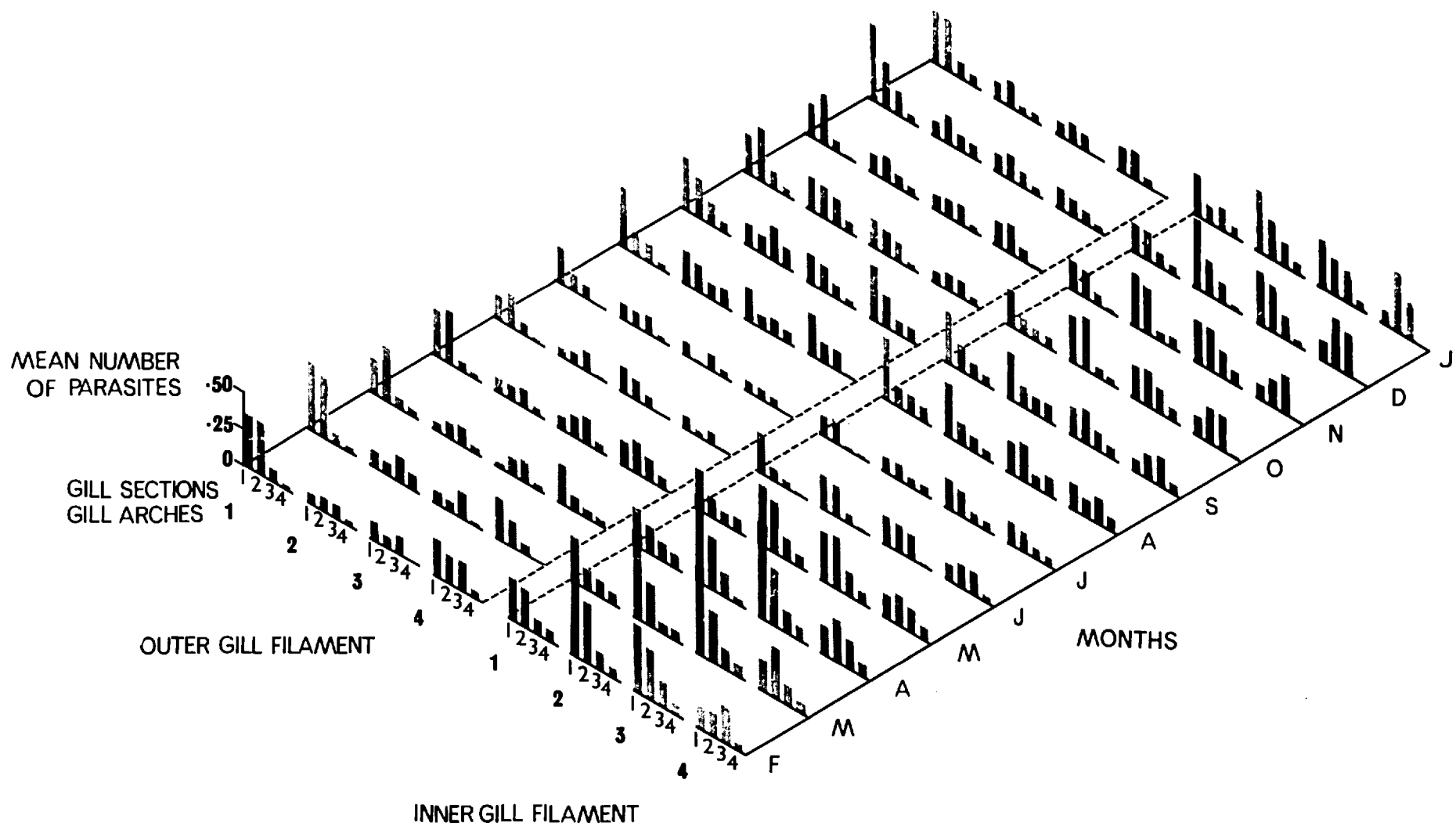
1

2

3

4

5

AGE GROUPS OF HOSTS

Fig (2.24)   The spatial distribution of D.paradoxum on the gills of

the bream (derived mean number of parasites per gill

section) in each month of the year with age groups of

hosts combined.

MEAN NUMBER
OF PARASITES

GILL SECTIONS
GILL ARCHES

OUTER GILL FILAMENT

INNER GILL FILAMENT

MONTHS

The distribution of the counts of the number of parasites per section, filament and arch:-

The frequency distributions of the number of parasites in the three different sampling units, sections, filaments and arches, were tabulated for each age group of hosts in each of the monthly samples.

No distinction was made between sections 1, 2, 3 and 4, all counts being tabulated in the frequency tables. Similarly counts for both filaments were combined and for all the four different arches. It was not possible to test the goodness of fit of these observed distributions to the theoretical models available in the computer programme TOPFIT because of restrictions imposed by low numbers of degrees of freedom arising from relatively small ranges of counts particularly for the sections.

To overcome this problem the variance/mean ratios were examined for each age group of hosts in each month of the year. Of the 180 calculated ratios only 11 were greater than 1.0 and these eleven were all less than 1.5. The dispersion of the parasites within these sampling units therefore tends to be regular or under dispersed. The eleven ratios greater than 1.0 were all section ratios indicating that a slight tendency to overdispersion occurs between gill sections. The analysis of variance also indicated "preference" for specific sections. The apparent regularity of the dispersion of the parasite is interesting and may well imply that the parasites are in competition for space on the gills, the presence of one parasite on a section, filament or arch decreasing the probability of establishment of other invading parasites. Regularity (p 150) was apparent in the total numbers of D.paradoxum per fish in the 3^+ hosts sampled in March 1969, and may well be a feature of the dispersion of the parasite not only within the host population , but also within the micro-environment.

Discussion of the dispersion of D.paradoxum on the gills of the bream

From the factorial analysis of variance the following points are established concerning the distribution of D.paradoxum on the different regions of the gills:-

1) Largernumbers of parasites on average are attached on the dorsal sections of the gills throughout the year.

2) The trend in (1) is not so marked in the younger age groups of fish during the summer months.

3) On average larger numbers of parasites are attached to the inner gill filaments during the year.

4) The trend in (3) is not so marked in the younger age groups of fish.

5) The differences between the number of parasites on the four gill arches are not significant.

6) There are significant differences between the number of parasites in each of the five age groups of hosts, older fish harbouring larger numbers of parasites.

7) There are significant differences in the number of parasites per age group of hosts between the months of the year.

The mechanisms determining the positioning of the parasite on the gills are most probably complex and a combination of several factors. Two basic mechanisms are suggested which might explain the distribution of D.paradoxum on the gills.

1) Water currents in the gill chambers of the host (PASSIVE)

The nature of water currents through the gills may determine completely where the parasite becomes attached on the gills. This could operate in two ways.

(a) Large volumes of water passing over specific gill areas could lead directly to more parasites becoming attached in those areas.

(b) Conversely a high flow rate could lead to less attachments, a disruption effect, so that areas subjected to low flow, for example to eddy currents, would be most available for successful attachment.

2) Behavioural response by the parasite (ACTIVE)

Although D.paradoxum is swept passively into the gill chamber (Bovet, 1967), the oncomiracidia may, once attached to the gill surfaces, actively migrate in response to various stimulii, such as strength of water flow, to preferred regions of the gill arches. It has been observed in this study and by other authors (Wiles, 1965; Bovet, 1967; Davies, 1967) that if adult D.paradoxum are detached from the gills they fail to reattach themselves. It thus seems that the couples are not capable of movement on the gills and thus any active migration would have to take place in the early stages of infection; that is in the diaporpal stage. A preference may occur for sheltered or exposed positions, or it may be connected with the nature of the blood flow via different regions of the gill hemibranchs and arches.

Since the parasite enters the host passively in the water currents, it seems probable that gill currents play some part in determining the parasite's distribution. The most relevant of the published research on teleost respiration is concerned with the varying pressures occurring in the buccal and opercular cavities, and the consequent respiratory currents over the gills as a whole (Hughes and Shelton, 1958). However, only a single attempt (Paling, 1968) has been made to determine the relative volume of water which passes over the four gill arches or between the five gill slits. Paling's work was carried out using the larval glochidial stages of fresh water mussels such as species of Anodonta. These glochidia were mixed randomly in tanks of water into which brown trout (Salmo trutta) were introduced for set periods of time.

Glochidia clamp onto the gills on contact with the gill tissues. Thus the author reasoned that the largest number of glochidia would be found on gill arches over which the largest volume of water flowed. His results suggested that the largest volume of water passed over the middle two gill arches. Different species of fish have widely differing respiratory currents (Hughes, 1960a, b; Hughes and Ballintijn, 1965) thus the water flow in the bream is not necessarily similar to that in the trout. Paling's work, although a novel approach to the study of gill current, contains many assumptions which may not be valid. The most outstanding of which relates to the attachment of the glochidia. Areas over which large fast flowing volumes of water pass might be sparsely covered with glochidia; the fast water flow preventing attachment in the same way as is argued in 1a above.

