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A study of the morphology, taxonomy and life history of trematodes of the genus *Phyllodistomum*, with notes on the incidence of other parasites of sticklebacks

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A STUDY OF THE MORPHOLOGY,
TAXONOMY AND LIFE HISTORY OF TREMATODES OF
THE GENUS PHYLLODISTOMUM, WITH NOTES ON THE
INCIDENCE OF OTHER PARASITES OF STICKLEBACKS.

A Thesis presented for the Ph.D. degree

of the University of

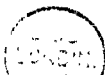
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ABSTRACT

Eighty-eight species have been directly assigned to the genus Phyllodistomum Braun, 1899 (Trematoda : Gorgoderidae). Many criteria utilised in species separation possess doubtful validity and some authors even consider that the characters separating Phyllodistomum from the genus Gorgoderina do not justify generic distinction.

In this investigation Phyllodistome specimens were examined in order to determine the extent of variation in a single species. The egg production rates of Trematodes recovered from Sticklebacks in Southern England are studied at varying temperatures both in vivo and under experimental conditions. The sizes of the eggs produced by Flukes of different ages are determined. The miracidium's reactions to the primary intermediate host (Sphaerium corneum) are recorded and the structure of the following larval phases described. The metacercariae are recovered from Insect nymphs and larvae.

The population density of Phyllodistomes collected from Fish in the sampling areas is compared on a seasonal basis. The effects of these flukes upon the host are discussed and the interrelationships between Phyllodistomum and Myxosporidia inhabiting the urinary system are reviewed. The incidence of other parasites recovered during the examination of the Fish are recorded. The closely similar life cycle of a Gorgodera sp. is experimentally established and each phase compared with the equivalent stage of Phyllodistomum. Differing growth rates but identical sucker papillary patterns were noted.

The value of taxonomic criteria utilised in this Family is discussed. The records indicate that the species studied in this

investigation differs from the only other detailed British record for a Phyllodistomum adult (P. simile(Thomas, 1958)). In view of the lack of information concerning the proportions attained by Phyllo-distomes in relation to their age; the extent of specific variation and host effects, this species can only be tentatively assigned to P. folium as recorded by Pigulevsky (1953).

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IN

ORIGINAL

Braun(1899)created the genus Phyllodistomum with Distomum folium v. Olfers, 1816 as the type species. Later Looss created the genus Spathidium for this same group, also placing D. folium v. Olfers, 1816 as the type species. By the law of priority the valid generic name became Phyllodistomum Braun, 1899. Looss(1901)created the Family Gorgoderidae and divided it into two subfamilies - the Gorgoderinae and Anaporrhutinae. At this time he placed only Gorgodera and Phyllodistomum in the former subfamily but in 1902 he also incorporated and created the genus Gorgoderina and Catoptroides, crediting the latter to Odhner(1902).

Following this early work several genera have been erected which have subsequently been considered as synonymous with Phyllodistomum. Yamaguti(1958)placed the following genera in this category: Catoptroides Odhner, 1902; Microlecithus Ozaki, 1926; Dendrorchis Travassos, 1926; Phyllochorus Dayal, 1938; Plesiodistomum Dayal, 1949 and Vitellarinus Zmeev, 1936. Previously Pigulevsky(1953)had suggested a new scheme of classification relegating Catoptroides Odhner, 1902; Phyllodistomum Braun, 1899; Vitellarinus Zmeev, 1936 and Microlecithus Ozaki, 1926 to the level of subgenera in the genus Phyllodistomum Braun, 1899. Slusarski(1958)however considered Pigulevsky's classification to be unsatisfactory and reflected Yamaguti's scheme in omitting the use of subgenera. He added to the list of synonyms of Phyllodistomum - Spathidium Looss, 1899 (which was omitted by Yamaguti(1958)) and Gorgotrema Dayal, 1938 p.p..

Pande(1937) and Kaw(1950) regarded Gorgoderina Looss, 1902 as a synonym of the more comprehensive genus Phyllodistomum.

The characters used to differentiate the genera Phyllodistomum, Gorgoderina and Gorgodera are basically associated with the body form and structure of the reproductive system particularly the testes. In the two former genera there are two testes whilst, in the latter, these structures are divided into nine lobes. Gorgotrema appears to be an interesting intermediate form possessing subdivided testes which are almost folliculate in structure. The Gorgoderinae are parasites of the urinary systems of either Fish or Amphibia and in the course of their evolution have given rise to two basic types of bodily construction. Ideally the Phyllodistomum genus, mainly parasitising Fish but also found in some Amphibia, attains a dorso-ventrally flattened form which may be posteriorly expanded to give a spatulate outline. Gorgoderina and Gorgodera are mainly parasites of Amphibia which have extended to produce an almost cylindrical characteristically lanceolate body. Gorgotrema parasitising Fish, possesses a spatulate body form equivalent to that described for Phyllodistomum.

