PROTOZOA PARASITIC IN FISH

by

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ABSTRACT

Trypanosomes have been reported from 195 of approximately 25,000 species of fish. It is emphasised that some behavioural feature must have effected contact with the leech intermediate host for a fish to be infected. Lack of such associations accounts for the rarity of trypanosomes, particularly in marine fish.

The morphology of bloodstream Trypanosoma tincae from the tench, Trypanosoma percae from the perch, and Trypanosoma granulosum from the eel is redescribed in detail, using mensural methods. The morphology of bloodstream Trypanosoma leucisci from the roach, and Cryptobia sp. from the tench, is described in part. It is concluded that they are all separate and discrete species exhibiting a strong host ~~rigidity~~ restriction.

Passaging T. tincae between tench in the laboratory showed that the course of infection varied considerably with temperature.

Also fish showed greater incidences and parasitemia levels in the summer than in the winter in the Berkshire pond studied. The incidences per fish species also varied.

Dividing T. tincae are described from the tench. The life cycle in the leech, Hemiclepsis marginata, is also described. The life cycle of Cryptobia sp. from the tench is discussed briefly. Both parasites were transmitted between tench by this leech, which was cultured in the laboratory. H. marginata was found to have an annual life cycle, with a habitat restricted to marginal reeds.

The behaviour of the leech and tench is described in relation to the transmission of T. tincae in the Berkshire pond. It is

proposed that transmission occurred when mature tench entered shallow water in warm weather, especially when spawning. At this time the numbers of H. marginata and their feeding activities were at a maximum. The leeches then infected the majority of 1 year old tench.

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INTRODUCTION

In this study of Protozoa parasitic in fish, a particular group of fish and of parasites was selected from the start. The fish were those occupying a Berkshire pond. The parasites were their haemoflagellates.

The identification of the parasites was a major problem in the light of contradictory evidence on the species question by earlier workers. A mensural approach was adopted in the hope that the quantitative results would be comparable to previous and future work. For comparative purposes, Trypanosoma granulosum from the eel was also studied.

The transmission of the haemoflagellates was investigated. Their life cycle in tench and in the leech intermediate host was studied experimentally. Trypanosomes were passaged between fish to investigate effect of temperature on the course of infection in tench.

Finally, the seasonal variation of the parasite was observed, and discussed in terms of temperature effects and the life cycles and behaviour of the fish and leech hosts.

The previous work on fish haemoflagellates is presented and discussed. The methods used in keeping fish and leeches as experimental animals are given in detail. The majority of the results are presented quantitatively, but movement and gross cytological features of the flagellates is also described. The major implications arising from this and previous work are discussed at the end of the thesis.

HISTORICAL REVIEW

I. GENERAL REVIEW OF TRYPANOSOMES AND CRYPTOBIA OF FISH.

The early work on trypanosomes and cryptobia parasitic in the blood of fish was reviewed by Laveran and Mesnil (1904, 1912). They state that motile haematozoan fish parasites were first observed in trout blood (Salmo trutta) by Valentin in Switzerland in 1841. Lebailly (1906) states that Valentin described "pseudopodial" movement, and that he classed them with the amoebae. The organisms were more likely, therefore, to be Cryptobia.

Next followed Remak's observations in 1842, when he reported motile protozoa from the blood of pike (Esox lucius). He described structures which could be interpreted as the undulating membrane. Therefore he was probably the first person to see a trypanosome, preceeding by one year the original description of the genus Trypanosoma from the blood of a frog by Gruby in 1843.

In Russia Gros (1845) reported "vermicules" with projecting ribbon-like folds from the blood of a number of fish, including the gudgeon (Gobio gobio), "rockling", perch (Perca fluviatilis), sterlet (Acipenser sp.), burbot (Lota lota), and tench (Tinca tinca). The sterlet and burbot are both anadromous fish. There are no freshwater "rocklings", so this was probably the first record of a trypanosome from a marine fish.

In the same year Berg repeated Remak's observations on the pike, finding 4 out of 5 infected. Then Chaussat (1850) in Paris found a haematozoan in the blood of barbel (Barbus barbus) which resembled the frog trypanosome.

No more reports were issued until the classical work of Mitrophanow (1883) in Russia. He described and illustrated Haematomonas cobitis from the giant loach, Misgurnus fossilis, and H. carassii from the crucian carp, Carassius carassius. From his lengthy descriptions and adequate illustrations, it is obvious that these parasites belong to the genus Trypanosoma.

Danilewsky (1885) working in the Ukraine, followed with a detailed examination of the blood of a number of freshwater fish. He found flagellates in the blood of carp (Cyprinus carpio), tench (Tinca tinca), giant stone loaches (M. fossilis, Nemacheilus barbatula), pike (E. lucius), perch (P. fluviatilis), and crucian carp (C. carassius). He noted two definite types which, in the light of his excellent descriptions, can now be interpreted as belonging to the genera Trypanosoma and Cryptobia. He also noted, but did not describe, multiplication in the blood by unequal binary fission.

Again in Russia, this work was followed by Chalachnikov (1888) who similarly noted the two different flagellate types from the blood of C. carpio, E. lucius, C. carassius, and Acerina vulgaris, the ruffe. He also noted multiplication of the flagellates by longitudinal division.

In 1901, Laveran and Mesnil recognised the two flagellate types already noted by Danilewsky and Chalachnikov and placed them into 2 genera. The first parasite was found in a pike (E. lucius) This they assigned to the established genus Trypanosoma Gruby, 1943, naming the species Trypanosoma remaki. The second flagellate type

which they recognised as having two flagellae was assigned to a new genus, Trypanoplasma. The type species was from the rudd, Scardinius erythophthalmus, and was named Trypanoplasma borelli. Plehn (1903) followed with a description of Trypanoplasma cyprini following an epizootic which, according to Hofer (1906), had caused serious mortalities amongst carp in German fish ponds.

The Léger (1904a) recorded Trypanoplasma borelli from the minnow, Phoxinus phoxinus, which, he said, was also highly pathogenic. Cross infection experiments by Laveran (1904) confirmed that the parasite from the rudd and minnow were the same species, although it was more pathogenic to the minnow. Léger (1904c) also described what he considered to be a different species from the loach, N. barbatula, naming it Trypanoplasma varium.

For two years the separate species concept was maintained, in spite of admitted morphological similarities. Brumpt (1906b) described four new species of Trypanoplasma: Trypanoplasma guernei from the miller's thumb, Cottus gobio, Trypanoplasma barbi from the barbel, B. barbus, Trypanoplasma abramidis from the bream, Abramis brama, and Trypanoplasma truttae from the trout, S. trutta.

Keysselitz (1906) disputed Brumpt's view, and claimed that the Trypanoplasma he observed from the following fish species were the one parasite, Trypanoplasma borelli: P. fluviatilis, A. cenua, L. lota, B. barbus, C. carpio, C. carassius, T. tinca, A. brama, Leuciscus idus (the ide), Squalius cephalus (the chub), S. erythrophthalmus, R. rutilus, and N. barbatula.

In addition to the uncertain status of the species, the status of the genus itself was questioned. Leidy (1846) had described Cryptobia helicis from the seminal vesical of a land snail. Crawley (1909) then demonstrated, on the grounds of morphological similarity, that Cryptobia from snails and Trypanoplasma from fish were cogenetic. Laveran and Mesnil (1912) separated them again on the basis of habitat differences. Reports by Léger (1905), Keysselitz (1906) and Martin (1910) of Trypanoplasma from the stomachs of marine fish, and by Swezy (1919) of Trypanoplasma as an ectoparasite of goldfish (Carassius auratus) extended the habitat range of the genus in fish. Thus modern workers(Katz 1951, Strout 1965, etc.) have accepted Crawley's (1909) merging of the two genera, giving the name Cryptobia priority over Trypanoplasma.

A number of Cryptobia species have been described from fish blood. Many are of doubtful validity. The shape and dimensions of a single species are so variable, that separation on morphological grounds alone is dubious. The indications are that the genus does not exhibit a tight host relation(Becker and Katz, 1965, 1966; Strout, 1965). It is likely that Keysselitz's (1906) view will prevail in the light of further work, and that many of the species will be merged.

Cryptobia in fish blood has a wide geographical distribution. It has been reported from Palearctic fish. In Asia the first record was Cryptobia ninaekohlyakimovi (Yakimoff), 1923, from the catfish Silurus glanis. The first British record was Cryptobia gurneyorum (Minchin) 1909,

from the pike, E. lucius. The first Oriental record was Cryptobia clariae(Mathis and Leger), 1911, from Clarias macrocephalus. The first Ethiopean record was a Cryptobia from Labeo macrostoma reported by Rodhain, 1908. The first Australasian record was Cryptobia parmae Mackerras and Mackerras, 1925, from Parma microlepsis. The first Nearctic record was Cryptobia salmositicae Katz, 1951, from Oncorhyncus kisutch. No Cryptobia have been described from Neotropical fish.

Whilst there have been many reports of Cryptobia from the blood of freshwater fish, often in mixed infections with trypanosomes, there have been few records from marine fish.

The first description of a trypanosome species from fish blood by Laveran and Mesnil (1901) began a decade of great activity in this field. In the same work they named the first species from a marine fish, Trypanosoma soleae from the sole, Solea solea. The following year, they (Laveran and Mesnil, 1902a) named the eel trypanosome, Trypanosoma granulosum. This had already been seen by Sabrazes and Muratet (1902a, b). Laveran and Mesnil (1902a, b) also further characterised the pike trypanosome. They inoculated blood from infected pike into apparently uninfected pike, and found that the trypanosomes multiplied to an infection peak after 3-4 weeks. They briefly described the unequal binary fission process of the dividing bloodstream trypanosomes. In the same papers they described similar cross inoculation work. They successfully transmitted T. granulosum between eels by blood passage. However, inoculations between different species of fish gave uniformly negative results. They stated that this fact justifies the description of different species of trypanosome for the different species of fish,

although the parasites themselves do not present any marked morphological differences.

Brumpt and Lebailly (1904) and Lebailly (1904, 1905) cited this principle as a justification for naming as separate species trypanosomes from a number of marine fish. Lebailly (1905), furthermore, tested the principle. He inoculated blood from a dab, Limanda limanda, infected with Trypanosoma limandae, into an eel, A. anguilla. T. limandae never appeared in the eel. The recognisably different T. granulosum did. Similarly three conger eels, Conger conger, inoculated with blood from A. anguilla heavily infected with T. granulosum remained negative. Trypanosoma platessae from the plaice, Platessa platessa, Trypanosoma callionymi from the dragonet, Callionymus lyra and T. granulosum from A. anguilla, were each inoculated into conger eels, C. conger, the gunnel, Pholis gunnellus, and the rockling, Giadropsarus tricirratus. All recipients remained negative. He also took C. lyra and P. platessa, each with patent infections of T. callionymi and T. platessae respectively, and inoculated them with the recognisably different species, T. granulosum and T. limandae. Neither recipient species developed infections with the latter two parasite species. Thus he concluded that trypanosomes of marine fish showed the same host restrictions as trypanosomes of freshwater fish, and that Laveran and Mesnil's principle was justified.

Since 1904, the finding of trypanosomes in a new fish species has, almost without exception, resulted in the naming of a new

trypanosome species. No further experimental work has been undertaken to test the basic premise.

At the beginning of the century, interest was also centred on the intermediate hosts for the two genera of fish haamoflagellates. Lebailly (1905) reviewed the early work, and stated that Leydig in 1857 found flagellates in the crop of the leeches Piscicola and Pontobdella. Doflein (1901), commenting on Leydig's observations, suggested that blood sucking leeches were the intermediate hosts of fish haemoflagellates. Léger and Brumpt then confirmed this hypothesis.

Léger(1904b) followed the development of Trypanosoma barbatulae, from the stone loach Nemacheilus barbatula, in Piscicola geomatra for 4 days. The trypanosomes divided by binary fission. He interpreted the division products as sexual forms, which themselves produced globular forms by a budding process. The production of metacyclic forms, and transmission back to a fish, was not investigated. In another report, Léger (1904c) described the development of Cryptobia varium from the same loach, in Hemiclepsis marginata.

Simultaneously, Brumpt (1904) inoculated the trypanosome-like flagellates, from the crops of wild H. marginata, into minnow, P. phoxinus, rudd, S. erythrophthalmus, and sticklebacks, G. aculeatus, with negative results. He also bred H. marginata experimentally and found that the flagellates were not transmitted from parent to offspring.

The following year, Brumpt (1905) reported that the Cryptobia of the roach, minnow, and miller's thumb, C. gobio, all developed in

P. geometra. Yet the Cryptobia from the carp, tench, beam, and "loach" only developed in H. marginata. He experimentally infected carp and miller's thumb with trypanosomes and Cryptobia using blood sucking leeches. He described the accumulation of metacyclic forms in the proboscis sheath, and their subsequent inoculation into a fish.

Brumpt (1906a) then described in detail the development of T. granulosum in H. marginata. Development did not proceed to metacyclic forms in Callobdella punctata, Hirudo troctina, and P. geometra. Later (Brumpt 1906b), he stated that Cryptobia from the "loach" developed only in H. marginata, whilst the morphologically similar Cryptobia from the barbel only developed in P. geometra. He also studied the development of the following freshwater fish trypanosomes; T. abramidis from A. brama, T. remaki from E. lucius, T. barbi from B. barbus, T. percae from P. fluviatilis, T. acerinae from Acerina cernua, the ruffe, T. granulosum from A. anguilla, T. danelewskyi from C. carpio, and T. phoxini from P. phoxinus. They all developed exclusively in H. marginata. None developed in P. geometra. He also reported the development of marine fish trypanosomes in leeches. T. soleae from S. solea, and T. cotti from Cottus bubalis, developed in Callobdella punctata. T. scyllii from Scyliorhinus stellaris, and T. raiae from Raia spp., developed in Pontobdella nuricata.

Keysselitz (1906) followed with a detailed study of Cryptobia borelli from a large number of freshwater fish. In a long report he described the development of sexual stages in P. geometra, an inter-

pretation which has remained unsubstantiated. He failed to transmit the parasite back to fish again either by feeding infected P. geometra or by the inoculation of crop contents. Keysselitz has been misquoted as studying the development of trypanosomes in P. geometra (Laveran and Mesnil, 1912; Wenyon, 1925; Qadri, 1952; Becker, 1967).

At the same time Robertson (1906b) studied the morphogenesis of T. raiae - like flagellates in the crop of Pontobdella muricata. Neumann (1909) followed by describing the development of trypanosomes from Raia punctata and R. oxyrhyncus in P. muricata. Robertson (1910) actually bred this leech, and elaborated on her earlier observations, reporting in detail on the development of T. raiae in the leech's crop. She followed in 1912 by describing the development of trypanosomes from A. brama, P. fluviatilis, S. erythrophthalmus, and Cryptobia and trypanosomes from Carassius auratus, the goldfish, in H. marginata. All the trypanosomes had identical cycles in the leech, and were transmitted to apparently uninfected goldfish. The morphology of the trypanosomes in the fish was not described, and the species were not named. In a personal communication (1968) she stated that the behaviour of the trypanosomes in different species of fish blood was similar, but very low numbers, even at the peak of infection between the fifth and twentieth days, prevented morphological studies. No division forms were seen.

In 1924, Tanabe described the development of Cryptobia and trypanosomes from the loach, Misgurnus anguillicaudatus, in the leech,

Hirudo nipponica. Qadri (1952, 1962a) studied the development of T. danilewskyi from C. carpio in H. marginata. He stated that development on P. geometra was"flecting and poorly active" (Qadri, 1952). Neither Tanabe nor Qadri attempted experimental transmission back to fish.

Finally, Becker and Katz (1965) reported the successful transmission of Cryptobia salmositica between torrent sculpins, Cottus rhotheus, and coho salmon, Oncorhyncous kisutch, through the agency of the leech Piscicola salmositica.

Following Laveran and Mesnil's (1902a, b) reports on experimental infections of pike with T. remaki, Brumpt (1905) stated that inoculation of trypanosomes either by syringe passage or by leech feeding gave rise to a short acute infection, followed by a long chronic phase. This acute phase may be superimposed on an existing chronic infection. During the acute phase, dividing forms were common.

However, fish with natural acute infections of trypanosomes have only rarely been encountered. In these, dividing trypanosomes have been reported as follows: T. remaki from E. lucius (Laveran and Mesnil, 1902a, b; Breindl, 1911), Trypanosoma sp. from Clarias angolensis (Dutton, Todd and Tobey, 1907), Trypanosoma sp. subsequently Trypanosoma gan'ei Rodhain, 1942, from Labeo macrostoma (Rodhain, 1908) Trypanosoma variabile from Raia punctata (Neumann, 1909), Trypanosoma sp. from Fluvidraco sp. (Ogawa and Uegaki, 1927), and Trypanosoma clariae var. batrachi from Clarias batrachus (de Mello and Valles, 1936). Rarely have dividing forms been illustrated and the complete

process has not been described.

In contrast, fish acutely infected with Cryptobia have been encountered more frequently, usually in fish farms during epidemics of Piscicola geometra (Doflein, 1901; Plehn, 1903; Hofer, 1906; Keysselitz, 1906; Nowicki, 1940; Ivasik, 1964). Dividing forms were rarely encountered, and were described by Laveran and Mesnil (1902a), Plehn (1903), and Keysselitz (1906).

Little work has been attempted on the ecology of haemoflagellates in fish. Brumpt(1905) reported that the majority of tench, carp, and pike he examined were infected with trypanosomes irrespective of age. Keysselitz (1906) however, found that 2 year old carp had heavier haemoflagellate infections than 1 year old carp. Sabrazes and Muratet (1902a, b) actually found all young eels negative and all older eels, from the same habitats, positive for T. granulosum. Similarly, Hasan and Qasim (1962) found only the larger Ophicephalus punctatus infected with T. punctati, and Breindl (1911) found only the larger Albumus lucidus infected with T. laverani. Horta (1910) however, found a higher incidence of T. chagasi in young Plecostomus punctatus than in adults. Dubinin (1952) found T. acipenseri in the majority of young sturgeon she examined, and yet all adults were negative. This she correlated with the migration of the adults to salt water, where the intermediate host was absent.

There has also been some work on the seasonal occurrence of these haemoflagellates. Mitrophanow (1883) noticed that trypanosomes in the

loach, M. fossilis, were less numerous in November and December than at other times of the year. Lingard (1904a, b) found the heaviest trypanosome infections of Trichogaster fasciatus, Ophicephalus striatus, Macronis seenghala, and M. tengara, in May and June in India. Similarly Castellani and Willey (1905) reported that Saccobranchus fossilis had the heaviest T. saccobranchi infections in August and September. Keysselitz (1906) found a higher Cryptobia infection rate in carp, tench, and bream in the summer than in the winter. Horta (1910) found that Plecostomus punctatus had heavier T. chagasi infections in warmer weather. Breindl (1911) reported in detail on the haemoflagellate infections of carp, tench, pike, eel, perch, giant loach, and bleak. He found that the highest incidence with the heaviest infections occurred in the summer. Becker and Katz (1966) worked out the epidemiology of Cryptobia salmositica. They found that adult coho salmon became progressively more infected during November and December as they entered freshwater to spawn. The parasite was transmitted by Piscicola salmositica from the reservoir hosts, the torrent sculpins. Finally Khayboulajev (1969b) found a higher degree of haemoflagellate infections in Caspian sea fish in spring and early summer, than in autumn and winter.

116 species of fish trypanosome have been reported to date. Most workers have followed Laveran and Mesnil's (1904) principle in describing a new trypanosome species for each new host. Because of low infection levels, many of the descriptions could apply to

almost any trypanosome. Some, indeed, were made from single parasites, and one, T. squalii Brumpt, 1904, was made from fresh material only.

The trypanosomes of the cartelagenous fish were considered by Lavier (1942) to be the most primitive. They have characteristics in common with trypanosomes of other vertebrates, having resemblances with T. grayi (see Hoare, 1931) from crocodile, T. avium (see Baker, 1956) from birds, and the trypanosomes of mammals of the subgenus Megatrypanum. The broad leaf-like body has a kinetoplast situated near the nucleus. The undulating membrane is tightly frilled, and the free flagellum is short. T. heptatreti Laird, 1948, from the primitive hag fish is typical of the type. As in trypanosomes of amphibia, bizarre variations exist. T. gargantua Laird, 1951, has a body length of up to 130μ.

Trypanosomes from marine teleosts also form a natural group. Those described by Lebailly (1905), Mackerras and Mackerras (1925), and Laird (1957) have elongated narrowly tapered posterior ends, with the kinetoplast situated well subterminally, generally where the body is wide enough to accomodate it. Unlike those from cartilagenous fish, the body is usually narrow enough for the nucleus to occupy its whole width.

Freshwater fish trypanosomes are difficult to categorise because of the morphological differences between many species. The situation is further complicated by polymorphism within individual species. There is one clearly defined group, however; these are the well documented species from the Cyprinidae. They are typified by a

long narrow body, with a blunt or sharply tapering posterior end, and a narrowly tapering anterior end. The kinetoplast is terminal or nearly so. The prominent undulating membrane crosses the body in a series of wide folds, and terminates in a long free flagellum. Species are difficult to separate on morphological grounds alone, although they are said to be restricted to few or single host species.

Trypanosomes from the other Palearctic families differ considerably from the "cyprinid" type, and from each other. Those from the pike (Esocidae) and eel (Anguillidae) are dimorphic, the smaller forms resembling the cyprinid types. T. perca, from the perch (Percidae) represents an offshoot of its own.

Trypanosomes of Neotropical, Ethopean, and Oriental freshwater fish may also form a natural group. The type is best shown in a series of 64 photographs of a single unnamed species from the Ethiopian fish, Periophthalmus koelreuteri, by Zupitza (1909). The smallest was the cyprinid type, the largest was the "cartelagenous-fish" type, with a complete size and morphological range in between. Accepting this considerable polymorphism for one species, it would not be difficult to synonymise the majority of species described from the tropics. Baker (1960) carefully described another polymorphic species, T. mukasai, noting its great host range, extending through the following orders: Ceratodiformes, Mormyriformes, Cypriniformes, Siluriformes and Perciformes. Formal synonymy would be justified if the trypanosomes were found experimentally not to be

restricted to individual host species.

So it is likely that, amongst the freshwater teleosts, trypanosomes of Palearctic fish are less polymorphic with a tight host restriction, and trypanosomes from the tropics are highly polymorphic with a loose host restriction. Therefore there may be a correlation between the parasites and the evolutionary state of their hosts. For both the Neotropical and Ethiopian fish faunas are rapidly speciating, the prime examples being the Neotropical Siluriformes, and the Ethiopean Cichlidae (Lagler et al, 1962). So the trypanosomes themselves could be speciating, but without yet reaching the tight host specificity of the more established Palearctic species from the more established hosts. Also, the affinities between the Ethiopean and Neotropical teleost faunas have long been recognised (Bertin and Arambourg, 1958). In spite of the complete lack of information on their intermediate hosts, similar affinities are possible between the respective fish trypanosome faunas.

Trypanosomes have been reported from the following 195 species of fish. They are listed under the major divisions proposed by Arambourg et al (1958). The teleost classification is that of Greenwood et al (1966). For each fish its freshwater (FW) or marine (M) habitat is shown, its most recent specific name and common name where known, the zoogeographical region and the reference for the trypanosome record.

I Class Asnatha; Order Myxiniformes
Fam. Eptatretidae (Hagfish)
Eptatretus cirrhatus; Hagfish M
Au. Laird, 1948; Laird 1951

II Class Chondrichthyes; Order a) Galeiformes
i) Fam. Orectolobidae: (Nurse sharks)
Hemiscyllium ocellatum; Shark M
Au. Laird, 1951; Mackerras and Mackerras, 1961

ii) Fam. Scyliorhinidae (Cat sharks)
Scyliorhinus canicula; Dogfish M
Pa. Henry, 1910
S. stellaris; Dogfish M
Pa. Laveran and Mesnil, 1902 a, c; Coles, 1914

iii) Fam. Carcharhinidae: (Requiem sharks)
Carcharias sp.; Shark M

Order b) Rajiformes
i) Fam. Platyrhinidae (Rays)
Zanobatus schoenleini; Electric Ray M
Et. Rangüe, 1967

ii) Fam. Rajidae (Rays and Skates)
Psammobatis microps; Skate M
Nt. Bacigalupo *et al*, 1948
Raia batis; Skate M
Pa. Coles, 1914
R. capensis; skate M
Et. Fantham, 1918
R. clavata; thornback ray M
Pa. Laveran and Mesnil, 1902c, 1904
R. erinacea; skate M
Na. Bullock, 1958
R. macrorhyncus; ray M
Pa. Laveran and Mesnil, 1904
R. mosaica; ray M
Pa. Laveran and Mesnil, 1902 a, c; 1904
R. nasuta; skate M
Au. Laird, 1957
R. ocellata; skate M
Na. Kudo, 1923
R. oxyrhyncus; long nosed skate M
Pa. Neumann, 1909
R. punctata; skate M
Pa. Laveran and Mesnil, 1902a, c; Neumann, 1909; Minchin and Woodcock, 1910; Yakimoff, 1912; Henry, 1913

Order c) Tropeniformes

Fam. Torpedinidae (Electric rays)

Torpedo marmorata; electric ray M

Pa. Sabrazes and Muratet, 1908; Lagarde, 1943

III Class Osteichthyes

Order a) Ceratodiformes

Fam. Lepidoserenidae (South American and African lungfishes)

Protopterus aethiopicus; African lungfish FW

Et. Baker, 1960.

Order b) Polypteriformes

Fam. Polypteridae (Birchirs)

Polypterus sp.; birchir FW.

Et. Neave, 1906

Order c) Acipenseriformes

Fam. Acipenseridae (Sturgeons)

Acipenser güldenstadti; Russian sturgeon FWM

Pa. Dubinin, 1952

A. nudiventris; Thorn sturgeon FWM

Pa. Dubinin, 1952; Khayboulajev, 1969a

A. ruthenus; sturgeon FWM

Pa. Dubinin, 1952

A. stellatus; starred sturgeon FWM

Pa. Dubinin, 1952

Huso huso; huso FWM

Pa. Dubinin, 1952

Order d) Amiiformes

Fam. Amiidae (Bowfins)

Amia calva; bowfin FW

Na. Clark, 1959

Order e) Anguilliformes

Fam. Anguillidae (Freshwater eels)

Anguilla anguilla; eel FW

Pa. Laveran and Mesnil, 1902; Sabrazes and Muratet, 1902 a, b, 1904 a, b, 1907; Brumpt, 1905, 1906 a, b; Lebailly, 1905; Manca, 1906; Keysselitz, 1906; Franca, 1907; Minchin, 1909; Breindl, 1911; Dunkerly, 1913; Ponselle, 1913 a, b; Kraneveld and Keidel, 1955; Bykovskaya-Pavlovskaya et al, 1962.

A. japonica; eel FW

Pa. Hoshina and Sano, 1957

A. reinhardtii; eel FW
Au. Johnston and Cleland, 1910; Mackerras and Mackerras, 1961
A. mauritania; eel FW
Au. Johnston and Cleland, 1910
Pa. Ogawa and Uegaki, 1927

Order f) Mormyriformes

Fam. Mormyridae (Mormyrids)
Gnathonemus victoriae FW
Et. Baker, 1960
Mormyrus kannume FW
Et. Baker, 1960

Order g) Salmoniformes

1) Fam. Salmonidae (Salmon, trout, etc.)
Salmo trutta ezenami; Eisenam trout FW
Pa. Khayboulajev, 1969a
2) Esocidae (Pikes)
Esox lucius; pike FW
Pa. Danilewsky, 1885; Chalachnikov, 1888; Laveran and Mesnil, 1902, 1904; Brumpt, 1906b; Keysselitz, 1906; Minchin, 1909; Breindl, 1911; Coles, 1914; Navrotsky, 1914; Yakimoff, 1928; Zalevskaya, 1950; Sluhei, 1960; Bykovskaya-Pavlovskaya et al, 1962; Shumela et al, 1963; Aligodjiev, 1969; Khayboulajev, 1969a
Lucius reticulatus; Pickerol FW
Na. Kudo, 1921
Esox reicherti; Amur pike FW
Pa. Dogiel and Achmerov, 1959
3) Fam. Aulopodidae
Aulopus purpurissatus; Sergeant Baker M
Au. Mackerras and Mackerras, 1925

Order h) Cypriniformes

1) Fam. Characidae (Characins)
- ; Ferreira fish FW
Nt. Fonseca and Vaz, 1928a
- ; Piava fish FW
Nt. Fonseca and Vaz, 1928a
2) Fam. Anostomidae
Prochilodus sp; FW
Nt. Fonseca and Vaz, 1929
Francisodoras marmoratus; FW
Nt. Fonseca, 1935

3) Fam. Cyrinidae (Carps, minnows, etc.)

Abramis brama; bream FW

Pa. Laveran and Mesnil, 1904; Keysselitz, 1906; Brumpt, 1906b; Minchin, 1909; Robertson, 1912; Nikitin, 1929; Zalevskaya, 1950; Qadri, 1952; Sluhei, 1960; Bogdanova, 1961; Koval, 1962; Markov and Kosareva, 1962; Bykovskaya-Pavlovskaya et al, 1962; Shumela et al, 1963; Aligodjiev, 1969; Khayboulajev, 1969a

Alburnus alburnus; Bleak FW

Pa. Breindl, 1911

Aristichthys nobilis; Chinese bighead FW

Pa. Chen, 1956b, Bykovskaya-Pavlovskaya et al 1962

Aspius aspius; Common asp FW

Pa. Zalevskaya, 1954; Bogdanova, 1961; Bykovskaya-Pavlovskaya et al, 1962

Barbus barbus; Barbel FW

Pa. Brumpt, 1906b; Keysselitz, 1906; Bykovskaya-Pavlovskaya et al, 1962; Ivasik, 1963

B. carnaticus; Barbel FW

Or. Lingard, 1904a

Blicca bjoerkna; Silver bream FW

Pa. Nikitin, 1929; Zalevskaya, 1950; Bykovskaya-Pavlovskaya, et al, 1962;

Carassius auratus; Goldfish FW

Pa. Petrie, 1905; Thompson, 1908; Robertson, 1912; Ogawa and Uegaki, 1927

Or. Mathis and Leger, 1911

C. auratus gibelio; goldfish FW

Pa. Dogiel and Achmerov, 1959

C. carassius; crucian carp FW

Pa. Mitrophanow, 1883; Chalachnikow, 1888; Keysselitz, 1906; Nikitin, 1929; Zalevskaya, 1950; Sluhei, 1960; Koval, 1962; Bykovskaya-Pavlovskaya et al, 1962; Shumela et al, 1962; Khayboulajev, 1969a

Chondrostoma nasus; Savetta FW

Pa. Zalevskaya, 1954; Bykovskaya-Pavlovskaya et al, 1962

Cyprinus carpio; carp FW

Pa. Danilewsky, 1885; Chalachnikow, 1888; Laveran and Mesnil, 1904; Brumpt, 1905, 1906b; Keysselitz, 1906; Breindl, 1911; Qadri, 1952, 1962a, b, c; Koval, 1962; Bykovskaya-Pavlovskaya et al, 1962; Vismanis and Peslak, 1963; Aligodjiev, 1969; Khayboulajev, 1969a

C. carpio haematopterus; Amur wild carp FW
Pa. Dogiel and Achmerov, 1969
Gobio gobio; gudgeon FW
Pa. Brumpt, 1906b; Ivasik, 1963
Labeo falcifer; FW
Et. Rodhain, 1908
L. macrostoma; FW
Et. Rodhain, 1908; 1962
L. victorianus; FW
Et. Baker, 1960
Leuciscus idus; ide FW
Pa. Keysselitz, 1906; Zalevskaya, 1950; Bogdonova, 1961; Bykovskaya-Pavlovskaya et al 1962; Khayboulajev, 1969a
Mylopharyngodon piceus; black amur fish FW
Pa. Chen, 1956a; Bykovskaya-Pavlovskaya et al 1962
Phoxinus phoxinus; minnow FW
Pa. Laveran and Mesnil, 1904; Brumpt, 1906b; Keysselitz, 1906; Delanoe, 1911; Ponselle, 1913b
Pseudaspius leptocephalus; amur asp FW
Pa. Dogiel and Achmerov, 1959
Rutilus rutilus; roach FW
Pa. Brumpt, 1906b; Keysselitz, 1906; Coles, 1914; Zalevskaya, 1950; Koval, 1962; Bykovskaya-Pavlovskaya et al, 1962; Abolarin, 1966; Khayboulajev, 1969a
Sarcochilichthys sinensis; Chinese lake gudgeon FW
Pa. Dogiel and Achmerov, 1959
Scardinius erythrophthalmus; rudd FW
Pa. Brumpt, 1906b; Keysselitz, 1906; Delanoe, 1911; Robertson, 1912; Nikitin, 1929; Zalevskaya, 1950; Sluhei, 1960; Koval, 1963; Bykovskaya-Pavlovskaya et al, 1962; Shumela et al, 1963; Aligodjiev, 1969; Khayboulajev, 1969a
Squalius cephalus; chub FW
Pa. Brumpt, 1906b; Keysselitz, 1906
Tinca tinca; tench FW
Pa. Danilewsky, 1885; Doflein, 1901; Laveran and Mesnil, 1904; Brumpt, 1905; Keysselitz, 1906; Minchin, 1909; Breindl, 1911; Ponselle, 1913b; Franchini, 1923; Nikitin, 1929; Zalevskaya, 1950; Barrow, 1955; Sluhei, 1960; Koval, 1962; Bykovskaya-Pavlovskaya et al, 1962; Shumela et al, 1963; Aligodjiev, 1969; Khayboulajev, 1969a

4) Fam. Cobitidae
Misgurnus anguillicaudatus; loach FW
Pa. Tanabe, 1924
M. fossilis; giant loach FW
Pa. Mitrophanow, 1883; Danilewsky, 1885; Briendl, 1911; Bykovskaya-Pavlovskaya et al, 1962; Khayboulajev, 1969a
M. taenia; spined loach FW
Pa. Zalevskaya, 1950; Bykovskaya-Pavlovskaya et al, 1962; Khayboulajev, 1969a
Nemacheilus barbatula; stone loach FW
Pa. Danilewsky, 1885; Leger, 1904b; Brumpt, 1905; Keysselitz, 1906; Ponselle, 1913b; Zalevskaya, 1950; Sluhei, 1960; Bykovskaya-Pavlovskaya et al, 1962; Shumela et al, 1963; Khayboulajev, 1969a

Order i) Siluriformes

1) Fam. Bagridae (Bagrid catfish)
Auchenoglanis biscutatus; FW
Et. Leboeuf and Ringenbach, 1910
Bagrus bayard; FW
Et. Neave, 1906
B. docmac; FW
Et. Prates, 1928; Baker, 1960
Chrysichthys auratii; FW
Et. Wenyon, 1909
Fluvidraco sp; FW
Pa. Ogawa and Uegaki, 1927
Liocassis ussuriensis; Ussurian catfish FW
Pa. Dogiel and Achmerov, 1959
Macrones cavasius; FW
Or. Castellani and Willey, 1905
Macrones sp. FW
Or. Lingard, 1904a
Pseudobagrus fluvidraco; FW
Pa. Dogiel and Achmerov, 1959

2) Fam. Siluridae (Eurasian catfishes)
Silurus glanis; Sheatfish FW
Pa. Keysselitz, 1906; Nikitin, 1929; Zalevskaya, 1950; Sluhei, 1960; Bogdanova, 1961; Koval, 1962; Bykovskaya-Pavlovskaya et al, 1962; Shumela et al, 1963; Khayboulajev, 1969a
Tandanus tandanus; Jewfish FW
Au. Johnston and Cleland, 1910; Mackerras and Mackerras, 1961

3) Fam. Schilbeidae (Schilbeid catfish)
Schilbe mystus; FW
Et. Baker, 1960
4) Fam. Clariidae (Labyrinthic catfish)
Clarias angolensis; FW
Et. Dutton, Todd, and Tobey, 1906
C. anguillaris; FW
Et. Wenyon, 1909; Bouet, 1909
C. batrachus; Maroof FW
Or. de Mello and Valles, 1936; Qadri, 1952, 1962a
C. fuscus; FW
Pa. Ogawa and Uegaki, 1927
C. gariepinus; Barbel FW
Et. Fantham, 1919; Dias, 1952
C. macrocephalus; FW
Pa. Montel, 1905
Clarias sp; FW
Et. Zupitza, 1909
Saccobranchus fossilis; Singhi FW
Or. Castellani and Willey, 1905; Pearse, 1932; Qadri, 1952, 1962b.
5) Fam. Malapteruridae (Electric catfish)
Malapterurus electricus; FW
Et. Rodhain, 1908, 1942
6) Fam. Mochokidae (Upside-down catfish)
Synodontis notatus; FW
Et. Leboeuf and Ringenbach, 1910
S. schall; FW
Et. Neave, 1906; Wenyon, 1909
7) Fam. Doradidae (Armoured catfish)
Rhinodoras dorbigny; FW
Nt. Fonseca and Vaz, 1928a
8) Fam. Pimelodidae (Pimelodid catfish)
Rhamdia quelen; FW
Nt. Botelho, 1907; Splendore, 1910
Pseudopimelodus zungaro; FW
Nt. Fonseca and Vaz, 1928a
9) Fam. Loricariidae (Armoured catfish)
Chaetostostoma sp. FW
Nt. Fonseca and Vaz, 1929
Loricaria piracicabae; FW
Nt. Fonseca and Vaz, 1929
Loricaria sp. FW
Nt. Fonseca and Vaz, 1928a
Otocinclus francirochai; FW
Nt. Fonseca and Vaz, 1928b

Plecostomus albopunctatus; FW
Nt. Fonseca and Vaz, 1928a
P. auroguttatus; FW
Nt. Splendore, 1910; Fonseca, 1935
P. margaritifer; FW
Nt. Fonseca and Vaz,1928a
P. punctatus; FW
Nt. Horta, 1910
P. regani; FW
Nt. Fonseca and Vaz, 1928a
P. strigaticeps FW
Nt. Fonseca and Vaz, 1928a
Plecostomus sp. FW
Nt. Fonseca and Vaz, 1928a

Order j) Gadiformes

1) Fam. Gadidae (Cod, hake, etc.)
Gadus callarias; cod M
Pa. Nikitin, 1927
Melanogrammus aeglefinus; haddock M
Pa. Henry, 1913
Physiculus bachus; Red cod M
Au. Laird, 1951
2) Fam. Macrouridae (Grenadiers)
Coelorhyncus australis; M
Au. Laird, 1951
Longiopodus leucopaecicus; M
Au. Laird, 1951

Order k) Gasterosteiformes

1) Fam. Gasterosteidae (Sticklebacks)
Gasterosteus aculeatus; 3-spined stickleback FW
Na. Becker, 1967
Pungitius platygaster; 9-spined stickleback FW
Pa. Khayboulajev, 1969a
2) Fam. Syngnathidae (Sea horses)
Syngnathus acus; sea horse M
Pa. Yakimoff, 1912

Order l) Channiformes

Fam. Channidae (Milkfish)
Ophicephalus maculatus; FW
Or. Mathis and Leger, 1911
Pa. Ogawa and Uegaki, 1927
O. obscurus; FW
Et. Wenyon, 1909

O. punctata; FW
Or. Hasan and Qasim, 1962
O. striatus; FW
Or. Lingard, 1904a, b; Mathis and Leger, 1911; Pearse, 1933; Qadri, 1952, 1955 1962c