In this present study the numbers of parasites on the gill arches were not significantly different. If water flow over the different gill arches varied then one would expect differences between the numbers or parasites on each gill arch, ignoring other influences. The observed distributions would seem to indicate that influences other than gill currents affect the distribution of the parasites.

There is little information available in the literature concerning the behaviour of the oncomiracidia of monogenes (Paling, 1969; Kearn, 1967). Bovet (1967) has shown that the oncomiracidia of D.paradoxum are positively phototropic, but are not sensitive to the presence of the host's skin or mucus. It seems probable therefore, that infection is passive but there is no information available on the sequence of events in selection for sites, once the parasite is inside the host.

The free swimming oncomiracidium has a number of sensory organs especially at the anterior end, including an eye spot. After attachment to the gill surfaces, the eye spot and some of the other sensory organs are lost. A number of sensory organs must be retained if the parasite

actively seeks a preferred site for attachment in the diaporpal stage.
The selection of a site for attachment by the parasite would aid in the
finding of another parasite for fusion, concentrating the larval stages
in certain areas.

The size of each region of the gills may also be important.
Although there are differences in size of the sections, filaments and
arches within a fish, they are small in comparison to the total area of
each region. In older fish they may have more influence since differences
will be larger in relation to the size of the parasite.

To summarize it seems probable that the observed distribution
of D.paradoxum on the gill surfaces could result from four prime factors:
(a) gill currents in the host, (b) behavioural response of the parasite,
(c) space available and (d) competition for this space. Space and
competition could be of overriding importance when infectations are
heavy or when the fish are small.

The interrelationships between the number of D.paradoxum per fish

and host and weather parameters

It has been demonstrated in this study that the intensity of infection of the bream with D.paradoxum increases with the age of the host. This relationship was investigated in greater detail by ortho- gonalised multiple regression analysis (p 83). This technique was also used to investigate the relationship between a variety of local meteorological variables and the mean number of parasites per age group of hosts throughout the year. The aims of this type of analysis are as described previously (p126).

Host features and parasite numbers

The host variables used in this analysis were as follows:-

Variable number 1) Weight (gms.)

2) Length (cms.)

3) Girth (cms.)

4) Operculum size (cms.)

5) Sex (dummy variable)

6) Maturity (dummy variable)

7. Age (years)

The raw counts of the number of parasites per host were transformed to $x_2 = \sqrt{(x_1+1)}$ (where x_1 = raw count). Each monthly sample was analysed separately. Principal component analysis of the above independent variables produced seven components. The interpretation of these components in terms of the original variables was achieved by the examination of the normalised latent vectors of each component. The structure of the components was very similar in each month of the year; thus these vectors are only shown for one month, January 1970 (Fig 2.25) and are typical of the other months. The seven components can be inter-

preted as follows:-

Component one.

The first component is made up of almost equal normalised latent vectors for each of the seven independent variables and measures the overall size and thus age of the fish. The weight for the sex variable is the smallest.

Component two.

The second component has a dominant large weighting due to the sex variable. Female fish have high component values, males intermediate values and immature fish low values.

Component three.

This component is made up primarily of a large weight due to the variable maturity which dominates the other variables. Mature fish have high component values while immature fish have low component values.

Component four.

This component is difficult to interpret. The variable weight has a high weighting in opposition to the weightings of the variables length, girth and operculum size.

Component five.

In component five the largest weights are attributable to the variable weight in opposition to age. Thus fish which are heavy for their age have high component scores and vice versa.

Component six.

This component contains large weights for the variables length and girth in opposition to operculum size. This component is difficult to interpret and was found to be unimportant in later analysis.

Component seven.

In component seven the variables length and girth have large positive and negative weights respectively, and thus the component seems

to measure shape. High component scores are assigned to fish with small fat bodies and low scores to long slim fish.

Since all the correlations between the transformed dependent variable and the independent variables were large within each monthly analysis (Table 2.30), the above components were used in the regression analysis. Far fewer significant correlation coefficients (r) occurred between the components and the dependent variable (Table 2.31). The percentage variances of the dependent variable accounted for by each of these components are shown in Table (2.32) and their values are illustrated in Fig (2.26) for each month of the year. The above percentages when summed over each component give the total percentage variance in the dependent variable accounted for by all the components, which is equivalent to the total variation accounted for by the original independent variables. These totals, when divided by one hundred, equal the square of the multiple correlation coefficients (R).

It is clear that within each month a significant amount of variation (Table 2.32) in the transformed numbers of parasites per host has been accounted for by the measured independent variables. As described previously (p 85), the components with significant correlations with the dependent variable (Table 2.31) were used in the subsequent multiple regression analysis. The significance levels of the r or R^2 values, depending on whether one or more components were used in the analysis, are shown in Table (2.33). The calculated standardized partial regression coefficients for each independent variable (a) using all the components and (b) using only the significant components are shown in Tables (2.34) and (2.35) respectively. For predictive purposes the coefficients in Table 2.34) should be used; however, since the original data was standardized the means and standard deviations of the variables are given in Table (2.16). The predictive equations are of the form

described previously (p. 80). Employing the standardized partial

regression coefficients calculated from the analysis using only the

significant components, coefficients of separate determination were

calculated. From these coefficients of separate determination,

"coefficients of effect" were calculated and thus the absolute per-

sentage effects of each independent variable in its association with

the dependent variable were derived (Fig. 2.27).

It can be seen (Fig. 2.27), as would be expected from the

previous analysis of distribution on the gills, that size of the host

is most important for the majority of the months. The correlation is

positive, thus the number of parasites on the gills increases with the

size of the fish. The variables' weight, length, girth, operculum size

and age all measure various aspects of size and thus contribute equally

to the criterion variables variance. In future studies the measurement

of only one of these independent variables would be sufficient to

predict the numbers of parasites per host if no other factors were

operating at that time. The apparent association of sex of the host

with the parasite numbers in January 1970 is difficult to explain and

may be due largely to chance variation.

Table 2.30   The correlation coefficients (r) between the transformed number of <u>D.paradoxum</u> per host and the host factors.

Independent Variable Month	Weight 1	Length 2	Girth 3	Operculum 4	Sex 5	Maturity 6	Age 7
1969 Feb	.5121	.7853	.7845	.7468	.6329	.4814	.7855
March	.4499	.7661	.7539	.7423	.4700	.5375	.7205
April	.4122	.6832	.6870	.6511	.6424	.4112	.6515
May	.6221	.8313	.8281	.8364	.7048	.6014	.8022
June	.8060	.9615	.9571	.9619	.8922	.8499	.9525
July	.9067	.9034	.8955	.9033	.6145	.9120	.8242
Aug	.9146	.9324	.9282	.8141	.3855	.9576	.9271
Sept	.8514	.8917	.8836	.9126	.5137	.8200	.8712
Oct	.8045	.9167	.9144	.9326	.6442	.7517	.9013
Nov	.8389	.8820	.8722	.9081	.4760	.7976	.8634
Dec	.7644	.9075	.9136	.8977	.7221	.7404	.8960
1970 Jan	.5909	.7861	.7848	.7875	.8144	.5844	.7403

Table 2.31   The absolute values of the correlation coefficients (r) between the number of <u>D.paradoxum</u> per host and the host components.

Independent Variable Month	Weight 1	Length 2	Girth 3	Operculum 4	Sex 5	Maturity 6	Age 7
1969 Feb	.7401****	.3214*	.0883	.4200**	.0424	.0728	.0010
Mar	.6978****	.0529	.0346	.5824****	.0883	.0001	.1216
Apr	.6417****	.3924**	.2083	.4353***	.0888	.0693	.1212
May	.8081****	.1705	.1985	.3178*	.1063	.0264	.0100
June	.9587****	.0387	.0979	.1463	.0360	.0728	.0224
July	.9120****	.2152	.0200	.2258	.0728	.1049	.0021
Aug	.9451****	.2466	.1131	.1063	.0458	.0877	.0283
Sept	.8923****	.1552	.0360	.1655	.0883	.1833	.0200
Oct	.9028****	.0374	.1192	.2095	.0883	.1252	.0173
Nov	.8823****	.1581	.0566	.1637	.0938	.2240	.0142
Dec	.8959****	.1780	.1122	.1916	.0985	.0794	.0283
1970 Jan	.7729****	.4061***	.1025	.1936	.0506	.0141	.0583

(**** P $<$ .001;   *** P $<$ .01;   ** P $<$ .05;   * P $<$ .10)

Table 2.32   <u>D.paradoxum</u>   The Percentage variance of the dependent variable accounted for by each host component.