The taxonomic chaos which is found in the literature dealing with this subfamily, existing particularly between Gorgoderina and Phyllodistomum, is primarily the result of two factors. The character involving the body form is not entirely satisfactory because intermediate stages are frequently encountered and despite the large numbers of species which have been described, the knowledge concerning the true value of most of the taxonomic criteria utilised is extremely poor. Few

life cycles have been experimentally established and there are a large number of cercarial forms described as associated with the family merely on the 'evidence' that the reproductive system at this stage possesses a comparative arrangement to that shown in some sexually mature Gorgoderids. This has resulted in Rhopalocercariae being included in a family which is also said to contain both Microcercous and Cystocercous (Macrocerous) cercarial types. The only life cycles which have been experimentally proven have involved the latter two cercarial groups. Early workers (Lühe (1901), Odhner (1911) and Nyebelin (1926) among them) attempted to link adult and larval forms purely on their structural similarity. The quality of the descriptions of cercarial forms has varied and has resulted, particularly in the case of some of the early work, in a series of subsequent attempted synonymies by various authors and the division of the original data under different cercarial names. Detailed historical surveys, discussions upon the validity of taxonomic criteria and numerous generic and specific keys may be found in abundance in the literature, adequately illustrating the difficulties which authors have, and still are experiencing in this field. Articles particularly associated with Phyllodistomum are written by Travassos (1922); Nyebelin (1926); Lewis (1935); Lynch (1936); Bhalerao, (1937); Byrd et al. (1940); Goodchild, (1943); Dawes, (1946); Kaw, (1950); Pigulevsky, (1953); Jaiswal (1957) and Yamaguti (1958). Authors who have dealt principally with cercarial types and their taxonomic position are Nyebelin, (1926); Miller, (1936); Goodchild, (1943); Fischthal, (1951); Coil, (1954); Thomas, (1958) and Rai (1964).

Since the creation of the genus Phyllodistomum 88 species have

been directly placed into this grouping giving the genus world wide distribution. Of these, many are considered to be of doubtful validity and have been reduced to the status of synonyms by subsequent authors. P. catostomi, P. cotti and P. umbrae were erected by Wu (1938) but apparently were never described. P. longicollis, referred to on page 206 of the book by Dogiel et al (1961) and in the index as a Phyllodistomum species, is apparently a misprint referring in the text to Proteocephalus longicollis. Kaw (1950) considered that in addition to these Phyllodistome species all those described for Gorgoderina should be added to the list.

It was the purpose of the present investigation to examine variation exhibited by populations of Phyllodistomum of a single species in order to check the validity of basic taxonomic criteria. A study on the extent of a single species variation had only been attempted previously by Groves (1945) with 25 specimens of P. solidum; Coil (1955) on 103 specimens of P.(C.) lacustre and Tonn (1961) with 25 P. bufonis. Phyllodistomes in this study were recovered from Sticklebacks in collection areas associated with the Thames drainage. These fish were readily available throughout the year, were practically ubiquitous and thrived under laboratory conditions. The population of trematodes from each area was measured and studied separately in the first instance to establish whether a single species was being examined. Analysis of the results indicated that this was the case and the degree of variation in a single British species could then be described in detail for the first time. Very little information concerning the living fluke or the effects of fixatives and dehydration

upon its measurements existed although species comparisons were readily made between material which had been treated in a variety of ways. Differential growth rates, although probably of specific significance, had not been studied to any extent in this genus and the effects of different host species and crowding had remained totally uninvestigated. In this work an experimentally established growth-series could not be obtained despite repeated attempts to infect the definitive host with large numbers of flukes. Studies were based upon mixed-age populations recovered from nature which introduced so many variables that statistics would not be usefully applied on a collection where the utilisation of a standard fixation technique had not eliminated contractile variation.

During this investigation another Gorgoderid was recovered which provided a useful point of comparison with the Phyllodistomum species. It was found to belong to the Gorgodera genus and a detailed comparison of the two trematodes at all phases of their life cycle strongly emphasised the differing growth rates and proportional changes which were taking place. It was during this comparison that it was discovered that the sucker papillary patterns in the two flukes were identical. In recent years it has been thought that the papillae might provide a recognisable specific character when so many other morphological features had failed to do so. The present discovery, however, indicated that this was unlikely. The life cycles of the two trematodes were also similar, involving Sphaeriid and Insect intermediary hosts (which in the latter case were sometimes identical). The two parasites diverged sharply at definitive host level when Gorgodera successfully

entered Amphibia and Phyllodistomum became established in Fish.