Order m) Synbranchiformes

Fam. Synbranchidae (Swamp eels)
Fluta alba; FW
Pa. Ogawa and Uegaki, 1927
Monopterus javanensis; FW
Or. Mathis and Leger, 1911
Synbranchus marmoratus; FW
Nt. Neiva and Pinto, 1926

Order n) Scorpaeniformes

1) Fam. Scorpaenidae (Scorpion fish)
Scorpaena ustulata; M
Pa. Neumann, 1909
2) Fam. Triglidae
Trigla corax; M
Pa. Neumann, 1909
T. gurnadus; M
Pa. Henry, 1913
T. lineata; M
Pa. Minchin and Woodcock, 1910
3) Fam. Cottidae (Sculpins)
Cottus bubalis; M
Pa. Brumpt and Lebailly, 1904; Lebailly, 1905
C. gobio; Miller's thumb FW
Pa. Brumpt, 1906b
C. gulosus; Riffle sculpin FW
Na. Becker, 1967
C. rotheus; Torrent sculpin FW
Na. Becker, 1967
Myoxocephalus octodecimspinosus; Longhorn sculpin M
Na. Becker, 1967
4) Fam. Agonidae (Poachers)
Agonus cataphractus; M
Pa. Henry, 1913

Order o) Perciformes

1) Fam. Serranidae (Sea basses)
Gilbertia semicincta; Half banded sea perch M
Au. Mackerras and Mackerras, 1925
Siniperca chuatsi; FW
Pa. Dogiel and Achmerov, 1959

2) Fam. Percidae (Perch)
Acerina cernua; ruffe FW
Pa. Chalachnikov, 1888; Brumpt, 1906b; Keysselitz, 1906; Bykovskaya-Pavlovskaya et al, 1962
Lucioperca lucioperca; Pike perch FW
Pa. Nikitin, 1929; Sluhei, 1960; Bogdanova, 1961; Bykovskaya-Pavlovskaya et al, 1962; Khayboulajev, 1969a
Perca flavescens; Yellow perch FW
Na. Fantham, Porter, and Richardson, 1942; Fantham and Porter, 1948
P. fluviatilis; perch FW
Pa. Danilewsky, 1885; Brumpt, 1906b; Keysselitz, 1906; Minchin, 1909; Breindl, 1911; Robertson, 1912; Coles, 1914; Franchini, 1923; Nikitin, 1929; Zalevskaya, 1950; Qadri, 1952; Barrow, 1955; Sluhei, 1960; Koval, 1962, Bykovskaya-Pavlovskaya et al, 1962; Shumela et al, 1963; Aligodjiev, 1969; Khayboulajev, 1969a

3) Fam. Carangidae (Jacks)
Lichia amia: Leervisch M
Et. Fantham, 1918

4) Fam. Sparidae (Sea breams)
Box salpa; bamboo fish M
Et. Fantham, 1919
Dentex argyrozona; M
Et. Fantham, 1919

5) Fam. Sciaenidae (Drums)
Macrodon malabaricus; FW
Nt. Botelho, 1907

6) Fam. Cichlidae (Cichlids)
Astatoreochromis alluaudi; FW
Et. Baker, 1960
Haplochromus cinereus; FW
Et. Hoare, 1932
H. humilior; FW
Et. Hoare, 1932
H. nubilus; FW
Et. Hoare, 1932

H. serranus; FW
Et. Hoare, 1932
Haplochromus sp; FW
Et. Baker, 1960
Tilapia esculenta; FW
Et. Baker, 1960
T. lata; FW
Et. Leger and Leger, 1914
T. mossambica; FW
Et. Dias, 1955
T. nilotica; FW
Et. Baker, 1960
T. variabilis; FW
Et. Baker, 1960
T. zilli; FW
Et. Wenyon, 1909

6) Fam. Pomacentridae (Blue fish)
Parma microlepis; White ear M
Au. Mackerras and Mackerras, 1925

7) Fam. Mugilidae (Mullets)
Mugil sp; Noke fish FW
Et. Neave, 1906; Wenyon, 1909

8) Fam. Labridae (Wrasses)
Thalassoma columna; M
Et. Wurtz and Thiroux, 1909

9) Fam. Mugiloididae
Parapercis colias; Blue cod M
Au. Laird, 1951

10) Fam. Blennidae (Combtooth blennies)
Blennius cornutus; Blenny M
Et. Fantham, 1930
B. pholis; Blenny M
Pa. Brumpt and Lebailly, 1904; Lebailly, 1905
Clinus anguillaris; Snake klipfish M
Et. Fantham, 1930
Ericentrus rubrus; M
Au. Laird, 1953
Tripterygium medium; M
Au. Laird, 1951
T. varium; M
Au. Laird, 1951

11) Fam. Callionymidae (Dragonets)
Callionymus dracunculus; Dragonet M
Pa. Brumpt and Lebailly, 1904
C. lyra; dragonet M
Pa. Lebailly, 1905; Henry, 1910, 1913

12) Fam. Gobiidae (Gobies)

Benthophilus macrocephalus; Caspian goby FW
Pa. Khayboulajev, 1969e

Gobius giuris; FW
Or. Castellani and Willey, 1905

G. niger; Black goby M
Pa. Brumpt and Lebailly, 1904; Lebailly, 1905

G. nudiceps; Dikkop M
Et. Fantham, 1919, 1930

Mesogobius batrachocephalus; Whip goby FW
Pa. Zalevskaya, 1954; Bykovskaya-Pavlovskaya et al, 1962

Neogobius fluviatilis; FW
Pa. Khayboulajev, 1969a

N. kessleri; FW
Pa. Khayboulajev, 1969a

N. ratan; FW
Pa. Khayboulajev, 1969a

Periophthalmus koelreuteri; FW
Et. Zupitza, 1909

13) Fam. Anabantidae (Climbing perches)

Anabas scandens; FW
Or. Mathis and Leger, 1911

Macropodus opercularis; FW
Pa. Ogawa and Uegaki, 1927

Trichogaster faciatus; FW
Or. Lingard, 1904a

T. trichopterus; FW
Or. Pearse, 1933

14) Fam. Osphronemidae

Macropodus viridiauratus; FW
Or. Mathis and Leger, 1911

15) Fam. Mastacembelidae (Mastacemblid eels)

Rhynchobdella aculeata; FW
Or. Lingard, 1904a

Order p) Pleuronectiformes

1) Fam Bothidae (Lefteye flounders)

Arnoglossus laterna; Scaldfish M
Pa. Lebailly, 1904, 1905; Henry, 1913

Bothus rhombus; Brill M
Pa. Lebailly, 1905

Zeugopterus punctatus; M
Pa. Henry, 1910

2) Fam. Pleuronectidae (Righteye flounders)
Caulopsetta scapha; Witch M
Au. Laird, 1951
Limanda limanda; Dab M
Pa. Brumpt and Lebailly, 1904; Lebailly, 1905
Pleuronectes flesus; Flounder M
Pa. Lebailly, 1904, 1905; Robertson, 1906a
P. platessa; plaice M
Pa. Lebailly, 1904, 1905; Robertson, 1906a
Rhombosolea plebia; Sand flounder M
Au. Laird, 1951

3) Fam. Soleidae (Soles)
Solea monochir; sole M
Pa. Yakimoff, 1912
S. solea; sole M
Pa. Laveran and Mesnil, 1901, 1904; Brumpt, 1906b; Lebailly, 1905; Henry, 1910; Coles, 1914

Order q) Tetraodontiformes

Fam. Balistidae (Triggerfish)
Balistes capriscus; Trigger fish M
Na. Saunders, 1959

Trypanosomes have been found in 140 species of freshwater fish, 50 species of marine fish, and 5 anadromous species from the Acipenseridae. They were found in all major zoogeographical regions, their distribution reflecting the distribution of workers in this field, i.e. records and therefore species were rare in the freshwater Nearctic and Australasian fish.

The fish which have been reported to contain trypanosomes are distributed as follows: 25% Perciformes, 19% Siluriformes, 18% Cypriniformes, and 9% Chondrichthyes. However, of all the known fish species, 40% are in the Perciformes, and about 25% in the Siluriformes and Cypriniformes taken together (Lagler et al, 1962). Thus the position is reversed with the trypanosome records, reflecting the almost exclusive freshwater nature of the Siluriformes and Cypriniformes. Also, the Siluriformes are typically mud-living fish, occupying habitats where associations with leech intermediate hosts are likely. Lagler et al (1962) also state that only 3% of all fish species are in the Chondrichthyes.

Of the freshwater fish, 24% of the species with trypanosomes were airbreathing. This high proportion reflects their ease of capture. Yet their mud-living still water habitat renders them more likely to leech attack.

Although workers did not generally record negative fish, it is clear that trypanosomes were found in the majority of freshwater species examined. This was also true of the Chondrichthyes, but not

of marine teleosts. In the Palearctic Atlantic, Henry (1913) found 10/51 marine species positive for trypanosomes. In the Palearctic Mediterranean the proportions were 4/60 (Neumann, 1909), 3/82 (Yakimoff, 1912), and 1/48 (Lagarde, 1943). In the Nearctic Atlantic, the proportions were 1/25 (Kudo, 1923), and 1/43 (Bullock, 1958). In the Australasian Pacific, the proportions were 3/27 (Mackerras and Mackerras, 1925), and 0/122 (Laird, 1958). These low incidences partly reflect the difficulty of finding trypanosomes in blood smears taken from scantily infected fish. However, the uniformity of the results must mean that few species were infected.

Of the 50 marine species found with trypanosomes, 32% were Perciformes, 13% Rajiformes, 10% Scorpaeniformes, and 10% Plueronectiformes. Many of the fish positive for trypanosomes were recovered from the litteral or intertidal zone, with representative species amongst the Soleidae, Cottidae, Triglidae, Blenniidae, Gobiidae, and Syngnathidae. Laird (1951) found trypanosomes in 10/51 New Zealand intertidal species.

The remaining fish positive for trypanosomes, with the exception of the requiem shark, Carcharias sp, were recovered from the inner sublitteral zone down to a depth of 50 meters. Marine leeches are rare below this depth (Epshstein, 1962). It is not surprising therefore, that Noble (1969) found no blood flagellates in fish below 100 meters.

Also, marine leeches cannot swim (Harant and Grassé, 1959). Therefore, varying degrees of association exist between these leeches and their hosts. For example, Branchellion spp, Calliobdella spp., Notostomobdella laeve, Pontobdella muricata, Stibarobdella macrothela, and Trachelobdella lubrica, are wandering "predators", and were reported from a wide range of sublittoral bottom living cartelagenous and teleost fish (Knight-Jones, 1962). Malmiana spp., Heptacyclus spp., and Sanguinothus pinnarum, have closer associations with intertidal fish, particularly of the families Blenniidae, and Cottidae (Epshstein, 1962; Gibson and Tong, 1969; Knight-Jones, 1940; de Silva and Burdon-Jones, 1961; Srivastava, 1966). Finally the close association between Hemibdella soleae and the Soleidae is well documented (Knight-Jones and Llewellyn, 1964; Llewellyn, 1965).

All these marine leeches are in the family Piscicolidae and, by each possessing an ensheathed proboscis, are theoretically capable of transmitting trypanosomes. Clearly more work on the intermediate hosts of fish trypanosomes is needed to back up ecological explanations of the occurrance of trypanosomes in certain groups of fish.

II. HISTORICAL REVIEW OF THE HAEMOFLAGELLATES OF THE TENCH, Tinca tinca.

a) Cryptobia

Danilewsky (1885) probably was the first to see Cryptobia in tench. He was followed by Brumpt (1905) who remarked that Cryptobia from tench developed in H. marginata, and not in P. geometra.

Keysselitz (1906) identified the parasite as Cryptobia borelli, and noted that tench usually contained mixed Cryptobia and trypanosome infections. He found in natural epidemics, the Cryptobia infections increased greatly over the trypanosome infections, which remained at constant low levels. He studied the cycle of development in P. geometra but failed experimentally to cause noticeable changes in the tench Cryptobia levels by the repeated feeding of up to 60 infected P. geometra. Minchin (1909) found mixed Cryptobia and trypanosome infections in all the tench he examined from Sutton Broad in Norfolk. He described large and small Cryptobia, and named the species Cryptobia keysselitzi. He made no measurements. Breindl (1911) found Cryptobia levels lower than trypanosome levels in Czechoslovakian tench infected with both parasites. Young tench were not infected with Cryptobia, and all fish were negative between November and March. He considered C. keysselitzi synonymous with C. cyprini Plehn, 1903. Nikitin (1929) recorded C. keysselitzi from Volga tench, and Bykovskaya-Pavlovskaya et al, (1962), quoted the measurements for the Cryptobia from tench of the river Dnieper given by Zalevskaya (1954). Koval (1962) also recorded C. keysselitzi from the Dneiper, and Khayboulajev (1969a) found C. keysselitzi in 14 out of 39 tench from the Caspian sea.

It is important to note at this stage that much of the Russian work was unobtainable. Zalevskaya (1954), for example, is a Ph.D. thesis at Kiev University. However, Shulman in a personal communication

told me that, in writing the section on Protozoa in Bykovskaya-Pavlovskaya et al (1962), he copied all the information on trypanosomes and Cryptobia in fish directly from the thesis of Zalevskaya (1954). Thus the information in the thesis is obtainable indirectly.

b) Trypanosomes

Danilewsky (1885) reported the first trypanosome from tench, and Doflein followed with a report in 1901. He considered the parasite to be T. carassii Mitrophanow 1883. Then Laveran and Mesnil (1904) described trypanosomes from 3 out of 6 tench, naming the genus T. tincae. In several parasites they reported two kinetoplasts and two flagella, butlater division stages were not found. Brumpt (1905) found that nearly all the tench he examined of ages between 1 and 10 years old were parasitised with trypanosomes. Keysselitz (1906) also reported high incidences of low chronic infections in German tench. Minchin (1909) found all tench examined from Sutton Broad infected, and gave an account of the nuclear structure. Breindl (1911) found trypanosomes were not as abundant in tench as in carp in Czechoslovakia. Young tench were uninfected. One older fish had an acute infection. From June to October, infections were regularly detected. In winter, the majority of fish were apparently negative, but in the spring a few positive fish were detected. Ponselle (1913b) reported the successful in vitro culturing of T. tincae in N.N.N., as did Franchini (1923). Nikitin (1929) reported the parasite in tench from the river Volga, and Zalevskaya (1950) reported all six tench

examined from the river Dneiper as having heavy infections. This she correlated with the still water and muddy bottom habitat of the fish. Barrow(1955), in a confusing paper, reported that the dominant tench in a tank at 20°C lost its trypanosome infections because its territorial activities prevented the other tench from feeding. He also investigated antibody production in trypanosome infected tench, but his results are not convincing. Sluhei (1960) recorded trypanosomes in tench from the river Donetz, and Koval (1962) recorded them from the Dneiper again. Bykhovskaya-Pavlovskaya (1962) reported the measurement of T. tincae from Zalevskaya (1954). Shumela et al (1963) recorded the parasite from the river Dneister, and Aligodjiev (1969) from the reservoirs of Dagestan. Khayboulajev (1969a) found the parasite in 5 out of 39 Caspian sea tench, a surprisingly low incidence, reflecting this workers'tendency to examine stained slides rather than fresh material.

III. HISTORICAL REVIEW OF THE HAEMOFLAGELLATES OF THE CRUCIAN CARP *Carassius carassius*.

a) Cryptobia

Chalachnikov (1888) is cited by Laveran and Mesnil as first seeing Cryptobia in the blood of crucian carp. Keysselitz followed in 1906, calling the parasite C. borelli, along with the Cryptobia from the other fish he examined. Chen (1956a) recorded Cryptobia

from the gills of crucian carp in China. He named the parasite C. branchialis.

b) Trypanosomes

Mitrophanow (1883) described and named T. carassii from crucian carp. Chalachnikow (1888) repeated his findings, as did Keysselitz in 1906. Nikitin (1929) recorded the parasite from Volga crucian carp, and Zalevskaya (1950) found all three crucian carp she examined from the river Dneiper infected. Sluhei (1960) reported T. carassii from the River Donetz, and Koval (1962) found it again in Dneiper crucian carp. Bykovskaya-Pavlovskaya et al (1962) reported the measurements of T. carassii from Zalevskaya (1954). Shumela et al (1963), found the parasite in the river Dneister, and Khayboulajev (1969a) found it in all three crucian carp he examined from the Caspian sea.

IV. HISTORICAL REVIEW OF TRYPANOSOMES OF THE PERCH, Perca fluviatilis.

Danilewsky (1885) first reported trypanosomes from perch, but they were not observed again until Brumpt (1906b) named the species T. percae, and described its development in Hemiclepsis marginata. Keysselitz (1906) also reported trypanosomes from perch, and Minchin (1909) carefully described T. percae which he found in almost all the perch examined from Sutton Broad. One was acutely infected. Breindl (1911) reported that 22/25 of the perch he examined between July and September were infected with T. percae, with higher infestation

rates in rivers than in lakes. Robertson (1912) repeated Brumpt's work on the life cycle of perch trypanosomes in H. marginata, and reported that the parasite infected clean goldfish via an infected H. marginata. Coles (1914) found that British perch were frequently infected with trypanosomes. In 1923, Franchini achieved some success with the culture of T. percae in N.N.N. In 1929, Nikitin reported the parasite from Volga perch. Qadri (1952) found T. percae in 2 out of 10 perch from Windemere and Essex. Both infections were low. Barrow (1955) reported trypanosomes from perch at Cambridge, Sluhei (1960) from the river Donetz, Koval (1962) from the Dnieper, Shumela et al (1963) from the Dniester, and Kligodjiev (1969) from the Dagestan reservoirs. Zalevskaya (1950) found 8 out of 15 perch infected in the river Dnieper, and Bykovskaya-Pavlovskaya et al (1962) reiterated the measurements for T. percae from Zalevskaya (1954). Abolarin (1966) found 9 out of 26 perch infected from the Shropshire Union Canal, and Khayboulajev (1969a) found 29 out of 64 perch infected from the Caspian sea.

V. HISTORICAL REVIEW OF THE HAEMOFLAGELLATES FROM THE ROACH, Rutilus rutilus.

a) Cryptobia

Brumpt (1905) was the first to report a Cryptobia from the roach, mentioning that its life cycle was completed in P. geometra. Keysselitz (1906) said the parasite in the roach he examined was

C. borelli. There were no further reports until that of Khayboulajev (1969a), who found that 6 out of 106 Caspian sea roach were infected with a Cryptobia which he named C. borelli forma rutili.

b) Trypanosomes

Brumpt (1906b) studied the development of trypanosomes from the roach in H. marginata to which he gave the name T. leucisci. Keysselitz (1906) also found trypanosomes in roach. Coles (1914) reported that trypanosomes occurred infrequently in roach, and Zalevskaya (1950) found only 3 out of 15 roach infected from the river Dnieper. Koval (1962) also reported the parasite from the Dnieper. Dogiel, Petrushevski, and Polyanski (1961) cite Dubinin (1952) as stating that Trypanosoma abramidis, restricted to the genus Abramis, occurs also in the blood of young, but not adult roach. In fact the original reference makes no mention of either T. abramidis or roach. Bykovskaya-Pavlovskaya et al (1962) repeated the measurements for T. leucisci from Zalevskaya (1954). Abolarin (1966) found 3 out of 17 roach infected from the Shropshire Union Canal, and Khayboulajev (1969a) found 1 out of 31 Caspian sea roach slightly infected with trypanosomes, which he named T. shulmani forma leucisci.

VI. HISTORICAL REVIEW OF TRYPANOSOMES OF THE EEL, Anguilla anguilla.

Sabrazes and Muratet (1902a, b) first reported trypanosomes from the eel. They found that adult freshwater eels were all infected, whilst small eels from the same habitat were not. Marine eels were also negative. They demonstrated the presence of small and large

trypanosomes, and, in 1904, observed dividing forms in blood maintained under a coverslip for up to 6 days. Eels kept their low chronic infections when maintained in aquaria for $1\frac{1}{2}$ years. In 1907 they demonstrated that the trypanosomes survived in the eel for up to 62 hours after its death. Laveran and Mesnil (1902a) described the trypanosomes they found in 6 scantily infected eels, and named the species T. granulosum. Lebailly (1905) described two varieties for the species. T. granulosum var. magna had a great size range, and was present in all 25 freshwater eels he examined. Actively dividing trypanosomes were present in heavy infections. T. granulosum var. parva was smaller, with a restricted size range. This was described from 3 marine eels, all heavily parasitised. Brumpt (1905, 1906a, b) described in detail the development of T. granulosum in H. marginata. Manca (1906) reported that 8 out of 9 freshwater eels were infected, but a further 8 from a briney march in Sardinia were not. Keysselitz (1906) reported trypanosomes from German eels, and Franca (1907) described further observation on development under the coverslip. Minchin (1909) found one heavily infected eel in Norfolk, in which the trypanosomes showed a large and continuous size range. He made no measurements. Breindl (1911) found that although all the eels he examined in the summer were positive for trypanosomes, all eels collected in January and February were negative. Dunkerly (1913) reported T. granulosum from Irish eels, and in the same year Ponselle (1913a) cultured the trypanosome on N.N.N. media. In 1943,

Lagarde found that all 44 A. anguilla collected from the Mediterranean were negative. Kraneveld and Keidel (1955) found that of the 125 Dutch freshwater eels they examined, 55% were infected with T. granulosum. They described the species as dimorphic, larger and smaller forms being present. Bykovskaya-Pavlovskaya et al (1962) reported the measurements of T. granulosum observed by Zalevskaya (1954) from the river Dnieper, the only Russian record.

Thus descriptions are available for the five trypanosomes and one Cryptobia species discussed. However the descriptions of the early workers could apply to almost any trypanosome. The later workers, largely Russian, have faithfully reported host incidences based on vast numbers of stained preparations. Only Zalevskaya (1954) and Khayboulajev (1969a) presented detailed measurements of the haemoflagellates they observed. Their measurements are compared with my own in the "Discussion" section.

It is also suprising that of all the incidences reported from the differing hosts, only Breindl (1911) was careful enough to note a seasonal incidence. The incidence per fish species is discussed later in the light of fish behaviour, but the investigation of this seasonal incidence has formed a major part of my own work. It is hoped by the end of the work, therefore, that a picture of the ecology of fish trypanosomes is presented, in addition to the essential groundwork on their morphology and taxonomy.

1. COLLECTION OF FISH

Five species of wild fish were examined for flagellate infections. These were the tench, Tinca tinca, the crucian carp, Carassius carassius, the roach, Rutilus rutilus, the perch, Perca fluviatilis, and the eel, Anguilla anguilla. These fish, with the exception of the eels, were collected from a flooded gravel pit managed by Trilakes Ltd., and situated at Yately, near Sandhurst in Berkshire. In the winter and spring, they were netted using a 2 inch mesh, 75 yard seine net of 6 feet depth. From May to July they were trapped in shallow water in perch traps (Macan and Worthington, 1951) consisting of a wire frame covered in chicken netting with an inverted cone inlet as in figure 1. For the rest of the summer, the fish were collected from anglers. In the autumn netting was resumed at the onset of frosts. Electric stunning methods (Smith, Franklin and Kramer, 1959) were employed to capture small fish. A 15 volt/13.3 amp D/C current was generated by a 225 watt AC/DC generator (Outboard Marine and Manufacturing Co. Canada) carried on the boat. Terminals led to a metal plate and a landing net. The circuit was completed when both were placed in the water as in figure 2. The stunned fish were collected from the surface. Finally 10 eels from the river Avon at Fordingbridge were supplied by the Dorset and Avon river authority, and 10 marine eels were purchased at Bracknell market.

Roach for leech-feeding experiments were trapped from the Silwood Park lake. This lake is sterile, being polluted by iron-loving bacteria from the Bagshot Sand deposits, and by Sunninghill gas works.

Fig. 1 Fish trap

Fig. 2 Electro-fishing at Trilakes

Accordingly the leech intermediate hosts for blood flagellates were found to be absent. So the uninfected roach were used to rear parasite free leeches.

Experimental parasite free tench of 10 to 15 centimeters were of Italian origin, where they had been reared commercially in leech-free environments. They were supplied by the London Aquatic Co. Ltd., 42 Finsbury Rd., London N.22; the Anglo Aquarium Plant Co., Wildwoods, Theobald Park Rd., Enfield Middlesex; Mr D.F. Leney, the Surrey Trout Farm, Haslemere, Surrey, and Mr F. Stott, Freshwater Fisheries Laboratory, Ministry of Agriculture, Fisheries and Food, Whitehall Place, London S.W.1.

The following initial details were taken of all fish on arrival at the laboratory:-

1) The length from the tip of the snout to the end of the central ray of the caudal fin.
2) Age, as estimated from the annual rings on the scales. For the scales increase in size as the animal grows, and the continual addition of material to the periphery leads to the appearance of a series of concentric rings. The annual rings are the concentrations of these growth rings during winter when the growth of the fish is slow (Graham, 1929).
3) Sex of tench from external appearances. Brotherton (1954) states that the pelvic fins of the male are larger than those of the female, and of a more spoon-like or "cockle-shell" shape.
4) Origin, method of capture, and date.

2. COLLECTION OF LEECHES

Hemiclepsis marginata (fig. 3) was found in the leaf bases of the marginal reeds of the Basingstoke canal at Fleet in Hampshire. Figures 4 & 5 show the habitat which is just above the rhizome underneath the first few layers of leaves. The leeches were collected from reeds of Scirpus sp., Typha sp., Phragmites sp., at all times of the year, with the addition of the water plantain, Alisma sp., in the summer. They were also collected periodically from Scirpus at Trilakes, and Typha at Slapton Ley in Devon.

Piscicola geometra (fig. 6) was collected from trout in the Wraysborough reservoir near Chelmsford in Essex, and by Mr G. Tatham from the Welham Park Trout Hatchery, Malton, Yorkshire. Occasional piscicolids arrived from anglers following an appeal in the Angling Times, and a questionnaire circularised to river boards and angling clubs.

On arrival, the lengths of the leeches were taken as they adhered in a relaxed state to the glass aquaria with both their suckers. Breeding state, source, and date were also noted.

3. MAINTENANCE OF FISH

The experimental and smaller wild fish were maintained singly in aerated tap water in 18 by 12 by 12 inches glass aquaria in constant temperature rooms. The temperatures were fixed at 10°C, 15°C, and 20°C, and there were 12 hour alternating light and dark periods. The larger wild fish were maintained in constant flow 40 gallon

Fig. 3 Hemiclepsis marginata x 5

Fig. 4 On removal from lake

Fig. 5 Pulled apart to show leech habitat

Fig. 6 Piscicola geometra x 5

Osmaniabs water storage cisterns housed at room temperature, and in a large open sunken concrete tank of 2 foot depth. Fish were fed with Tubifex sp. supplemented with "Riverpride" fish food supplied in pellet form from Stambridge Fisheries in Essex.

Disease was largely prevented and controlled using the following methods adapted from van Duijn (1967).

Fungus: the infected fish were bathed daily for half a minute in a 0.006% solution, and maintained for up to two weeks in a 0.004% solution of Malachite Green. Infected parts were painted with tincture of iodine.

Bacteria: infected fish were removed and destroyed.

Ichthyophthirius multifilis: all fish were initially kept in quarantine after arrival for one month in a 0.01% Chloramine T solution (Sodiumpara-toluenesulphonchloramide). Infected fish were treated by maintenance in 0.04% Methylene Blue solution.

Chilodonella cyprini and Costia necatrix: the same prophylactic measures were observed as for I. multifilis. C. cyprini were eliminated by daily immersions for 30 minutes in 2% sodium chloride solution. C. necatrix was treated similarly using 0.05% formalin solution.

Strict hygiene was practised. Empty tanks were scrubbed with Ajax, and steralised using cooking salt followed by Hibitane treatment (Chlorhexidine diacetate, I.C.I. Ltd.). They were then washed in running water for two days.

4. MAINTENANCE OF LEECHES

H. marginata was maintained with ease in the laboratory and the constant temperature rooms, in contrast to *P. geometra* which was never successfully maintained. The leeches were cultured in aerated pond water in large glass sweet jars in total darkness. Optimum temperatures were 15°C and 20°C. *H. marginata* were removed from their containers using glass rods covered with fish mucous, and transferred into tanks containing live roach of 10-18 cms, or experimental tench, for feeding. To maintain the colony it was necessary to feed at least once a month.

5. RECOVERY OF FLAGELLATES FROM FISH

a) Anaesthetics

Fish were anaesthetised using a 1:10,000 solution of MS 222 (Tricaine methanesulphonate, Sandez Ltd.) after the technique of Randall and Smith (1967). They were removed after about 3 minutes at stage II, plane 2 anaesthesis (McFarland, 1959), i.e. at total respiratory arrest as indicated by the complete cessation of opercular movement. Recovery followed in fresh tap water.

b) Collection of Blood

For routine blood sampling, the anterior gill arch on the left hand side of the fish was blotted dry, and pricked with a sterile needle. Blood was drawn up into an Aimer micropipette (Aimer Products Ltd., London N.W.1.). A 0.0025 ml. portion was placed on one slide, the rest, about 0.02 ml., on another. After allowing

clotting to take place, the large and small drops were covered with 24 x 24 mm and 18 x 18 mm thin coverslips respectively. Blood was also removed using a blood diluting pipette, enabling the measurement of heavy flagellate infections and red blood cell counts. Blood smears were taken at speed because of the rapid clotting properties of fish blood. After bleeding, the punctured area soon clotted, and was cleaned with tincture of iodine.

Larger quantities of blood were withdrawn under anaesthesia from the retro-orbital sinus. A sequestrinised Johnston's sterile disposable syringe fitted with a 20 grade needle was inserted into the posterior side of the top of the orbit at an angle of 45 degrees, the needle inclined down the length of the fish. Care was taken not to puncture the eye or the cranial cavity. Recovery always followed.

c) Estimation of flagellate numbers

For the initial examination of blood and diagnosis of infection, a simplification of Strout's (1962) method was employed. The larger drop of clotted blood was examined after half an hour under phase contrast at x 100 magnification. The flagellates, where present, were detected swimming in the serum between the clotted red blood cells. Flagellates present in the 0·0025 ml drops were counted. Heavy infections were estimated using a haemacytometer counting slide with Neubauer ruling. The blood was previously diluted with isotonic citrate saline consisting of 0·65% sodium chloride and 0·5% sodium citrate in solution (Diamond, 1965).

d) Concentration techniques - centrifugation

Approximately 1 ml of blood removed from the retro-orbital sinus was diluted with an equal part of phosphate buffered saline (pH 7·2) containing 100 units per ml. of Heparin, and placed in a centrifuge tube. The whole was spun for 3 minutes at 620 g in an M.S.E. Minor centrifuge. The supernatent and top red blood cell layer containing the flagellates were removed, and spun at 2180 g for 5 minutes. The supernatent was then discarded, and the red blood cells and immediate covering layer retained. The mixture was shaken to resuspend with the flagellates, and taken up into unibore "break-off" 0·02 ml Benjamin haematocrit tubes (Harshaw Chemicals Ltd., Daventry, Northants.). These were spun for 5 to 10 minutes in an M.S.E. Minor microhaematocrit centrifuge head at 1590 g until separation of the red blood cells was achieved. The haematocrit tubes were cut at a point just below the surface of the red cell layer, and the overlaying pale area containing the flagellates and white blood cells retained.

e) Concentration techniques - anion exchange

Trypanosomes from perch were separated from blood using an anion-exchange method (Lanham, 1968). The blood was extracted from the retro-orbital sinuses using syringes containing 200 units of dried heparin per ml 0·05% sodium chloride solution. The mixture was then diluted with at least 3 parts of the following solution containing 0·005 molar sequestrin and 30 units per ml. heparin in phosphate-saline-glucose buffer of pH 8·0. The buffer consisted of 285 ml. 0·2 m $Na_2 HPO_4$, 15 ml. 0·2m Na $H_2 PO_4$, 300 ml 0·85% NaCl, and 400 ml

2·5% glucose. The diluted blood was filtered through a tea strainer to remove debris, and then fractionated. The DEAE (DE 52) cellulose had been previously equilibrated with P.S.G. buffer adjusted to pH 8·0 with 10% orthophosphoric acid. After 6 washes, the equilibrated slurry was packed into a short wide column of 6·0 by 2·0 cms. by passing it through a glass buckner funnel containing a sintered disc of porosity 0, with a Whatman No. 41 filter in the base. The head of red blood cells was run to enter the column, which was now eluted with buffer to harvest the trypanosomes. As the above buffer gave poor separation, its ionic strength was increased by adding 0·1m NaCl. Separation was also improved with slight suction. These two modifications, however, gave rise to some haemolysis, and slight elution of the red blood cells. The eluant was spun at 1630 g for 20 minutes in large centrifuge tubes in an M.S.E. freezing centrifuge at 2°C. After centrifugation, the supernatent was removed, and the "button" of trypanosomes and cell debris at the bottom retained.

6. RECOVERY OF FLAGELLATES FROM LEECHES

The leeches were relaxed in water containing a crystal or two of chlorbutol (Diamond, 1965), and washed. The crop was punctured by a single longitudinal dorsal incision after Robertson (1906), the leech resting in a wax depression flooded with 4% glucose. Hoare (1940) stated that isotonic glucose gave good results with Geimsa's stain. The crop contents were removed using a fine pipette, and examined both fresh under phase, and stained. Flagellates in the proboscis sheath were examined by cutting the integument below the

anterior sucker, and drawing the sucker away from the rest of the body. The proboscis in its sheath was left protruding from the leech's body.

For studies on the life cycles of the flagellates, experimentally-bred clean H. marginata were fed on infected fish. Batches were then sacrificed at intervals: 4 hourly for 24 hours; 6 hourly for the next 24 hours, and 12 hourly thereafter.

7. EXPERIMENTAL INFECTIONS OF FISH

Fish were infected experimentally by syringe-passaging blood from infected fish, and by allowing infective leeches to feed on the experimental fish. In the former method, infected blood was diluted with citrate saline, and the number of flagellates estimated. Not more than 0.5 ml of diluted blood was then introduced into another anaesthetised fish by intraperitoneal inoculation into the flank between the pelvic and pectoral fins, and below the lateral line. The inoculation site before and after the injection was treated with tincture of iodine.

With leech-induced infections, the experimental fish was simply introduced into the large sweet jar containing the infected leech, and the onset of feeding observed. After feeding the leech was examined for infectivity. The fish was similarly examined at fortnightly intervals.

8. FIXATION AND STAINING OF FLAGELLATES

a) Smears Smears from fish blood, the leech gut contents, and fish

tissues were air-dried, and fixed for 1 minute in methanol. Gurr's improved R 66 Geimsa's stain was used in varying concentrations. Thin smears were stained in 2 drops per ml. buffered distilled water at pH 7·2 for 20 minutes. Thick blood smears and tissue smears were stained similarly for periods of up to 45 minutes, or overnight in a 1 drop per ml. concentration. Laird (1957) has adequately defended the routine of air-dried smears and Geisma's stain for fish trypanosomes. Cryptobia, however, did not fix satisfactorily by this method. So they were routinely fixed in Carnoy's fluid, and stained with Geisma. Other methods of fixation and staining were used for comparative cytological work on the flagellates. Fixatives used were osmic acid vapour (Minchin, 1909b), formol saline, Carnoy's and Schaudinn's fluids. Stains used were Azure A (Vickerman, 1960), Geisma after NHCl hydrolysis for 6 minutes at 60°C following fixation in Carnoy's fluid, and Heidenheim's iron haematoxylin.

b) Sections Whole leeches and small pieces of fish tissue were fixed in Carnoy's fluid or aqueous Bouin's fixative. The material was dehydrated in alcohol. Initially it was cleared in xylol or xylol and chloroform, but subsequently Supercedrol was used for the final dehydration and clearing. Material was embedded in paraffin wax of melting point 56°C. Sections were cut at 4μ and 6μ, and stained using the ~~Geisma~~ Giemsa Colophonium method of Shortt and Cooper (1948), Ehrlich's haematoxylin and eosin, or Heidenheim's iron haematoxylin.

9. DRAWING AND MEASUREMENTS OF FLAGELLATES

A camera lucida was used to project the image viewed at x 2500 magnification onto graph paper ruled in millimeters. Complete line drawings of selected flagellates were made. However, for the purposes of measurement only, the circumference of the nucleus and kinetoplast were drawn. Then two parallel lines denoting the width of the body at the level of the nucleus, and elsewhere if wider, were drawn. The posterior and anterior extremities of the body, the centre of the kinetoplast, the centre of the nucleus, and the anterior tip of the flagellum were shown by drawing lines at right angles to the main axis of the body. A line was then drawn down the centre of the body from the posterior extremity to the tip of the trypanosome flagellum. With Cryptobia the central body line and the two flagellae from their points of departure from the kinetoplast, noting where they leave the body, were drawn. The measurements were then made by running along the central lines and flagellae with a map measurer (fig. 7) calibrated in millimeters (Curvimeter No. 56, from W.G. Pinner & Co., Birmingham).

Thirteen measurements were made for each trypanosome, and fourteen, with the additional flagellum, for each Cryptobia as follows: the distance from the posterior extremity to the centre of the kinetoplast (PK), the centre of the kinetoplast to the centre of the nucleus (KN), hence the posterior extremity to the centre of the nucleus (PN), the centre of the nucleus to the anterior extremity (NA), hence the length of the body (LB), the length of the free flagellum or flagellae (LF),

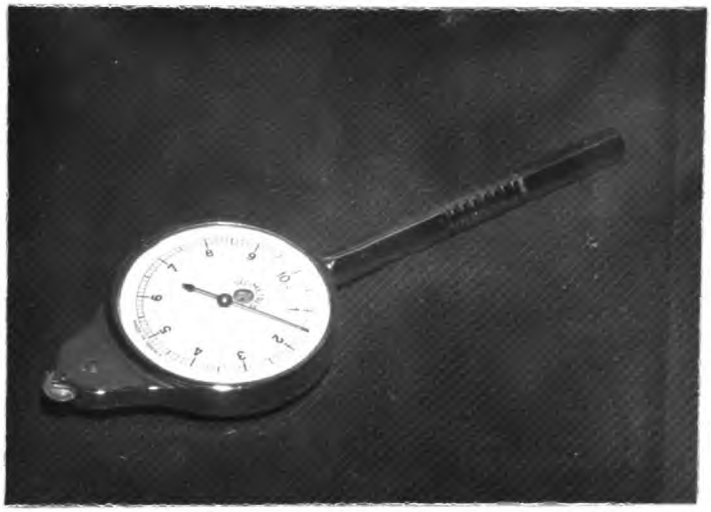


Fig. 7 Map measurer

hence the total length of the trypanosome (TL). The nuclear index (NI) was computed by dividing PN by NA. The maximum breadth of the body was measured (BB), as was the length and breadth of the nucleus (LN, BN), and the size of the kinetoplast (LK, BK), using the map measurer and the squares on the graph paper.

10. PHOTOGRAPHY

Flagellates were photographed using high contrast Recordak Microfile type 5669 film (Kodak Ltd.) and printed on low contrast Multigrade paper (Ilford Ltd.).

11. MORPHOMETRIC TECHNIQUES

a) Frequency histograms

Length-frequency histograms were plotted of the blood stream trypanosomes to determine monomorphism or polymorphism. Monomorphism is defined here as the existance within a trypanosome species of a single morphological form of the trypomastigote stage in the fish blood, the variation of which is statistically expressed in the form of a monomodal frequency curve of variation (adapted from Diamond, 1965). If necessary some distributions were subjected to a probit analysis (Southwood, 1966) to check further for mono- or polymodality. The position of the nucleus was selected as an important taxonomic criterion. Accordingly an IBM 7094 computer was programmed with nuclear indexes and body length measurements to determine whether the nuclear position relative to the body length of the trypanosome was

characteristic for any one species.

b) Dice-Leraas Diagrams

With this method (Dice and Leraas, 1936), the mean, the range, the standard deviation (σ), and twice the error of the mean ($2\sigma_m$), of a set of measurements may be depicted in graphic form. A vertical line is drawn to indicate the mean, black retangles are placed on either side to represent the standard deviation, and white rectangles inside them to show twice the standard error of the mean. This method was modified for use in biometrical studies of trypanosomes by Davis (1952), and has been used in such studies by Hoare (1956, 1959), Diamond (1965), Davis (1969), and Keymer (1969).