Component Months	1	2	3	4	5	6	7	Total $(100 \times R^2)\%$
1969 Feb	54.77	10.33	0.78	17.64	0.18	0.53	0.00	84.23***
Mar	48.70	0.28	0.12	33.92	0.78	0.00	1.48	85.30***
Apr	41.18	15.40	4.34	18.95	0.79	0.48	1.47	82.78***
May	65.30	2.91	3.94	10.10	1.13	0.07	0.01	83.37***
June	91.92	0.15	0.96	2.14	0.13	0.53	0.05	95.10***
July	83.18	4.63	0.04	5.10	0.53	1.10	0.00	94.57***
Aug	89.32	6.08	1.28	1. 13	0.21	0.77	0.08	98.87***
Sept	79.63	2.41	0.13	2. 61	0.78	3.36	0.04	88.95***
Oct	81.50	0.14	1.42	4. 39	0.78	1.57	0.03	89.84***
Nov	77.86	2.50	0.32	2.68	0.88	5.02	0.02	89.27***
Dec	80.26	3.17	1.26	3.67	0.97	0.63	0.08	90.06***
1970 Jan	59.74	16.49	1.05	3.75	0.32	0.02	0.34	81.71***

(*** $<$ P 0.001)

Table 2.33   <u>D.paradoxum</u>   Significance of r or R^2 calculated in the multiple regression analysis using only the significant components.

Months	r	R^2	F-ratio	d.f.	Probability
1969 Feb	–	.8274	41.54	3/26	$<$ .001
Mar	–	.8262	64.17	2/27	$<$ .001
April	–	.7553	26.75	3/26	$<$ .001
May	–	.7540	41.38	2/27	$<$ .001
June	.9587	–	–	28	$<$ .001
July	.9120	–	–	28	$<$ .001
Aug	.9451	–	–	34	$<$ .001
Sep	.8923	–	–	38	$<$ .001
Oct	.9028	–	–	38	$<$ .001
Nov	.8823	–	–	33	$<$ .001
Dec	.8959	–	–	40	$<$ .001
1970 Jan	–	.7623	64.74	2/41	$<$ .001

Table 2.34   <u>D.paradoxum</u>   Standardized partial regression coefficients calculated from the multiple regression analysis using all the components.

Independent Variables Month	1 Weight	2 Length	3 Girth	4 Operculum	5 Sex	6 Maturity	7 Age
1969 Feb	-.940	1.027	1.020	-.518	-.093	-.248	.358
Mar	-1.428	-.536	0.910	1.730	-.269	-.439	1.271
April	-1.213	-1.339	3.222	- .656	.121	-.442	.860
May	- .623	.948	.778	.239	.146	-.421	-.326
June	- .323	1.154	-.555	.793	.213	.055	-.376
July	.371	.839	.597	-.890	.015	.643	-.577
Aug	.293	-.946	.168	.049	-.037	.614	.898
Sept	- .601	.529	-.843	1.912	-.122	.332	-.322
Oct	- .287	.315	-.878	1.879	-.095	-.179	.098
Nov	-. 626	.313	-1.182	2.415	-.146	.229	-.155
Dec	-.683	.300	1.305	-.586	-.006	-.115	.640
1970 Jan	-.616	1.334	-.196	.252	.270	-.378	.119

Table 2.35   <u>D.paradoxum</u>   Standardized partial regression coefficients calculated from the multiple regression analysis using only significant components.

Independent Variable Month	1 Weight	2 Length	3 Girth	4 Operculum	5 Sex	6 Maturity	7 Age
1969 Feb	-1.081	.521	.468	.345	-.021	-.114	.528
Mar	-1.087	.725	.648	.471	-.328	-.244	.289
Apr	-1.218	.667	.535	.435	.115	-.222	.324
May	- .672	.462	.389	.405	.169	.073	.361
June	.138	.150	.149	.150	.137	.134	.148
July	.135	.148	.147	.148	.117	.133	.143
Aug	.153	.164	.165	.146	.100	.145	.164
Sept	.141	.146	.146	.146	.104	.126	.146
Oct	.137	.147	.147	.146	.105	.132	.147
Nov	.140	.146	.145	.146	.101	.124	.145
Dec	.138	.146	.146	.145	.100	.134	.145
1970 Jan	-. 069	.119	.119	.127	.561	.001	.081

Fig (2.25) The interrelationships between the number of D.paradoxum

per host and the host parameters.

The Normalised latent vectors of each component for

January 1969. (Figures in brackets represent the percentage

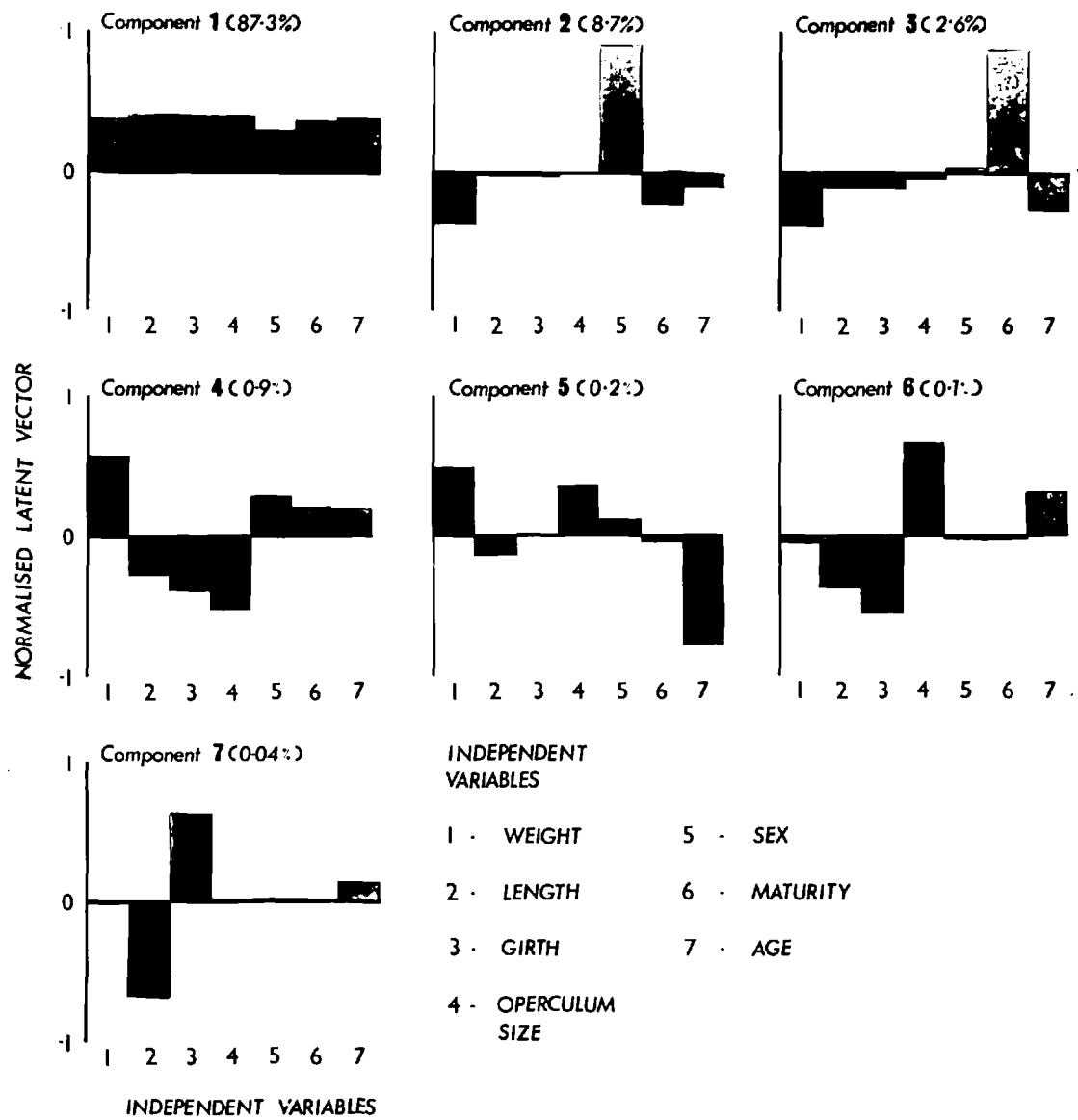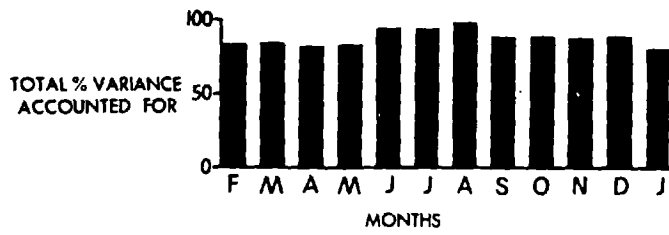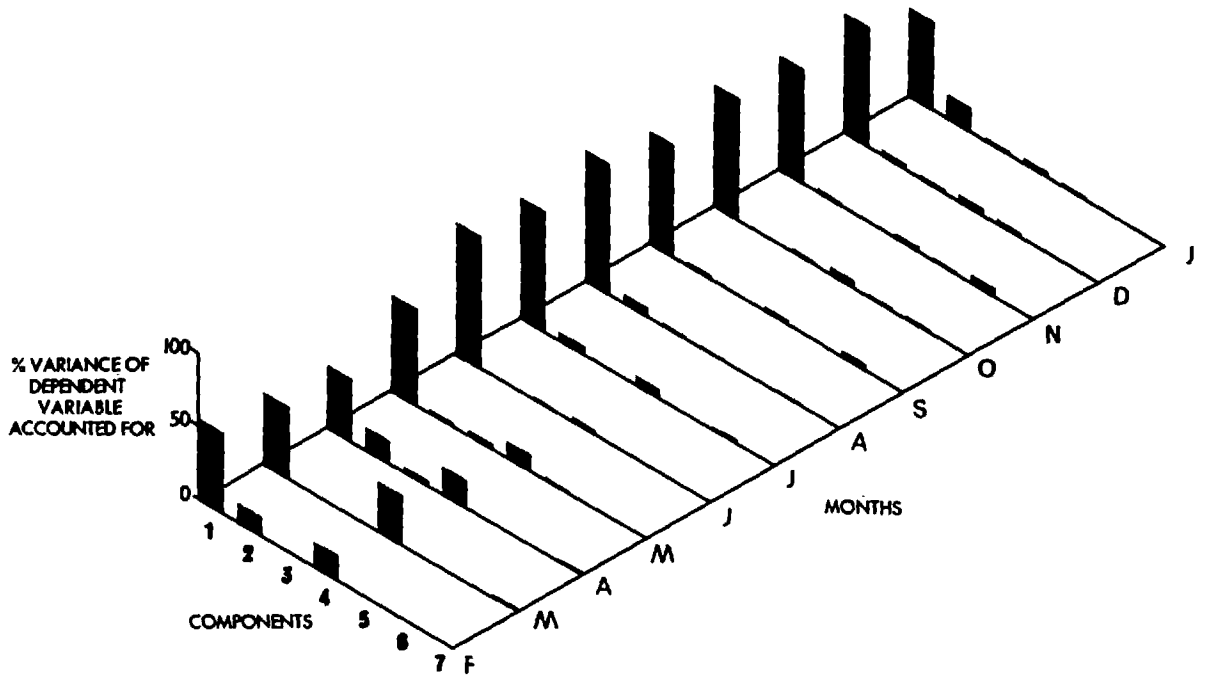of the total variation accounted for by each component)