The interrelationships between the urinary flukes and the general condition of the host, especially when it is heavily infected by other parasites, is another feature which has not received much attention in the literature. Their unusual location generally shields them from direct competition with most parasites, whilst a noticeable exception occurs in the Myxosporidia. Nyebelín (1926) noted an antagonistic relationship between Myxidium lieberkuhni infesting the urinary system of Pike and a species which he referred to as P. folium. Sticklebacks are prone to a large number of infections and act as carriers in other fish populations in some cases. They proved to be ideal material for the study of dense infections of Phyllodistomum and many other parasitic associations of varying intensity. The location of the Phyllodistomes within the urinary system would appear at first sight to represent a probable centre for marked pathological reaction on the part of the host. It is surprising to find that in dense infections reaching from 72 - 92 per Stickleback the pathological picture is so restricted. This is probably the reason why this factor has largely been overlooked in the literature and was only briefly mentioned for Phyllodistomum species by Choquette (1947) and Thomas (1958).

Despite such a large species list for this genus, few species have been recorded from the British Isles. The situation has been confused by several authors assuming that fish species identical with those found in Europe will carry identical parasites when recovered in the British Isles. This has resulted in the production of species lists

for this country including Phyllodistomes which have not been obtained from this area to date. The only detailed description which has been given for an adult British form was reported by Thomas (1958) who obtained P. simile from Salmo trutta in Wales. A comparison with Thomas's findings is made throughout this investigation at all stages of the life cycle, but principally in order to check the possibility that the obvious size difference of the adult trematodes was not simply a reflection of a discrepancy in the sizes of the respective definitive hosts.

The report is arranged so that the life cycle of the trematode can be followed from the time of egg development to the adult egg-laying fluke. For the first time a life cycle involving an insect intermediary host has been described for Great Britain. The species differs from P. simile at all stages, but records concerning the adult phases particularly require careful interpretation to demonstrate the underlying differences which are of specific value. In the last Section (10) an evaluation of all the characters which are used to separate both genera and species in the Gorgoderinae is made. From the results obtained during this study it appears that considerably more work will have to be carried out in this field before the taxonomic situation can be successfully clarified. Most morphological characters possess little specific value when taken either singly or in conjunction with other factors. In only one case (Pigulevsky (1953)) has an author attempted systematically to define trematodes according to the proportions attained at different ages. This type of approach would

appear to be essential if morphological distinctions are to be retained as a basis for species separation. Life cycle studies will undoubtedly aid the process of identification amongst species which, by means of parallel or convergent evolution, have become so closely similar in the adult phases.

SECTION 1 THE EGG

a) Method of investigation.

The development of the miracidium was studied in utero on living material. The most useful intra-vital stain was found to be neutral red.

b) The development and structure of the egg.

Immediately prior to their release, the oocytes lie clustered around the origin of the oviduct which is commonly situated close to the centre of the ovary. They vary from a spherical to an ovoid form and possess an average maximum diameter of 0.0204 mm. Laterally, within each egg cell, there lies an elongate, basophilic body which may represent a food reserve. When mounted, the oocytes shrink to an average size of 0.0153 mm. in diameter, which is similar to that recorded by Willey and Koulish (1950) for mounted material obtained from Gorgoderina attenuata Stafford.

Following their release from the ovary, the oocytes enter a spherical expansion of the oviduct which may possibly function as an ovicapt. (The female reproductive system is illustrated on page 19 diagram 1). They then pass into a larger, contractile, tapered chamber, the receptaculum seminis. It is here that spermatozoa are primarily concentrated, although they also occur sporadically throughout the length of the uterus. Laurer's canal arises from the oviduct immediately following the receptaculum. It is a short duct which curves slightly to open via a small ovoid pore immediately dorsal to either the right or left vitelline gland. The siting of the opening