12. DEEP FREEZE PRESERVATION OF FLAGELLATES

A modification of the technique of Cunningham et al. (1963) was used. Taking aseptic precautions throughout, 1 ml. of blood was removed from the retro-orbital sinuses of a tench heavily infected with trypanosomes, and lightly infected with Cryptobia. Heparin (10 units per ml. saline) prevented clotting. Sufficient glycerol was added to give a final concentration of 7·5% by volume. The mixture was agitated, and dispensed into capillary tubes which were then flame-sealed. The tubes were then placed in a universal culture bottle, and precooled in dry ice at -80°C in a thermos flask overnight. After this the bottle was put into a Union Carbide vapour phase liquid nitrogen refrigerator, and stored at -179°C. Three capillaries were removed after 6 months. The contents of one were

examined fresh and stained. The contents of the other two were each inoculated into 2 experimental uninfected tench maintained at 20°C.

RESULTS

I. BLOOD FLAGELLATES FROM THE TENCH, *Tinca tinca*.

1. Wild Tench

The habitat of the wild Tench was a landscaped flooded gravel pit of 5 acres, with a maximum depth of 15 feet. It was a rich, eutrophic, water body, with shallow shores fringed by marginal reeds as in figure 8.

The behaviour of the tench accounted for the variation in trapping methods. From October to early May, they rested in a state of dormancy at the bottom of the lake. From May to July they ventured into shallow water to spawn, so they were trapped in the marginal reed beds. Table I shows the age of tench in the montly samples. In June and July samples consisted mostly of 2 year old fish. These readily entered the traps. The winter samples contained a greater proportion of older fish that were big enough to be lifted out of the bottom mud by the seine net. Up to mid-October, the activity of anglers prevented netting. So, as the tench only occasionally ventured into the traps during periods of hot weather, the anglers themselves supplied the fish. All methods, including electric stunning, failed in the search for 1 year old tench. One specimen was netted. Fry, or 0 year old tench, were captured with a plankton net from July to September.

In all, 120 tench from 0 to 7 years old were examined for blood flagellate protozoa.

Fig. 8 Trilakes

Table I. Age classes of leech collected per month 1967-1969.

AGE	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.	TOTAL
0	-	-	-	-	-	-	7	15	20	-	-	-	42
1	-	-	-	-	-	-	-	-	-	-	-	1	1
2	-	2	-	4	-	8	7	9	-	4	-	2	36
3	-	4	-	5	-	2	2	5	-	1	-	4	23
4	-	4	-	1	-	-	2	1	-	2	-	3	13
5	-	-	-	-	-	-	-	1	-	3	-	-	4
7	1	-	-	-	-	-	-	-	-	-	-	-	1
TOTAL	1	10	0	10	0	10	18	31	20	10	0	10	120

2. Wild Leeches

a. P. geometra

Over 1000 P. geometra were collected from fish of the Wraysborough reservoir and the Welham Park fish hatchery. In addition small numbers were sent from fish captured in Buckinghamshire, Northamptonshire, Lancashire, Oxfordshire, and Surrey, following the 'Angling Times' letter. The habitats were large oligotrophic lakes and rocky rivers, and fast flowing trout streams. In the smaller water bodies the heaviest infections of fish occurred in the summer. In the Wraysborough reservoir and the Welham Park fish hatchery, the heaviest infections occurred in the winter. The preferred fish species were brown and rainbow trout (Salmo trutta, S. irideus). P. geometra were also found on barbel (Barbus barbus), bream (Abramis brama), bullhead

(Cottus gobio), carp (Cyprinus carpio), chub (Squalius cephalus), dace (Leuciscus leuciscus), gudgeon (Gobio gobio), minnow (Phoxinus phoxinus), pike (Esox lucius), roach (Rutilus rutilus), and tench (Tinca tinca). No P. geometra were found on or off the fish at Trilakes.

The lengths (0.9 cms to 3.4 cms) of P. geometra varied little over the year. Most of the leeches in every sample laid cocoons in the aquaria either on arrival, or after feeding.

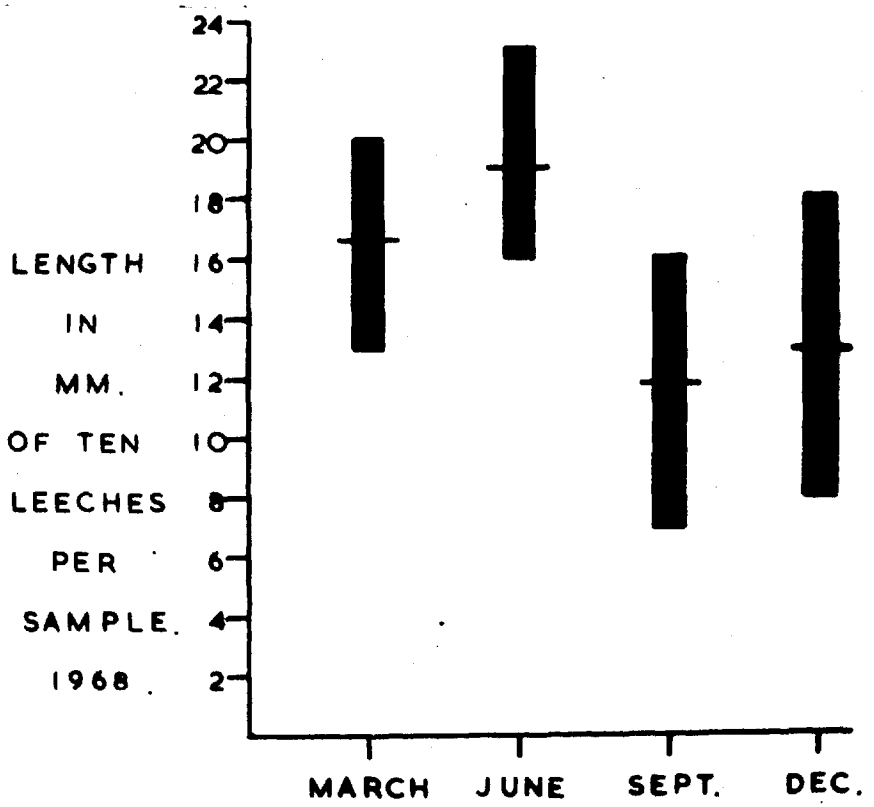
b. H. marginata

This species was never found naturally infecting a fish. Its restricted habitat was the leaf bases of reeds on the margins of ponds, lakes, and slow moving rivers. In the winter months they were found hidden amongst the rhizomes which supported the dead leaves. A days collection would produce 10 H. marginata at best from the three waters. Thus continuous sampling was not possible from Trilakes because the removal of so many reeds would ruin the landscaping. However 6 H. marginata were collected in November, and 10 the following December during annual weed-clearing operations. 18 H. marginata were collected from Slapton Ley in two successive Septembers. Continuous sampling was possible, however, from the Basingstoke Canal, and a total of 40 leeches, with 10 in each sample, were collected in March, June, September, and December. Then 74 H. marginata were collected from three sources.

The means and ranges of the lengths of the canal samples are given in graph 1. Some small dead H. marginata were found in March

GRAPH 1.

H. marginata from the Basingstoke canal.



so low temperature was a likely factor in the "cut-off" in the lower size ranges between December and March. 1 adult was brooding eggs when collected in March. In the June collection, 3 were carrying offspring, and 5 were brooding eggs. The September and December leeches were all immature.

3. Maintenance of Tench

All tench were found to require subdued light, or preferably total darkness. So to prevent agitation on the part of the fish, aquaria were shaded, tanks were covered, and black polythene bags were floated on the water in the concrete pond, under which the tench congregated. Feeding was reduced at 15°C, while at 10°C and below they ate nothing. Thus at low temperatures, continuous blood sampling resulted in death unless the temperature was raised, and feeding initiated. Disease problems were always greater at 20°C. Accordingly the fish were quarantined on arrival at 20°C, and treatments carried out at this temperature. Tench soon became acclimatised, so there were few problems of fungus growing on damaged areas. Their oxygen requirements were the lowest of all the fish species studied, though running water was necessary in the fibre glass tanks and concrete pond.

4. MAINTENANCE AND BREEDING OF LEECHES

a) P. geometra

This species was never successfully maintained in the laboratory for any length of time. The majority died after laying cocoons within

a week of arrival; the rest died following a single feed on an experimental fish. Again they died after cocoon deposition. They all died within days unless maintained in heavily oxygenated pond water. Tap water was lethal. Temperatures and light were not critical. It is likely that maintenance in continuously flowing pond water would be more successful. Transmission experiments between fish were not possible.

Many thousands of cocoons were laid at an average of 5 per leech, at temperatures ranging from 5°C to 25°C. However, less than 1% hatched after a minimum of 23 days at 20°C, and a maximum of 48 days at 5°C. No young survived to take a first feed. So flagellate life cycle studies with this leech were not conducted.

b) H. marginata

In contrast, the successful maintenance and breeding of H. marginata under experimental conditions enabled the life cycles of the leeches and their trypanosomes to be studied. Shortage of time and material prevented comprehensive repetition of the early work by Leger (1904b), Brumpt (1905), Keysselitz (1906), Robertson (1912), Martin (1913), and Tanabe (1924), on the development of Cryptobia in this leech.

Breeding only occurred experimentally at 15°C or over. At this temperature a colony was initiated from the 10 H. marginata in the March sample from the Basingstoke canal. 5 leeches were fed on an experimental tench the day after collection. They all laid eggs after 10-26 days, which hatched 67-88 days after the feed. The rest

of the leeches were not fed. One was already brooding eggs, and 2 laid eggs at 14 and 30 days, which hatched at 59 and 78 days after the original collection. The other 2 leeches contained no meal on collection: they were thin and green as opposed to fat and red. They died without laying eggs.

A further colony was iniated at 20°C with the 10 leeches from the June sample. The 3 carrying offspring, and 2 not brooding eggs, were fed on arrival. The eggs were laid after 3 and 12 days, and hatched 8 and 19 days after feeding. The other 5 leeches were brooding eggs and were not fed. These eggs hatched within 14 days.

Up to 88 eggs were laid and attached by each leech to their undersides. The "mother" rythmically fanned the rows of eggs by undulating body movements, and remained throughout the "gestation period" attached to the same place. They did not feed during this period. Enforced movement caused them to drop some of their eggs, most of which hatched independently. After hatching, the "mothers" carried their offspring onto a fish. There the infants dispersed and fed with alacrity, although they were only 2 to 4 millimeters in length. The "mothers", however, left the fish after attempting to feed, and invariably died.

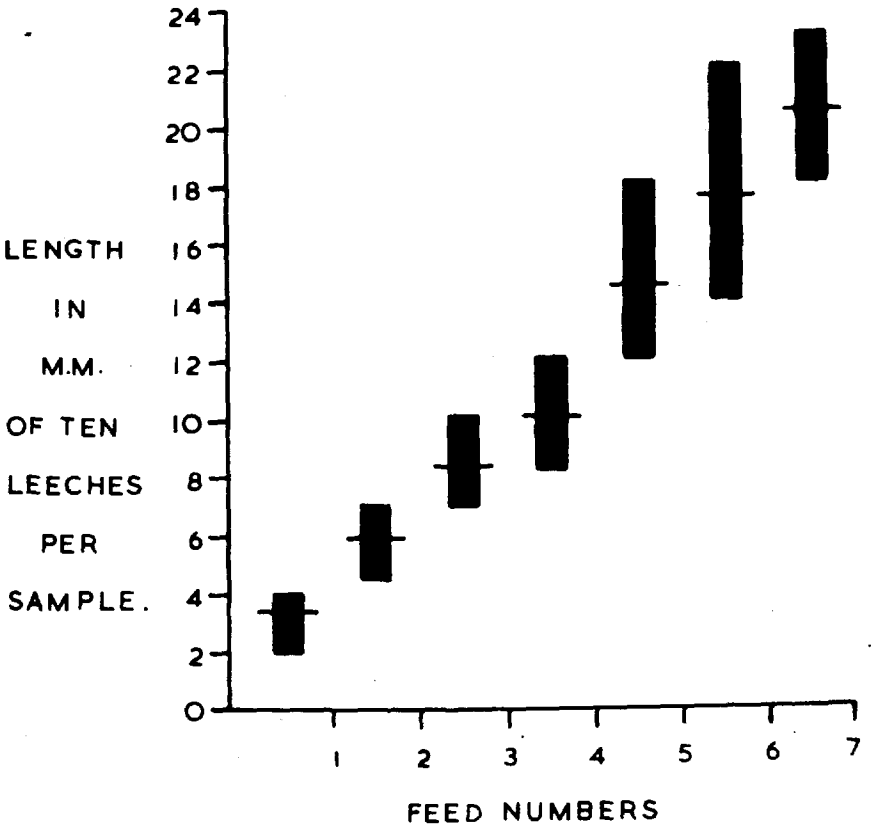
Feeding of hungry H. marginata was straightforward. Unlike P. geometra which only exhibited a tactile response to the fish, H. marginata reacted to the presence of a fish by reaching movements with their anterior end, and looping "walks" in the general direction of the fish. Recently gorged H. marginata showed no such reaction.

All hungry leeches found the fish within a few minutes, and feeding lasted about 4 hours at 20°C, 12 hours at 15°C, and 1-2 days at 10°C and 5°C. Unlike P. geometra which only tended to leave the fish to lay cocoons or die, H. marginata left the fish immediately after feeding, and rested on the walls of the aquarium. Preferential feeding sites were those that the leech was likely to reach first, i.e. fins, ventral surface, and mouth. They successfully resisted the deliberate attempts of the fish to eat them by attaching to, and feeding on, the lips and inside of the mouth.

The rate of digestion of the blood meal varied with temperature, though the sizes of the leech and blood meal were important. As a rule, the red blood cells were still evident in the crop at 5 days at 20°C, and at 7 days at 15°C. The meal was digested at 20°C in 7 days by a small leech with a small meal, and in up to 25 days by a large leech with a large meal. At 15°C the meal was digested in 7 to 42 days. At 5°C the meal was digested after up to 72 days, and survival time without feeding at this temperature was up to 8 months. Survival time was directly dependent on size, the largest leeches living longest.

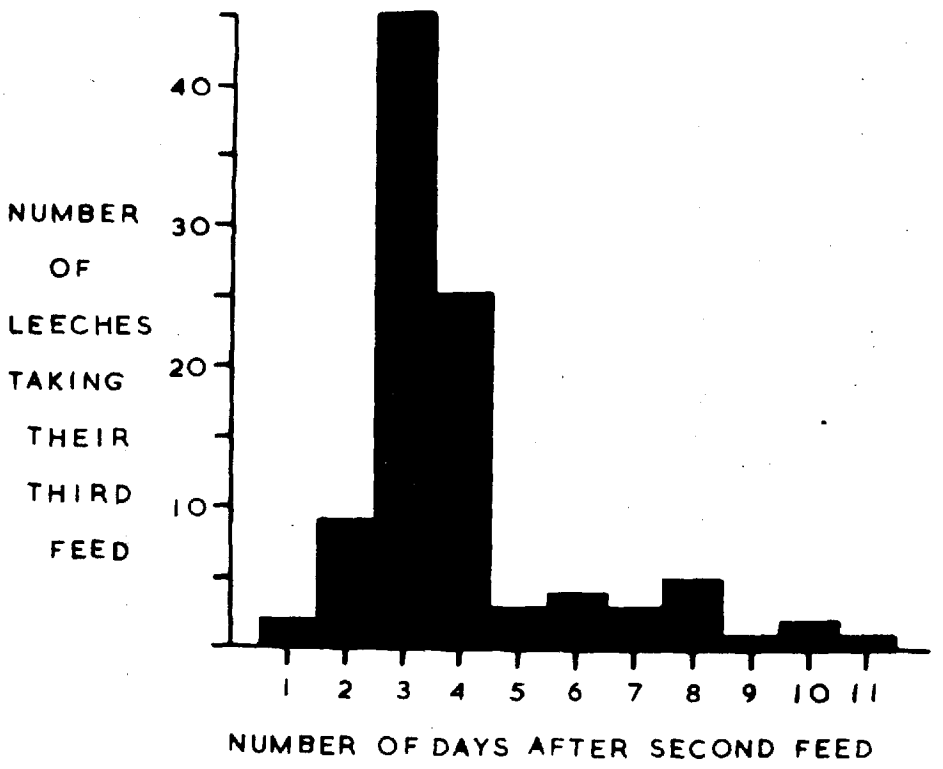
Initially young leeches were fed only after they had digested their previous meal. Graph 2 shows the lengths achieved after each feed for 10 leeches at 15°C. The digestion times averaged 15 days for the first feed to 29 days for the sixth feed. After this sixth feed the H. marginata copulated, laid eggs and hatched their eggs without feeding further. There was never any transfer of flagellates

H.marginata : experimental feeding.



GRAPH 2.

Feeding to maturity.



GRAPH 3.

Feeding frequency.

from parent to offspring.

However, as the H. marginata collected from the wild were rarely found without a blood meal, the feeding rate was further investigated experimentally. 100 young H. marginata were invited to feed at daily intervals after their second meal. Every day feeding leeches were removed attached to the fish, and placed in another container. In this way daily feeding batches were removed and counted. The results in graph 3 show that 45% of the leeches fed on the third day after their previous feed, although they were still gorged with blood, and hardly able to "walk". Thus, in the wild, the feeding rate probably depends as much on the availability of fish, as on temperature and size. Also it is likely that many more partial feeds are taken than the 6 suggested in the first experiment.

5. RECOVERY OF FLAGELLATES FROM TENCH

a) Incidence in Trilakes

The incidence of Cryptobia and trypanosomes varied with the age of the tench, as shown in table II. Fish at age 0 were fry. No flagellates were found from the 42 fry examined. The scales of the single 1 year old tench collected showed that it had already passed through one complete winter. As it was collected the following December, it was about 18 months old. It had a patent Cryptobia infection, and a sub-patent trypanosome infection. Scanty trypanosomes appeared in the blood after 42 days at 20°C. The older age groups had high infection rates, with 81% having mixed Cryptobia and

trypanosome infections.

Table II Age infection rates of tench 1967/1968

Age	No. of Tench	Trypanosomes		Cryptobia		Mixed	
		No +ve	% +ve	No +ve	% +ve	No +ve	% +ve
0	42	0	0	0	0	0	0
1	1	(1)	(100)	1	100	(1)	(100)
2	36	35	97	34	94	33	92
3	23	20	87	21	91	19	83
4	13	9	69	11	85	6	46
5	4	4	100	4	100	4	100
7	1	1	100	0	0	0	0
Total	120	(70)	58	71	59	63	53
2-7	77	69	90	70	91	62	81

Table III Age infection rates of tench November to March

1	1	(1)	(100)	1	(100)	(1)	100
2	8	7	88	6	75	5	63
3	13	10	77	11	85	9	69
4	8	5	63	7	88	4	50
7	1	1	100	0	0	0	0
Tot. (2-7)	30	23	77	24	80	18	60

b) Seasonal variation

The flagellate infections of the tench varied seasonally, both in the number of fish showing patent infections, and in the level of these infections. Table III shows that in winter and spring, only

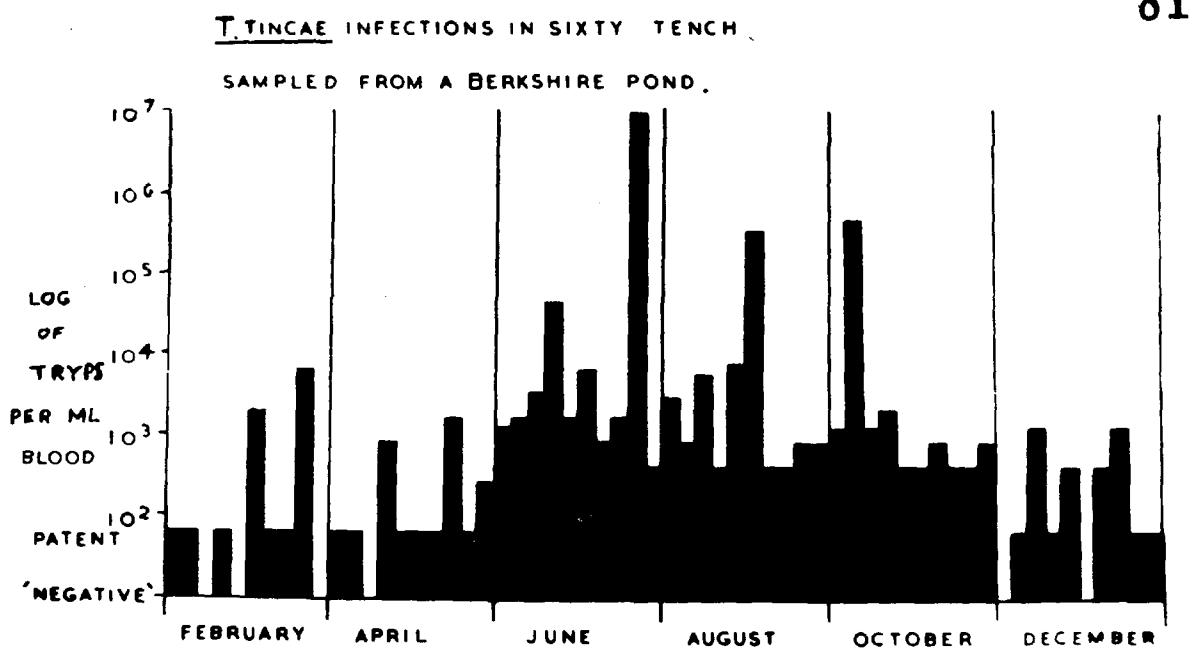
60% of the 2 to 7 year old tench had detectable mixed flagellate infections. In the summer and autumn, however, table IV shows this figure raised to 98%.

Table IV Age infection rates of tench June to October

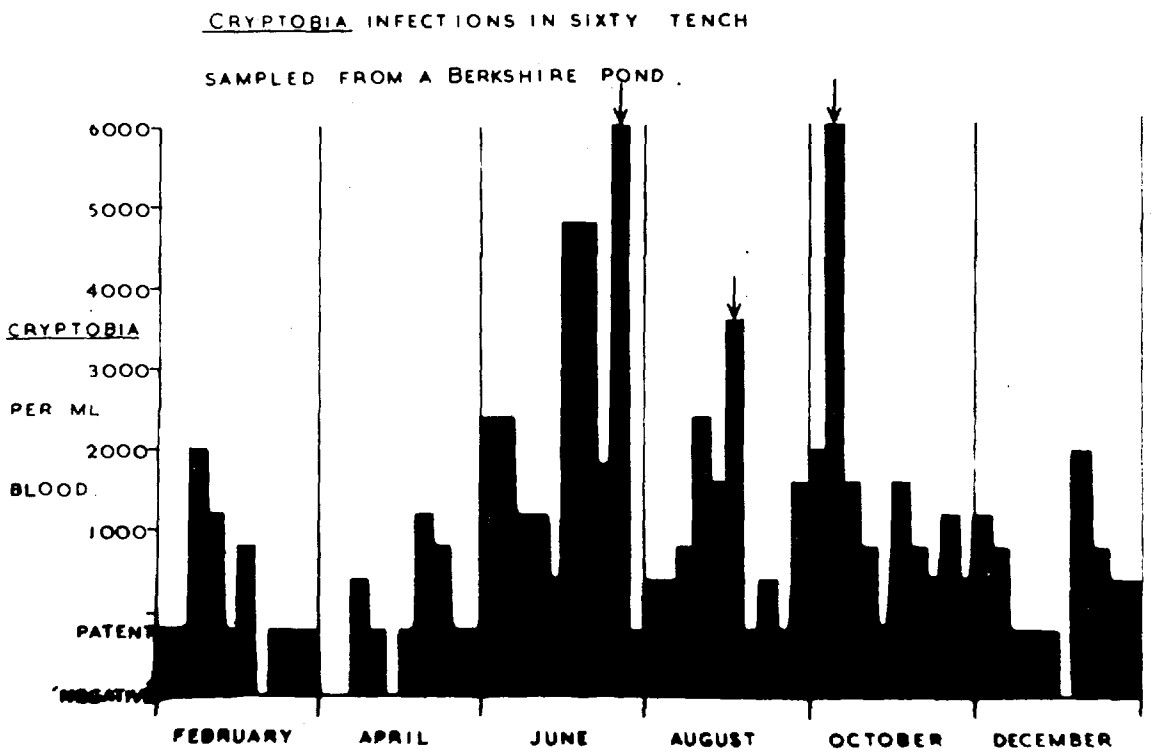
Age	No. of Tench	Trypanosomes		Cryptobia		Mixed	
		No +ve	% +ve	No +ve	% +ve	No +ve	% +ve
0	42	0	0	0	0	0	0
2	28	28	100	28	100	28	100
3	10	10	100	10	100	10	100
4	5	4	80	4	80	4	80
5	4	4	100	4	100	4	100
Tot. (2-5)	47	46	98	46	98	46	98

That the levels in addition to the incidence of patent infections also varied is shown in graphs 4 and 5. The two graphs give the Cryptobia and trypanosome infections of 10 tench taken from Trilakes every two months throughout 1968. Thus a total of 60 infection rates are shown. The histogram for each two monthly sample begins with the youngest, and ends with the oldest tench. The trypanosome levels in graph 4 are plotted on a log. scale, because in June, August, and October, 1 fish in each sample was acutely infected. The other 27 fish in these months covering summer and autumn all had measurable chronic infections of trypanosomes and Cryptobia. On graph 5, the

GRAPH 4.



GRAPH 5.



arrows denote those fish with acute trypanosome infections. 2 of those fish had the heaviest Cryptobia infections. A similar pattern arose in 17 tench of 2 to 4 years old collected during July and August of 1967. 2 of them were acutely infected with trypanosomes at levels of approximately 10^6 tryps. per ml. of blood. These two fish also showed comparatively heavy Cryptobia infections. For the winter and spring sample taken in February, April, and December 1968, the flagellate infections showed lower incidences and levels. Graph 4 shows that there were no fish acutely infected with trypanosomes in these months. When the infections were detectable in a large drop of blood, but not in the small drop of 0.0025 ml, the infection is shown as patent on the histograms. When no flagellates were detectable even in the large drop of blood, the infections are shown as "negative", i.e. they were sub-patent or negative. In fact of the 30 tench collected during the winter and spring months of 1968, only 33% had measurable Cryptobia infections, and 53% measurable trypanosome infections. Yet of the 30 tench from the summer and autumn months of that same year, 83% had measurable Cryptobia infections, and 100% measurable trypanosome infections.

Thus there was an age variation in the flagellate infections. Fish under 1 year old were uninfected. However there were also seasonal variations in the levels and rates of infections. More fish were patently infected, and had higher parasitemia levels, in the summer and autumn, than in the winter and spring.

In order to investigate this seasonal variation further, the

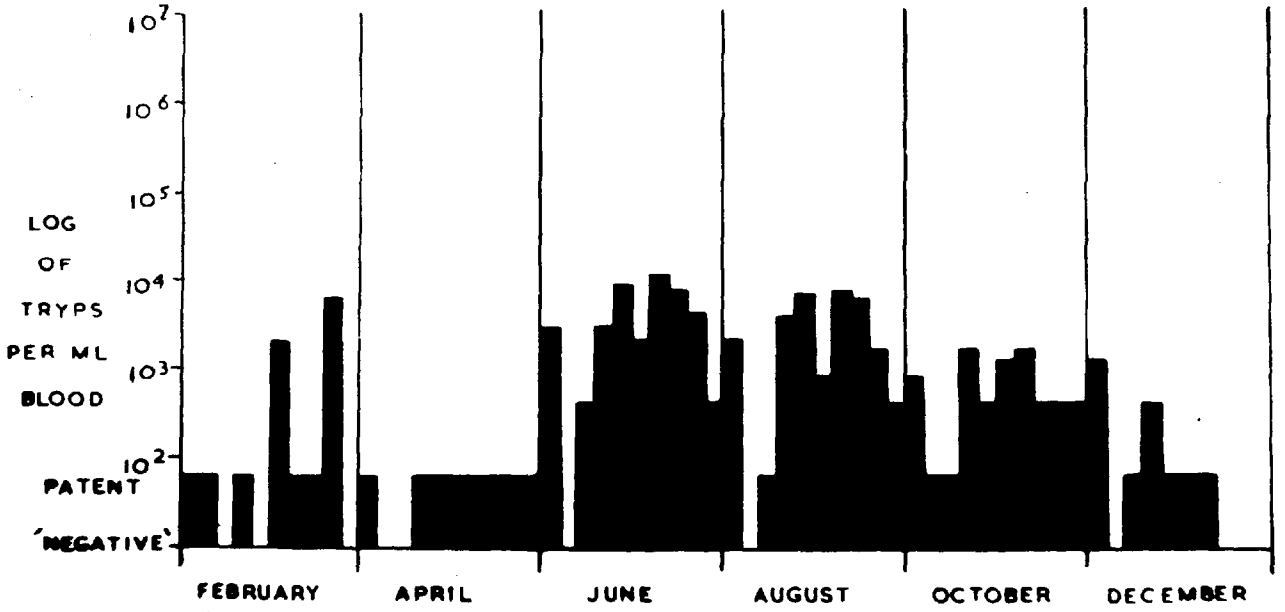
10 fish from the 1968 February sample were maintained throughout the rest of the year in a large concrete tank full of water. There they were exposed to similar climatic conditions as the tench in Trilakes, without the complicating factor of leeches in the environment. The 10 fish, distinguished individually by cotton threads sewn into their fins, were sampled for flagellate infections every two months. The results, shown in graphs 6 and 7 are therefore directly comparable with the results in graphs 4 and 5 from the Trilakes tench. Graph 6 shows that the trypanosome infections increased over the summer, and by December had decreased again. Sub-patent infections became patent, and some became sub-patent again. No infections, however, were in excess of 10^4 trypanosomes per ml. of blood. So, unlike the tench from Trilakes taken over the same period, there were no acute infections. The Cryptobia infections, shown in graph 7, also remained at lower levels than those in the tench from Trilakes. In graph 8 the mean Cryptobia infections of the two monthly samples from each source are compared. The winter and spring infections are similar, but in summer and autumn, and particularly in June, the Trilakes tench were more heavily infected. Significantly, H. marginata was present in Trilakes, but absent from the concrete pond.

Graph 9 compares the mean two-monthly trypanosome infections of the tench from the concrete tank taken arithmetically, with the mean montly air temperatures at Silwood Park during 1968. The tenuous similarity between the two plots will be amplified later in

GRAPH 6.

T TINCAE INFECTIONS IN TEN TENCH MAINTAINED

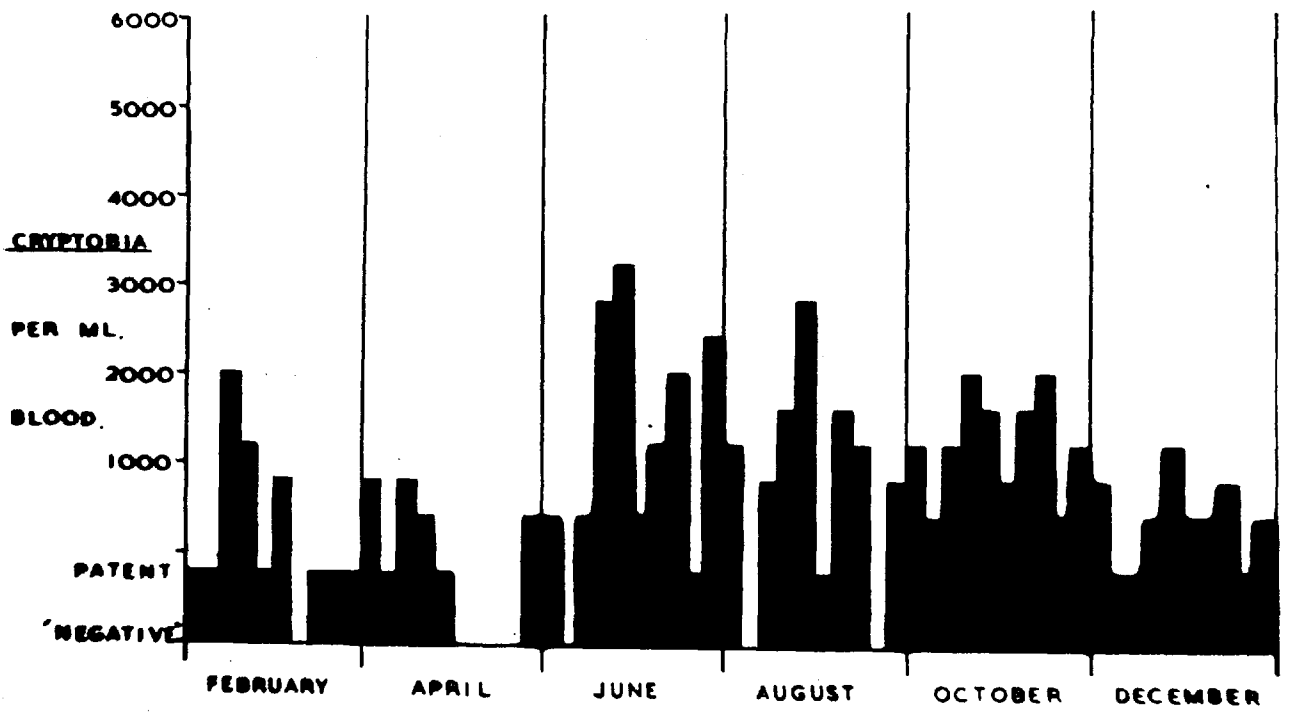
OUTSIDE IN A LEECH FREE ENVIRONMENT.



GRAPH 7.

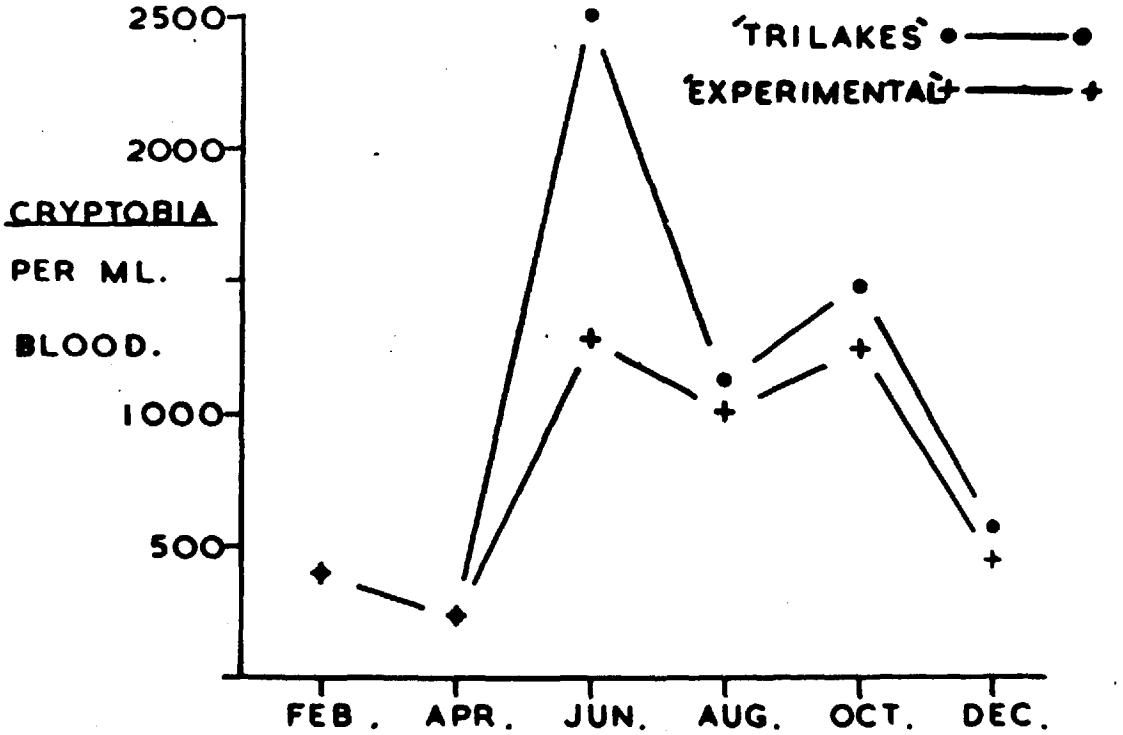
CRYPTOBIA INFECTIONS IN TEN TENCH MAINTAINED

OUTSIDE IN A LEECH FREE ENVIRONMENT.

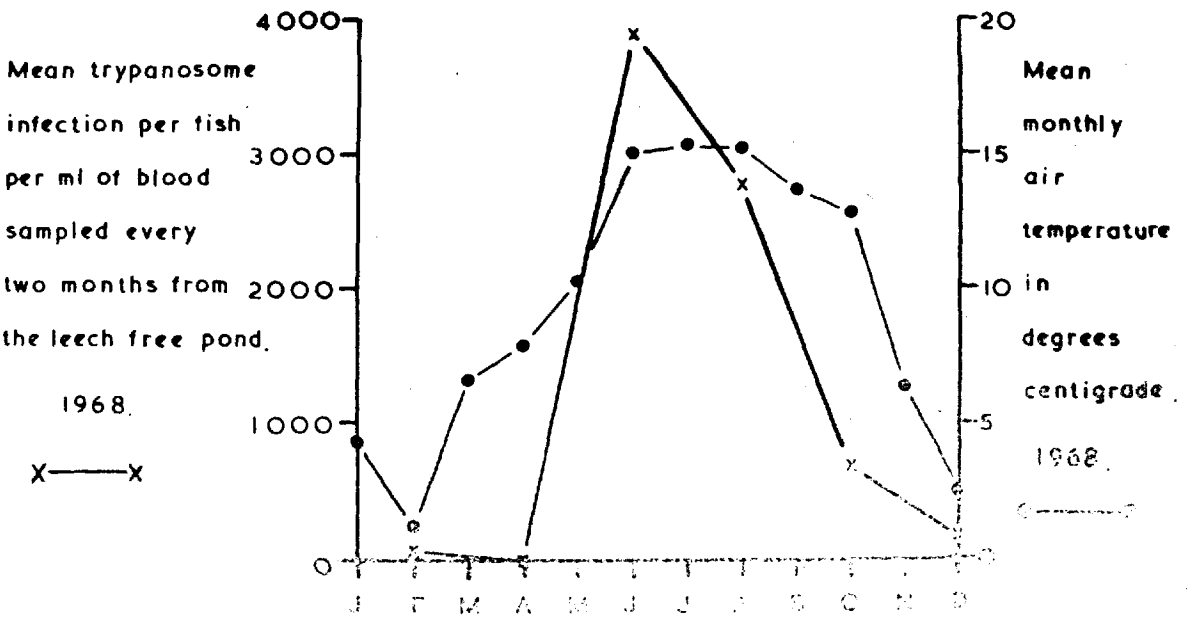


GRAPH 8.

MEAN TWO MONTHLY CRYPTOBIA LEVELS FROM THE 1968 TENCH SAMPLES



GRAPH 9.



the light of controlled experiments at different temperatures. For the moment, however, the June increase in trypanosome infections is seen more dramatically and more accurately than on graph 4 where the infections are plotted logarithmically.