Fig (2.26)   The interrelationship between the number of D.paradoxum

per host and the host parameters.

The percentage variance of the dependent variable and

the total variation, accounted for by the components.

% VARIANCE OF
DEPENDENT
VARIABLE
ACCOUNTED FOR

COMPONENTS

MONTHS

TOTAL % VARIANCE
ACCOUNTED FOR

F M A M J J A S O N D J

MONTHS

Fig (2.27)   The interrelationship between the number of D.paradoxum

per host and the host parameters.

The percentage 'effects' of the independent variables

in their association with the dependent variable

(analysis incorporating the significant components)
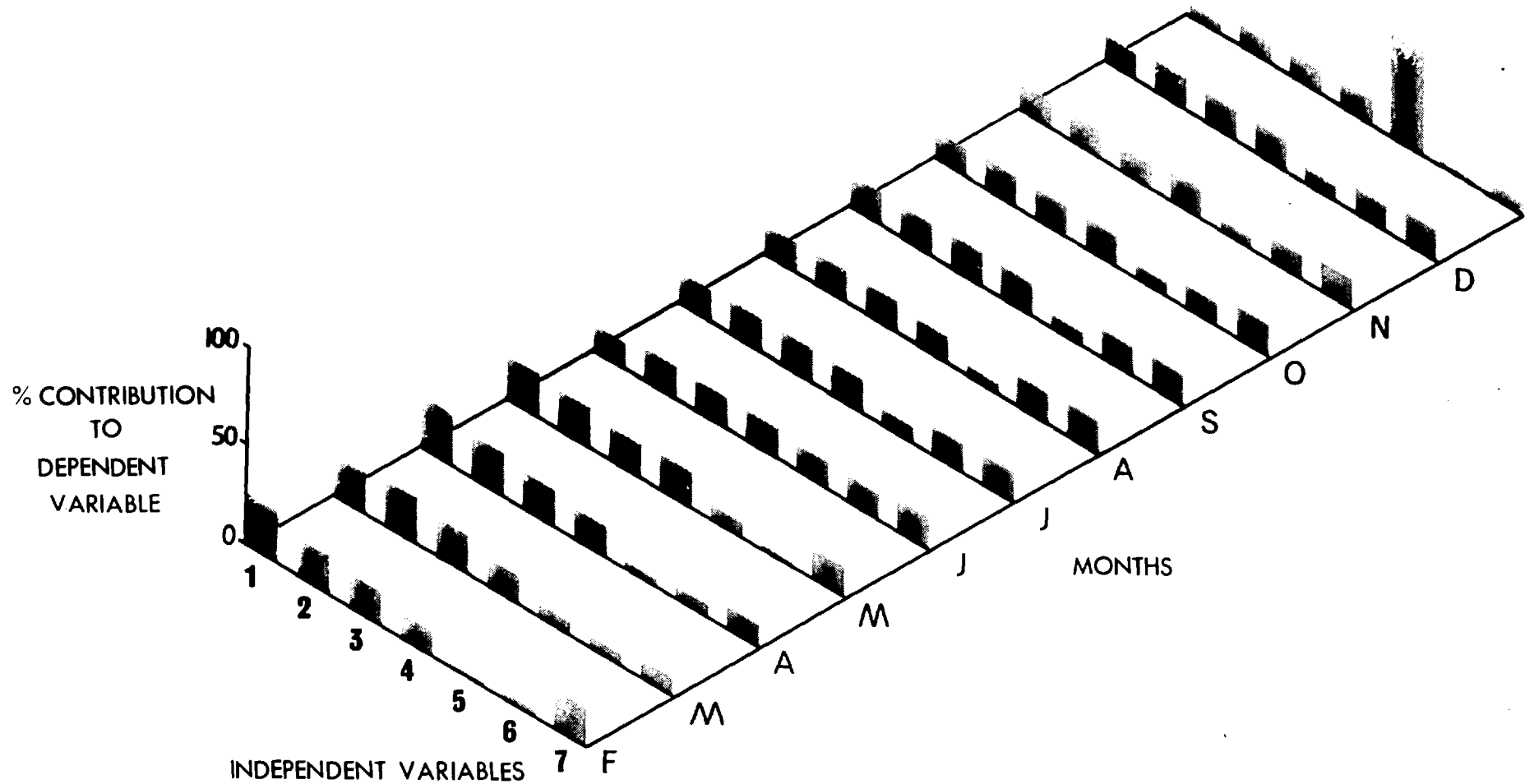
Weather factors and parasite numbers

The weather variables used in this analysis were the same as described previously (p139) in connection with C.laticeps, thus the same components were used in the analyses (p139, Fig 2.14) The 0 age group of hosts were uninfected throughout the year and were not included in the analysis. The correlation coefficients between the dependent variable and the independent variables, between the dependent variable and the components and the percentage variation and total variation accounted for by the components are shown respectively in Tables (2.36), (2.37) and (2.38) Fig (2.28). Since a significant amount of variation is accounted for by the components (Table 2.38), components with significant correlations (Table2.37) with the dependent variable were used in the subsequent multiple regression analysis. The significance of the amount of variation accounted for in these new analyses are shown in Table (2.39). The calculated standardized partial regression coefficients for each independent variable (a) using all the components and (b) using only the significant components are shown in Tables (2.40) and (2.41) respectively. For the purpose of constructing predictive equations the means and standard deviations of the original variables are listed in Table (2.23).

It is clear from the absolute percentage effects (percentage contribution to the dependent variable) (Fig 2.29), that the observed contributions of each weather variable differs for each age group of hosts. Water temperature (variable 3) stands out as important in its association with the dependent variable in the 1st, 2nd, 3rd, 5th and to a lesser extent the 4th age group of hosts. It is interesting to note that the correlations for the first to the fourth age groups are negative while that for the fifth age group is positive. The influence of day length and sunshine on the mean number of parasites in the fourth age group of hosts is difficult to explain but may be related to the hosts' behaviour. Possible explanations for these observed correlations will be discussed later.

Table 2.36   The correlation coefficients (r) between the selected
              weather factors and the mean number of D.paradoxum per host.

Independent Variable	Rainfall	Sunshine	Water Temperature	Day length	Range of water temperature
	1	2	3	4	5
Age group					
1	-.0529	-.5536	-.7788	-.6112	-.3837
2	-.1138	-.4481	-.8026	-.3646	-.5575
3	-.1421	-.3589	-.6952	-.3586	-.3529
4	-.2391	-.5846	-.2290	-.5384	-.6050
5	-.1610	.1264	.5181	.2865	.0234

Table 2.37   The absolute values of the correlation coefficients (r)
              between the mean number of D.paradoxum per host and the
              weather components.