6. FLAGELLATES FROM WILD LEECHES

a) P. geometra

53% of the P. geometra from the Wraysborough reservoir contained epimastigote flagellates. However, they were so unlike the developmental stages of fish trypanosomes observed by myself and other authors, that, after an initial attempt at culture and their failure to infect experimental tench, further study of them was abandoned. Moreover, examination of the brown and rainbow trout, perch, and rudd, of the reservoir failed to reveal any trypanosomes.

Samples examined from all the other P. geometra collected were negative, except for 3 of the 17 sent from Culton Park lake, in Lancashire. These were positive for Cryptobia. 6 of the sample which remained alive were fed on an experimental tench, which remained negative. After feeding all the leeches were sacrificed, and were also found to be negative.

b) H. marginata

All the H. marginata that were dissected from all three sources containing a blood meal also contained the developmental stages of fish trypanosomes. 96% of the leeches examined in the

summer months, and 50% of those examined during the winter months contained blood meals. Only those from Trilakes with blood meals contained mixed trypanosome and Cryptobia stages, with the Cryptobia greatly outnumbering the trypanosomes in any single leech. As the majority of H. marginata were used for breeding and longevity experiments, few were examined initially for flagellates. However, xenodiagnosis was used with experimental tench. No trypanosome infections resulted from the feeding of wild leeches on tench, although the majority of leeches were subsequently found to contain flagellates. However, the leeches that had lost their blood meals prior to feeding on the experimental tench were invariably negative on examination after the experimental feed. One Cryptobia infection resulted in an experimental tench from the feeding of 10 H. marginata collected from Trilakes in December. This infection was subsequently utilised for blood passage and leech transfer experiments.

All the wild H. marginata containing flagellates in the crop also had long slender flagellates in their proboscis sheaths. Figure 9 shows the structure of the gut of H. marginata, and figure 10 shows a whole mount of the anterior end showing the proboscis resting in its sheath. The proboscis was a coiled muscular tube extending above the oesophagous. The oesophagous and crop formed a sack-like structure extending into many diverticulae when distended with blood. Flagellates were never found at a level below the crop, which was separated from the intestine by a tight sphincter. The intestine itself only contained amorphous matter, which formed into

FIG. 9.

Digestive system of Hemiclepsis marginata

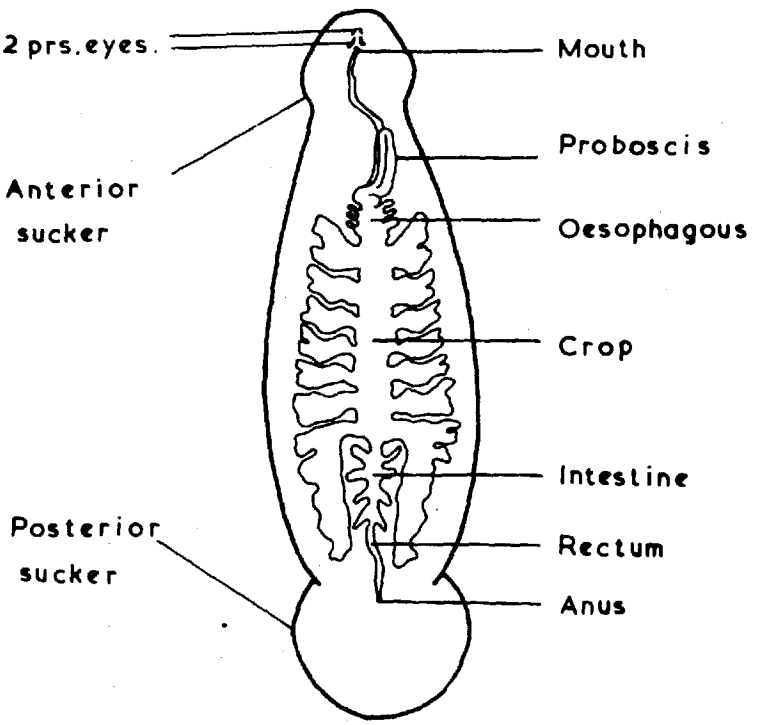


Fig. 10 Whole mount of the anterior end of H. marginata x 20

faeces in the rectum.

7. FLAGELLATES FROM EXPERIMENTALLY BRED H. marginata.

a) Cryptobia

Because of low numbers of Cryptobia in the fish, a comprehensive repeat of the early work on the development of Cryptobia in Hemiclepsis marginata was not possible. However, 15 leeches at 20°C were fed on the experimental tench that had developed Cryptobia infection from the wild Trilakes leeches. It was the third feed for the experimentally bred leeches, so they were a convenient size (7·0 to 10·0 m.m.).

They were also previously uninfected. 1 leech was sacrificed daily for 5 days after feeding. Observations on the early developmental stages of Cryptobia were hampered by the very low numbers present. The original infection in the fish had been barely detectable. So only after 3 days were Cryptobia from the leech crop found in stained preparations, and only after 5 days were they present in any numbers. For the first 2 days, observations with phase contrast showed that small sickle-shaped forms predominated, with about 25% dividing. Developmental stages are shown in plate I, figures a-e. Figure a shows a dividing form at day 3. 2 bar-shaped kinetoplasts are present at the anterior end, with the nucleus just below them. These forms were up to 15μ in length, dividing to produce two offspring of about 9μ in length, and 2μ wide at the thickened anterior end. The movement, in contrast to the amoeboid movement of blood

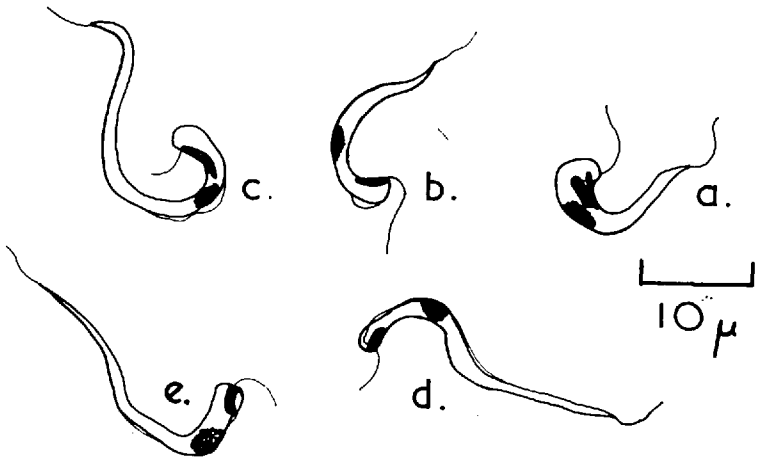


Plate I. Cryptobia sp. from the tench in Hemiclepsis marginata.

stream Cryptobia, consisted of a jerky bending of the rigid body, with the undulating membrane acting as a keel along the long convex side of the body. The short thin free flagellar played no obvious part in movement.

On day 4, longer slender forms, as in figure b, appeared. On day 5 and in all subsequent examinations of experimental infections and in natural infections the slender forms predominated. However, the smaller dividing stock always remained. The slender forms, shown in figures c to e did not divide. They attached themselves to the crop walls or particles of matter, or even to each other, by their thicker anterior ends. The violent flexing of their slender posterior ends, and the rippling of the undulating membrane, enabled them to move forward and change direction at some speed, if they were not attached to anything. On the fifth day after their infective feed, long slender Cryptobia up to 27μ long were present in the proboscis sheath, attached in clumps to the wall.

Of the other 10 infected leeches 5 were each fed on experimental tench starting on day 2. The fourth and fifth leeches feeding on days 5 and 6 produced Cryptobia infections in their tench. The other 5 leeches were retained for reinfection experiments. These showed that by periodically allowing these leeches to feed before they had completely digested their blood meals, they maintained their infections and the ability to pass them on to tench. However, when allowed to digest a blood meal completely, they lost their infectivity, and subsequent examination showed them to be negative.

Therefore, in Cryptobia infections obtained from a tench at 20°C, there was a basal dividing stock, producing slender infective forms in the proboscis sheath by the fifth day. The infection, and the multiplication of the small forms, lasted for as long as the leech contained a blood meal.

b) Trypanosoma Tincae

As heavily infected tench were available, a more complete study of the development of T. tincae in H. marginata was possible.

In the two principal experiments, one at 15°C, the other at 20°C, the 30 leeches fed on acutely infected fish for each experiment had already fed twice. Therefore the infected blood meals taken on the third feed yielded enough material for study. The feeding of large numbers of larger leeches prejudiced the survival of the heavily infected experimental tench and thus large leeches were not chosen for the experiment. In later confirmatory work, however, leeches of all size ranges were used, from newly hatched leeches to leeches with 5 previous feeds. The only differences observed involved the differing trypanosome survival times. These survival times corresponded with the differing digestion times for the blood meal. Table V gives the blood meal digestion times, and therefore the differing survival times of T. tincae, in H. marginata of varying age in feed numbers at 15°C. Digestion of the blood meal was completed when no red or brown colouring remained in the crop.

The cycle of development of T. tincae took place initially in the crop and is shown in plate II, and in figures 12 to 22.

Table V Digestion times of H. marginata blood meals at 15°C.

Feed number	Time in days	
	Minimum	Maximum
1	7	38
2	8	15
3	10	20
4	9	31
5	8	25
6	13	42

Unchanged blood stream trypanosomes from the blood meal are shown in figure 11 and plate II, figures a, b, and c. Figure c being a dividing form.

On entering the crop, the medium and large trypanosomes immediately underwent a change in their posterior ends, as in plate II d, e and 12 and 13. The whole posterior end enlarged becoming swollen, losing its narrow wedge shape. The kinetoplast also enlarged, moved anteriorly and became laterally displaced. The cytoplasm of the posterior region stained a deep violet-blue with Geisma's stain, as in figure 13. The nucleus migrated posteriorly as in figure e. Under phase the characteristic vermiform body movements of the bloodstream type slowed down, the trypanosome rotating around its rigid posterior end.

The smaller bloodstream trypanosomes did not show these changes

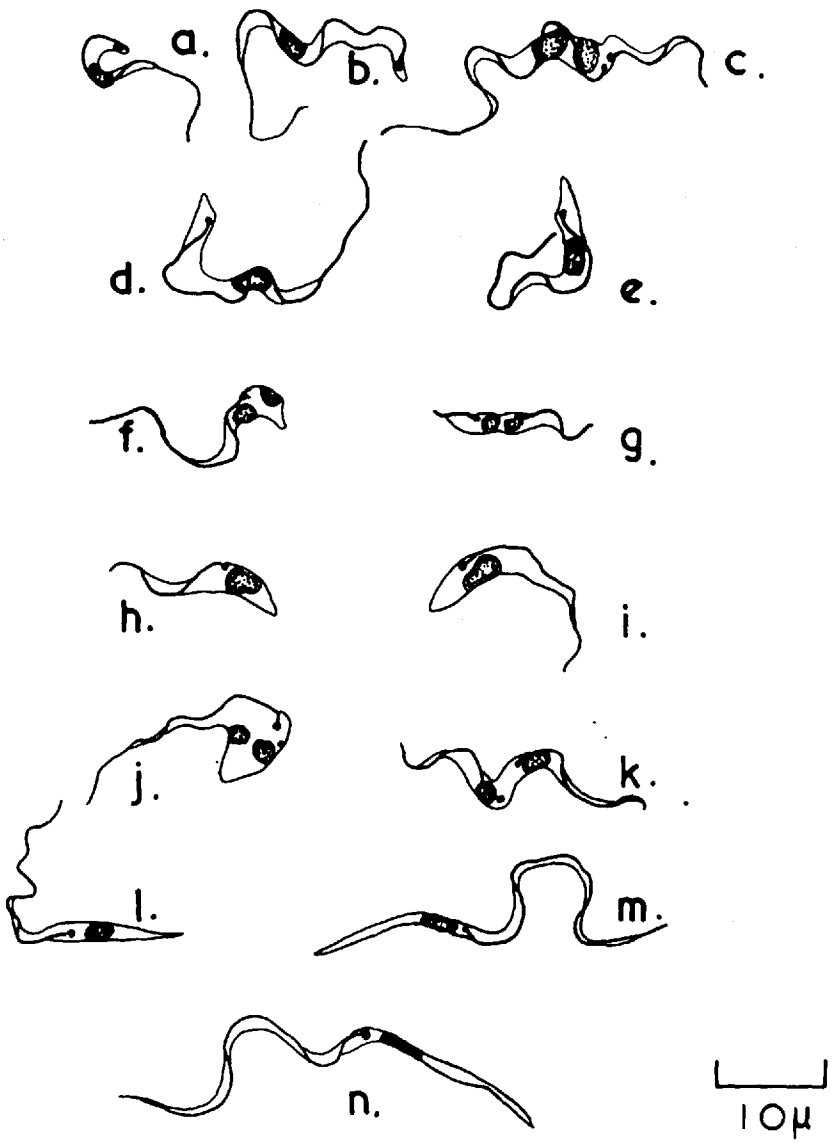


Plate II. T.tincae in H.marginata.

Fig. 11 Bloodstream form

Fig. 12 Altered blood-stream form

Fig. 13 Altered blood-stream form

Fig. 14 1st division

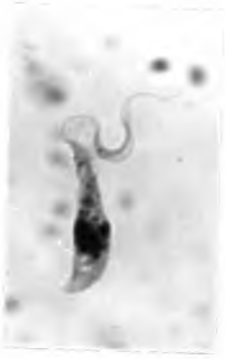


Fig. 15 1st epimastigote

Fig. 16 1st epimastigote

Fig. 17 2nd division

Fig. 18 2nd division

Fig. 19 2nd epimastigote

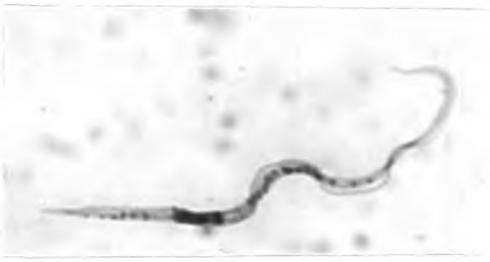


Fig. 20 2nd epimastigote

Fig. 21 2nd epimastigote

Fig. 22 Metacyclic form

until they had grown in size.

After this change in morphology, the first division occurred. First the nucleus divided, one of them passing beneath the kinetoplast into the swollen posterior end, as in figures f and 14. Then the kinetoplast divided in the region between the two nuclei. One kinetoplast retained its original flagellum, now much shorter. The other kinetoplast produced a new flagellum, eventually extending beyond the cytoplasm past the second nucleus. At this stage the two nuclei and their kinetoplasts remained closely associated in the middle of the cytoplasm, as in figure g. The two ends of the flagellate began to wave about independently, pivoting at the central region between the nuclei. Eventually the junction was broken, and the two equal sized small flagellates were released, the minimum size being 7μ. The division was therefore an equal binary fission.

As the kinetoplast was along-side or just anterior to the nucleus, the offspring were epimastigote forms. They are shown in figures h and i, and 15 and 16. They were easily distinguished from the original bloodstream forms. They had comparatively rigid bodies, being slightly curved to a point anterior to the nucleus where the first wave of the undulating membrane crossed over the body. Although one side of the body was convex, the other side was either straight, or only slightly concave. The only real movement was that of the undulating membrane, rippling up the narrowly tapering anterior end to the short free flagellum. The

organism tended to stay in the same place, the short free flagellum and undulating membrane probing in different directions, trailing the cumbersome posterior end behind in a zig-zag motion.

The epimastigotes grew in length, the posterior end becoming pointed as in figure 16. The cytoplasm remained slightly granular, with more dense staining matter in the nuclear region. After growing to a maximum of 34μ in body length, and 4μ in breadth, the second division occurred. Here the kinetoplast divided before the nucleus. Figure 17 shows this second division at the early stage after kinetoplast division, with the second free flagellum just protruding. Nuclear division is taking place. The division sequence is shown in figures j and k, and 17 and 18, and was also an equal binary fission. After nuclear division, the two nuclei drew apart, taking with them their associated kinetoplasts and flagella. Again, as in figures k and 18, there was a flexing of the organism around the region between the nuclei. The first and second divisions could be easily distinguished by the relative size of the organisms. Comparing figures f and j, the second division is larger and broader than the first division. In the later stages, the two connected daughters became considerably lengthened before separation, as seen in figure k . Eventually, when the union broke, two equal sized long slender epimastigotes were released, as shown in figures 1 and 19. The smallest were 21μ in body length, by 1.5μ, with a free flagellum of 1 to 12μ.

In addition to their narrow tapering shape and long length,

they were distinguished from the first epimastigotes by their capacity for movement. Although the region posterior to the kinetoplast remained rigid, the waves passed rapidly along the undulating membrane, with the free flagellum of variable length probing in all directions. Thus the movement was "spirochaete"-like, and progressive movement was rapid, with abrupt changes of direction. So, unlike the earlier forms which tended to remain in the same place amongst the crop contents, these forms tended to aggregate against the crop walls. This was particularly evident in sectioned material.

No further division occurred. The second epimastigotes became greatly elongated and narrowed, as in figures m and n, and 20 and 21. The cytoplasm retained its granular nature, the granules simply becoming spaced out longitudinally. The kinetoplast remained in the region of the nucleus, generally in an anterior position.

Eventually these forms were found in the proboscis sheath. They were seen in fresh preparations under phase contrast moving along the crop walls and passing up the narrow lumen of the proboscis, spilling out over the top, and remaining in the sheath. Gently squashing the leech forced open the oral aperture, and the slender epimastigotes swam out.

Smears from the proboscis sheath revealed only slender epimastigotes of the types shown in figure n and 22. They had a maximum body length of 61μ with a minimum breadth of 0·7μ, with a short or non existent free flagellum of 0-5μ. A small proportion

of abherrent forms were encountered with two nuclei or two kinetoplasts. Division never proceeded further than this.

The sequence of these events is shown in table VI and VII. At 20°C there were no typical bloodstream forms left after 8 hours, and the altered bloodstream forms had disappeared by 36 hours. The first division was at its peak at 20 hours, the maximum number of

Table VI Percentage forms of T. tincae in H. marginata

	HOURS AFTER FEEDING									DAYS						
20°C	2	4	8	12	16	20	24	30	36	2	3	4	5	6	7	8
BS	84	53	15	-	-	-	-	-	-	-	-	-	-	-	-	-
ABS	15	44	76	87	68	35	10	1	-	-	-	-	-	-	-	-
1st DIV	1	3	5	8	16	19	16	6	2	-	-	-	-	-	-	-
1st EPI	-	-	4	5	16	43	58	60	38	10	2	1	-	-	-	-
2nd DIV	-	-	-	-	-	3	12	18	20	10	5	4	1	-	-	-
2nd EPI	-	-	-	-	-	-	4	15	40	80	93	95	99	100	100	100
Pb													+	+	+	+

Table VII 15°C

Key: EPI - Epimastigote; BS - Bloodstream; ABS - Altered bloodstream; DIV - Division form; Pb - proboscis sheath, positive

BS	91	70	36	10	2	-	-	-	-	-	-	-	-	-	-	-
ABS	9	30	61	82	77	67	50	34	5	1	-	-	-	-	-	-
1st DIV	-	-	3	5	11	17	23	18	13	4	-	-	-	-	-	-
1st EPI	-	-	-	3	10	16	25	34	55	45	20	13	7	3	1	-
2nd DIV	-	-	-	-	-	-	2	8	10	15	19	16	3	3	1	-
2nd EPI	-	-	-	-	-	-	-	6	16	35	61	81	90	94	98	100
Pb															+	+

first epimastigotes at 30 hours, and the second division at its peak at 36 hours. The minimum period for completion of the cycle was 24 hours, when the first 2nd epimastigotes was produced. These however were not present in the proboscis sheath until the firth day, which corresponded to the disappearance of the last intact red blood cell.

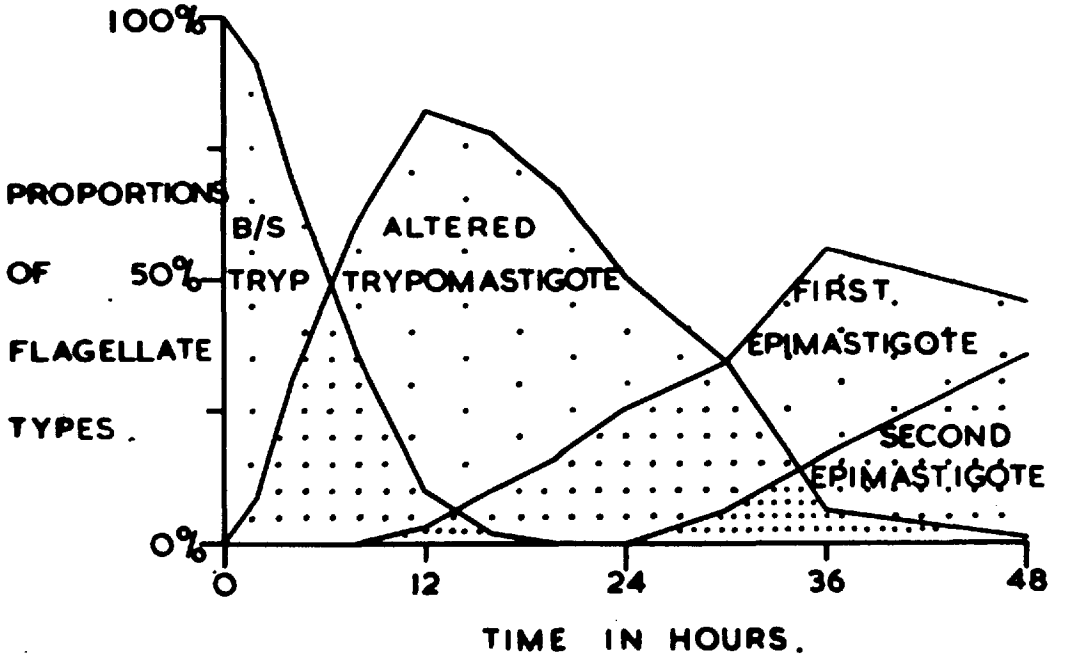
The differences in the early rate of development at 15°C and 20°C are shown in graphs 10 and 11, where the relative proportions of bloodstream, altered bloodstream, first epimastigotes, and second epimastigote flagellates up to 48 hours after feeding are plotted. At 15°C development, therefore, proceeded more slowly. Table VII shows that there were no flagellates in the proboscis sheath before the seventh day after feeding at 15°C.

In both experiments leeches were sacrificed at daily intervals for up to 20 days, and were found to lose their infections once they had digested their meals. After the break down of the red blood cells, red fluid containing elongate crystals and cell debris persisted. This darkened in colour, and gradually emptied out of the crop into the intestine. The last regions to empty were the long posteriorly extending seventh cauae shown in the diagram in figure 10. There, and in the proboscis sheath, the trypanosomes persisted. However, when the crop contents turned from brown to dark green as green algae and bacteria flourished, the trypanosomes from both the crop and the proboscis sheath dramatically disappeared. Leeches refed on uninfected tench at this stage

GRAPH 10.

EARLY DEVELOPEMENT OF T. TINCAE

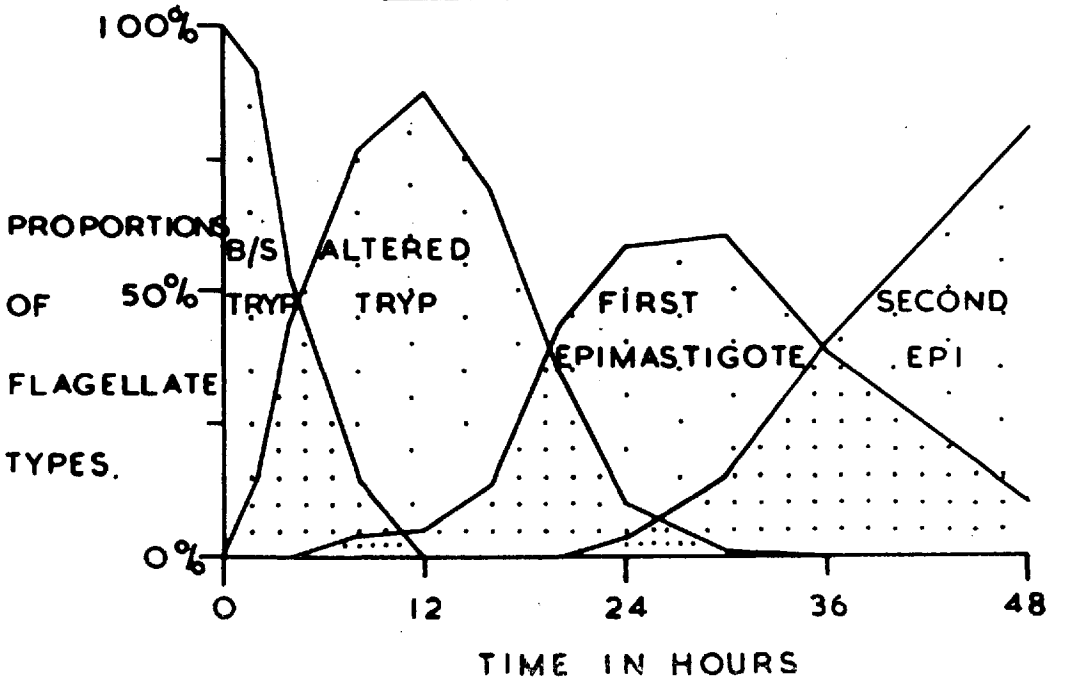
IN H. MARGINATA AT 15°C.



GRAPH 11.

EARLY DEVELOPEMENT OF T. TINCAE

IN HEMICLEPSIS MARGINATA AT 20°C.



remained uninfected, as did the tench.

Leeches at 20°C were fed on uninfected tench at 5, 10, 15 and 20 days after being infected. Tench developed infections on the first two occasions, but not after feeds by the leeches at 15 and 20 days, although flagellates were still present in the crop and proboscis sheath 15 days after its original feed.

Similarly leeches at 15°C were fed on uninfected tench at 5, 10, 15 and 20 days after being infected. This time the only leech that produced infection in the tench had the 10 day infection. The 5 day and 20 day leeches had no proboscis sheath infections, but the 15 day leech had.

Confirmatory work showed that at 15°C, leeches could infect tench after carrying their infections from 7 to 14 days. They produced the infections described in the next section.

A further group of 8 leeches were refed on uninfected tench at 10 daily intervals at 15°C after their initial infection feed. They produced an infection in a tench after the first 10 days, but not on subsequent feeds. Two leeches were sacrificed after each feed, i.e. at 20 days, 30 days, 40 days and 50 days after the original infective feed. All the leeches sacrificed were infected with only long attenuated epimsstigotes in their crops and proboscis sheaths, although an increasingly higher proportion of involution and dead forms were present as time went on. So, in contrast to Cryptobia, T. tincae did not keep its infectivity with a continued replenishment of the blood meal. In fact the infective

period was short, only extending a few days after the initial appearance of long attenuated epimastigotes in the proboscis sheath.

Finally, an experiment was undertaken to check whether there were really only two divisions in the developmental cycle of T. tincae in H. marginata, and not undetected "stem" cells maintaining the production of long attenuated forms. 2 groups, each of 5 leeches, were fed on two tench with low patent infections of T. tincae. One leech from each group was sacrificed after 2, 4, 6, and 10 days. Precisely the same pattern of development in the leech was observed in fresh material, but stained material was difficult to observe because of the low numbers of flagellates present. One leech from each group was refed at day 10, and sacrificed after a further 10 days. There were still considerably fewer numbers of long attenuated epimastigotes present in the crop and proboscis sheath of these two leeches than in the equivalent leeches that had fed on heavily infected tench. In fact the low infections in these leeches corresponded with the comparatively low numbers of trypanosomes present in wild H. marginata, in contrast to the larger numbers of Cryptobia, where mixed infections occurred.

8. CHARACTERISTICS OF LEECH INDUCED FLAGELLATE INFECTIONS IN EXPERIMENTAL TENCH.

The morphology of the bloodstream stages of the flagellates is described in section 15. In the present section, the infection curves of the flagellates in the tench resulting from infected

leech feeding are described.

a) Cryptobia

Initially 2 tench were infected with Cryptobia by the feeding of individual leeches that had themselves been infected at 20°C. These leeches had fed on the experimental tench that had gained its Cryptobia infection from the feeding of 10 H. marginata collected from Trilakes in December.

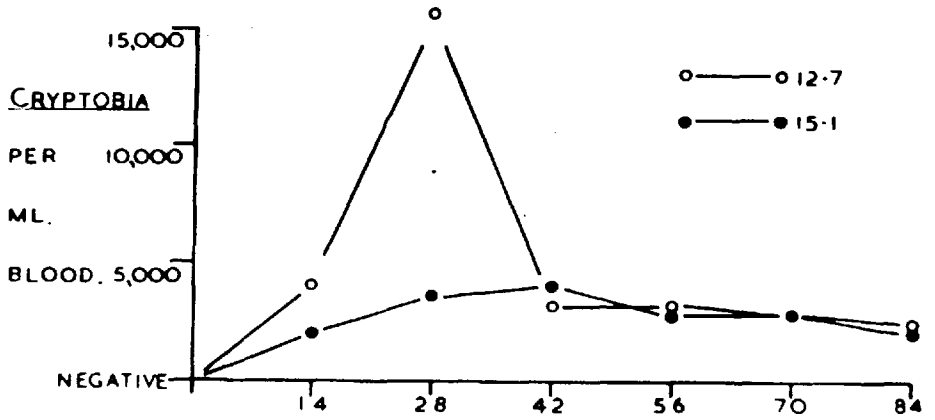
The Cryptobia infections of the 2 tench are shown in graph 12. Two controls attacked by uninfected leeches remained negative. In this, and subsequent graphs plotting infections of experimental tench, the lengths of the tench in centimeters are given on the right hand side. The Cryptobia levels in fortnightly blood samples, plotted arithmetically, are given for 84 days in graph 12. A peak of 4.0×10^3 Cryptobia per ml. of blood was reached in the larger fish after 42 days, and a peak of 1.56×10^4 Cryptobia per ml. of blood was reached in the smaller fish after 28 days. The differing sizes, no doubt, effectively resulted in the smaller fish having a larger dose.

Neither of these infections was acute. In fact the peak of 1.56×10^4 Cryptobia in a millilitre of blood is a rate of 39 Cryptobia counted in the 0.0025 ml. drop of blood. Even at this level, the Cryptobia were barely detectable in blood smears.

Accordingly an experiment was devised to attempt to raise the Cryptobia numbers in the tench for morphological studies. The

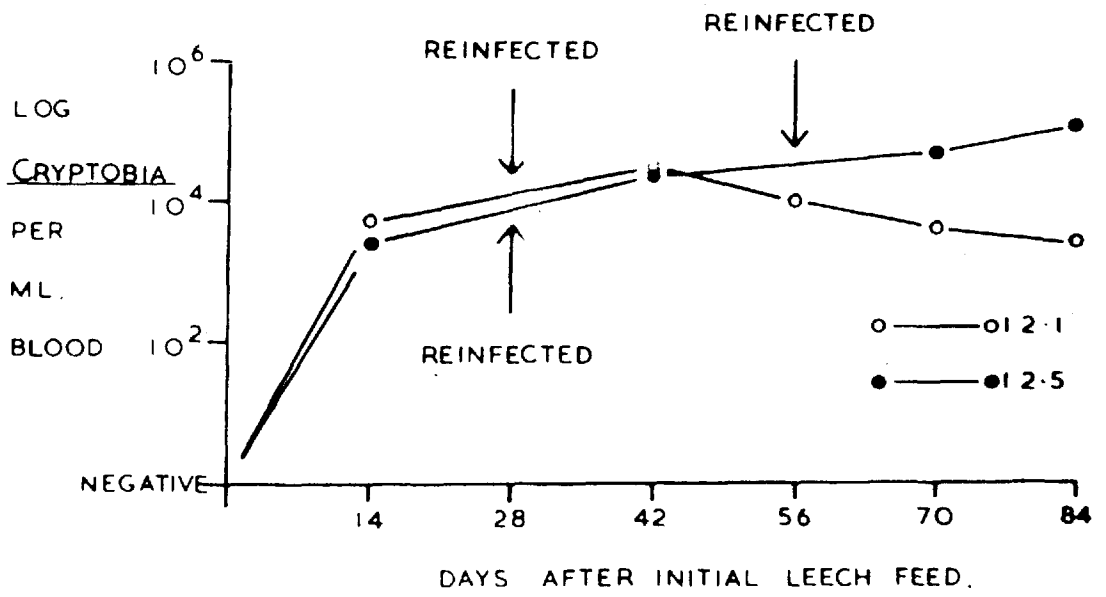
GRAPH 12.

LEECH INDUCED INFECTIONS OF 2 TENCH WITH CRYPTOBIA AT 20°C.



GRAPH 13.

REPEATED LEECH INDUCED CHALLENGES TO 2 TENCH INFECTED WITH CRYPTOBIA AT 20°C.



capacity of Cryptobia to retain their infectivity in the leech was investigated at the same time.

Two tench were infected by the feeding of single infected leeches. The resulting Cryptobia infections are shown in graph 13. On day 28, both fish were reinfected, and on day 56 one fish was reinfected, whilst the other was attacked by an uninfected leech as a control. Graph 13 shows that reinfecting the tench with single infected leeches had the effect of steadily building up the Cryptobia numbers in the bloodstream. After the 12.5 cm tench had been reinfected a second time, its infection increased to a level of 1.16×10^5 Cryptobia per ml. of blood after 84 days. At this time it was so anaemic that it did not recover from the anaesthetic needed to take the blood sample. The morphology of the bloodstream Cryptobia obtained from this fish is described in section 14, and the pathology of the Cryptobia infection is described in section 12.

During the course of this experiment it was found that H. marginata remained infected with Cryptobia provided that it was refed before it had completed digestion of the blood meal. It made no difference if it was fed on infected or uninfected tench, except that bloodstream Cryptobia were recovered from the crop immediately after the feed on the infected fish. H. marginata also maintained their Cryptobia when fed on uninfected roach. After such a feed the Cryptobia were still infective to tench, as indeed they still were after several feeds on uninfected tench.

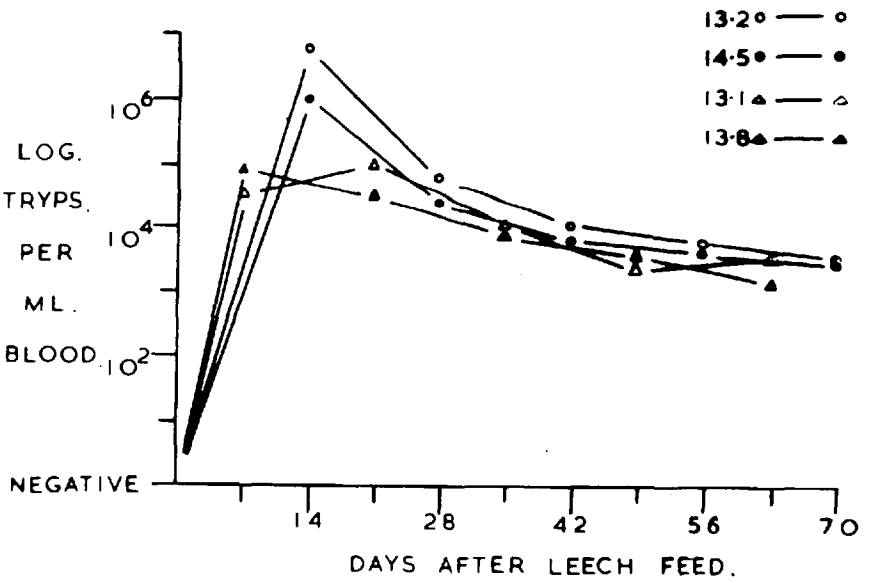
b) T. tincae

There was some difficulty in transmitting T. tincae infections from the leech to tench because, as already mentioned in section 7, the leeches were only infective for a short period. This was in direct contrast to the situation described above with Cryptobia. However, infections were produced in tench by the following leeches fed singly: 2 leeches at 20°C, one feeding at 5 days and the other at 10 days after being infected, and 3 leeches at 15°C, one feeding at 7 days, another at 10 days, and the third at 14 days after being infected. In addition a further 8 leeches infected 8 tench at 15°C after 10 days. Those 8 tench were sacrificed at intervals in the search for tissue developmental stages of T. tincae. Of the 5 tench retained that had leech induced trypanosome infections, one was kept back as a "stock" fish to be used for leech reinfection experiments so that infections induced in tench could be challenged. Therefore the infections of four tench were monitored at fortnightly intervals, and are shown in graph 14. Whether attacked at 15°C or 20°C, immediately after the leech feeding was completed, the fish were removed to 20°C. Four control fish were similarly monitored, having been attacked by uninfective leeches, and remained uninfected.

As graph 14 shows the readings began at day 7 with the 13.1 and 13.8 cm fish, and at day 14 with the 13.2 and 14.5 cm fish. A peak of 6.0×10^6 and 1.0×10^6 trypanosomes per ml. of blood were present in the 13.2 and 14.5 fish respectively. After the short acute phase

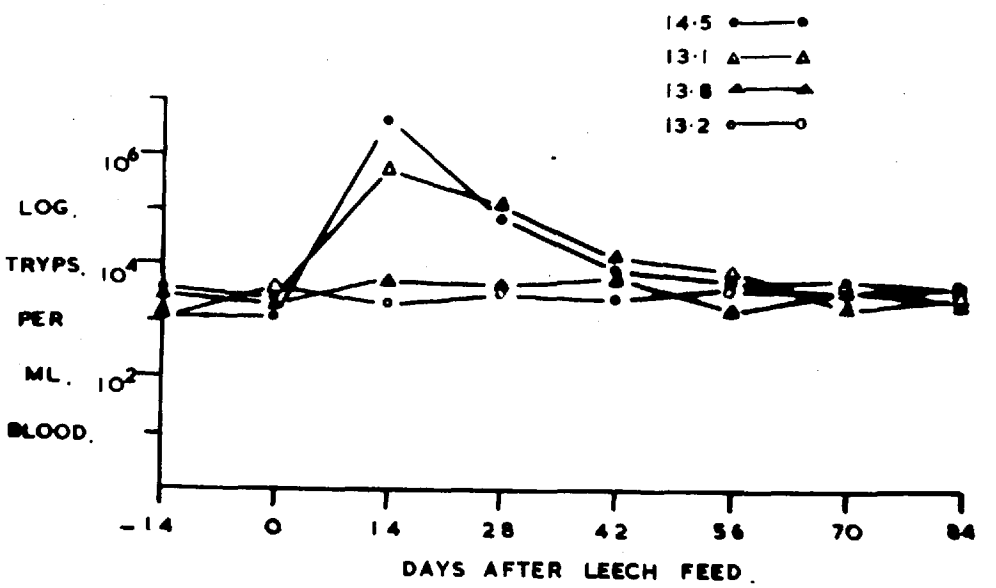
GRAPH 14.

LEECH INDUCED INFECTIONS OF TENCH WITH T.TINCAE AT 20°C.



GRAPH 15.

LEECH INDUCED CHALLENGE INFECTIONS OF TENCH WITH T.TINCAE AT 20°C.



only detectable at 14 days after leech feeding, all the infections of all four fish settled down to chronic levels of between 10^3 and 10^4 trypanosomes per ml. of blood.

After the chronic infections had been maintained largely unchanged for 84 days, a challenge experiment was initiated. Leeches were fed on the chronically infected tench that had been retained as a stock fish. Two of these leeches, after they had been infected for 10 days, were each fed on the 14.5 and 13.1 cm. tench. The tench had, themselves, been infected for 94 days. In addition to the two fish attacked by infected leeches, the other two fish of 13.8 and 13.2 cms were attacked by single uninfected leeches. After feeding all leeches were sacrificed to see if trypanosomes were present.

Those chronically infected fish attacked by infective leeches developed acute infectios, again only for a short period as shown in graph 15. The peaks of these infections were strikingly similar to the critical infection peaks. The 14.5 cm fish had the highest peak after 14 days of 2.4×10^6 trypanosomes per ml. of blood, whilst the 14 day peak for the 13.1 cm fish was 4.4×10^5 trypanosomes per ml. of blood.

Thus leech induced T. tincae infections in tench at 20°C develop to an initial short acute phase, followed by a chronic phase. Challenge results in a similar short acute phase.

9. LEECHES FEEDING ON FRY

In spite of the small quantities of blood, 42 fry were

examined for flagellate infections. As recorded in table II no fry were infected. Xenodiagnosis was employed with another 23, using the smallest H. marginata available. All leeches were fed singly on individual fry. All the fry died before the completion of feeding, even when leeches of 2 m.m. length were used. All the leeches remained negative.

10. LEECHES FEEDING ON DEAD FISH

Twenty H. marginata were fed on acutely infected experimental tench at 15°C in batches of 5 every 12 hours after death up to 48 hours. They were sacrificed at 5 daily intervals after feeding, starting on the second day. 100% infections were recovered from the 12 and 24 hour groups, 80% from the 36 hour group, and 20% from the 48 hour group.

11. BLOOD PASSAGE EXPERIMENTS

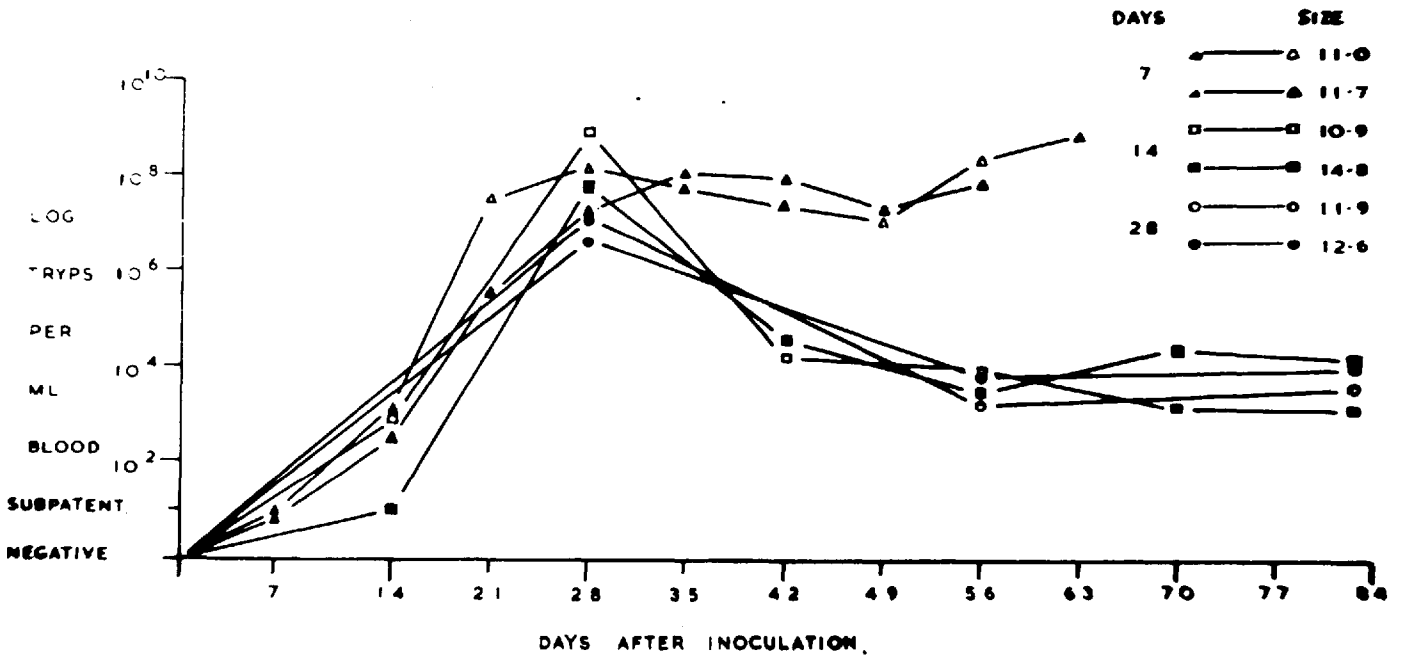
It was found at an early stage that trypanosomes could be easily passaged from wild to experimental tench, and thenceforth between experimental tench. Cryptobia, however, was never successfully syringe-passaged to give patent infections.

a) Frequency of blood readings

Approximately 200 T. tincae were inoculated from a chronically infected wild Trilakes tench into two uninfected tench of 11.0 and 11.7 cms at 20°C. The trypanosome levels in the blood were measured at 7 day intervals, and the results are shown in graph 16. The smaller

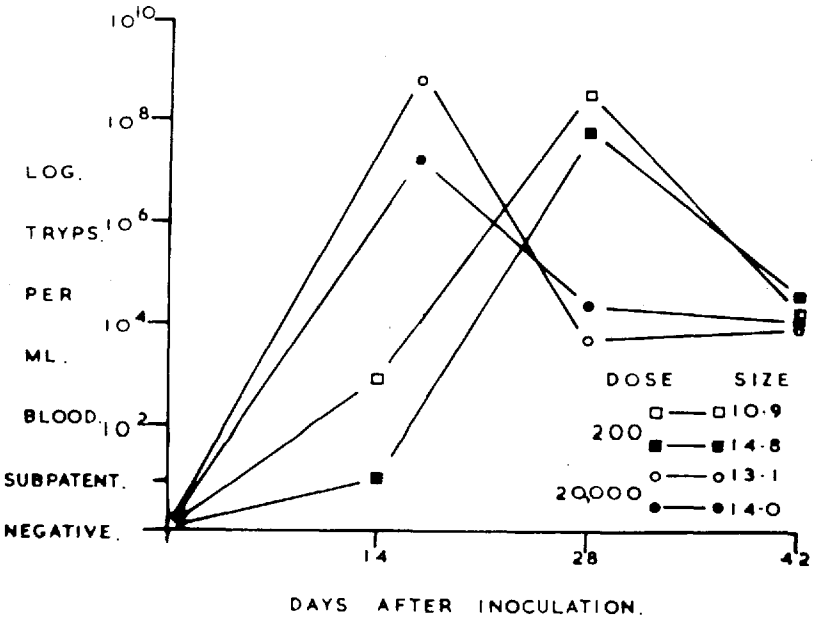
GRAPH 16.

INFECTIONS OF 6 TENCH INOCULATED WITH 200 T.TINCAE AT 20°C.
EXAMINED AT WEEKLY FORTNIGHTLY AND MONTHLY INTERVALS.



GRAPH 17.

INFECTIONS OF 4 TENCH INOCULATED WITH
200 AND 20,000 T.TINCAE AT 20°C.



fish developed a heavy acute infection after 28 days with a level of 1.06×10^8 trypanosomes per ml. of blood. The larger fish had an infection of 5.20×10^7 tryps. per ml. after 35 days. As graph 16 shows, neither fish recovered: after a slight dip, the infections increased again after 49 days. The 11.7 cm tench died on day 56 with an infection of 6.40×10^7 tryps. per ml., and the 11.0 cm tench died on day 66, having had an infection of 2.02×10^8 tryps. per ml. blood on day 63. Both fish were anaemic at death, with red blood cell counts below 0.5×10^6 erythrocytes per ml. blood.

Accordingly the blood sampling technique was revised, and blood for future experiments was only taken in small quantities from the 5th and smallest branchial artery. Also 4 more tench were similarly inoculated with aprox. 200 trypanosomes from the same chronically infected wild tench, and 4 controls inoculated with 0.5 ml. each of citrate saline. The controls did not develop trypanosome infections. The other 4 tench did, however. The infections of 2 were monitored at 14 days intervals, and the other 2 at 28 day intervals. As graph 16 shows, the 4 fish survived, the infection peaks at 28 days all being at acute levels varying from 1.75×10^6 to 4.40×10^8 tryps. per ml. of blood. Although the fish examined at 14 day intervals both had higher peaks, it was decided to continue examining fish at such intervals, in spite of the effect that the removal of blood may be having on the course of infection. The 4 fish both developed similar chronic infections, so the effects of bleeding, if any, were not lasting.

b) The effect of different sized inocula

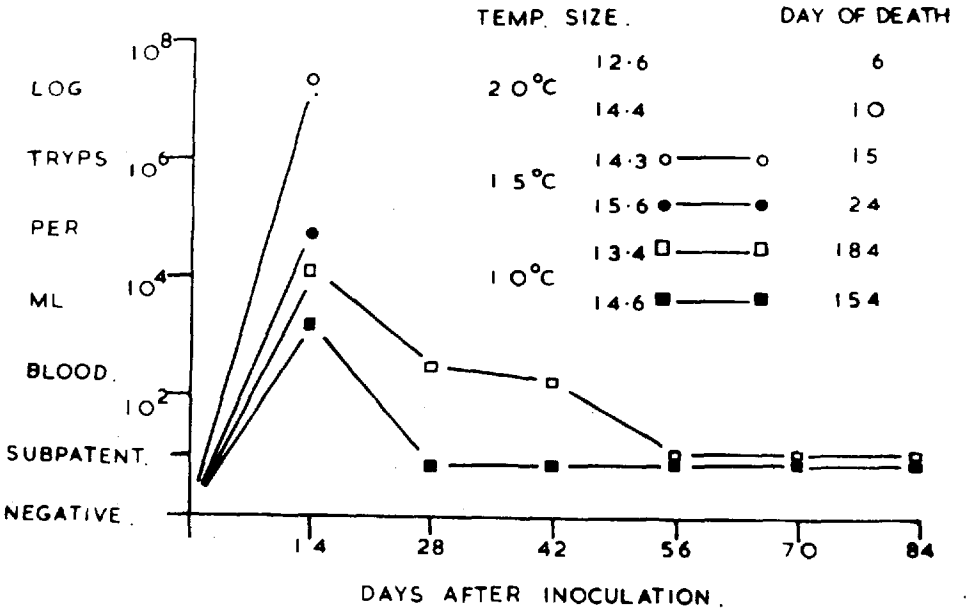
The capture of wild tench heavily infected with T. tincae enabled higher numbers to be inoculated. 2×10^4 and 2×10^5 T. tincae were inoculated respectively into 2 groups of 2 experimental tench at 20°C. Two controls, inoculated with 0.5 ml. citrate saline, did not develop infections. The infection curves of the 2 tench inoculated with 2×10^4 trypanosomes are shown in graph 17, where they are set against the curves already obtained from the 2 tench inoculated with 2×10^2 trypanosomes. 14 days after being inoculated, one of the latter 2 fish was negative, and the other barely patent. Yet the 2 fish inoculated with 2×10^4 trypanosomes both had peaks of 2.51×10^8 and 1.60×10^7 tryps. per ml. of blood at 14 days. Of the 2 tench inoculated with 2×10^5 trypanosomes, one measuring 12.6 cms. died on the sixth day, and the other measuring 14.4 cms. died on day 10. Both had heavy trypanosome infections on post-mortem examination. Thus the heavier the inoculum, the more rapidly the acute phase developes. With a massive inoculum, the tench die.

c) Temperature variations

1. A further 4 tench were inoculated with 2×10^5 trypanosomes, and, along with one control at each temperature, were inoculated with citrate saline and remaining uninfected; 2 of them were maintained at 15°C, and 2 at 10°C. The infections are shown in graph 18, where the results for the 20°C tench with the same inocula are given for comparison. At 15°C both fish died with heavy trypanosome infections.

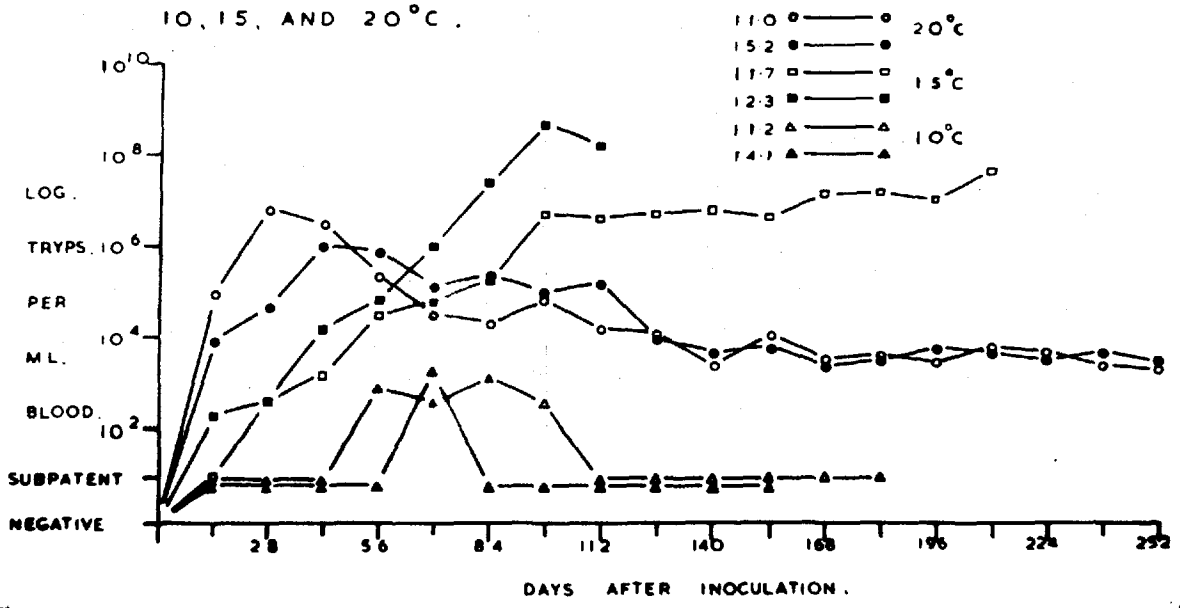
GRAPH 18.

INFECTIONS OF 6 TENCH INOCULATED WITH 200,000 T. TINCAE AT 10, 15, AND 20°C.



GRAPH 19.

INFECTIONS OF 6 TENCH INOCULATED WITH 2000 T. TINCAE AT 10, 15, AND 20°C.



The smaller fish died at 15 days, having had 2.10×10^7 tryps. per ml. of blood at 14 days. The larger fish died at 24 days, having had 5.20×10^4 tryps. per ml. of blood at 14 days. At 10°C, the heaviest infections occurred at 14 days, with a level of 1.32×10^4 tryps. per ml. of blood for the smaller tench, and 1.48×10^3 tryps. per ml. of blood for the largest. Thereafter the infection of the larger tench became "subpatent". After being barely patent for two readings, the infection of the smaller fish became "subpatent" after 56 days.

Thus size of fish was important with large inocula, the larger fish living longer. Temperature, however, was also important. At 10°C the trypanosome hardly developed. The fish survived longer at 15°C than at 20°C.

2. To investigate the temperature effects using sub-lethal inocula, 6 experimental tench were each inoculated with 2×10^3 T. tincae, and 3 controls with citrate saline. Two infected tench with one control were each maintained at 20°C, 15°C, and 10°C. The controls did not develop infections. The infections of the other 6 fish are presented in graph 19. At 20°C, the two infections followed an acute, then a chronic course from which they did not deviate for 252 days. The peak for the smaller tench was 4.0×10^6 tryps. per ml. of blood after 28 days. The larger tench at 20°C had a peak of 1.0×10^6 tryps. per ml. of blood after 42 days. At 10°C the infections remained either subpatent or barely patent. The maximum number of trypanosomes was 5 in the 0.0025 ml. drop of

blood of the 14.1 cm fish after 70 days. This fish died on day 157, the smaller fish dying on day 184. The control at 10°C died on day 81. At 15°C the infections slowly reached an acute level, and remained there. The 11.7 cm fish died on day 212, and the larger fish died on day 112. Both fish had steadily developed anaemia prior to death. The control of 11.8 cms at 15°C died after 133 days.

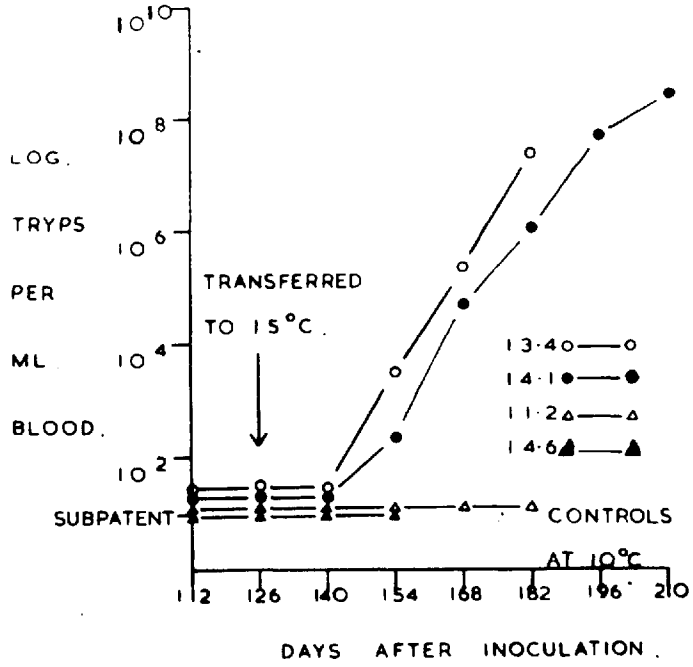
3. In order to investigate the apparently negative or barely patent infections at 10°C further, 2 more tench were inoculated with 2×10^3 T. tincae. The 2 controls were the 11.2 cm tench that had been inoculated with 2×10^3 T. tincae at 10°C, and the 14.6 cm tench that had been inoculated with 2×10^5 T. tincae at 10°C. Up until day 126, only occasional trypanosomes had been detected. So, on this day, 2 of the tench were transferred to 15°C, as shown in graph 20. After a further 28 days, infections appeared in both fish. The smaller fish died on day 183, having had an infection of 3.20×10^7 tryps. per ml. of blood on day 182. The larger fish died on day 215, having had an infection of 2.10×10^8 tryps. per ml. of blood on day 210. Both fish were anaemic at death.

Thus the two infections had been subpatent rather than negative at 10°C.

4. Lastly, the effect of reducing the temperature for 2 chronically infected tench were investigated. The initial infections of the 4 fish used in this experiment have been shown in graph 16. The continuation of their infections is shown in graph 21. 98 days after inoculation, 2 of the 4 tench were transferred to tanks at

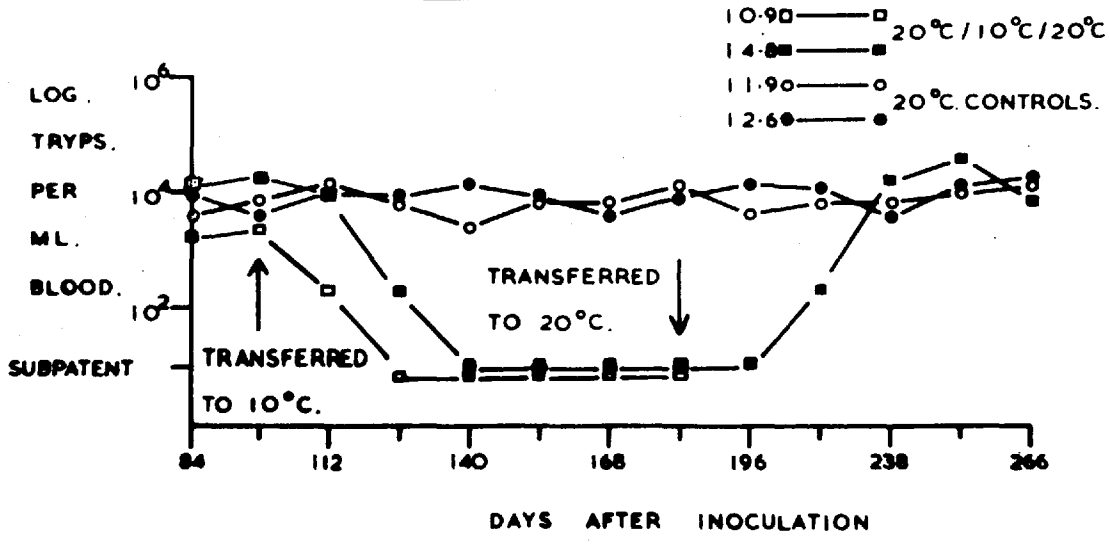
GRAPH 20.

INFECTIONS OF 4 TENCH INOCULATED WITH T. TINCAE INITIALLY AT 10°C.



GRAPH 21.

CHRONIC INFECTIONS OF 4 TENCH INOCULATED WITH 200 T. TINCAE.



10°C. The infection of the smaller fish became "subpatent" in 28 days, and that of the larger fish in 42 days. The controls at 20°C, on graph 21, maintained their chronic infections. After 84 days, the 2 fish at 10°C were returned to 20°C, as they were showing signs of weakening. The smaller fish died 3 days after the transfer. The larger fish developed a chronic infection after 28 days which did not become acute.

12. PATHOLOGY OF FLAGELLATE INFECTIONS

There was no evidence that the flagellates harmed the leeches in any way. The pathological effects on the tench were difficult to assess, because normal tissues have never been formally characterised. No observations on the leucocytes were made, as they could not be identified. Erythrocyte counts were made of all wild and experimental tench on arrival at the laboratory and during the course of flagellate infections. Uninfected, subpatently infected, and chronically infected tench at 20°C and the wild had counts varied between 0·99 and 2·37 x 10^6 erythrocytes per cu. m.m. The mean was 1·71 x 10^6 erythrocytes per cu. m.m. of blood. Acutely infected wild tench, and acutely infected experimental tench at 20°C showed counts well within this range. There was no great reduction at the peak of infection. Indeed some fish even increased their red blood counts. However, the counts were steadily reduced to a state of anaemia in the following cases: all tench permenantly at 10°C with or without trypanosomes; in

all tench at 15°C long periods; in one experimental tench at 20°C with a heavy Cryptobia infection; and in the two tench at 20°C examined at weekly intervals. When the count dropped to below 0.5×10^6 erythrocytes per cu. m.m. of blood, the fish was deemed anaemic, and recovery never followed. External symptoms were pale organs and large numbers of "normoblasts" in the blood. These were circular erythrocytes with large, often distorted or dividing nuclei, No other external or internal symptoms were observed in tench infected with blood parasites.

Trypanosomes selectively invaded tissues in acute infections. Muscle fluid was as heavily infected as the blood, particularly so after death. Next in infection levels was the tissue fluid of the spleen and the kidneys and the coelomic, pericardial and cerebro-spinal fluid. The lowest number were found in the liver, and in the gut mucosa. Nervous tissue, bone, and eyes were never infected. Venous blood from the retro-orbital sinus contained higher trypanosome concentrations than arterial blood from the branchial artery. Peripheral blood from capillaries in the muscle, skin, and fins contained the most.

13. DEEP FREEZE PRESERVATION OF FLAGELLATES

After preservation for 6 months, T. tincae and Cryptobia regained their motility in full after 20 minutes at room temperature. In stained preparations, 90% of the flagellates observed were normal, as were the majority of erythrocytes. The diluted contents

of two capillaries were inoculated into 2 experimental tench at an approximate rate of 10^2 trypanosomes per fish. They both developed trypanosome infections with peaks at 28 days, at which time they were used for leech feeding experiments.

14. MORPHOLOGY OF CRYPTOBIA FROM THE TENCH

a) Movement

Movement was forwards, with frequent backward retreats. Preceeded by the probing anterior free flagellum, the body varied its shape considerably, bending and twisting behind the "anvil shaped" anterior end. The shallow waves of the undulating membrane passed down the body to the second free flagellum at the tapering posterior end. In degenerate forms amoeboid, and spiral and coiling movements predominated, with one or other free flagellum generally attached to a red blood cell.

b) Cytology

As in plate III and figures 23 to 25, the fixed and stained Cryptobia were elongate, flattened, rounded anteriorly, and tapering posteriorly. The cell was frequently sickle-shaped, as in figures a and i. The nucleus, not visible in fresh material, was an aggregation of light red granules with Geisma's stain. An endosome showed up with Heidenheim's iron haematoxylin stain. The nucleus was usually sub-anterior and lateral in position. Exceptionally, as in figure c, it was posterior in position. The kinetoplast was visible under phase in fresh material, and stained a deep purple

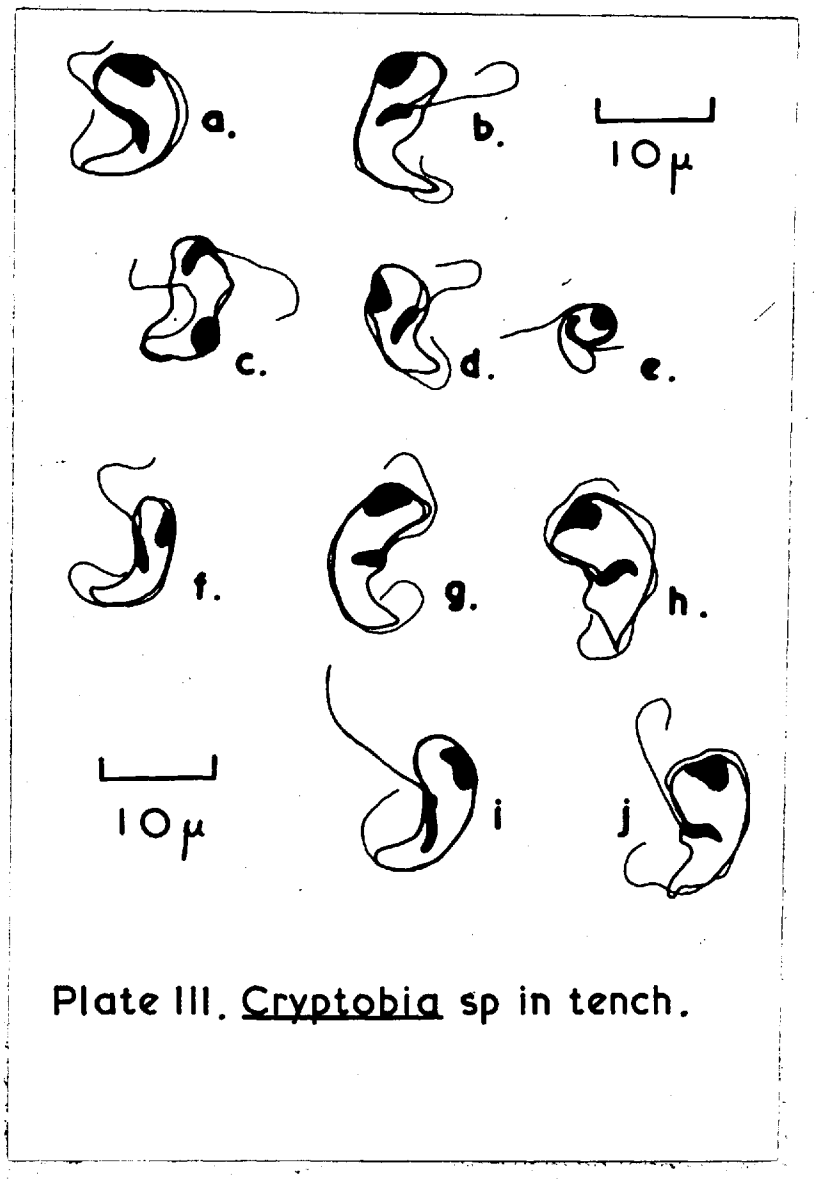


Plate III. Cryptobia sp in tench.

Cryptobia sp. from T. tinca x 1500

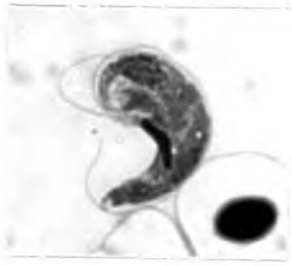


Fig. 23 Bloodstream Cryptobia

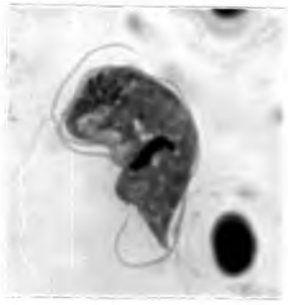


Fig. 24 Bloodstream Cryptobia

Fig. 25 Bloodstream Cryptobia

with Geisma's stain. It was usually situated about halfway down the body, and displaced laterally towards the concave surface of the cell. The shape varied from bar-shaped, as in figure d and f, curved as in figure b, boomerang-shaped as in figure 23, and "S - shaped as in figures h and j. Often one end was pressed against the edge of the cell, as in figures g and j. At this point the fine free flagella emerged. The anterior flagellum proceeded straight to the outside. The posterior flagellum was attached by a narrow undulating membrane passing round the anterior end of the body. The cytopl sm was alveolar and dense, with scattered small dark red granules as in figure 20, and vacuoles as in figure 24. There was often a clear broad streak extending from the anterior end towards the kinetoplast, as in figures 23 and 25.

c) Dimensions

Fifty Cryptobia fixed in Carnoy's fluid and stained in Geisma's stain were measured from the arterial blood of one tench. Their dimensions are given in table VIII. The kinetoplast index is a measure of the position of the kinetoplast. It is computed by dividing the distance from the centre of the kinetoplast to the posterior end by the distance to the anterior end.

Table VIII shows the considerable range of the measurements. The smallest Cryptobia, shown in figure e, was short and broad. However the larger forms were broad as in figure h, or narrow as in figure g, depending on the state of movement at fixation.

d) Division stages

No dividing forms were observed either in the blood or tissues of the tench.

Table VIII

Dimensions of 50 Cryptobia sp. from T. tincae in microns.

	Mean	Range	
Body length	13.8	7.1	17.4
Body breadth	5.2	2.8	6.8
Anterior flagellum	13.3	5.0	16.4
Posterior flagellum	8.3	5.9	10.9
Kinetoplast length	3.7	2.8	5.0
Kinetoplast breadth	0.8	0.3	1.2
Kinetoplast anterior end	6.0	1.6	8.4
Kinetoplast posterior end	7.3	4.0	12.4
Kinetoplast index	1.32	0.89	8.0
Nucleus length	3.4	2.2	4.3
Nucleus breadth	2.0	1.6	3.1
Nucleus to anterior end	2.7	1.6	9.3
Nucleus to posterior end	10.9	0.9	15.5
Nuclear index	4.06	0.13	8.6

15. MORPHOLOGY OF Trypanosoma tincae FROM THE TENCH.

a) Movement

The various stages seen in movement are shown in the figures in plate IV. There were two types of movement, "travelling" and "stationary" movements. In the first type, the body is stretched so that the curves are more gentle, as in figures i, l, and m. The free flagellum probed in all directions ahead of the cell, and the

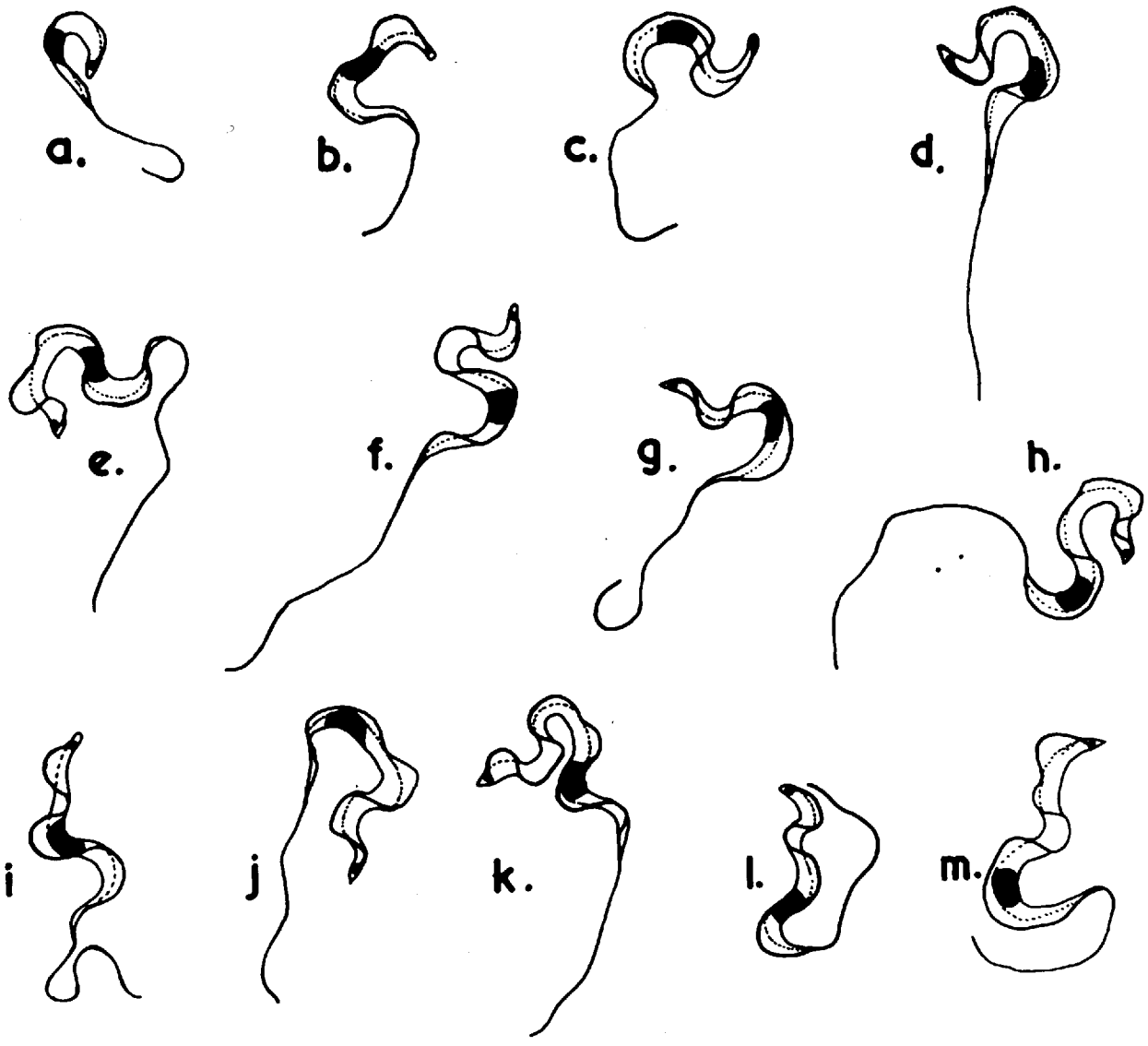


Plate IV. Bloodstream T. tincae.

10 μ

curved body reflected the waves of the broad undulating membrane, as in figures d, g, and k. Frequent changes of direction were caused by sharp bends in the body, as in figures a and j. Overall movement was forwards, with frequent retreats. "Stationary" movements occurred when the cell was trapped in a pool of serum, and nearing death. It tended to stay in the same place, flexing and twisting its body violently, as in figures e and h.

b) Cytology

Anteriorly the thin body tapered gradually. Posteriorly it tapered sharply to a blunt point. The subterminal medium sized kinetoplast was generally associated with a vacuole, situated more anteriorly, as in figure 29. The prominent undulating membrane passed forward, crossing the body one to three times. The nucleus occupied the full width of the body, taking up its curvature. An endosome was detected using Heidenheim's iron haematoxylin, and Geisma's stain after HCl hydrolysis. It was acentrally situated. The nucleus was characteristically situated in the anterior half of the middle third of the body. The cytoplasm, staining light blue with Geisma's stain, was more dense and dark blue laterally, as in figures 26 and 27. It contained scattered granules staining violet with Geisma's stain. Their number and position varied with individual cells. Granules were always present, particularly between the nucleus and the kinetoplast.

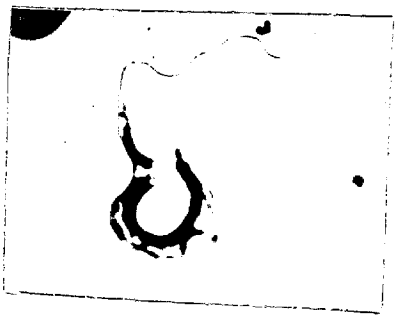


Fig. 26 Bloodstream T. tincae

Fig. 27 Bloodstream T. tincae

Fig. 28 Bloodstream T. tincae

Fig. 29 Bloodstream T. tincae

c) Division stages

In spite of detailed examinations of tissues at all stages of leech induced, passaged, and natural infections, no division forms specific to any tissues were found. However, trypanosomes dividing by unequal binary fission were found both in the blood, and tissue fluid. This process is illustrated in plates V to VII, and in figures 30 to 41.

At the beginning of the process, the kinetoplast of the largest forms grew, and moved anteriorly and laterally (plate V, figures a to c, figure 30). The whole posterior end of the cell became thickened and more truncate. The kinetoplast divided into two (plate V, figure d, figure 31), and a second flagellum grew our from the posterior kinetoplast (plate V, figures e and f, figure 32). The single vacuole associated with the original kinetoplast also divided into two (figures 32 and 33). Then a portion of cytoplasm grew out with the second flagellum (plate V, figures h and k, figure 33). The nucleus moved posteriorly as this arm of cytoplasm lengthened (plate V, figures i and j and plate VI, figures a and b). Then the nucleus divided (plate VI, figure c, figure 34). The anterior nucleus moved anteriorly (plate VI, figures d to f, figure 35), the posterior nucleus migrated underneath the two kinetoplasts with the arm of cytoplasm (plate Vi, figures g to j, figure 36). The second flagellum and cytoplasm were growing throughout, and the daughter trypanosome was now recognisable (plate VII, figures a to c, figure 37).