Components	1	2	3	4	5
Age group					
1	.6791***	.0100	.1876	.3569	.0794
2	.6181**	.1640	.1459	.6430**	.1304
3	.5109*	.1113	.1311	.5474*	.1170
4	.5549*	.2404	.1679	.5941**	.0412
5	.2846	.1830	.1852	.5827**	.4948

(*** $P < .01$; ** $P < .05$; * $P < .10$)

Table 2.38   D.paradoxum   The percentage variance of the dependent
              variable accounted for by each weather component.

Component	1	2	3	4	5	Total $(100XR^2)\%$
Age group						
1	46.12	.01	3.52	12.74	0.63	63.02
2	38.21	2.69	2.13	41.35	1.70	86.08**
3	26.10	1.24	1.72	29.97	1.37	60.39
4	30.79	5.78	2.82	35.30	0.17	74.80*
5	8.10	3.35	3.43	33.96	24.48	73.30*

(** $P < .01$; * $P < .05$)

Table 2.39   <u>D.paradoxum</u>   Significance of r or R^2 calculated in the multiple regression analysis using only the significant components.

Age group	r	R^2	F-ratio	d.f.	Probability
1	.6791	-	-	10	$< .01$
2	-	.7956	17.51	2/9	$< .001$
3	-	.5607	5.74	2/9	$< .05$
4	-	.6609	8.77	2/9	$< .01$
5	.5827	-	-	10	$< .05$

Table 2.40   <u>D.paradoxum</u>   Standardized partial regression coefficients calculated from the multiple regression analysis using all the components.

Independent Variables Age group	1 Rainfall	2 Sunshine	3 Water temperature	4 Day length	5 Range of water temperature
1	-.016	.422	-.838	-.346	.002
2	.011	-.217	-1.258	.891	-.143
3	-.243	-.153	-1.158	.573	.242
4	-.079	-.468	.915	-.597	-.569
5	-.688	-2.343	.946	1.457	.480

Table 2.41   <u>D.paradoxum</u>   Standardized partial regression coefficients calculated from the multiple regression analysis using only the significant components.

Independent Variables Age group	1 Rainfall	2 Sunshine	3 Water temperature	4 Day length	5 Range of water temperature
1	-.011	-.212	-.203	-.207	-.153
2	-.135	.306	-1.265	.313	-.085
3	.116	.266	-1.072	.272	-.069
4	-.143	-.634	.832	-.633	-.175
5	-.132	-.452	.979	-.455	-.049

Fig (2.28)   The interrelationship between the mean number of

D.paradoxum per age group of hosts and the weather

parameters.

The percentage variance of the dependent variable

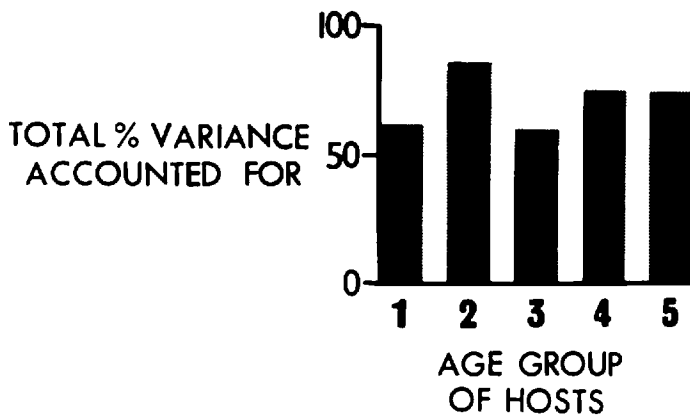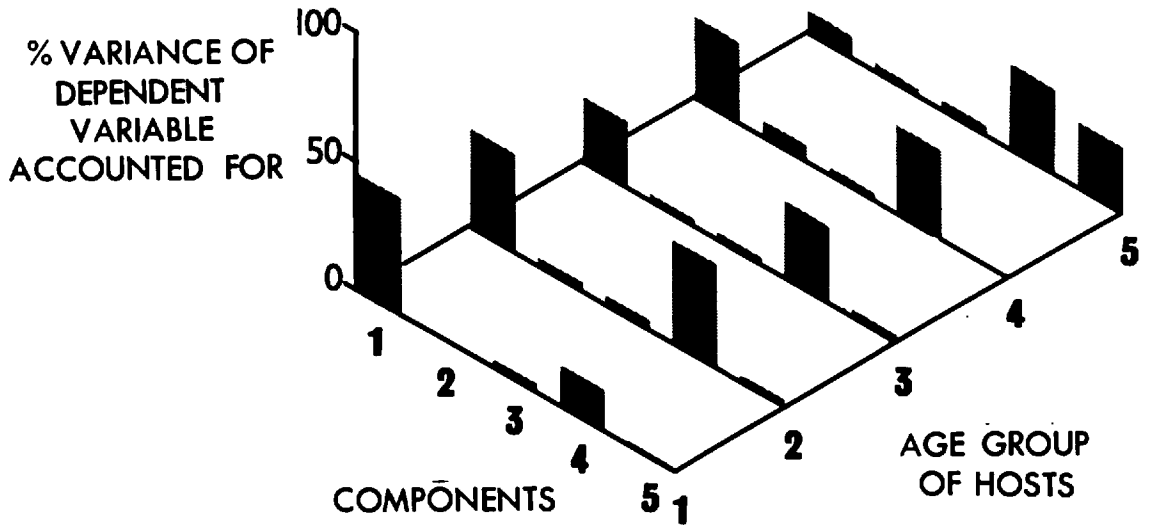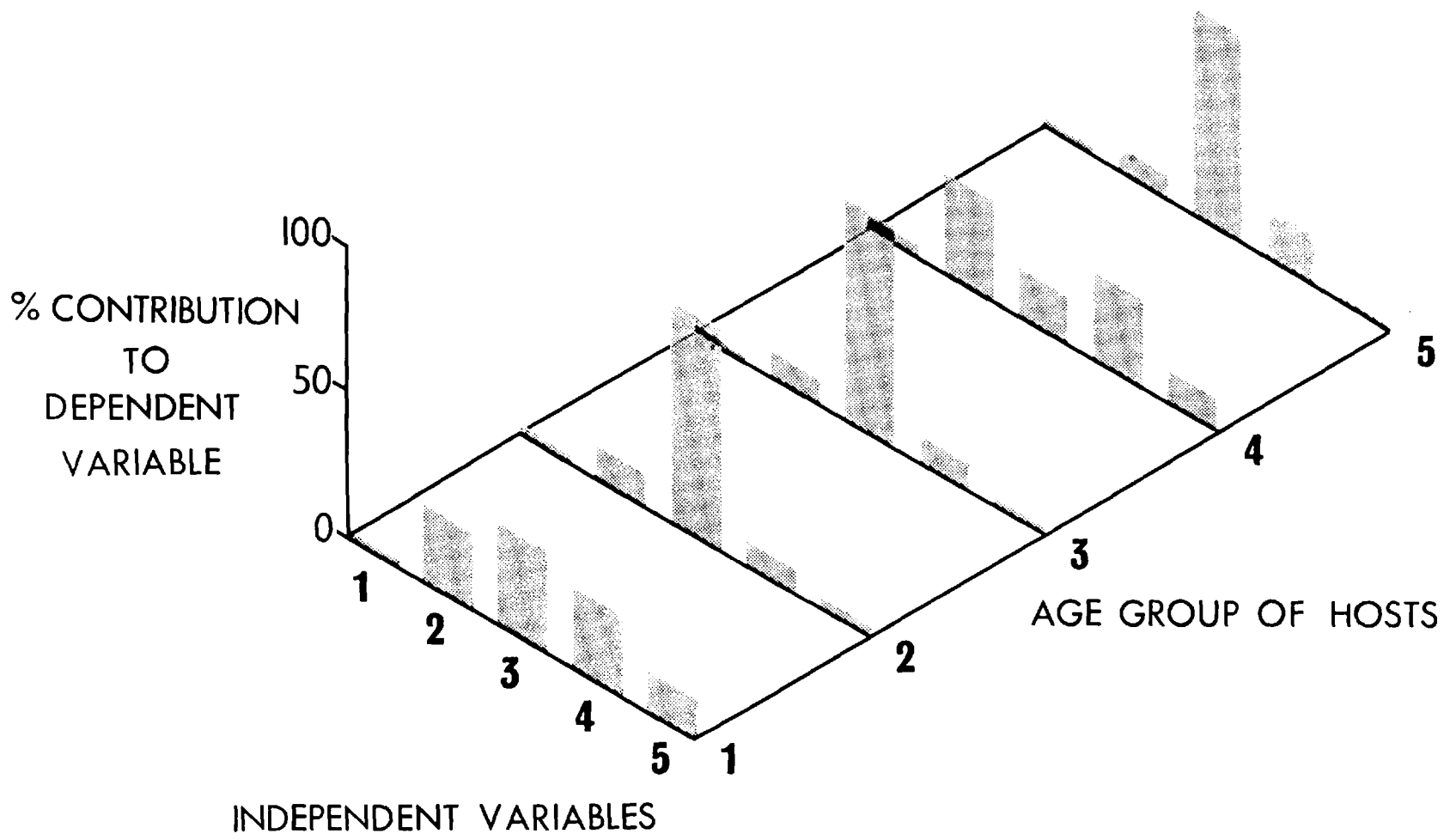and the total variation accounted for by the components.