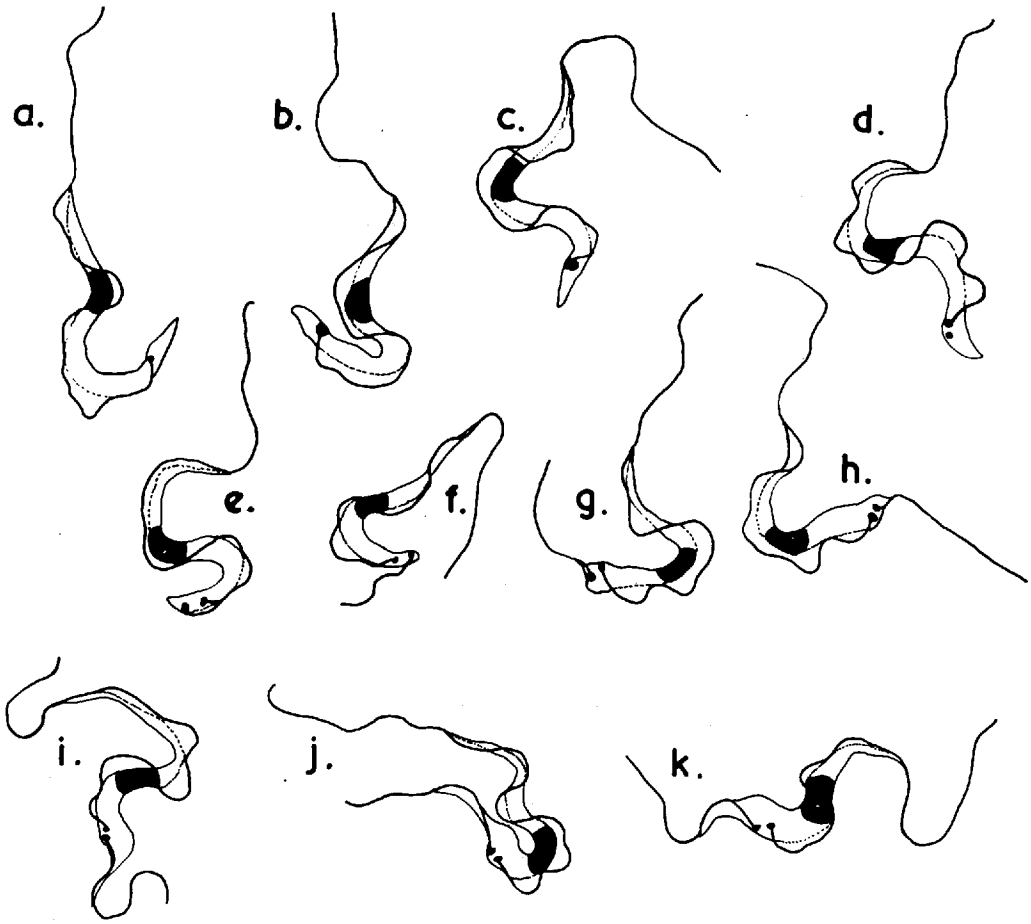


Plate V. Dividing bloodstream T.tincae. 10 μ

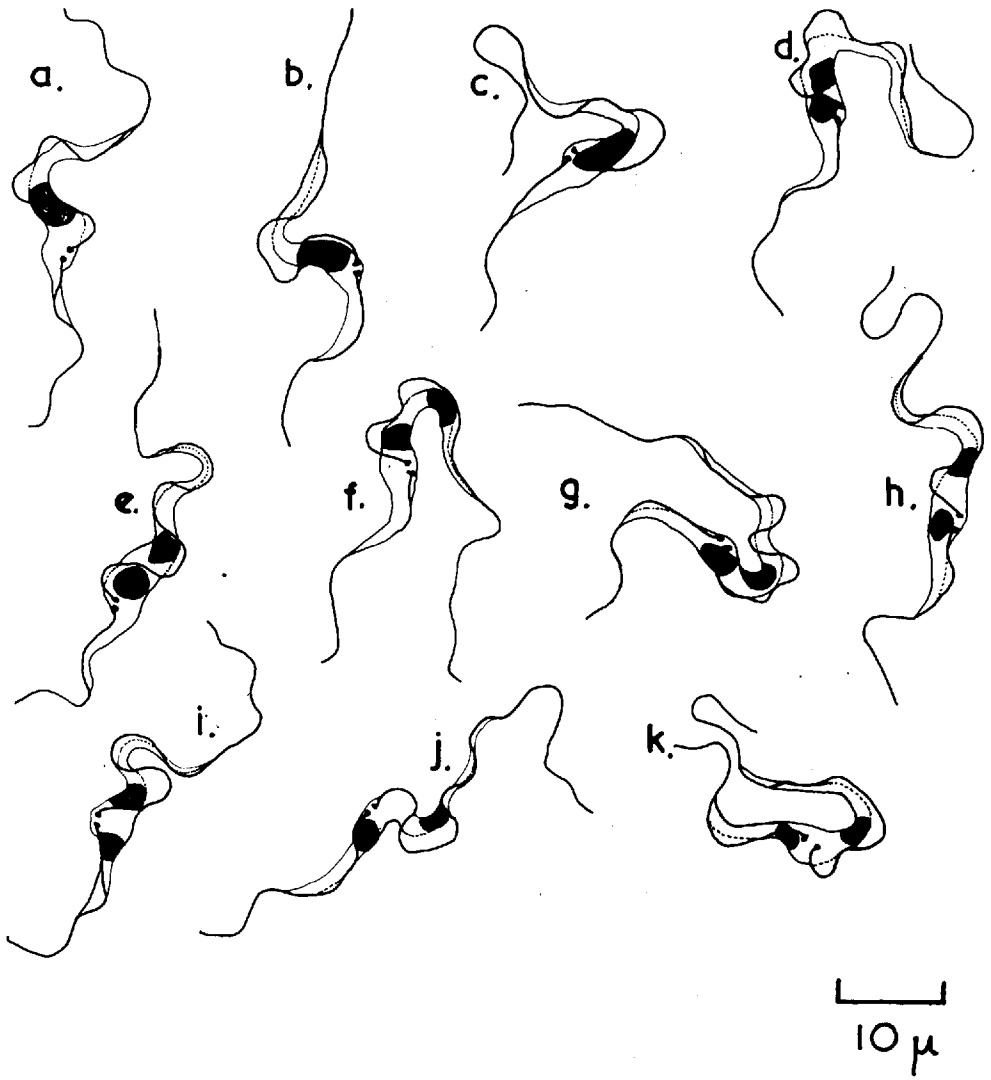


Plate VI. Dividing bloodstream T. tincae.

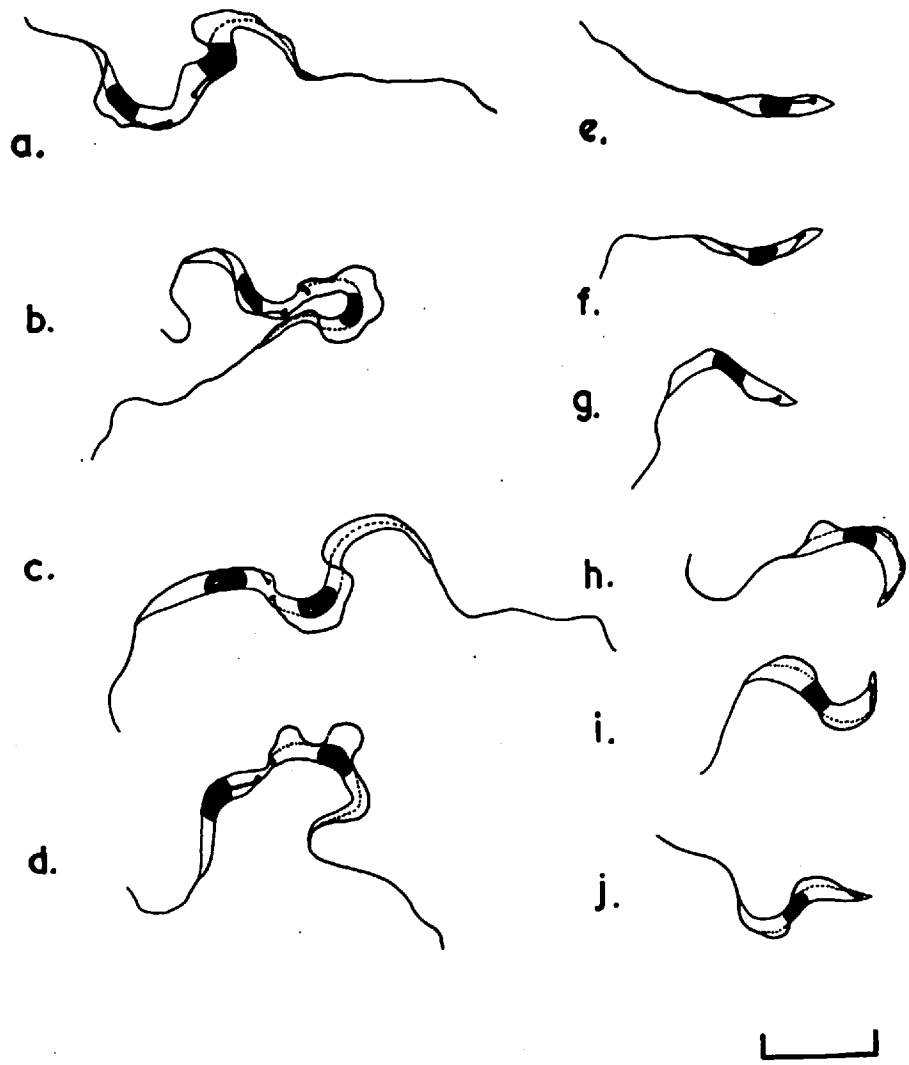


Plate VII. Dividing bloodstream *T. tincae.* 10μ.

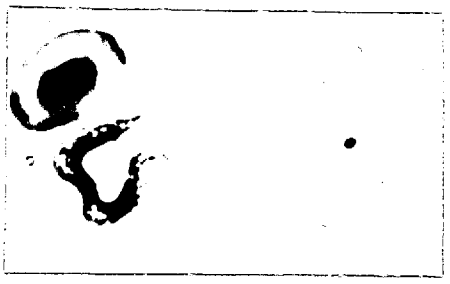


Fig. 30 Dividing form

Fig. 31 Dividing form

Fig. 32 Dividing form

Fig. 33 Dividing form

Fig. 34 Dividing form

Fig. 35 Dividing form

Fig. 36 Dividing form

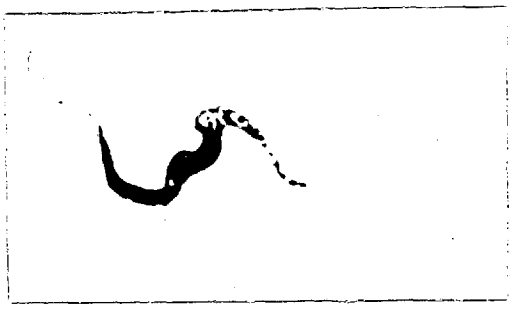


Fig. 37 Dividing form

Fig. 38 Dividing form

Fig. 39 Small form

Fig. 40 Small form

Fig. 41 Small and large form

Finally, a narrow union remained between "mother" and "daughter" as the kinetoplasts separated, and the cytoplasm between them narrowed (plate VII, figure d, figure 38). The unequal nature of the division was clear at this stage. Then the two cells separated. At first the daughter was quite rigid, with no undulating membrane, and moving feebly using its free flagellum (plate VII, figure e). Then the undulating membrane developed, and the cell was able to bend its body (plate VII, figures f and g). Figure 39 shows this stage with a pronounced vacuole associated with the kinetoplast. Then the undulating membrane became clearly differentiated from the body (plate VII, figures h to j, figure 40). From now on the trypanosome grew, its posterior end becoming blunted, and the nucleus moving to a more anterior position, as in figure 41.

These dividing forms were encountered in chronic and acute infections from wild and experimental tench. Just before and during the peak of the acute phase, they represented up to 6% of the bloodstream forms. In the post acute chronic phase the figure was 1% or less. The commonest forms were those with two flagella and those with two arms of cytoplasm. The rarest were those undergoing kinetoplast and nuclear division.

d) Dimensions

The considerable size range of bloodstream T. tincae are seen in plate IV. Measurements of non-dividing forms are given in table IX. Using these measurements, further attempts to

characterise the species were made.

Table IX

Dimensions of *T. tincae* from *T. tinca* in microns.

	Number	Mean	Range	
Posterior end to kinetoplast	200	0.8	0.4	2.3
Posterior end to nucleus	200	12.8	5.1	20.4
Anterior end to nucleus	200	7.8	5.1	12.1
Length of nucleus	200	2.4	1.4	3.3
Breadth of nucleus	200	1.3	0.7	2.2
Length of body	400	22.8	10.1	33.3
Nuclear index	200	1.71	0.53	3.77
Length of flagellum	400	14.6	5.1	23.5
Total length	400	37.2	18.8	49.3

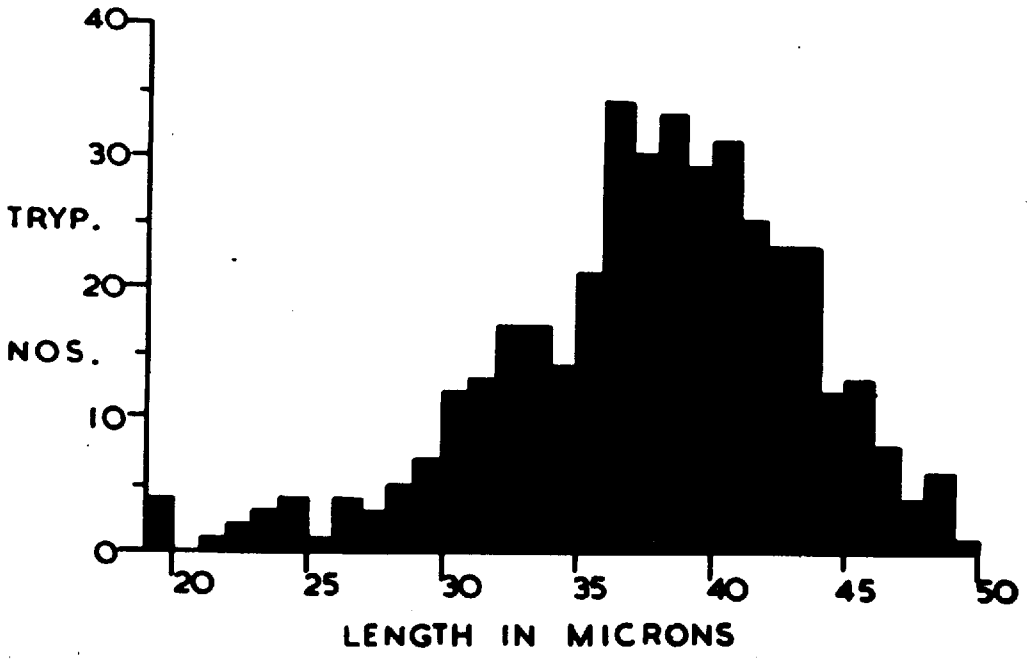
The frequencies of 400 total lengths and body lengths are given in graphs 22 and 23. Near normal distributions are present in both, with biases in favour of the larger forms. However, to see if two or more distributions were overlapping, the body length frequencies were plotted on a probit distribution, shown in graph 24. As it was not possible to distinguish significantly different slopes on this graph, different populations, if present, could not be separated on body lengths alone. So other criteria were selected.

First the length of the free flagellum was considered, but was not found to vary significantly with any other feature of the trypanosome. Long and short free flagella were present in both long and short trypanosomes, with no statistical pattern

GRAPH 22.

TOTAL LENGTH / FREQUENCY HISTOGRAM

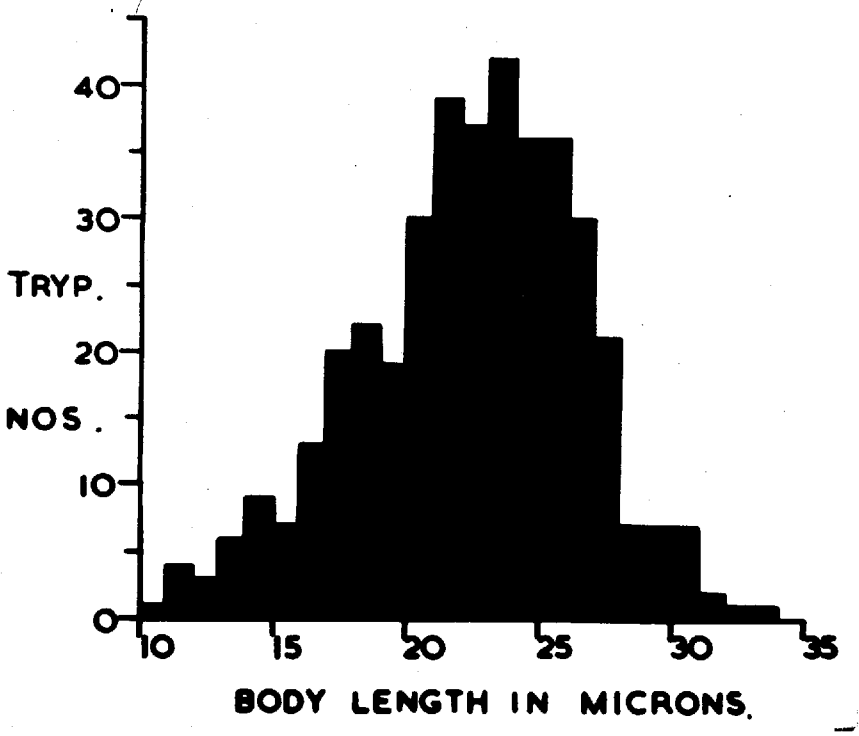
OF 400 TRYPANOSOMA TINCAE.



GRAPH 23.

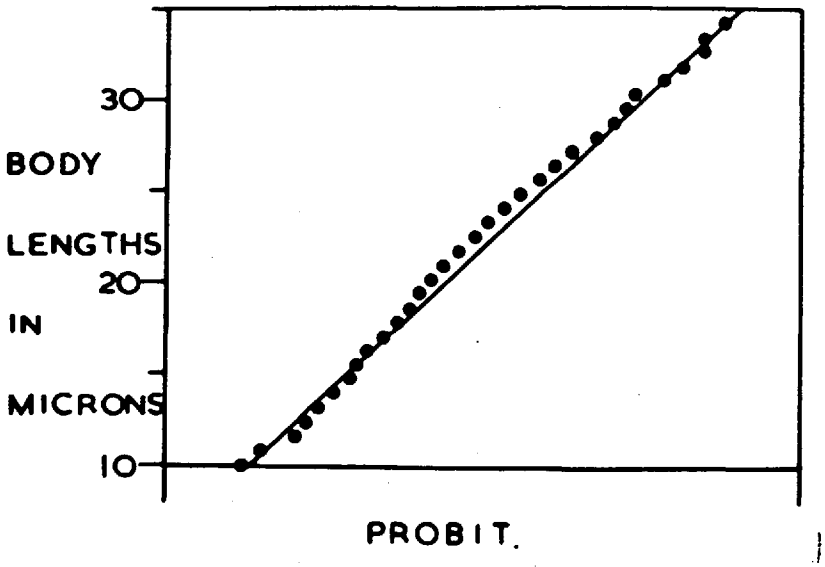
BODY LENGTH / FREQUENCY HISTOGRAM

OF 400 TRYPANOSOMA TINCAE.



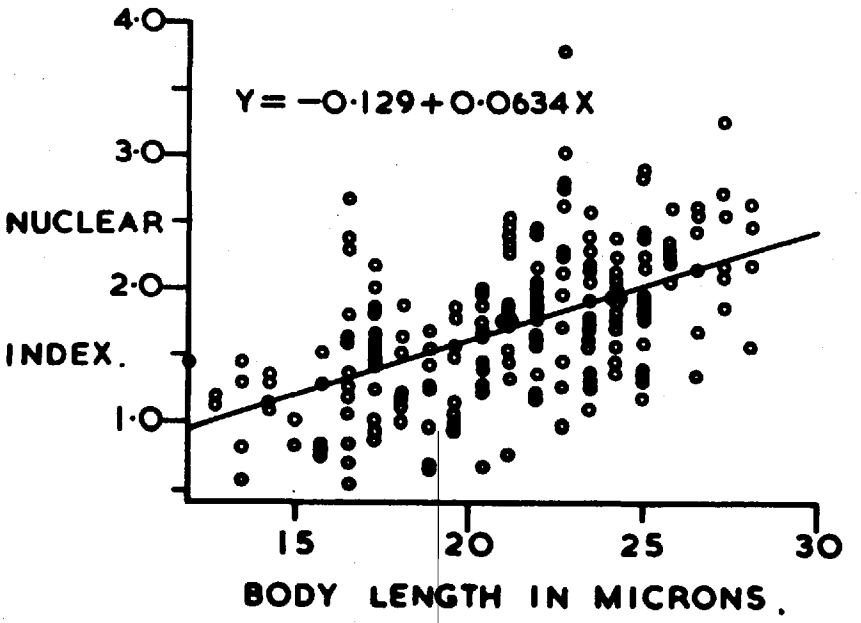
GRAPH 24.

PROBIT VALUES FOR THE BODY LENGTHS OF 400 T. TINCAE.



GRAPH 25.

RELATIONSHIPS OF NUCLEAR INDICES AND BODY LENGTHS OF 200 T. TINCAE.



emerging. Therefore, the length of the free flagellum, and hence the total length of the flagellate, was rejected as a useful criterion for either distinguishing populations within a species, or for distinguishing between species themselves.

Finally, in the absence of any further gross morphological differences, the position of the nucleus in the body was selected. Graph 25 shows the results of plotting the nuclear index against the body length of 200 T. tincae. It was found that the nucleus moved towards the anterior end of the trypanosome the larger it became by a factor of 0.0634. This was the regression coefficient, b, which had a highly significant correlation coefficient, r, of 0.5501. As this was a feature pertaining to the whole population examined, separate populations cannot be distinguished by the position of the nucleus. Therefore, in the lack of any other characters separating groups within the species, Trypanosoma tincae was a single, monomorphic species.

II. BLOOD FLAGELLATES FROM THE CRUCIAN CARP, Carassius carassius.

1. T. tincae

In attempts to investigate the host specificity of T. tincae, 5 uninfected crucian carp were trapped from the Silwood Park lake, and maintained at 20°C. Ten H. marginata carrying infections of T. tincae and Cryptobia were fed on two carp, 7 days after the leeches had been infected at 20°C. Both carp developed slight Cryptobia infections, showing that transfer

of flagellates had occurred. However, no T. tincae were detected at any stage. So 2 x 10^3 T. tincae were inoculated into two further crucian carp at 20°C. Again no trypanosomes were recovered. The last carp was inoculated with 2 x 10^5 T. tincae at 20°C, and T. tincae was recovered in small numbers, as shown in graph 26. No dividing trypanosomes were observed. On day 28, approximately 1 ml. of blood was removed, and inoculated into 3 experimental tench. All 3 developed acute T. tincae infections. On day 36, the carp died of anaemia.

2. Cryptobia

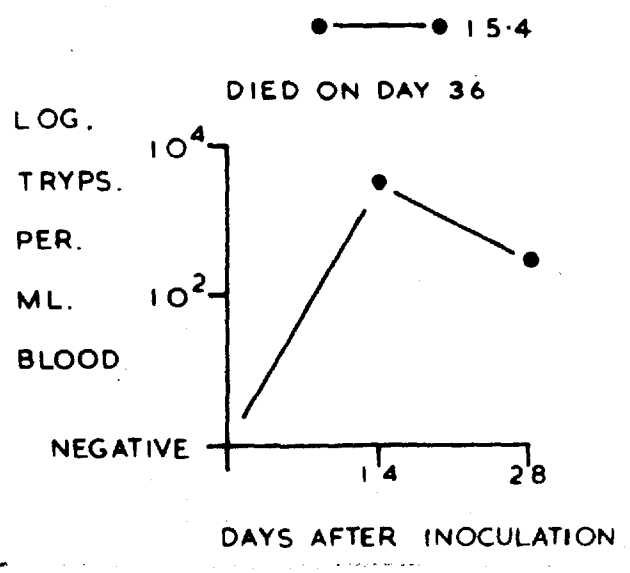
Ten crucian carp were captured from Trilakes. The details are given in table X. 50% were infected with Cryptobia, infections being principally detected during the summer and autumn. All three age groups, including one year old fish, were infected. In fresh material this Cryptobia resembled that from the tench. No stained preparations were recovered.

3. T. carassii

As table X shows, 70% of the crucian carp were infected with trypanosomes. All three age groups were infected, the chronic infections being heavier in the summer. In fresh material, the trypanosome was broader than T. tincae, with a narrower undulating membrane, and shorter free flagellum. The large kinetoplast often protruded from the "beak-shaped" posterior end. Movement was also different, the flagellate undergoing violent contortions whilst tending to stay in the same place. Because of the low

GRAPH 26.

INFECTION OF 1 CRUCIAN CARP WITH 200,000 T.TINCAE AT 20°C.



GRAPH 27.

BODY LENGTH / FREQUENCY HISTOGRAM OF 100 TRYPANOSOMA CARASSII.

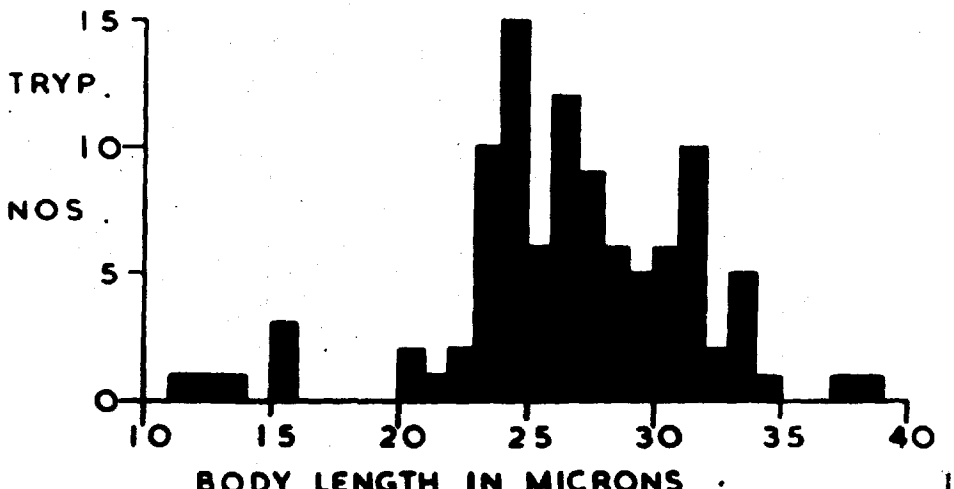


Table X

Flagellate infections in crucian carp

Date	Length (cms)	Age (years)	Cryptobia/ml	Trypanosomes/ml
21/10/68	7.8	1	2.0×10^2	-
21/10/68	9.0	1	-	4.0×10^2
4/ 6/69	8.7	1	8.0×10^2	4.4×10^3
21/10/68	10.4	2	-	4.0×10^2
13/ 2/69	11.6	2	-	-
13/ 2/69	11.8	2	2.0×10^2	-
4/ 6/69	15.2	3	2.4×10^3	2.8×10^3
4/ 6/69	15.7	3	6.0×10^2	2.0×10^3
21/10/69	16.0	3	-	3.2×10^3
13/ 2/69	16.1	3	-	+

infections, stained preparations were observed with difficulty. Accordingly smears were taken from the centrifuged blood of the 3 year old carp measuring 16.0 cms. It was therefore possible to observe and measure 100 trypanosomes.

T. carassii, as shown in plate VIII and figures 42 and 43 was a long, broad trypanosome. The posterior end was sharply tapering, "beak-shaped" (figures a, c, d, and j, figure 42), or evenly tapered (figures b, i, figure 43), or even blunt and rounded (figures e, g, h). The large sub-terminal

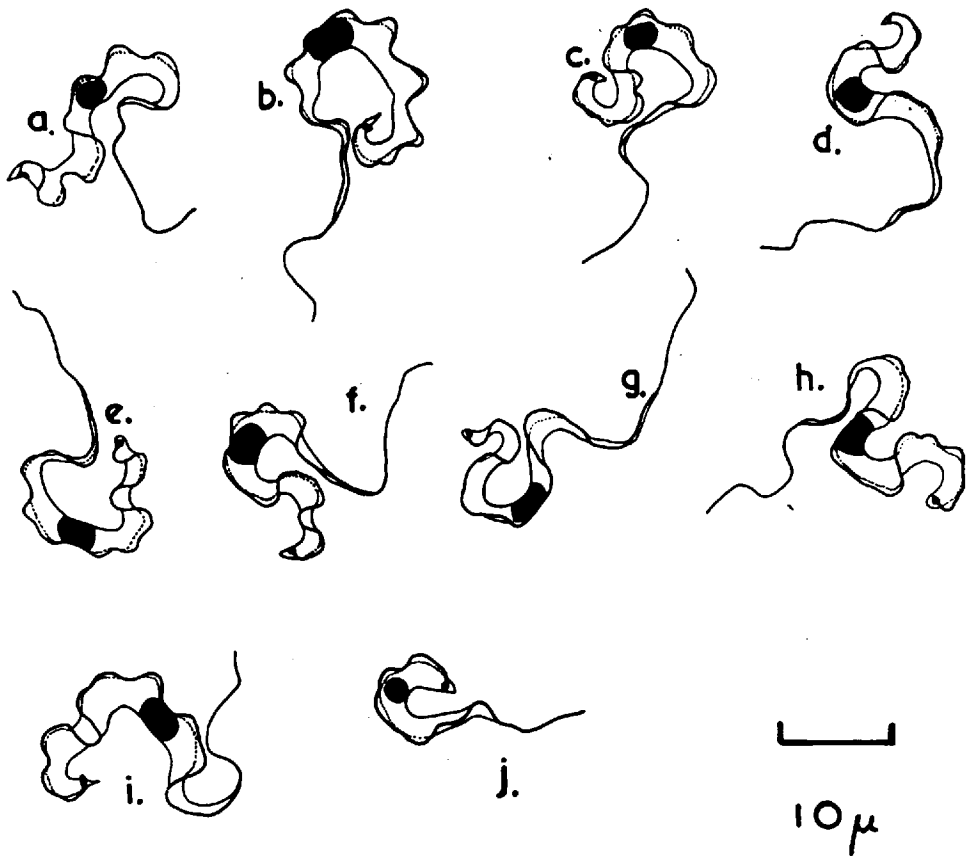


Plate VIII. Bloodstream T. carassii.

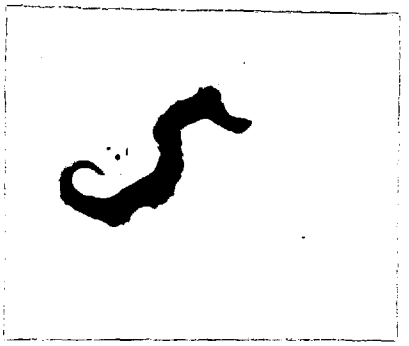


Fig. 42 Bloodstream T. carassii

Fig. 43 Bloodstream T. carassii

kinetoplast sometimes protruded from the body, as in figures b and i, and figure 42. The undulating membrane passed in a series of narrow folds to the trailing free flagellum. The nucleus was usually as broad as the body, taking up the body curvature. It varied in position around the centre of the body. In 22% of the flagellates it was in the posterior half. The anterior end tapered gently. The cytoplasm stained a dense blue with Geisma's stain, and contained a few large violet granules.

The dimensions of 100 T. carassii are given in table XI, and the body length frequency plotted on graph 27. In spite of the low number measured, the distribution was normal. The lengths of the flagellum was plotted against the body lengths, and found to be very variable, following no set pattern. Similarly no statistical correlation was found to exist between the nuclear indices and the body lengths. Therefore, in the absence of any other gross differential features, the species is considered monomorphic.

4. Division stages

No blood flagellate division stages were encountered.

5. Host specificity

Two experimental tench were inoculated with approximately 10^2 T. carassii from the 15·2 cms. carp. Two further tench were inoculated with approximately 10^3 trypanosomes from the centrifuged

blood of the 16.0 cms carp. All four tench, maintained at 20°C, failed to develope blood flagellate infections.

Table XI

Dimensions of 100 T. carassii from C. carassius in microns.

	Mean	Range	
Posterior end to kinetoplast	0.3	0.2	0.6
Posterior end to nucleus	11.6	5.6	22.0
Anterior end to nucleus	12.2	5.3	20.8
Length of nucleus	2.4	1.2	3.4
Breadth of nucleus	1.5	0.9	2.1
Length of body	26.2	11.5	38.1
Nuclear index	1.19	0.62	1.80
Length of flagellum	13.4	5.3	20.5
Total length	37.8	18.9	51.5

III. BLOOD FLAGELLATES FROM THE PERCH, Perca fluviatilis

1. Cryptobia

No Cryptobia were found in perch.

2. Trypanosoma percae

Ten perch were captured from Trilakes. Their details are given in table XII. 70% were infected with T. percae, all age groups showing low chronic infections, with the smallest and largest fish infected least. In fresh material the movement varied with the size. The smallest and thinnest flagellates twisted in a snake-like fashion between the red blood cells, preceeded by the free flagellum, with the shallow waves of the undulating membrane passing along its body length. The medium

forms moved more slowly, the prominent undulating membrane giving them an unwieldly appearance. The crook-like posterior end was dragged along behind. The large broad forms tended simply to coil up, waving the rigid bent posterior end. Occasionally transient longitudinal grooves were visible.

Stained preparations were difficult to study because of the scanty infections. Accordingly the blood of two perch was passed through a DEAE cellulose column. The perch were both 2 years old, measuring 12.5 and 13.3 cms. As a result of successful separation and concentration, it was possible to observe and measure 100 trypanosomes.

As plate IX and figures 44 to 47 show, there was considerable variation in size from long and thin to short and broad. In the thinnest (figures a, b, c) the kinetoplast was central, subterminal or rarely terminal (figure b), the posterior end was gently tapering or blunt, and the undulating membrane closely applied to the body. As the body becomes broader, the undulating membrane developed further, and the kinetoplast became laterally displaced (figures d, e, f), and figure 44. Then there was a broadening of the posterior half of the flagellate (figures g, h, and i, and figure 45). Also the posterior extremity became crook-like (figures g, k, l and figure 46). In the largest forms the posterior "crook" was lengthened (figures m, n, and figure 47), the kinetoplast well subterminal, and the nucleus more anterior in position. The undulating membrane was closely applied to the

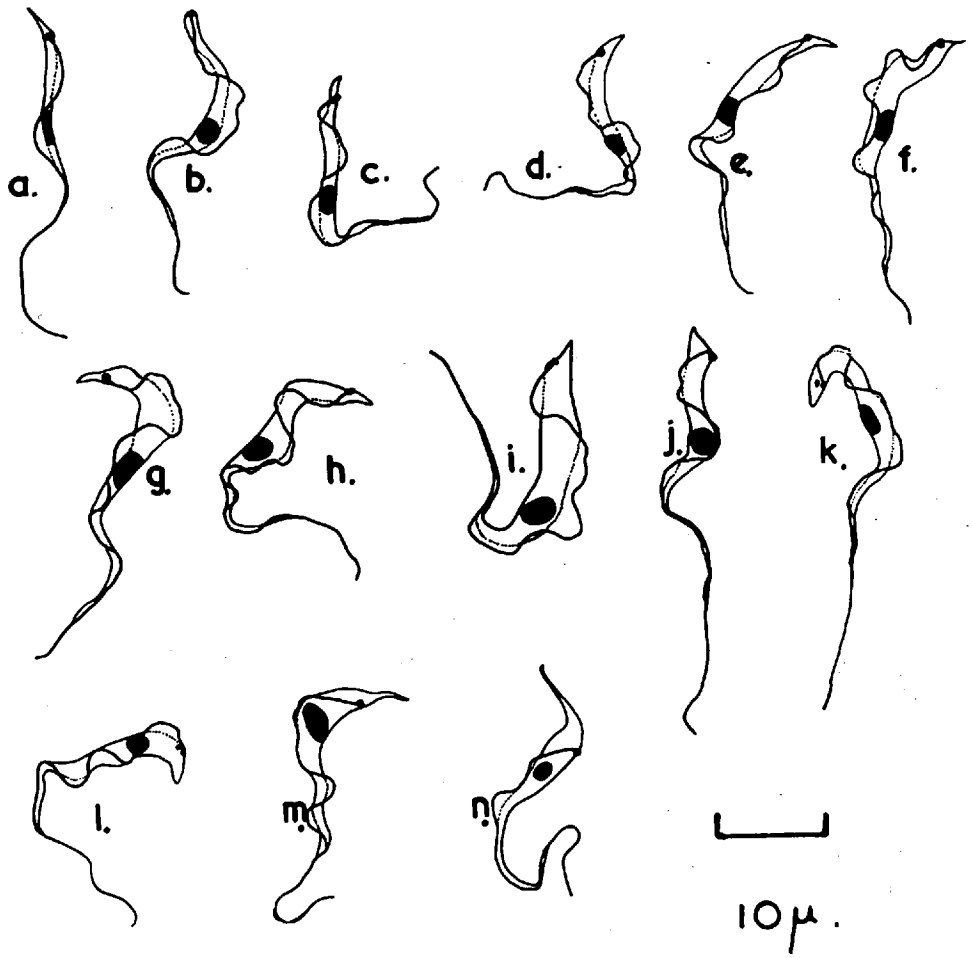


Plate IX. Bloodstream T.percae.

T. percae from P. fluviatilis x 1500

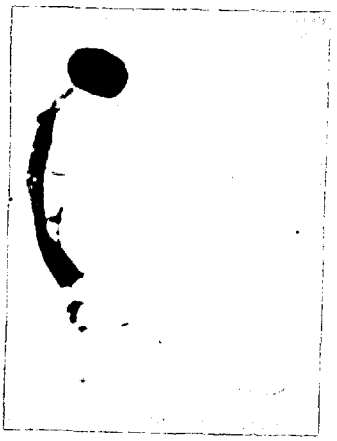


Fig. 44 Bloodstream
T. percae

Fig. 45 Bloodstream
T. percae

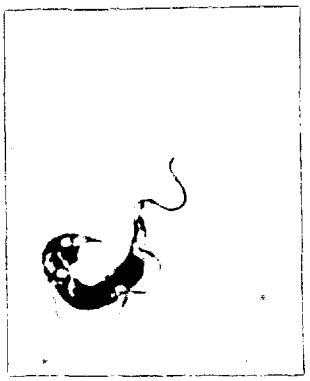


Fig. 46 Bloodstream
T. percae

Fig. 47 Bloodstream
T. percae

body again. The length of the flagella varied considerably with all forms.

Table XII

T. percae infections in perch.

Date	Length (cms)	Age (years)	Trypanosomes/ml blood
21/10/68	6.9	1	8.0×10^2
16/12/68	7.6	1	-
21/10/68	12.1	2	8.0×10^2
28/ 5/69	12.5	2	6.4×10^3
28/5/69	13.3	2	2.8×10^3
28/5/69	13.8	2	-
21/10/68	16.9	3	9.2×10^3
27/ 8/68	17.7	3	2.0×10^3
27/ 8/68	18.2	3	-
27/ 8/68	18.4	3	+

The kinetoplast was small. Rarely two were seen in the broader forms (figures k and l). This was the only evidence of division. The nucleus was as wide as the body in thin forms, and was round or oval in the broad forms. The anterior end always tapered considerably. The cytoplasm varied from dense

blue with Geisma's stain in the thinner forms (figure 44) particularly posterior to the nucleus. In the broader forms (figure 45), there were dense blue patches near the posterior part of the nucleus, but characteristically there were numerous small violet granules. In the broadest forms, the cytoplasm was much less dense (figures 46 and 47). There were no granules, but there were many small vacuoles. Longitudinal grooves were occasionally present on the surface of the body.

The dimensions of 100 T. percae are given in table XIII and the body length frequency plotted in graph 28. The distribution of body lengths was normal, and, in the light of the continuous gradations of characters mentioned above, T. percae is considered a monomorphic species.

As a further test for the homogeneity of the species, the nuclear indices were plotted against the body lengths. The results in graph 29 show that the nucleus moved towards the posterior end of the trypanosome the longer it became by a factor of 0.0609. This was the regression coefficient, b. The correlation coefficient, r, of -0.6182, was highly significant.

3. Division stages

Apart from the forms described with two kinetoplasts, no further division stages were found.

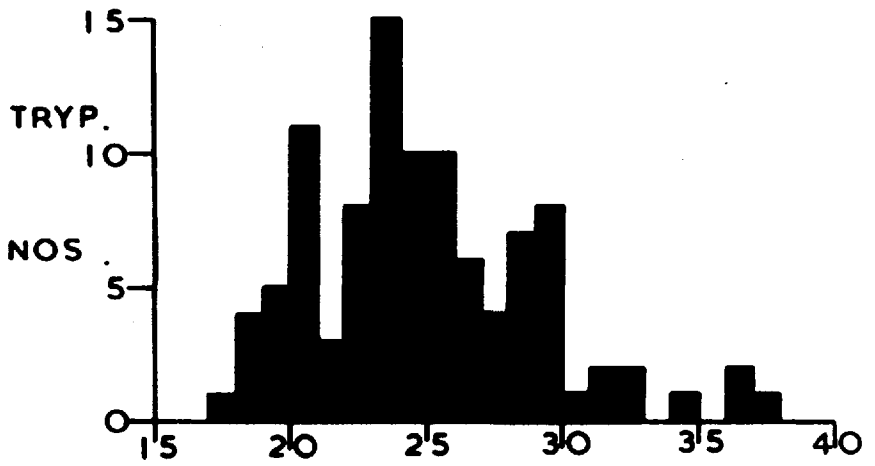
4. Host specificity

Four tench inoculated with approximately 10^2 T. percae from two perch of 16.9 and 17.7 cms failed to develope trypanosome

GRAPH 28.

BODY LENGTH / FREQUENCY HISTOGRAM

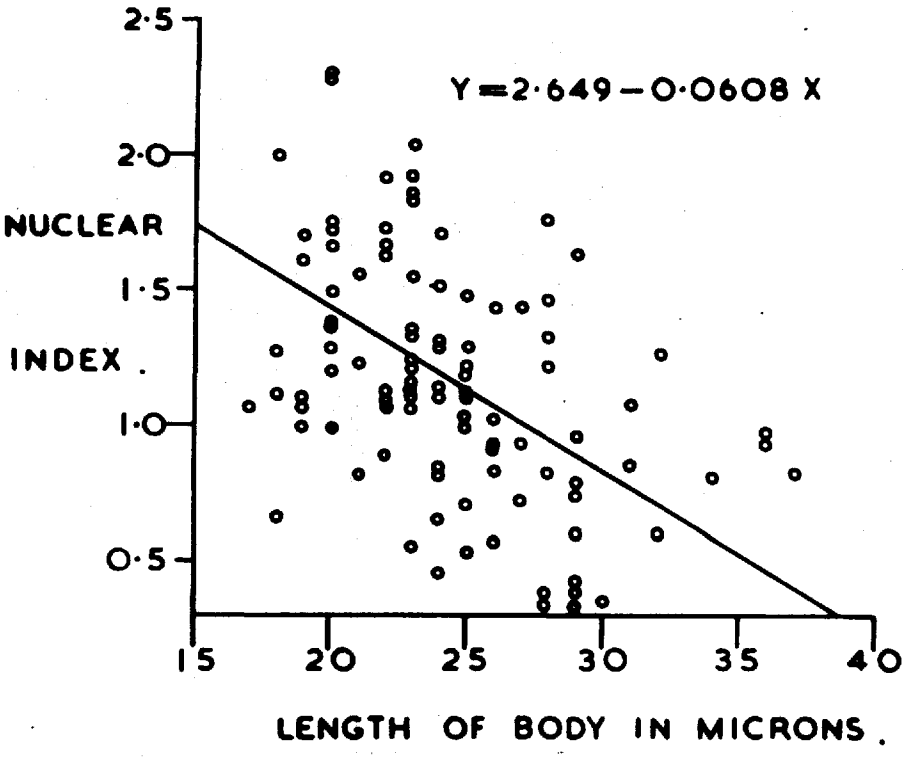
OF 100 TRYPANOSOMA PERCAE.



GRAPH 29.

RELATIONSHIPS OF NUCLEAR INDICES

AND BODY LENGTHS OF 100 T. PERCAE.



infections.

Table XIII

Dimensions of 100 T. percae from Perca fluviatilis in microns.

	Mean	Range	
Posterior end to kinetoplast	2.8	0.2	5.6
Posterior end to nucleus	12.5	7.1	18.0
Anterior end to nucleus	11.9	5.9	22.0
Length of nucleus	2.6	1.9	4.0
Breadth of nucleus	1.5	0.9	3.4
Length of body	24.4	17.7	36.6
Breadth of body	1.7	0.9	4.0
Nuclear index	1.16	0.34	2.3
Length of flagellum	10.7	4.3	18.6
Total length	35.0	23.6	46.2

IV. BLOOD FLAGELLATES FROM THE ROACH, Rutilus rutilus.

1. Cryptobia

Of the 10 roach examined from Trilakes, 20% were infected with Cryptobia. The low infection levels are shown in table XIV. Living material resembled the Cryptobia from the tench. No stained preparations were recovered, and no division forms were seen.

2. Trypanosoma leucisci

Only one of the roach collected from Trilakes was infected with trypanosomes. Table XIV gives the details. In fresh material, the trypanosomes resembled T. tincae. However the posterior end was more pointed, the kinetoplast longer, and the undulating

membrane more closely applied to the body. In general appearance it was very thin, and vermiform, with snake-like movements.

As the infections were so low blood was centrifuged to obtain stained preparations. Unfortunately small quantities were used because the rest was inoculated from the one patently infected fish into tench. Only three trypanosomes were recovered, one of them being shown in plate X and figure 4g. The large kinetoplast was situated terminally or subterminally at the cone-shaped posterior end. The undulating membrane passed up the thin body in a series of shallow waves. The nucleus was situated centrally, and was as broad as the body. The anterior end tapered slowly to a long free flagellum. The cytoplasm was pale blue with Geisma's stain, and contained a few violet granules. The dimensions of the 3 trypanosomes are given in table XV. No division stages were encountered.

3. Host specificity

Approximately 10^2 trypanosomes from the one infected roach were inoculated each into 2 experimental tench at 20°C. Both failed to develope infections.

V. BLOOD FLAGELLATES FROM THE EEL, Anguilla anguilla

1. Cryptobia

No Cryptobia were found in eel.

2. Trypanosoma granulosum

Ten marine eels were purchased at Bracknell market. They

10 μ

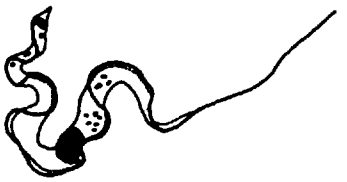


Plate X. Bloodstream

T. leucisci.

T. leucisci from R. rutilus x 1500

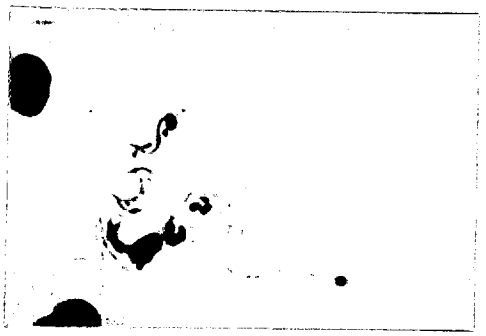


Fig. 48 Bloodstream T. leucisci

Table XIV

Blood flagellates infections in roach

Date	Length (cms)	Age (years)	Cryptobia/ ml. blood	Trypanosomes/ ml. blood
21/10/68	9.9	2	-	-
21/10/68	10.1	2	-	-
21/10/68	11.9	3	2.0×10^3	-
21/10/68	12.3	3	-	-
21/10/68	12.5	3	3.2×10^3	2.0×10^3
28/ 5/69	12.5	3	-	-
21/10/68	12.6	3	-	-
28/ 5/69	12.9	3	-	-
28/ 5/69	13.1	3	-	-
28/ 5/69	15.2	4	-	-

Table XV

Dimensions of three T. leucisci from Rutilus rutilus in microns

	Mean	Range	
Posterior end to kinetoplast	0.8	0.1	1.6
Posterior end to nucleus	17.6	16.4	19.5
Anterior end to nucleus	16.1	13.6	18.3
Length of nucleus	2.8	1.9	3.4
Breadth of nucleus	1.8	1.2	2.2
Length of body	33.7	30.1	36.0
Nuclear index	1.1	0.92	1.2
Length of flagellum	18.5	17.4	20.8
Total length	52.2	50.9	53.3

had originated from Holland. No blood flagellates were found. Ten further eels were collected from the River Avon, and records of their trypanosome infections are given in table XVI.

Table XVI

T. granulosum infections in eels

Date	Length (cms)	Trypanosomes/ml. blood
21/ 4/67	42.1	1.92×10^4
21/ 4/67	42.6	2.0×10^6
21/ 4/67	43.5	1.08×10^4
21/ 4/67	45.5	4.4×10^3
2/ 9/68	47.4	-
2/ 9/68	48.2	9.2×10^3
21/ 4/67	48.9	3.2×10^3
21/ 4/67	51.3	3.2×10^3
21/ 4/67	54.0	4.0×10^3
21/ 4/67	68.5	4.0×10^2

The eels were not aged, because of the difficulty of reading their small scales. 90% were infected with trypanosomes, the smaller eels having the heavier infections. One eel was acutely infected. Two distinct trypanosome types were present in fresh material. The first, which was more frequent, was filiform

with a narrow undulating membrane passing rapidly down the body in many tight folds. The cone-shaped posterior end was flicked from side to side as the trypanosome moved in a snake-like manner between the blood cells, preceeded by the long free flagellum. The second type of trypanosome was very long, with a broader body, and a blunt posterior end. The undulating membrane was even more clearly adherent to the body. Locomotion was slower, the cell twisting and coiling on itself, and generally staying in the same place. Both types were present in the acute infection. Only one large form was recovered from one of the chronic infections.

In the stained preparations from the acutely infected eel, the smaller form accounted for 96·4% of the trypanosomes. It resembled T. tincae, but was more uniform. It is shown in plate XI (figures a to c,) and in figure 49. The posterior end was cone-shaped (figure b), sharply tapering (figure 49), or blunt (figure a). The kinetoplast was medium sized, and usually situated subterminally. The nucleus was usually as broad as the body, and was situated in its middle third. The anterior end of the body was quite sharply tapering. The almost clear blue cytoplasm contained numerous small violet and red granules. The dimensions of 192 of these small forms are given in table XVII.

The large form, shown in figures d and e and in figure 50, had a longer, broader body, with a comparatively short free flagellum. The undulating membrane was so closely applied to the

T. granulosum x 1500

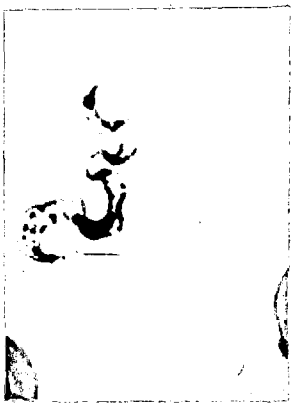


Figure 49.

Figure 50.

body that it was barely detectable in stained preparations. The kinetoplast was large and situated subterminally. The nucleus was also very large with two regions showing different staining reactions with Geisma's stain. One part was an opaque dark red, the other was an amorphous dark pink (figures d and e). The nucleus was situated much more posteriorly in the large forms than in the small forms. However the most characteristic feature about the large form was its opaque blue cytoplasm (figure 50). There were no granules. Occasional vacuoles were present, with

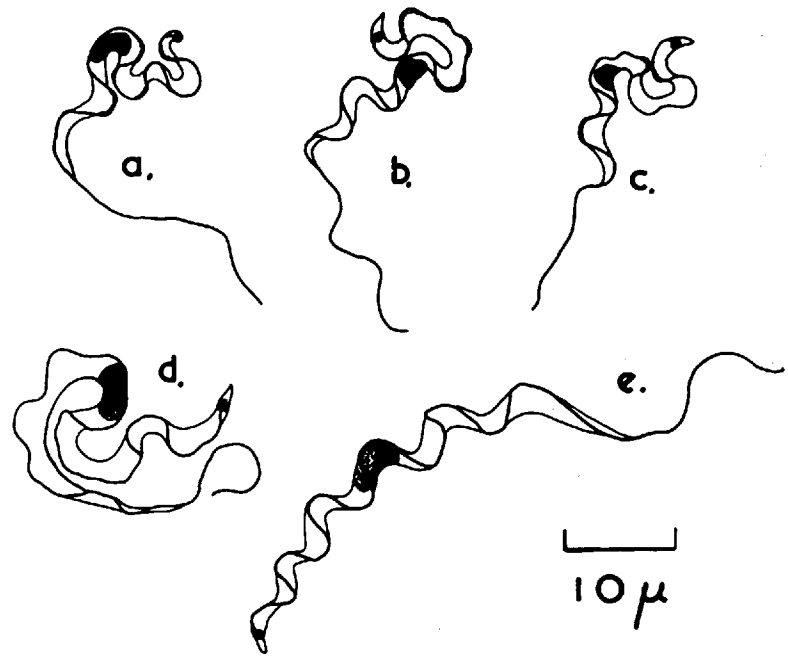


Plate XI. Bloodstream T.granulosum.

a large vacuole associated with the kinetoplast. The dimensions of 8 of these forms are given in table XVII.

The body length frequencies of 200 T. granulosum are given in graph 30. The group of large forms at the top end of the length axis are clearly distinguishable from the 192 small forms, which show a normal distribution. No intermediates in either body length or cytoplasmic staining were found. T. gran losum, therefore, is a dimorphic species.

To characterise the species further, the flagella lengths were plotted against the body lengths. Great variation was present, but the large forms tended to have shorter flagella. The nuclear indices were plotted against the body length. As the 8 large forms was too small a sample for statistical analysis, a regression coefficient was calculated for the 192 small forms. The results in graph 31 show that the nucleus moved towards the posterior end of the body the longer it grew by a factor of 0.0349. This, the refression coefficient had a highly significant correlation coefficient, r, of -0.2889.

3. Division stages

Rare larger forms of the small variety had 2 kinetoplasts.

4. Host specifity

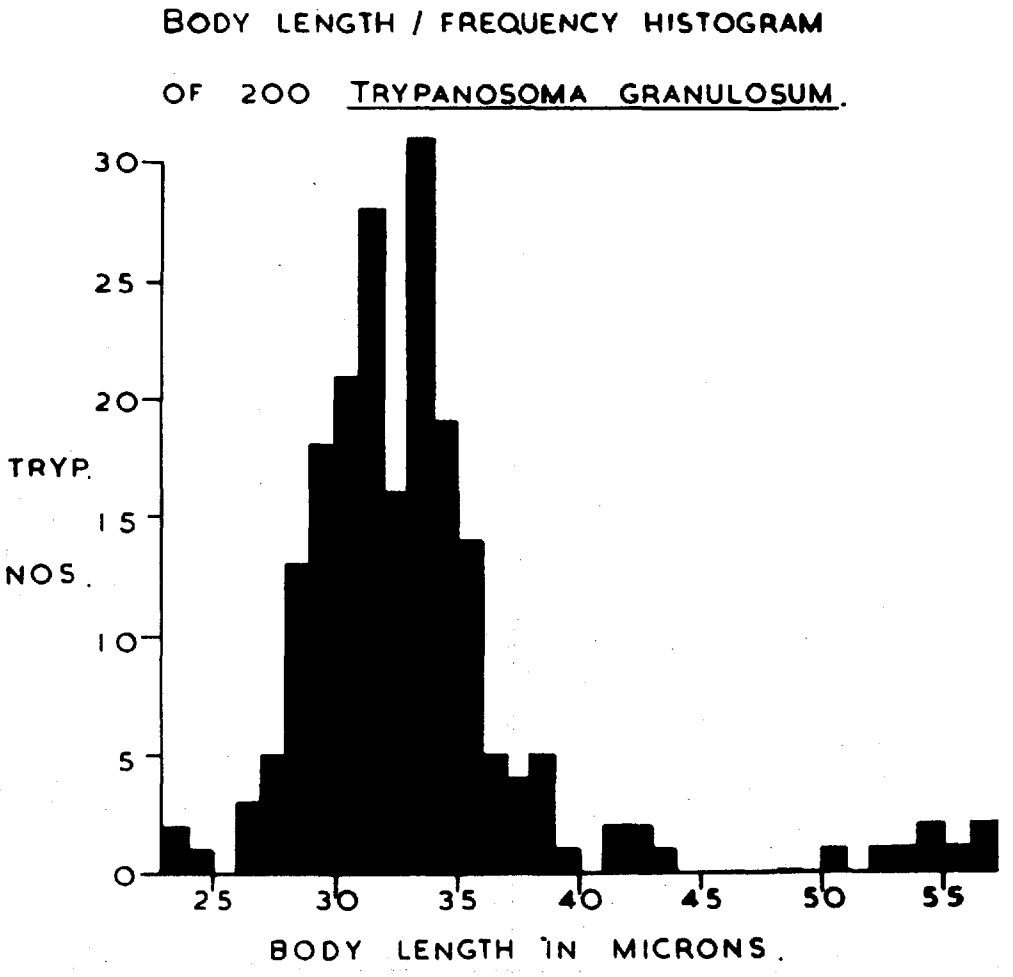
Four tench inoculated with approximately 10^2 T. granulosum from the 48.2 cm. eel at 20°C failed to develope trypanosome infections.

Table XVII

Dimensions of T. granulosum from A. anguilla in microns

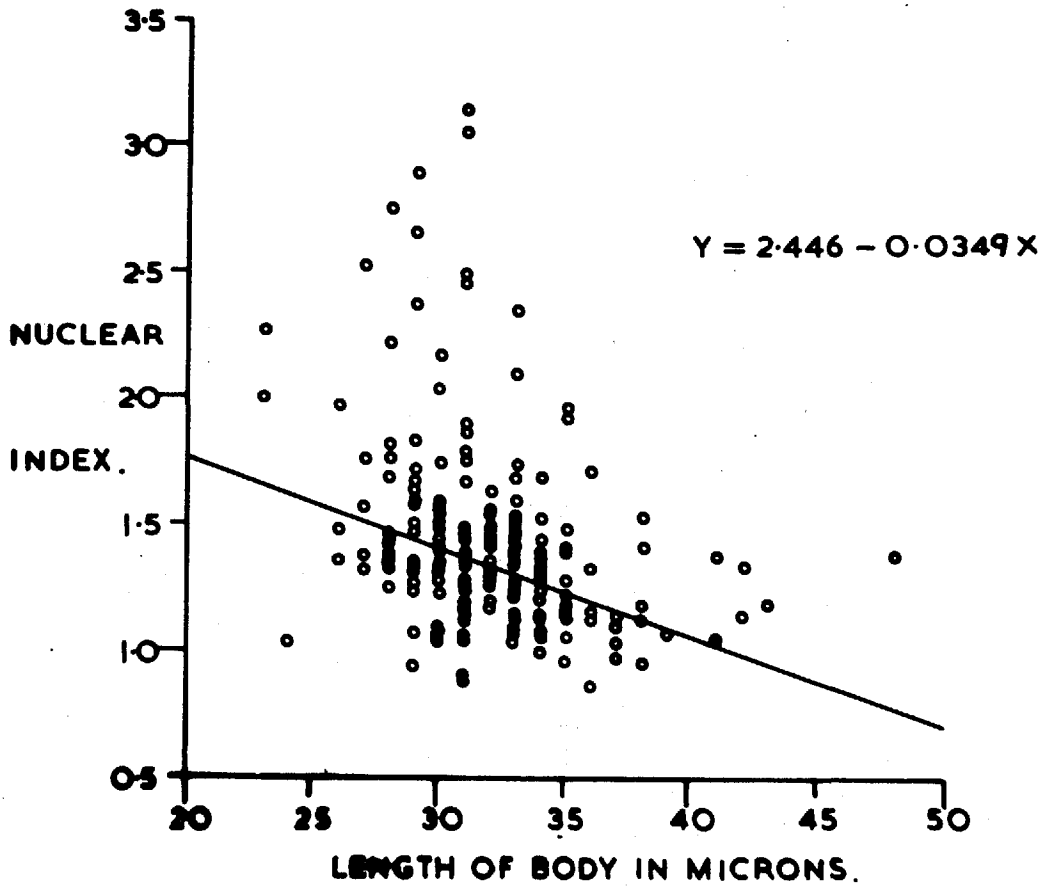
	192 Small forms			8 large forms			200 T. granulosum		
	Mean	Range		Mean	Range		Mean	Range	
Posterior end to k'plast	1.5	0.3	3.4	1.6	0.9	2.5	1.5	0.3	3.4
Length of kinetoplast	0.7	0.3	1.2	0.8	0.6	0.9	0.7	0.3	1.2
Breadth of kinetoplast	0.6	0.3	1.2	0.7	0.6	0.9	0.6	0.3	1.2
Posterior end to nucleus	18.6	12.1	23.9	23.7	20.5	27.9	19.1	12.1	27.9
Anterior end to nucleus	13.1	7.1	19.8	30.5	28.2	32.6	14.4	7.1	32.6
Length of nucleus	3.5	2.2	5.0	5.1	4.7	5.9	3.6	2.2	5.9
Breadth of nucleus	1.2	0.9	1.6	2.0	1.6	2.5	1.2	0.9	2.5
Length of body	32.1	23.3	43.5	53.8	49.9	56.1	33.0	23.3	56.1
Nuclear index	1.33	0.86	3.13	0.78	0.75	0.9	1.31	0.75	3.13
Breadth of body	1.2	0.9	1.9	2.0	1.6	2.5	1.3	0.9	2.5
Length of flagellum	19.5	11.5	28.2	19.3	14.6	23.3	19.5	11.5	28.2
Total length	51.6	39.7	69.1	73.1	66.3	79.4	52.5	39.7	79.4

GRAPH 30.


BODY LENGTH / FREQUENCY HISTOGRAM
OF 200 TRYPANOSOMA GRANULOSUM.
TRYP.
NOS.
30
25
20
15
10
5
0
25
30
35
40
45
50
55
BODY LENGTH IN MICRONS.

GRAPH 31.

RELATIONSHIPS OF NUCLEAR INDICES AND BODY LENGTHS OF 192 T. GRANULOSUM.



VI. SUMMARY OF THE MORPHOLOGICAL DIFFERENCES BETWEEN THE FIVE TRYPANOSOME SPECIES IN THE VERTEBRATE.

The morphological differences summarised in table XVIII show that the species can be separated on morphological grounds alone. Most have several characteristics peculiar to each species:

T. tincae
1) Wide undulating membrane with few folds,
2) Kinetoplast vacuole present,
3) Nucleus central in small forms, anterior in large forms.

T. carassii
1) Deep blue cytoplasm,
2) Large kinetoplast,
3) Centrally situated nucleus.

T. percae
1) Large breadth of body variations,
2) Large forms with a "crook-like" posterior end.

T. granulosum
1) Dimorphic,
2) Long slender small forms,
3) Large forms with deep blue cytoplasm.

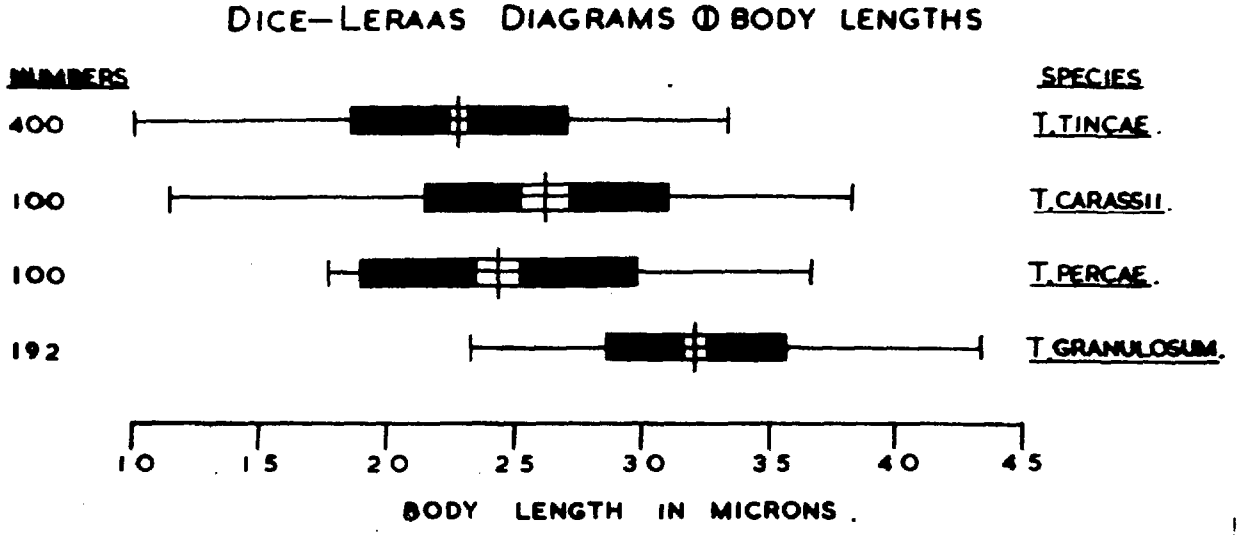
Using the Dice-Leraas diagrams, it was possible to quantify the differences in the body lengths and nuclear indices. In graph 32, the means of the body lengths of the four species are quite

Table XVIII

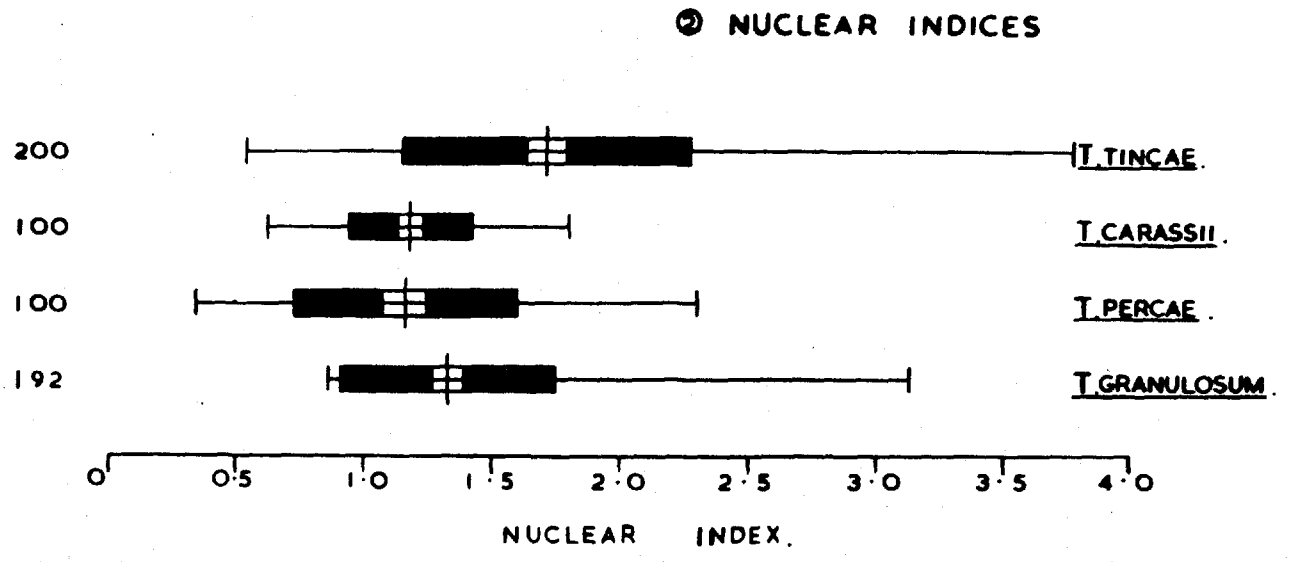
Morphological differences of trypanosome species

	T. tincae	T. carassii	T. percae	T. leucisci	T. granulosum	
1) Host	T. tinca	C. carassius	P. fluviatilis	R. rutilus	A. anguilla	
2) Movement	Sinuous	Coiling	Sinuous-coiling	Sinuous	Sinuous	Coiling
3) Post. end	Sharply tapered	Beak-shaped, blunt	Tapered - crook-shaped	Cone-shaped	Tapering	Blunt
4) K. plast	Small + vacuole	Large, no vacuole	Medium - small	Medium	Medium	Large
5) Und. memb.	Wide	Narrow	Narrow-broad-narrow	Narrow	Narrow	Very narrow
6) Nucleus:						
a) small	Central	Central	Anterior	-	Anterior	Posterior
b) large	Anterior	Central	Central	-	Posterior	Posterior
c) b	+ 0.0634	-	- 0.0608	-	- 0.0349	-
7) Cyto. Geisma	Light/dark blue	Deep blue	Dense blue-light blue	Pale blue	Pale blue	Dense blue
8) Granules	Many	Few	None	Few	Many	None
9) Body length μ	10.1-33.3 (22.8)	11.5-38.1 (26.2)	17.7-36.6 (24.4)	30.1-36.0 (33.7)	23.3-43.5 (32.1)	49.9-56.1 (53.8)
10) Body breadth μ	0.7-2.2 (1.3)	0.9-2.1 (1.5)	0.9-4.0 (1.7)	1.2-2.2 (1.8)	0.9-1.9 (1.2)	1.6-2.5 (2.0)
11) Flag. length μ	5.1-23.5 (14.6)	5.3-20.5 (13.4)	4.3-18.6 (10.7)	17.4-20.8 (18.5)	11.5-28.2 (19.5)	14.6-23.3 (19.3)
12) Total length μ	18.8-49.3 (37.2)	18.9-51.5 (37.8)	23.6-46.2 (35.0)	50.9-53.3 (52.2)	39.7-69.1 (51.6)	66.3-29.4 (73.1)
13) Nos.	400	100	100	3	192	8

GRAPH 32.



GRAPH 33.



different. No white areas overlap, and even some of the black areas are separated. Therefore all four species can be separated as different populations on body length alone. Some, by inspection, more different than others, e.g. T. tincae is quite clearly separable from T. granulosum.

Graph 33 shows the nuclear indices plotted in the same way. Those of T. carassii and T. percae are strikingly similar. The nuclear indices of T. tincae, however, are clearly separable from the rest.

DISCUSSION

I. THE CHARACTERISTICS OF FISH HEMOFLAGELLATES IN THE VERTEBRATE

a) Characterisation of species

Moshkovski (1969) discussed the concept of a protozoan species in terms of a system. Eidology, as he called his approach, involved the definition of every biological character of the species. The species system, then, consists of a sum total of genetically discrete characters. Unfortunately, whilst this approach has an attractive theoretical integrity, it has little practical application. However, the point is made that forms should not be described as new species because they show a single differential trait, a difference of a single locus. Huxley (1942) defined the species as a biologically discontinuous group. He implied that a series of biologically, and by inference, genetically differentiating characters must be used.

Hoare (1967) in his taxonomic treatment of the mammalian trypanosomes remarked that, in practice, allied groups are separated as species when the characters distinguishing them do not intergrade, so that there is a definite gap or discontinuity between them. As many of the characters he uses are not quantifiable, they are necessarily subjective. Accordingly Killick-Kendrick's (1969) personal comment is apt:-

"A species is a taxon defined as a species by a competent taxonomist".

For, not only are some of the characters used necessarily interpretive, but the minimum number of characters taken for a species to assume respectability is also subjective, and largely a matter of

convenience. It is a mistake, therefore, to disqualify a protozoan species because its complete life cycle is not known; but it is also a mistake to use a single criterion in species differentiation. Therefore, Laveran and Mesnil's (1904) principle that the occurrence of trypanosomes in specific hosts justifies the creation of separate species is rejected. Supported by other characters, however, the host range of a species is an important differentiating factor. After all, Mayr (1955) pointed out that occurrence in more than one host species is restricting gene flow just as much as is isolation on an island. Speciation in host specific parasites is, therefore, biologically and genetically the same kind of phenomenon as is geographic speciation.

Applying the above principles to the fish trypanosomes I have studied, four of the species at least retain their integrity.

Brumpt (1906b) found that T. percae developed exclusively in the crop and proboscis sheath of H. marginata. T. granulosum, however, developed principally in the intestine. In addition, therefore, to the morphological characters outlined previously, particularly the dimorphic nature of T. granulosum, and the peculiar shape of the posterior end of T. percae, these two trypanosomes are clearly separate species.

T. tincae and T. carassii are less easy to separate. Subjective characters such as the dense blue cytoplasm of T. carassii, the many granules of T. tincae, the differences in the undulating membrane, and their host specificity, are selected. Unfortunately T. tincae will infect the crucian carp, but only if a very high inoculum is given.

The absence of division forms suggested that the trypanosomes were not developing. The few trypanosomes observed in the infection had not assumed the characters of T. carassii.

In the Dice-Leraas diagrams of the body lengths, the black rectangles, representing the standard deviations, for the populations of T. tincae and T. carassii did not overlap. Hoare (1956) stated that in these cases, at least 84% of each population is separable one from the other. Such differences, he states, are of subspecific order. However, the nuclear indices of the two species were even more different. Also the nucleus of T. tincae occupied a statistically defined position depending on the body length. The nucleus of T. carassii did not.

Therefore, in the light of these characters, T. tincae and T. carassii are retained as separate species.

Unfortunately, the status of the Cryptobia found remains in doubt. Cryptobia from the different fish species resembled one another, but detailed comparisons were not possible because of the low numbers observed. The suspicion remains, however, that Keysselitz (1906) was right in considering them all one species.

The morphometric examination of the bloodstream trypanosomes showed that quantifiable characters were the most valuable, because direct comparisons between bloodstream populations could be made. Some characters were more valuable than others. For example, the length of the free flagellum, and therefore the total length of the

trypanosome was not a valuable character. Baker (1960) also made this point, but unfortunately presented T. mukasai as a dimorphic species on the basis of a total length frequency histogram.

In plotting body length frequencies, the presence of one or more populations can be deduced. For example, using the criterion of body length, one population of T. tincae, T. carassii, and T. percae was present. In each histogram the distribution was normal. This was further demonstrated in the probit analysis of the body length of T. tincae. Hoare (1956) separated stumpy, intermediate, and slender forms of T. evansi in this way. The body length frequency histogram of T. granulosum confirmed that the species is dimorphic. Unfortunately it was not demonstrated whether there were two independently dividing populations, as Breindl (1911) found with the dimorphic T. remaki from the pike. If so, interesting problems of speciation would have arisen. Also, it would be valuable to test the temperature dependence of the dimorphism. Barrow (1954) found that a newt trypanosome, T. diemyctyli, was dimorphic at lower temperatures, and monomorphic at higher temperatures. He offered no explanation.

The position of the nucleus was demonstrated as an important taxonomic criterion. Further experimental work is needed to confirm that the regression coefficient for the nuclear index plotted against the body length is statistically invariable for the species.

However, taking body length and nuclear indices, it was possible to give quantitative integrity to bloodstream trypanosome populations. Coupled with further gross morphological and cytological details,

species were defined. Further work on their biology may give the species further integrity. The complete absence of such work, however, need not necessarily disqualify a species description on bloodstream trypanosomes alone.

b) Comparisons with previous descriptions

Cryptobia from the tench.

The few Cryptobia Minchin (1909) observed in tench he differentiated into large and small forms, following Keysselitz's (1906) interpretation that they were gametes destined to copulate in the crop of the leech. Such differentiation in tench blood was found neither by myself nor other workers. Breindl (1911) considered the Cryptobia from carp and tench to be identical, with anterior free flagella of 16-18μ, and posterior free flagella of 8-10μ. Zalevskaya (1954) in Bykovskaya-Pavlovskaya, et al. (1962), repeated Minchin's (1909) drawings, and gave a body length of 12.1-21.3μ for tench Cryptobia. This was similar to my own range of 7.1-17.4μ. On the other hand they found the two free flagella were very short, measuring 4-5μ (anterior) and 2-3μ (Posterior). My measurements were 5.0-16.4μ and 5.9-10.9μ respectively. Their dimensions given as whole numbers make it likely that she encountered staining difficulties with the thin flagella which prevented accurate measuring. She found a body width of 4.1-7.5μ, favourably comparing with my measurements of 2.8-6.0μ. She found, similarly, that the nucleus was situated posteriorly. Khayboulajev (1969a) measuring again an unknown number of Cryptobia

from Caspian sea tench found a body length range of 16.6-19.4μ, with a breadth of 4.2-7.6μ. From the narrow body length range, it is clear he measured few Cryptobia. Whilst he was probably correct in naming the species C. borelli, there is little reason from the description he supplied to accept his naming of a new variety C. borelli n. forma tincae.

T. tincae

Laveran and Mesnil (1904) gave a total length of about 35μ in their original brief description of T. tincae from the tench. They also noted the blunt cone-like posterior end, the wide folds of the undulating membrane, and the relatively long free flagellum. These features apply also to the trypanosome I have found from the tench. Minchin (1909) enlarged on certain cytological details, noting numerous "coarse" granules, and a prominent endosome. Zalevskaya's (1954) measurements for the species were given in full by Bykovskaya-Pavlovskaya, et al. (1962). She found a body length range of 27-39μ. The body length range of the 400 T. tincae I measured was 10.1-33.3μ. As the dimensions she gave for all species were in excess of the dimensions given by other workers, a miscalculation is suspected. Khayboulayev (1969a) found a body length range of 12-21μ in an unspecified number of individuals, well within my own range of measurements.

T. carassii

Mitrophanow (1883) gave the total length of T. carassii as 30-40μ. Zalevskaya (1954) in Bykovskaya-Pavlovskaya, et al. (1962),

and Khayboulajev measured trypanosomes from the crucian carp. They provided recognisable descriptions of T. carassii. The body length range was 38·2-51·2μ and 24·1-30·1μ respectively. Certainly Khayboulajev's measurements fit the range I found of 11·5-38·1μ better. Again Zalevskaya's (1954) measurements were greater than and not even overlapping with mine. However, they both mention the dark blue cytoplasm amongst other cytological details. Thus the trypanosome from Trilake's crucian carp broadly fits previous descriptions for T. carassii for which further details have now been given.

T. percae

Brumpt's (1906b) original description of T. percae could apply to almost any trypanosome, although he did give the total length as 57μ. Minchin (1909) gave some cytological details for the species. He noted that the cytoplasm stained more densely in the broader forms, which occasionally exhibited longitudinal striations which he called myonemes. Unfortunately in his drawings it is geometrically impossible for them to be continuous down the length of the body as he suggested in the text. In my own preparations of T. percae, similar striations are interpreted as wrinkles in the flat broad body. Phase contrast observation showed them to be of transient nature. Baker, in a personal communication (1969) interpreted the myonemes of bird trypanosomes similarly. He could not detect them in electron micrographs (Baker and Bird, 1968). Breindl (1911), observing low numbers of T. percae, distinguished narrow and broad forms. My own work demonstrated a continuous gradation getween these forms, and that the species is not dimorphic.

Qadri (1952) also described the two forms, but he gave the body lengths of an unspecified number as 29.0-32.5μ, a surprisingly narrow range. Zalevskaya (1954) in Bykovskaya-Pavlovskaya, et al. (1962), and Khayboulajev (1969a) gave more complete descriptions of T. percae. They gave body length ranges of 46.0-61.4μ and 27.0-35.3μ respectively. Again the range from my own measurements of 17.7-36.6μ fits Khayboulajev's (1969a) observations better. Again Zalevskaya's (1954) measurements seem too high. Neither worker mentioned the characteristic crook-like posterior end. However, the species from Trilakes perch was clearly T. percae, and further details have been given.

T. leucisci

Zalevskaya (1954) in Bykovskaya-Pavlovskaya et al. (1962), measured trypanosomes from roach, calling them T. leucisci Brumpt, 1906 on the basis of occurrence only. Zalevskaya (1954) found the species to be dimorphic, with the body length range of the smaller variety 30-36μ, and that of the larger variety 42-46μ. Khayboulajev (1969a) measured few individuals from a single lightly infected roach, and found the body length range to be 26-33μ. He renamed the species T. shulmani n. forma leucisci for reasons not given. I found the body length range of three trypanosomes from the roach was 30.1-36.0μ. In the light of the low numbers observed, there is little point in naming the trypanosome from Trilakes roach anything other than T. leucisci.

T. granulosum

Minchin (1909) and Kraneveld and Keidel (1955) perpetuated the misconception that Lebailly (1906) described the magna and parva varieties of T. granulosum from fresh water eels. In fact Lebailly (1906) described the magna variety as a single monomorphic form having a great and continuous size range, and present only in freshwater eels. The parva variety was a smaller form from marine eels. Thus, when Kraneveld and Keidel (1955) recognised the dimorphic nature of T. granulosum from freshwater eels, they mistakenly believed that Lebailly (1906) had done so before them. Minchin (1909), Breindl (1911) and Zalevskaya (1954) in Bykovskaya-Pavlovskaya, et al. (1962), had previously described a continuous size range for T. granulosum in freshwater eels on the basis of a few trypanosomes. However, my own observations, like Kraneveld and Keidel's (1955), showed the freshwater species to be dimorphic. Most of the above previous workers briefly described the salient cytological details. Zalevskaya (1954) gave a body length range of 25-70μ. Accepting her tendency to exceed the top end of the normal range, her measurements are similar to the body length range I found of 23·3-56·1μ. The trypanosomes from the river Avon eels were undoubtedly T. granulosum, with a dimorphic character, for which further details have now been given.