Fig (2.29)   The interrelationship between the mean number of

D.paradoxum per age group of hosts and the weather

parameters.

The percentage 'effects' of the independent variables

in their association with the dependent variable

(analysis incorporating the significant components)

Discussion:-

A number of conclusions can be made concerning the population
dynamics of D.paradoxum.

1) The intensity and incidence of infection varies with the
age of the host and the time of the year.

2) The seasonality of these variations in intensity and
incidence are dissimilar in immature and mature fish.

3) The parasite does not infect fish of the 0 age group but
is present in the hosts of the other age groups throughout the year,
although it is lost from the young 1^+ fish during the mid summer months.

4) The parasites are regularly distributed between hosts
which might indicate competition for space within the fish. A tendency
to over dispersion is observed in mature fish.

5) The parasite has a maximum life span of between four and
five years.

6) Gravid parasites are present throughout most of the year
but large numbers are gravid in the summer months.

7) A decrease in the parasite population size occurred from
February 1969 to February 1970, and is most probably correlated with a
corresponding decrease in the host population.

8) The inner gill hemibranch and the dorsal gill sections
are more frequently occupied by D.paradoxum than other areas of the gills.

9) There is a high positive association between the number of
parasites per host and the physical size of that host.

10) The size of the parasite population is negatively correlated
with the water temperature in the 1st, 2nd, 3rd and to a lesser extent the
4th age groups of fish, whereas in the 5th age group of hosts the
relationship is positive.

A few of these points require further clarification.

The close correlation between the size of the host and the number of parasites present per host is the most consistent feature arising from the analysis of the D.paradoxum data. The size of the fish can be measured equally efficiently by a number of morphometric features as illustrated earlier. Three possible explanations for the link between number and size are suggested: 1) behavioural differences between fish of different sizes 2) volume of water flowing over the gills and 3) gill surface area.

The two latter explanations seem the most probable and have already been discussed (p180). The first explanation seems unlikely, since it appears probable that infective oncomiracidia are randomly dispersed within the environment. Water currents would serve as a horizontal dispersal agent while the vertical migration of the larvae towards the light (Bovet, 1967) would serve as a vertical dispersal agent. Thus, fish of all ages would most likely have an equal probability of encountering an infective stage of the parasite.

The correct explanation is more likely to be a combination of surface area available and volume of water passing over that area. Given a fixed density of infective stages within a habitat, it is probable that a simple relationship exists between the numbers of parasites per host, volume of water passing over the gills and the available gill area for attachment. (Gill area is closely correlated with length, girth, weight and thus with the physical size of a fish) Such a relationship would become more complex when saturation of the available gill area occurred, resulting in competition for space.

A further interesting feature of this study of D.paradoxum is the apparent high mortality rates of the parasites in the young age groups of hosts during the mid summer months, paralleled by a stable population in the 4th age group and an increased population in the 5th

age group of hosts. This pattern of infection is unusual since
D.paradoxum can live for approximately four years and large numbers
of infective stages are available in the summer months. Thus the pattern
in the 5th age group would be expected throughout the other age groups of
hosts. It was shown previously however, that correlation existed between
water temperature and numbers of parasites per host. Although the gravel
pit studied was relatively shallow, the deepest parts being approximately
twelve feet deep, a thermocline exists in the water between the surface
and bottom layers during the mid summer months. In this period the
differences between bottom and surface layers was approximately three to
four degrees centigrade. This thermocline exists, partially due to the
small size of the gravel pit and also to its sheltered position, summer
winds not causing a great deal of mixing of the water layers. Bovet (1967)
demonstrated experimentally that the upper lethal temperature for
D.paradoxum on the gills of bream was between twenty-two and twenty-four
degrees centigrade; at these temperatures the parasite detached from the
gills and died. The water temperatures recorded in this study (Fig 1.1)
were taken at a depth of one foot, and rose to above $22^{o}C$ in the mid
summer of 1969. Thus it seems plausible to suggest that these water
temperatures would cause mortalities in the parasite population, especially
in the young fish which inhabit the surface layers of the water. The
parasites harboured by the older bottom dwelling bream may never experience
these lethal temperatures due to temperature differences between bottom
and surface layers of the water.

The population size of the parasites within each age group of
hosts will depend upon the changes in input and output of the parasites.
The input or recruitment of parasites would seem to be greater during the
summer months due to the higher percentage of gravid worms during this
period. The output or mortality seems to depend on such factors as

competition for space, natural mortalities at the termination of the parasites' natural life span and the influence of environmental factors such as water temperature. Both input and output factors are time varying as indicated by the observed patterns of intensity and incidence of infection. They also vary in the different age groups of hosts and thus the seasonal patterns of population change in the host-parasite system are complex and determined by the interactions and main effects of a variety of factors. If a simultaneous input to and output from a host are occurring at a given moment in time, then the observed pattern is a reflection of the dynamic equilibrium of the system.

General Discussion

The sampling procedures and methods of analysis employed in this study have been orientated towards the detection and monitoring of changes in the population size and structure of both host and parasite throughout the year.

The relationships between host, parasite and changes in the external environment are complex. The system describes a multivariate biological problem including the interactions of three basic sets of variables consisting of host, parasite and environmental components. The main directions of these interactions can be represented diagrammatically as follows:-
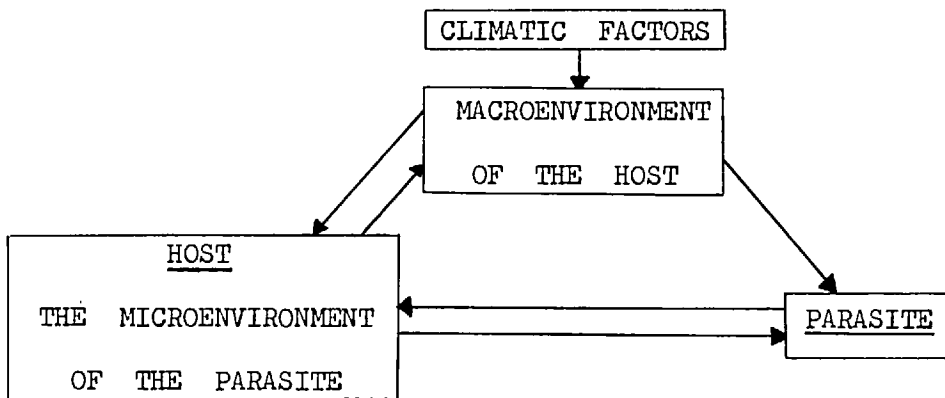
Perhaps one of the most fundamental points of interest both to fish biologists and parasitologists is the nature and direction of the influence between the size of the host and the parasite populations.

It has been shown that a decrease in the host population over the year of study was associated with a corresponding decrease in the parasite population. This poses the question of whether it is the host population which determines the size of the parasite population or the parasite population which determines the size of the host population. Alternatively there could be an interaction between the effects of both populations on each other modified by the influence of climatic factors.

For C.laticeps, the input of parasites into the host is
determined by the feeding behaviour of the fish and the availability
of infective stages. Feeding behaviour has been shown to vary with
age of the host, and in some cases with time of the year, thus
influencing the seasonal variation of the parasite population size in
each age group of hosts. For any one age group, little variation
would be expected in feeding behaviour between February 1969 and
January 1970. (This assumption may not be valid on a longer time scale
because the availability of food organisms could be changed by
alterations to the environment). Thus, when the host population
decreases, the intensity of infection per fish would have to increase
to maintain a stable parasite population size. For example, in
February 1969 the mean number of parasites per host, for the second
age group, was 0.54 (an estimated total of 2503 parasites in the age
group). To maintain a total of 2503 parasites in the third age group
of fish in January 1970 the intensity of infection would have had to be
increased to 1.50; in fact the observed value was 0.83. Little
variation had occurred in the availability of tubificids (16.1 per
sample in February 1969 and 15.5 per sample in January 1970) and in the
availability of infected tubificids (8.3% in February 1969 and 7.1% in
January 1970). Since the numbers of tubificids and the proportion of
those infected did not vary between the two sample dates it can be
reasonably assumed that the number of infective stages available per
fish was increased. This did not lead to an increased intensity of
infection which might indicate an asymptotic carrying or pick up
capacity per fish. It thus seems probable that the decrease in the
adult parasite population size has directly resulted from the decrease
in the host population. It is unlikely that the decrease in the fish
population was due to the influence of the parasite, since there is no

evidence to suggest that the host is damaged at the levels of intensities of infection encountered.

It also seems that the survival rate of the parasite is associated with a density independent factor, water temperature, which is particularly important during the warm summer months (Kennedy, 1971). Thus, the observed population size of the parasite would seem to be controlled by the combined effects of host population size and environmental factors.

A similar situation applies for D.paradoxum. However, from the evidence concerning the parasite dispersion between and within hosts, it seems that there is density dependent competition for space on the gills. Thus, the number of parasites on the gill surface area is directly related to the total area of the gill surfaces. It follows that the fewer the hosts present, the smaller the total number of parasites in the environment. Water temperature also seems to be important, particularly in decreasing the parasite population on the young fish during the summer months. (It is interesting to note in this case that the host has a wider range of tolerance than the parasite it harbours). Thus the parasite population is again regulated by host population size and environmental factors. However, it may be true that where heavy infections occur, the parasite causes some mortalities in the fish population, thus the mechanisms controlling the parasite population size are most probably the interaction effects of host, parasite and environment.

A further point of interest in connection with size of the parasite population lies in the reproductive capacity and maturation cycles of the two parasite species.

Both parasites' populations are at a minimum size during the mid summer months; in the case of D.paradoxum due to the adverse effects

of high water temperatures, and in the case of C.laticeps due to a
combination of changes in feeding habits of certain age groups of
hosts and also to the temperature dependent mortality rates of the
adult parasites. However, although both populations are at a minimum
in summer, this is the precise period when the largest proportion of
the total population of parasites are gravid. The coincidence of
minimum population size and the peak of reproductive capacity may
result in the prevention of hyper-infection of the host population,
thus preventing the extinction of both hosts and parasites. A subtle
mechanism of balance thus seems to be operating.

It is readily apparent from the results presented in this
study that the size of the parasite population within a host depends
on changes in input and output of the parasites. Input is influenced
by the availability of infective stages and by the feeding habits of
the host, while output is controlled by the failure of parasites to
establish, rejection of the parasites by the host and natural
mortalities. These above factors will in turn be affected by density
dependent factors such as crowding and competition for space and
density independent factors such as the influence of weather variables.
The observed parasite population size within a host at any one period
of time will be the dynamic equilibrium between input and output and
thus the seasonal variation in the parasite's population size will be
controlled by temporal changes in these factors.

The dispersion of the parasite within the host population is
of fundamental importance in ecological studies, and can be used as
the basis of a quantitative assessment of the nature of parasitism
(Crofton, 1971). It has been shown that models used to describe
parasite dispersion are not only empirical descriptions but also

provide information about the fundamental biological processes

involved in a host-parasite relation. The two species of parasite

studied illustrate two different patterns of dispersion. The model

presented for C.laticeps proposed that the overdispersion of the

parasites within the host population is due to many factors, the most

important of which is the aggregated distribution of the infective

stages of the tapeworm. The observed distribution of adult parasites

was well described by the negative binomial distribution. The

dispersion of D.paradoxum was not so clearly defined, but the observed

underdispersion was associated with competition for available space.

It is interesting to note that when the distribution model

for a particular species of parasite within a given habitat is constant,

the quantitative relationships between the percentage infection of the

host population and the mean number of parasites per host can be

determined.

For the Poisson series the probability of finding an uninfected

host is simply $P_o = e^{-\bar{x}}$ where $\bar{x}$ is the mean number of parasites per host.

If the mean is greater than 5.0 parasites/host, the probability of a

single fish being infected is 0.00674, and thus being uninfected is

0.99326 and the percentage infection of the host population is then

99.326%. Therefore, if the parasite dispersion is adequately described

by the poisson series and the mean number of parasites per host is $>$ 5.0

then the vast majority of the host population will be infected.

For the positive binomial the probability P_o of finding an

uninfected host is $P_o = (1-\frac{\bar{x}}{k})^k$ where k is the maximum possible number

of parasites which can occur within the host. k in the case of D.paradoxum

will vary with the age of the fish since older fish have large gill

surfaces for attachment of the parasite. In this present study k was

positively associated with the mean $\bar{x}$. The nature of the relationship

between P_o and $\bar{x}$ is thus similar to the poisson distribution. The
following values indicate the nature of this relationship.

$\bar{x}$	k	P_o	% infection
5	10	.00098	99.902 %
5	20	.00320	99.680 %
5	30	.00420	99.580 %
5	50	.00500	99.500 %
10	50	.00001	99.999 %
20	50	.00000	100.000 %

Thus an increase in k results in an increase in P_o and a
decrease in the percentage of the population infection. An increase
in $\bar{x}$ results in a decrease in P_o and thus an increase in the percentage
of the population infected. The influence of k on P_o is slight, however.
When dispersion is described by the positive binomial, the relationship
between P_o and $\bar{x}$ is further complicated by the introduction of the
parameter k; however in general for means greater than 5.0 the chances
of finding an uninfected host are small.

In the case of an overdispersed population of parasites,
described empirically by the negative binomial distribution, the
relationship between P_o and $\bar{x}$ is as follows (Bliss and Fisher, 1953).

$$P_o = \left(1 + \frac{\bar{x}}{k}\right)^{-k}$$ where k is a parameter varying inversely

within the hosts (Waters, 1959). In the case of this distribution as
shown earlier (p106) large means are required before the vast majority
of the population are infected, especially when the value of k is low
and thus aggregation is high $\underline{/}$ as $k \rightarrow \infty$ the Poisson series is approached$\underline{7}$.

The relationship between P_o and $\bar{x}$ is illustrated by the following values.

$\bar{x}$	k	P_o	% infection
5	0.5	0.3016	69.84 %
10	0.5	0.2180	78.20 %
100	0.5	0.0706	92.94 %
100	1.0	0.0099	99.01 %
100	2.0	0.0004	99.96 %

Therefore an increase in $\bar{x}$ results in a decrease of P_o and an increase in the percentage of the host population infected. An increase in k with $\bar{x}$ constant results in a decrease in P_o and an increase in the percentage of the host population infected.

From the above values $\bar{x}$ and P_o it can be seen that the relationship between the incidence and intensity of infection depends on the dispersion of the parasites between their hosts. The relationship is simple in the case of the binomial and poisson distribution. However, in the case of overdispersion described by the negative binomial, the degree of aggregation of the parasites is important in determining the proportion of the host population which is infected. The higher the aggregation of the parasites with a constant mean, the fewer hosts are infected.

These relationships are important in parasitological studies since, following a preliminary survey to determine the best fitting distribution model to the observed data, it would be possible to measure solely the number of infected hosts to provide estimates of the parasite population size. This has the additional advantage that it is easier and probably more accurate to determine presence or absence of a species, than to count their numbers. However, care must be taken in the use of such relationships between percentage infection and mean number of parasites per host, since in the case of the negative and positive

binomial distributions other parameters, so called measures of aggregation and dispersion, are also involved in the relationship. If these vary during the course of a survey, then conclusions based on the initial estimates of the parameter could be inaccurate.

It was mentioned previously in this discussion that the biological problem of host-parasite relations was essentially multivariate. This type of relationship can be most effectively analysed by the use of multivariate procedures and thus preserves information on a number of factors and their interactions which cannot be incorporated into univariate (unifactor) analysis. The development of the theory of these procedures has outpaced the practical applications of the techniques, with the result that only a few examples of their applications to biology are available in the published literature. (Jeffers, 1967; Alcock, Lovett and Machin, 1968; Barkham and Norris, 1970; Boratynski and Davies, 1971) Most examples of the application of multivariate techniques have been in the fields of numerical taxonomy (Sokal and Sneath, 1963; Blackith and Blackith, 1968; Rohlf and Sokal, 1962). However, it seems that the analysis of ecological data would be greatly aided by the use of these techniques. In part some of this lack of practical application has stemmed from difficulties in computation. However, the greater availability of electronic digital computers has removed this obstacle. The main difficulty has been in the interpretation of the results in terms of the biological processes under study.

The value of one such technique, orthogonalised multiple regression, has been demonstrated in this study. This method would seem to solve many of the problems involved in the use of multiple regression in ecological studies, primarily that of removing intercorrelations between supposedly independent variables by the transformation of the original variables to new orthogonal variates. A new coefficient E,

"the coefficient of effect" has been proposed to overcome difficulties of interpretation.

An extension of orthogonalised multiple regression is a more general technique known as canonical analysis (Kendall, 1957). By the use of canonical correlation analysis, it is possible to find the maximum correlations between linear functions of two sets of variables. Thus, instead of using just one dependent variable in an analysis, a number can be incorporated. Canonical analysis reveals the system of correlations underlying the two sets of variables and makes no distinction between dependent and independent variables, a distinction which is often difficult to make in the analysis of biological systems. This type of technique would seem to be suited to the analysis of host parasite relations. Similarly it could be used to investigate the relations between host and environment, or parasite and environment. Few examples exist in the literature of the application of this technique (Buzas, 1967; Morrison, 1967; Barkham and Norris, 1970), primarily due to the inherent difficulties in interpretation of the correlations, since more than one set of significant correlations may be extracted from the two sets of data. More than one set of correlations may also have sensible biological interpretations which can be based on previous knowledge, so the selection of the correct set of correlations is difficult and often arbitrary. However, it seems that future research in ecology could benefit from the application of this method since it does provide information of the relations between sets of variables and eliminates the problem of dependence and independence.