Thus T. tincae, T. carassii, and T. percae from Trilakes tench, crucian carp, and perch, are demonstrated as separate discrete species. They show considerable differences from one another, and from

T. granulosum from river Avon eels.

c) The kinetoplast vacuole

An interesting detail of the morphology of fish trypanosomes is the vacuole associated with the kinetoplast. This was reported from fish trypanosomes by Robertson (1906), Splendore (1910), Breindl (1911), Yakimoff (1912), Fantham (1919), Pearse (1933), Qadri (1952), Becker (1967), and Ranque (1967). I have found it particularly evident in dividing T. tincae in the tench bloodstream. Ranque, in a personal communication (1967) demonstrated a structure he called the flagellum reservoir in electron micrographs of T. boissoni from the skate Zanobatus schoenleini. This he equated with a vacuole he saw under the light microscope. Preston (1969) described the cytopharynx of cultural forms of T. raiae. Unfortunately he did not take electron micrographs of the bloodstream forms from the skate. It is possible that this vacuole, seen under the light microscope associated with the kinetoplast in fish trypanosomes, is, in fact, the ~~cytopharynx~~ reservoir. It would therefore be involved in feeding (Brooker, 1965; Preston, 1969).

d) Host specificity of fish trypanosomes

I found that T. carassii, T. leucisci, T. percae, and T. granulosum, unlike T. tincae, did not infect tench. Only when given as a massive inoculum did T. tincae show in a crucian carp. The infection was never acute, and dividing forms were not seen. What few forms were observed resembled T. tincae rather than T. carassii.

Thus Laveran and Mesnil's (1904) observations on host specificity were confirmed. Unfortunately, they did not list the fish species they cross inoculated. However in a later work (Laveran and Mesnil, 1912), they stated that inoculations from carp to gudgeon, and gudgeon to goldfish always failed. They did succeed twice out of 23 in inoculating the carp trypanosome into the closely related goldfish. They gave no further details.

It is possible, then, that trypanosomes will pass between closely related fish, as I showed with the tench and crucian carp. Dogiel et al. (1961) mentioned that fish trypanosomes were less spcific when their hosts were young. They advanced a theory of a higher degree of resistance in adult fish. Their dubious reference to Dubinin (1952) has already been discussed. They may have had access to other work.

This still does not clarify the work of Robertson (1912), who transmitted trypanosomes from perch (order Perciformes) and bream (order Cypriniformes) to goldfish (order Cypriniformes) via H. marginata. Three explanations are possible. Firstly she was using previously infected goldfish, though this is unlikely with such a careful worker. Secondly, genuine transmission occurred, and the trypanosomes, all of the same species as she stated, developed in goldfish. This is unlikely in the light of my demonstration of the identities of T. percae from the perch, and T. carassii from the crucian carp, a fish closely related to goldfish. Also my own and Laveran and Mesnil's (1904) observations show that species of fish trypanosomes

show a close host restriction. Thirdly, it was possible that the trypanosome species entered goldfish, and did not develop fully. She gave no details of the course of infection of goldfish in her 1912 paper, but her personal communication of 1968 stated clearly that the numbers were too low in all fish to allow morphological examination of stained preparations. The trypanosomes from the different fish resembled one another in the fresh state.

Therefore, it is concluded that the bream and perch trypanosomes probably did enter goldfish. Because acute infections did not develop as both Brumpt (1905) and I found with trypanosome transfer by leeches, the parasites in fact retained their host specificity.

e) Division stages

T. tincae is exceptional amongst the fish trypanosomes in that dividing bloodstream forms were regularly found in low natural chronic infections. All the other reports of dividing fish trypanosomes to date were from natural or experimentally induced acute infections. Even these reports have been rare (see historical account). I did not find division stages in the acute T. granulosum infection, and in the acute infections of Cryptobia in tench. It is likely, therefore, with other fish trypanosomes, that division occurs in the tissues. The trypanosomes probably become so large that they are retained in the smaller capillaries, and divide there. The thesis of Qadri (1952) and Khayboulajev (1969b) that fish trypanosomes do not divide in their vertebrate hosts is rejected.

The division sequence of T. tincae in the bloodstream has been presented in detail because division of this type that has not been reported before. T. theileri also shows unequal binary fission, but it divides in the epimastigote form. Division forms of fish trypanosomes have previously been incompletely described, so comparisons are difficult. The coverslip observations of dividing T. granulosum by Sabrazes and Muratet (1904a, b) almost certainly represented the initial stages in the invertebrate. Laveran and Mesnil (1902a, b) described the cytoplasmic division of T. remaki as "sub equal", but gave no further details.

f) Course of infection

As found by Laveran and Mesnil (1902a, b) and Brumpt (1905), inoculation of trypanosomes into a fish gave rise to a short acute infection, followed by a long chronic phase. The brevity of this acute phase partly accounts for the rarity of acutely infected fish from the wild. The chronic phase of T. tincae infections was very persistent. This bears out Bray's (1967) statement that, typically, trypanosomiasis is a chronic or relatively chronic disease in its chief hosts, never leading to recovery. Incidentally no true relapses were ever seen in chronic infections observed for up to 18 months.

There are two possible explanations for the short acute phase in T. tincae infections. Firstly the trypanosome may be losing its reproductive ability. Yet dividing forms were seen in chronic infections. Secondly, there is an immune response resulting in the

reduction of trypanosome numbers. Yet infected leech challenge in the laboratory transformed the chronic infections to parasitemia levels comparable to the initial acute phase. This also probably happens in the wild. Table II shows that 97% of the 2 year old tench examined had patent trypanosome infections. Yet 10% of the tench of 2 years old and above examined during the summer months had acute infections. The majority of these must have been reinfections.

Thus some part of the immune reaction limiting the initial acute infection must be short-lived. This is suggestive of a humoral factor. Humoral antibodies produced by fish against bacterial infections were demonstrated by Smith (1940), Cushing (1942), and Bisset (1948). They also reviewed the extensive literature on the subject. Barrow (1955) attempted to demonstrate agglutinating antibodies produced by tench against their trypanosome infections. His work, however, is unconvincing.

In contrast Cryptobia infections were characteristically low grade, so Cryptobia undoubtedly has a slow reproductive rate. This low reproductive potential probably explains why syringe passaging from fish with low grade infections never gave rise to patent infections in the recipient fish. Laveran and Mesnil (1902a, b) with their syringe transfer of C. borelli between rudd found that heavy infections never developed. It is possible that the recipient rudd were already subpatently infected, the Cryptobia becoming patent under laboratory conditions.

More Cryptobia, then, were probably inoculated by H. marginata than by syringe passaging for the latter method never resulted in patent infections. Repeated feeding of infected H. marginata gave rise to a heavy infection of Cryptobia in an experimental tench. So the low grade immune reaction presumably able to counteract the usual low grade infections was ineffective.

Yet heavy Cryptobia infections were regularly reported from carp and tench in fish farms (see historical account), generally associated with epidemics of Piscicola geometra. In these cases, unlike the Trilakes tench, the Cryptobia infections were always heavier than the trypanosome infections. If, as Keysselitz (1906) states but does not prove, P. geometra is the intermediate host for the Cryptobia of carp and tench, these heavy Cryptobia infections could result from the characteristic feeding habits of P. geometra. For, unlike H. marginata, P. geometra remains on the same host, repeatedly taking blood meals, and therefore repeatedly inoculating Cryptobia.

g) Incidence of haemoflagellates in wild fish species

As mentioned in the historical account, previous authors have already reported variable incidences of trypanosomes according to the species of fish. Tench, crucian carp, and eel were reported up to 100% infected. The majority of perch were infected. Roach were rarely infected. A similar balance was reported for Cryptobia, except that it has never been observed in perch and eel. My own results support the above observations.

Two factors may be operating. Firstly the fish may have differing responses to the haemoflagellates. Roach, for example, may have infections that are nearly always subpatent. They may even be able to eliminate the parasites completely. The trypanosomes of tench, on the other hand, remain patent in chronic infections, at least during the summer months.

Secondly, certain species of fish may be more susceptible to leech attack than others. The guts of eels examined during their summer feeding activity contained a wide variety of shallow water food (Sinha and Jones, 1967). Opuszynski and Leszczynski (1967) found that leeches made up 15% of the food of freshwater eels. The defence mechanism used by H. marginata of attaching to the fish's mouth has already been mentioned. Couch (1887) reported that eels rested near the surface supported by weeds in shallow water during warm weather. Thus regular contact with H. marginata in the reed beds would be expected. Tench, perch, and crucian carp spawn in reeds (Balfour-Browne, 1906), and the young continue their early development there (Bracken and Kennedy, 1967). Roach, on the other hand, are the only mid-water and surface feeding members of the group (Hartley, 1947). In Trilakes they moved in shoals over the open lake, and were never caught in the traps situated in the reeds. Whilst their spawning habits are not well documented, it is fair to state that contact with H. marginata would only be rare.

h) Experimental haemoflagellate inoculations

At 20°C, the classical pattern resulting from leech or syringe

inoculations of trypanosomes was a short acute phase, followed by a chronic phase. The peak of the acute phase varied between 10^6 and 10^8 trypanosomes per ml. of blood, corresponding well with the levels in acutely infected fish from the wild. Varying the size of the inoculum shifted the peak forwards or backwards; it did not alter the height of the peak significantly. Similarly varying the size of the experimental tench had roughly the same effect. With a massive inoculum of 2×10^5 trypanosomes, the tench died.

Because of the comparative uniformity of the peaks, an immunological explanation is likely. It is possible that a given high antigen titre acts as a trigger to antibody production.

Laveran and Mesnil (1902a, b) with their experimental inoculations of low numbers of T. remaki into pike at room temperatures found the peak of the acute phase between 3 and 4 weeks after inoculation. This compares well with the peaks at 28 days with inoculations of 200 T. tincae at 20°C shown in graph 16.

At 10°C, T. tincae infections remained patent or subpatent; an acute phase did not develop. This temperature was clearly below the optimum for multiplication of the trypanosome. Qadri (1952, 1962c) found the optimum development in culture higher for the tropical fish species, T. striati, than for the temperate fish species, T. winchii. At low temperatures, both parasites developed more slowly than at high temperatures.

However, at 15°C, T. tincae developed slowly to an acute level

from which the fish never recovered. Barrow (1958) found a similar situation with newts inoculated with T. diemyctyli. At 10°C, the infection barely became patent, if at all. At 20°C and 25°C, the infection became heavy, and then was largely eliminated. At 15°C, the trypanosomes slowly developed to a heavy parasitemia, eventually killing the newts. He offered no explanation for this series of events.

It has long been known that environmental temperature will influence antibody production in cold blooded animals. Good and Papermaster (1964) reviewed the literature. Bisset (1946) found that the "antibacterial defences" of goldfish increased with temperature. At high temperatures they rapidly eliminated the increased number of bacteria. At low temperatures, however, the bacteria remained in low numbers, and were not eliminated.

In 1948, Bisset went on to show that frogs inoculated at 8°C did not produce serum agglutinins to Salmonella typhosa. When transferred without further inoculation to 20°C, they began to produce agglutinins. If inoculated at 20°C, the serum agglutinin titre was high. When transferred to 8°C the titre became very low, but was restored to the original high titre on returning to 20°C. He concluded that the aquisition of the potential for antibody production was distinct from antibody production itself. In poikilotherms, the second stage is more affected by temperature.

Kreuger and Twedt (1963) extended Bisset's work. They showed

that frogs inoculated with Salmonella typhosa at all temperatures responded by antibody production. The antibodies, however, were retained in the splenic red pulp cells at 4°C, but were released at 26°C. Therefore temperature acted on the release mechanism of such preformed antibodies.

Therefore, in tench at 20°C, the rapid development of T. tincae was probably countered by antibody release. At 10°C, the parasite was incapable of developing properly, so the lack of antibody did not matter. When removed from 10°C to 15°C, or when inoculated at 15°C, the parasite developed more slowly than at 20°C, but unchecked. Admittedly, tench barely fed at 15°C, and were therefore less able to replenish blood removed for sampling then at 20°C.

As these performances at different temperatures were so easily reproducable, it would not be difficult to investigate the fish's immunological response, and therefore prove or disprove this immunological interpretation of events.

The characteristics of chronic infections with temperature changes is also interesting. When removed from 20°C to 10°C, chronic infections became subpatent. In the one surviving fish that was taken back to 20°C again, the infection became patent. This was clearly a reflection of the ability of the trypanosome to develop at different temperatures. It also explains why, in all the fish examined from Trilakes, more patent infections were found in the summer months. The experiments in the concrete pond also showed how the patent infections rose and fell at a chronic level under the influence of

temperature.

In the historical account, I have shown that all workers reported heavier haemoflagellate incidences and infections during the warmer months. The work of Khayboulajev (1969b) requires comment. For, in an intensive survey of fish of the Caspian sea, he found a high degree of haemoflagellate infections in spring and early summer, and a decline towards autumn. Yet in Trilakes, and in the concrete tank, the peak of the infections was in June. The lowest infections were recorded in April, as shown in graph 9. However, in the Caspian sea, the winters are warmer, and the summers very hot, with mean water temperatures of 28°C and more (Bauer, personal communication, 1969). It may be that the high temperatures were beyond the optimum development temperatures of the haemoflagellates, as Qadri (1952) found with cultural forms. Clearly the Caspian winter is equivalent to the Trilake's spring, and the Caspian spring the Trilakes summer. Therefore Khayboulajev's results and mine are not conflicting.

These seasonal and temperature observations also show that just because no haemoflagellates are recovered, the fish is not necessarily negative, particularly if it is examined during the winter. With development of serological tests, this problem may be surmounted. Another method is to keep the fish at a higher temperature, and the haemoflagellates, if present, will almost certainly appear.

Finally, chronically infected tench transferred back again to 20°C do not develop acute infections. Yet when reinfected by

infected H. marginata, acute infections invariably result. Earlier a short lived humoral antibody system was postulated as reducing the original acute infection to a chronic level. It may be that the trypanosomes in the subsequent low grade infections are antigenically different from those in the acute phase, and do not stimulate production of the original antibodies. They may also have a lower capacity for reproduction than the original antigenic trypanosomes. Antibodies to the subsequent antigenic trypanosomes clearly prevent the trypanosomes from developing acutely. Thus when the leech presents the original antigenic type, the original short lived antibodies are no longer present. An acute infection, therefore, results.

Clearly fundamental problems of poikilotherm immunology have been raised which can only be elucidated by further experimentation. However, the advantages of this temperature controlled system in presenting the parasite in the tench to H. marginata in the early summer are of vital importance, and are discussed later.

II. THE CHARACTERISTICS OF FISH HAEMOFLAGELLATES IN THE INVERTEBRATE

a) The invertebrate host of Cryptobia

Brumpt (1905) found that Cryptobia from the blood of freshwater fish developed in both H. marginata and P. geometra. Like Robertson (1912) and myself, he successfully transmitted Cryptobia back to fish again using infected H. marginata. Nobody has convincingly infected fish with Cryptobia using infected P. geometra although Becker and

Katz (1965) successfully used the related Piscicola salmositica to transmit Cryptobia salmositica between fish. They comment, however, that other mechanisms must also be involved in the transmission of Cryptobia in epidemics in trout hatcheries where leeches were absent. Although they discount physical contact between the crowded fish, this could be the mechanism in the light of reports by Lingard (1904), Chen (1956b), and others, of Cryptobia living ectoparastically on the gills of fish.

b) Cycle of development of Cryptobia

It is not difficult to understand how Keysselitz (1906) arrived at his description and interpretation of the development of C. borelli in P. geometra which included sexual stages since unconfirmed. He encountered similar difficulties as I in observing the low numbers present in the leech in the early stages. Also he was influenced by the work of his compatriots Prowazek and Schaudinn who, he cites, in 1903 and 1904 respectively, described trypanosome life cycles in terms of sexual stages. Furthermore Léger (1904b) described the incomplete development of C. barbatula in H. marginata in similar terms. Ever since, the sexual cycle of C. borelli has remained unconfirmed.

My own results were similar to those of Brumpt (1905), Robertson (1912), Martin (1913), and Tanabe (1924), who described only the continuous multiplication of small comma-shaped forms. These produced elongated Cryptobia, which migrated to the proboscis sheath. Robertson (1912) found this migration occurred as early as the

sixth day after feeding. I observed a comparable time of five days. She reported that only exceptionally was H. marginata cleared of Cryptobia on taking a subsequent blood meal. My results showed that H. marginata maintained Cryptobia infections for as long as the crops contained blood meals. This contrasted with the cycle of development of T. tincae in H. marginata: Cryptobia had unlimited division in the leech while T. tincae only divided twice. Thus it is not surprising that from Trilakes the H. marginata which contained mixed Cryptobia and trypanosome infections, had considerably greater numbers of Cryptobia. In the tench examined, the situation was reversed.

c) Invertebrate host of trypanosomes

This was discussed at length in the historical account. H. marginata has been demonstrated as an intermediate host by myself and other authors. The only account of trypanosomes developing in P. geometra by Léger (1904a) is unconvincing. Brumpt (1906b) and Qadri (1952) stated uncompromisingly that the freshwater fish trypanosomes they studied did not develop in P. geometra.

d) Cycle of development of T. tincae

The restricted cycle of development consisting of only two divisions seems peculiar to T. tincae. Brumpt (1906a) followed the development of T. granulosum in H. marginata but did not describe the division sequence in detail. Neither did Robertson (1912), though she did suggest that an ever present residue of broad, nearly spherical trypanosomes maintained the trypanosome infection in H. marginata. I did not observe such forms in the developmental cycle of T. tincae.

They are characteristic, however, of marine fish trypanosomes developing in Pontobdella muricata (Neumann, 1909; Robertson, 1906, 1910; Preston, personal communication, 1968).

Tanabe (1924) stated that active division of the Japanese roach trypanosome in Hirudo nipponica resulted in the production of long slender forms after 3 days. Division then ceased, and the trypanosomes disappeared by the 14th day. This is similar to the T. tincae division cycle. Unfortunately he did not state how many divisions preceeded the metacyclic forms. In 3 days, only a few successive divisions could have occurred.

Qadri (1952, 1962a) studied the cycle of the carp trypanosome, T. danilewskyi, in H. marginata at an unspecified temperature. He stated that no dividing forms were seen in the leech from 7 days onwards. My results show that second division forms were still present on the 6th day at 15°C.

Therefore, unlike Robertson (1912), Tanabe (1924) and Qadri (1952, 1962a) found no stem cells maintaining the production of metacyclic forms.

In Qadri's (1952, 1962a) elaborate series of figures, he demonstrattd with T. danilewskyi the smaller 1st division forms and the larger 2nd division forms I have described in T. tincae. He did not make the point, however, that the trypanosome only divided twice before the metacyclic stage. Nobody has reported dividing metacyclic fish trypanosomes.

Brumpt (1906a) and Qadri (1952, 1962a) found that metacyclic forms appeared in the proboscis sheath after 5 days. T. tincae appeared in the proboscis sheath as metacyclic trypanosomes after 5 days at 20°C, and 7 days at 15°C. Tanabe (1924) did not describe trypanosomes in the proboscis sheath. Robertson (1912) stated that no trypanosomes were found in the proboscis sheath until after the disappearance of blood in the crop. She did not elaborate on this. I found that the appearance of trypanosomes in the sheath corresponded roughly with the disappearance of intact red blood cells in the crop. However, she probably meant the disappearance of red or brown crop contents, remarking that flagellates took as long as 30-35 days to appear in the sheath in some leeches. These observations are contrary to those of Brumpt, Qadri and myself. I found clearly that the T. tincae infection was lost when the blood meal was digested. The metacyclic foms had reached the sheath a long time before this. Conversely Robertson supported this observation by stating in the same paper that H. marginata lost its T. remaki infections with the complete digestion of the blood meal after 10 days, at 25°C. Different species of fresh water fish trypanosomes clearly behave differently in this respect.

Qadri (1952) stated that the metacyclic trypanosomes of T. danilewskyi migrated to the proboscis sheath because they were unable to migrate backwards to the intestine. Yet Brumpt (1906a) found a major part of the development of T. granulosum took place in the

intestine, a feature shared with the marine trypanosome, T. raiae (Preston, personal communication, 1968). However Brumpt (1906b) went on to state that T. granulosum was peculiar in this respect. Of the other freshwater fish trypanosomes he studied, none developed in the intestine. Robertson (1912), Tanabe (1924), Qadri (1952, 1962a), and I also found no trypanosomes in the intestine. Thus the ability of T. granulosum to survive in the intestine indicates an affinity with marine fish trypanosomes. After all its host, the eel, is found in both fresh and salt water.

Most fish trypanosomes may well be digested in the intestine. Jennings and van der Lande (1967) found powerful digestive enzymes in the intestine, but not the crop, of H. marginata. Metacyclic forms may pass through both anterior and posterior apertures of the crop, but are only found intact in the proboscis sheath. There need not be any migratory stimulus at all. Their great powers of locomotion randomly directed would eventually take them up the lumen of the proboscis into the sheath.

The developmental cycles of trypanosomes of freshwater fish are quite characteristic. They differ from trypanosomes of marine fish in the factors already mentioned. They also differ from trypanosomes of amphibians and aquatic reptiles which develop in leeches. In these types, long slender metacyclic forms have not been described. Instead, forms resembling bloodstream trypanosomes develop (Robertson, 1909; Nöller, 1913; Franca, 1915; Barrow, 1953; Lehmann, 1952, 1958;

and Diamond, 1965). Also like the trypanosomes of marine fish, there is a stock of small, rounded, or even amastigote forms multiplying by binary or multiple fission which i turn give rise to the metacyclic forms. It is possible, therefore, to postulate an affinity between trypanosomes of marine fish, of amphibians, and of reptiles that develop in leeches. The trypanosomes of freshwater fish thus far described could be an isolated group on their own.

There is one significant similarity between trypanosomes of freshwater fish and, T. diemyctycli from a newt. Barrow (1953) found that when the leeches, Batrachobdella picta digested their meals of amphibian blood, they lost their trypanosomes.

III. THE LIFE CYCLE AND HABITS OF H. marginata

Harding (1910) stated that H. marginata was distributed throughout the greater part of Europe. Bykovskaya-Pavlovskaya, et al. (1962), extended the range to parts of Asia. At a local level Mann and Watson (1954) stated that it was common in the hard water lakes and ponds in Berkshire, thriving in smaller water bodies and was widespread in Great Britain. It was, nonetheless difficult to find. Wilkialis (1964) reported that H. marginata occurred in proportions of 0·75%-2% of all leeches in Polish ponds, and also (Wilkialis, 1968) in reed beds in the middle and lower reaches of rivers. Mann (1955, 1962) was more specific. He did not find it in soft standing water and fast running water, the characteristic habitats of P. geometra.

This would explain the rarity of reports of trypanosomes in the Salmonids which occupy these two habitats.

Bennike (1943) found H. marginata only in the bigger sheath bearing swamp plants, as was my experience. Herter (1936) similarly found the leech between "plant parts" near the shore. In a series of experiments he demonstrated that H. marginata was photo-negative, becoming photopositive only when hungry.

H. marginata was described as a fish parasite by all the above workers. Its life cycle, however has never been described.

Whitman (1878) and Brumpt (1900) were the first to breed the leech in the laboratory. Herter (1936) found leeches with young in Germany between May and mid-August. In Denmark, Bennike (1943) found them in June and July, stating like Harant and Grassé (1959), that the minimum breeding temperature was 16°C. Warwick and Mann (1960) found breeding H. marginata in Scotland in July, the only British record.

I found breeding to occur at a minimum of 15°C in the laboratory. It occurred naturally from March to July in the Basingstoke canal. On the basis of the canal samples and experimental observations, an annual life cycle for H. marginata is proposed, and outlined in figure 51.

The eggs are laid in late spring and early summer when the water temperature rises to 15°C or more. The eggs and subsequent offspring are brooded by the adults in the protective shelter of the

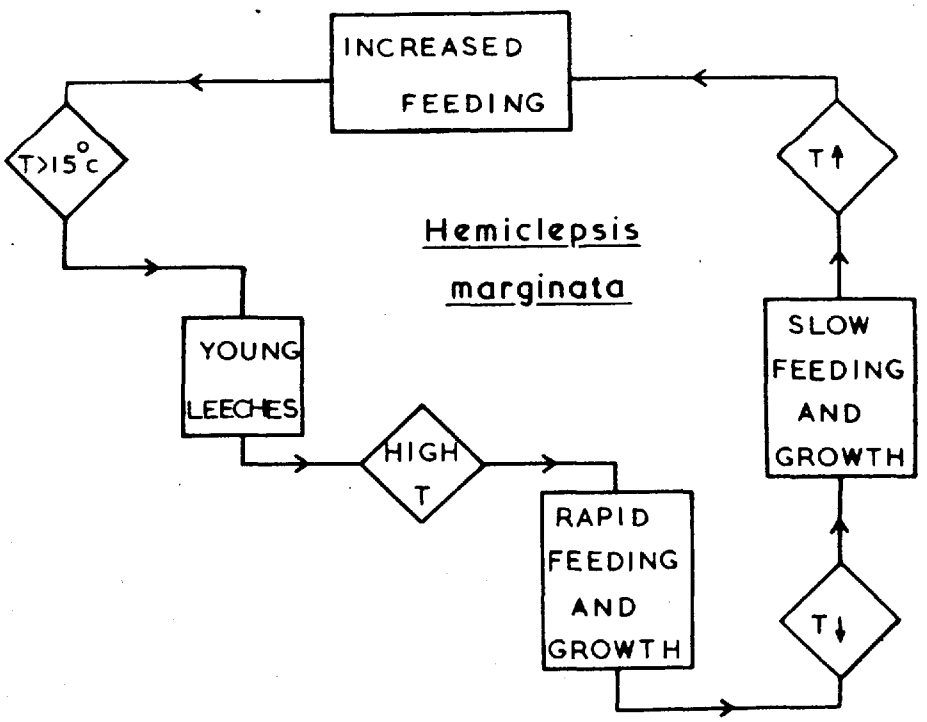


FIG. 51. Life cycle of H. marginata.

leaf-bases of the reeds. After hatching, the "mother" exhibits a hunger response, moves out onto the surface of the reed stem, and attaches to a fish. The offspring leave the mother, scatter all over the fish, and drop off at varying intervals after feeding. The adult leech dies, the young return to the shelter of the reeds and, during the summer, a maximal period of feeding and growth occurs. During the winter, feeding is reduced and may even cease, the leeches being capable of surviving starvation for considerable periods. However, as I have shown, this is a capacity of the larger leeches only. Also in the winter, their shallow water habitat makes them vulnerable to ice, from which they cannot survive (Herter, 1936). In the spring, activity is increased again prior to breeding.

IV. THE LIFE CYCLE AND HABITS OF THE TENCH

A proposed annual behavioural pattern for 2 year old and older tench deduced from my own and other workers observations is illustrated in figure 52.

Jenkins (1936) reported that tench wintered in bottom mud. There they entered a state of dormancy, and were gradually covered by rotting debris as it fell to the bottom (Steck and Franz, 1914). I found they left this habitat during May, when they first entered traps situated in the reeds of Trilakes. Berg (1964), Bracken and Kennedy (1967), Rosa (1958), and Varley (1967), stated that spawning occurs between May and August at a minimum water temperature of 18°C. The eggs were attached to weeds in shallow water, particularly amongst reeds of

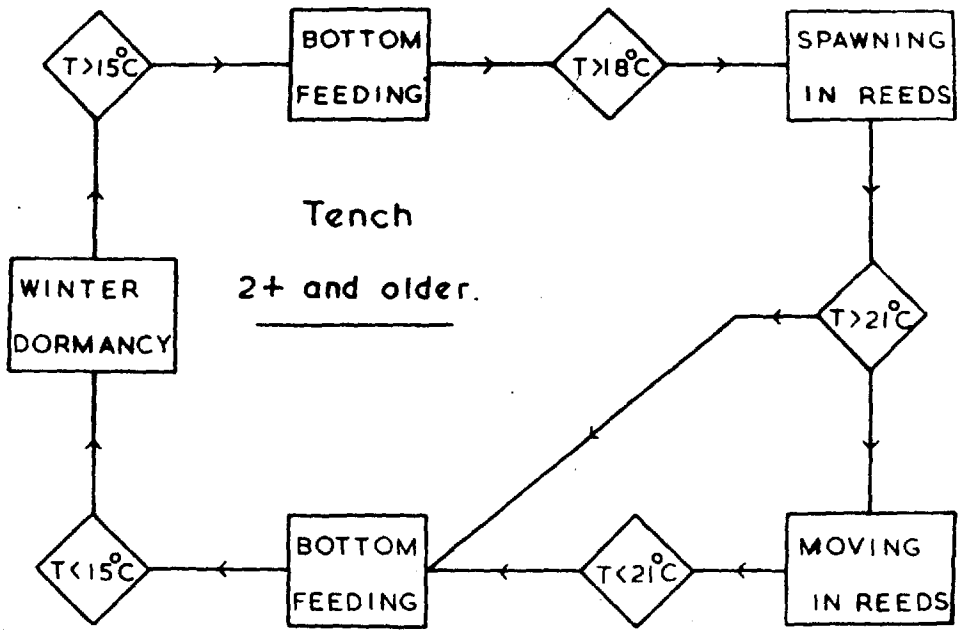


FIG. 52. Annual behaviour of mature tench.

Scirpus spp. (Bracken and Kennedy, 1967). There the tench clean themselves after spawning (Walker, 1955). During warm summer weather they tend to feed in shallow water amongst the marginal reeds (Stech and Franz, 1914; Walker, 1962; Taylor, 1967). For the rest of the summer and early autumn they feed on the bottom. This ceases in late September when the water temperature never rises above 15°C (Rosa, 1958). At this time they enter the dormancy period.

Thus the only time when these older tench and H. marginata can make contact is during spawning, and in subsequent warm summer weather. A possible contact situation may arise when dead or dying tench float to the surface, and are blown by the wind into the marginal reeds. It is interesting to recall that H. marginata became infected with T. tincae from tench that had been dead for up to 48 hours.

Little is known about the habits of tench during their first year. Varley (1967) stated that the fry stage fed on diatoms and zooplankton amongst marginal vegetation all through the first summer following hatching. Tench fry collected with a plankton net from Trilakes were undoubtedly doing the same. After this their habits become a mystery until the following summer. Then, 1 year old tench, by now up to 8 cms in length (Schindler, 1957), can be captured from amongst weed and rushes in shallow water with a sweep net (Marlborough and Wheeler, personal communications, 1968). Such tench, however, are never caught by anglers (Brotherton, 1954;

Torbett, 1961). They must either be feeding in shallow water, or on very small food, or both. Weatherly (1959), examined gut contents of tench of varying sizes. Those up to 10 cms were in their first full summer. Their guts contained small crustaceans, insect nymphs, and larvae, and the occasional small molluscs. These are all characteristic of shallow water. The guts of larger tench contained predominantly larger molluscs and annelids, indicating a bottom living habit. In 1961 he found that large tench aggregated in the shade in daylight. Small tench, in contrast, dispersed in an independent fashion in shallow water illuminated by strong sunlight. Large tench only behaved like this when spawning.

On the basis of the above work, a tentative behavioural pattern for young tench is proposed in figure 53. The winter dormancy is assumed because in this country tench are near the northern limits of their distribution (Berg, 1964).

V. EPIZOOTIOLOGY OF *Trypanosoma tincae*

A hypothetical scheme for the transfer of *T. tincae* between tench and *H. marginata* is presented in figure 54. The black arrows represent the precise points in the respective cycles that transfer occurs.

I have confirmed Brumpt's (1904) observations that trypanosome infections were not transmitted between parent leech and its offspring. Therefore, the young leeches produced in early summer are uninfected.

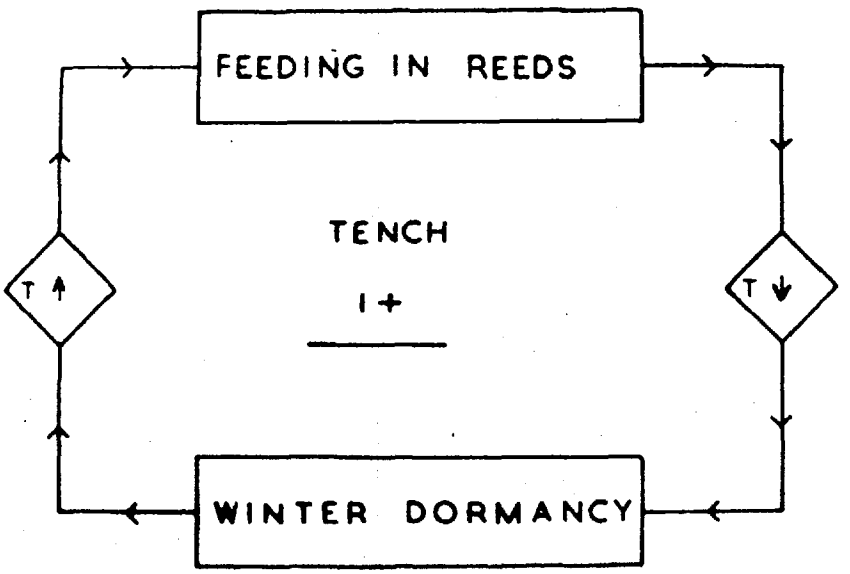
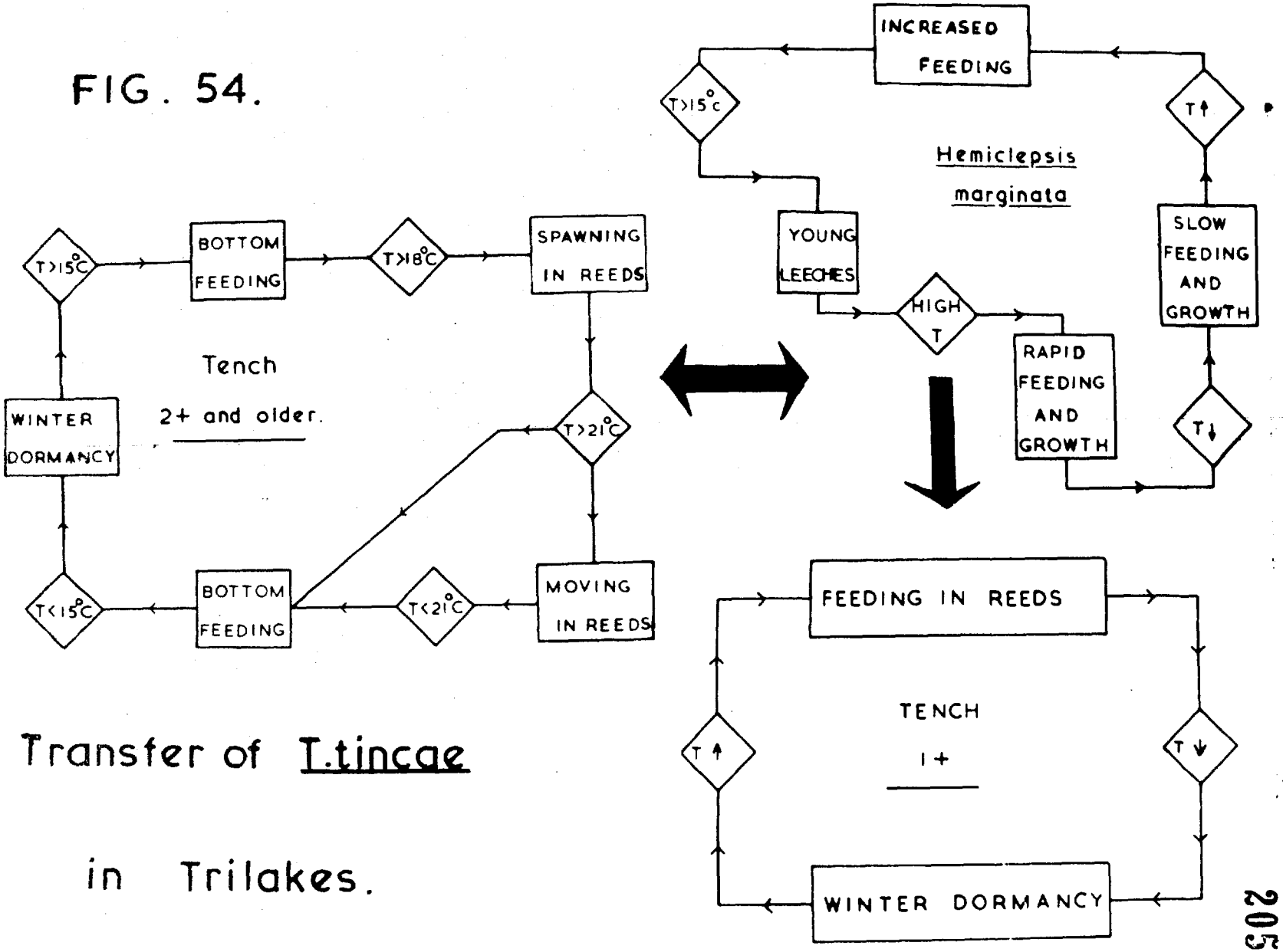


FIG. 53. Annual behaviour of 1+ tench.

FIG. 54.



Transfer of T.tincae

in Trilakes.

Breeding of H. marginata occurs at a minimum temperature of 15°C, spawning of tench at a minimum of 18°C. Therefore, the young leeches would be already hatched by the time the tench leave the bottom of the pond to spawn in reeds where the leeches live. The young leeches are carried by their "mother" onto the tench which have patent chronic infections of T. tincae. The infections became patent with the onset of warmer weather. The young leeches become infected. They can then superinfect a proportion of later spawners, and mature tench that remain in the shallows during hot weather. These fish would become acutely infected for a short period, and were represented by the 10% acutely infected tench collected from Trilakes during the summer. I have already shown that the majority of these acutely infected tench had heavier Cryptobia infections thatn the fish chronically infected with T. tincae. Cryptobia transfer, therefore, would also take place in this way.

I have also shown that 97% of the 2 year old tench examined from Trilakes had patent T. tincae infections. They could not have been infected as fry, because feeding by even the smallest H. marginata killed them. All fry examined were in any case uninfected. Therefore tench must gain their infections as 1 year old fish. I have postulated that, during the summer, they feed in shallow water. There they are open to attack by the young H. marginata infected with T. tincae. The course of infection undoubtedly follows that described in experimental tench, of between 1 and 2 years old.

The only other attempt to elucidate the epizootiology of fish haemoflagellates was by Khayboulajev (1969b). He only observed reproduction of fish haemoflagellates in leeches during the winter. Accordingly he was led to believe that the flagellates were transferred to the Caspian sea fish in spring, and back again to the leeches in autumn. The fish were clear of parasites, therefore during the winter. Recourse to his thesis (Khayboulajev, 1969a) showed that he was dealing with Piscicola geometra. He had no experimental evidence that P. geometra transmitted either trypanosomes of fish or Cryptobia. Furthermore the photographs in his thesis demonstrating the flagellates in P. geometra more closely resembled leech spermatozoa and the cyst-like bodies he described (1969b) as containing the flagellates looked like spermatophores.

Therefore, I reject Khayboulajev's interpretations. I prefer to believe, in the light of my own evidence, that freshwater fish trypanosomes exist primarily in their vertebrate host, with only transitory development in their invertebrate host, H. marginata.

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