Helminth parasite ecology has for a long time lagged behind other fields of ecology, largely due to a lack of (a) quantitative precision in the collected data and (b) detailed statistical analysis of collected data. It would seem possible, however, from the evidence and

results presented in this study to construct deterministic mathematical

models to aid in understanding the mechanisms operating in specific host/

parasite systems. This type of approach has been pioneered by Watt (1961,

1963, 1964a, 1964b) and Holling (1963, 1964, 1965) in the field of insect

ecology and is based on the construction of realistic mathematical models
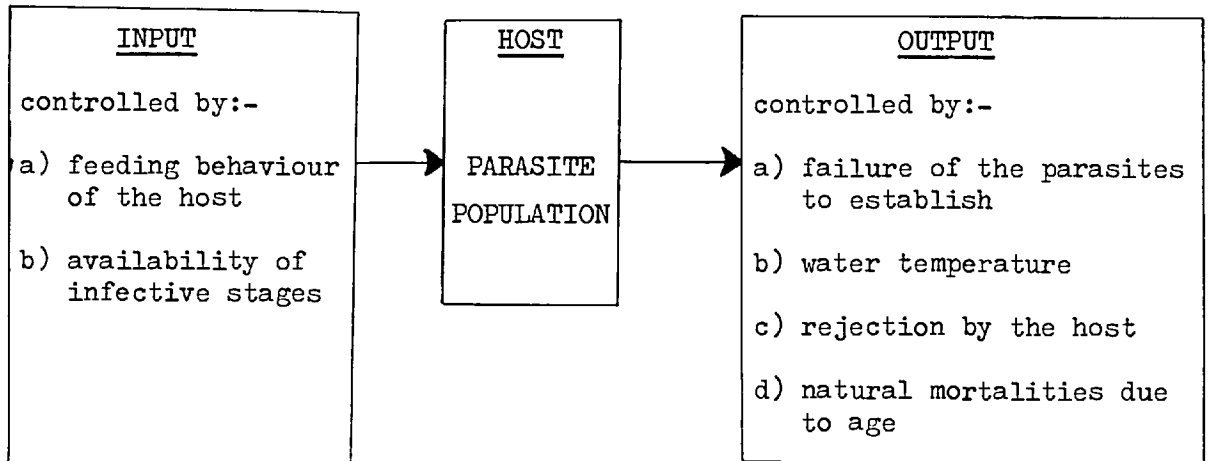
for use in computer simulation studies.

The primary emphasis in this study has been the identification

and evaluation of a few biologically important factors through the

analysis of empirical data, which provides the basic information for the

construction of a deterministic model. The biologically significant

variables which are selected by statistical analysis can be used to

formulate functional mathematical relationships between criterion and

predictor variables. From numerous functional relationships (sub-models)

an overall model can be constructed for use in simulation studies. The

model can then be used to:-

a) examine the extent to which a realistic biological appraisal

of the situation has been achieved and whether it is successfully expressed

and   b) explore a wider range of conditions than those represented by

the collected data, and thus to consider the consequences of long term

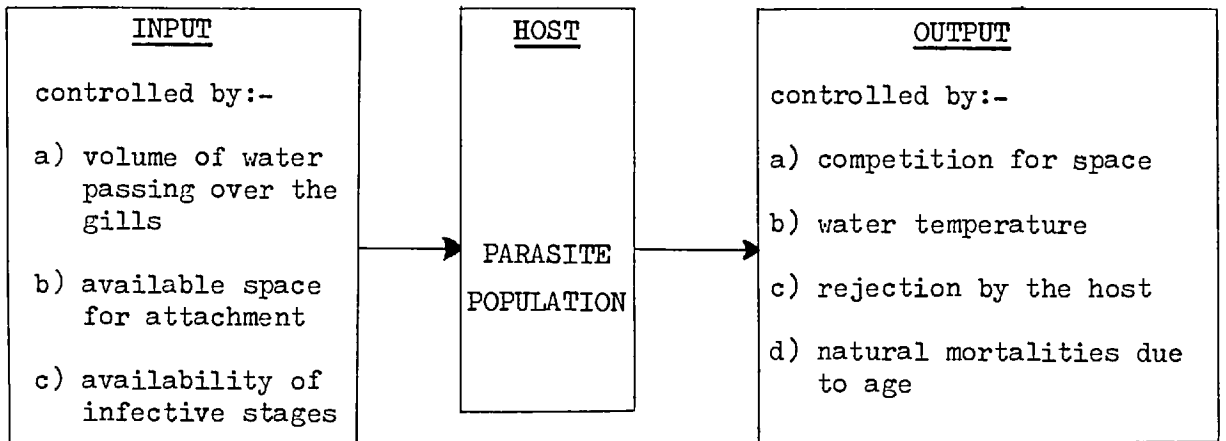changes in host population size and climatic factors.

In this present study it is hoped that the basic key factors

operating in the host parasite relations have been illustrated. These

are summarized in the flow diagrams for the input and output of

D.paradoxum and C.laticeps in Fig (2.30). The construction of deterministic

models to describe host parasite systems would seem to be a realistic and

important approach in future work on parasite ecology. The ultimate success

of such an approach could only be determined by long term studies.

Fig (2.30)   Flow diagrams illustrating the factors controlling

input and output of parasites in the bream.

a)   Caryophyllaeus laticeps:-

INPUT	HOST	OUTPUT
controlled by:-		controlled by:-
a) feeding behaviour of the host	PARASITE POPULATION	a) failure of the parasites to establish
b) availability of infective stages		b) water temperature
		c) rejection by the host
		d) natural mortalities due to age

b)   Diplozoon paradoxum:-

INPUT	HOST	OUTPUT
controlled by:-		controlled by:-
a) volume of water passing over the gills		a) competition for space
b) available space for attachment	PARASITE POPULATION	b) water temperature
c) availability of infective stages		c) rejection by the host
		d) natural mortalities due to age

Summary

1) The spawning behaviour, development, growth and food of the bream
population studied were typical for the species in European waters.

2) The population structure was dominated by a large 1967 year class
of fish which in February 1969 formed 73.2% of the total population.

3) Although the fish population decreased in size over the year of
study due to fishing and natural mortalities, particularly in the
1967 year class, the biomass of the total population increased.

4) The increase in biomass of the population was largely due to the
good growth of the younger age groups of fish; the older mature
fish, however, had a poor year for growth compared with previous
years.

5) Ten species of parasites were recorded from the bream consisting
of one Protozoon, three Monogenes, one adult Digene, three larval
Digenes, one Annelid and one Crustacea. Two species <u>Diplozoon</u>
<u>paradoxum</u> and <u>Caryophyllaeus laticeps</u> were selected for detailed
study.

6) The relationship between the mean number of parasites per host
(intensity of infection) and the percentage of the host population
infected are formulated for the Positive binomial, Poisson and
Negative binomial distributions.

7) The distribution of the adult stage of <u>C.laticeps</u> in the fish host
and the distribution of the tubificids infected with larval parasites
were adequately described by the negative binomial distribution. A

fundamental model for the generation of this distribution of

parasites in the fish was derived from the contagious distribution

of the infective stages of the parasite.

8) The distribution of D.paradoxum within the hosts was shown to be

under dispersed in the majority of cases examined. This distribution

could be described by the positive binomial model and was explained

in terms of competition for space on the gills of the host.

9) Mature C.laticeps were shown to have a preference for the mid regions

of the fish intestine.

10) D.paradoxum was shown to be more frequently encountered on the dorsal

sections of the gill and on the inner gill hemibranchs; no differences

were observed between gill arches. The distribution of the parasites

on the gills is probably related to water currents and space available.

11) Both parasites showed seasonal fluctuations in the size of their

respective populations; these fluctuations were non synchronous in

the different age groups of hosts.

12) Both parasites were capable of producing eggs throughout most of the

year, although a peak in production occurred in the warm summer months.

13) A decrease in the total population size of both C.laticeps and

D.paradoxum during the year of study was associated with a correspond-

ing decrease in the host population size.

14) C.laticeps was shown experimentally to have an adult life span in the

fish host of approximately one month at 12°C. The maximum life span

of D.paradoxum was estimated to be between four and five years.

15) The numbers of both D.paradoxum and C.laticeps per host were shown to be positively correlated with the size and age of the host. This was, in the case of C.laticeps, due to feeding behaviour which differed in each age group of fish throughout the year, and was also different in female and male fish during the spawning season. The correlation of D.paradoxum numbers with size was associated with the area of gill surface available for attachment.

16) High water temperatures were shown to be associated with a decreased survival rate of parasites for both D.paradoxum and C.laticeps within the host.

17) The application of a relatively new method of analysis, orthogonalised multiple regression, and the interpretation of the results is demonstrated. The advantages of this method over the more standard multiple and stepwise regression procedures were shown.

18) A new coefficient E, "the coefficient of effect" is proposed to aid in the interpretation of the relations between predictor and criterion variables.

19) Future directions of research in helminth parasite ecology are discussed.

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