18

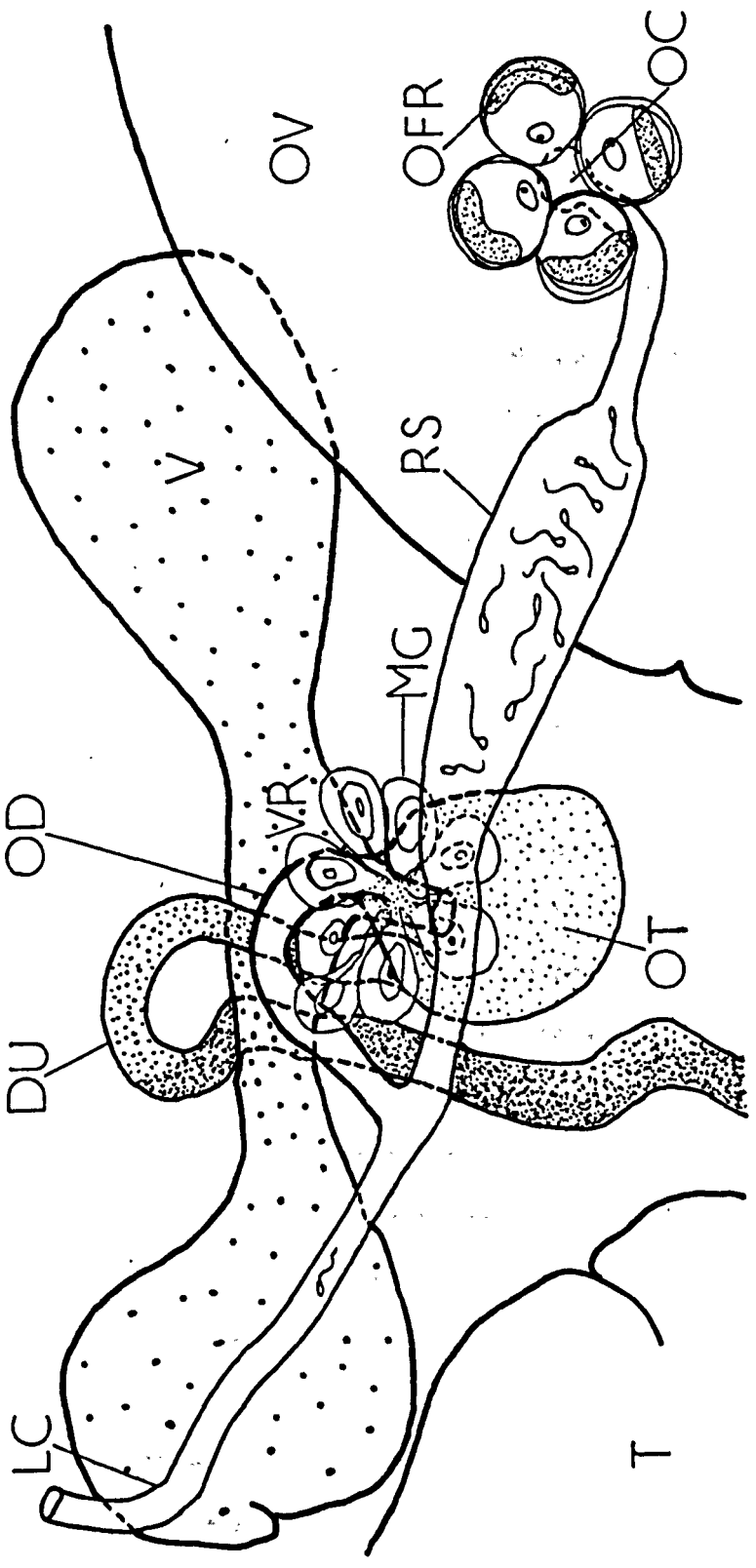
DETAILS OF THE FEMALE REPRODUCTIVE SYSTEM IN THE REGION OF OÖTYPE
(Dorsal View).

In this diagrammatic representation the majority of the ducts, the vitelline glands and their associated channels are drawn devoid of content and the ascending uterine limb is omitted for the sake of clarity.

Abbreviations:

| | | |
|-----|---|----------------------------|
| DU | = | descending uterine limb |
| LC | = | Laurer's canal |
| MG | = | Mehlis' gland |
| OC | = | ovicapt |
| OD | = | oviduct |
| OFR | = | food reserve of the oöcyte |
| OT | = | oötype |
| OV | = | ovary |
| RS | = | receptaculum seminis |
| T | = | testis |
| V | = | vitelline gland |
| VR | = | vitelline reservoir |

(Information for this diagram was obtained from both living and mounted specimens).



PHYLLODISTOMUM - REPRODUCTIVE SYSTEM. ①

bears no relation to the position occupied by the amphitypic ovary. The act of copulation was never observed so it cannot be definitely stated whether Laurer's canal or the genital atrium are the main sites for sperm transference (if, in fact, this act takes place at all.) In heavy infections, particularly in the hosts' ureters, flukes were found crawling over one another in positions which would have facilitated transference at either point. Self-fertilisation however is obviously a common feature since hosts infected by a single trematode containing fertile eggs were regularly recovered. In such cases, bearing in mind the lack of a cirrus or penis, the most probable copulatory site would be the genital atrium.

Sperm entry probably occurs prior to the egg cells' passage into the oötypic area which is a terminal, thin-walled expansion of the oviduct. Upon reaching this chamber, each ovum becomes surrounded by several vitelline cells. These are shed from the lateral vitelline glands and enter the oötype ventrally via the common vitelline duct. Each vitelline or 'yolk' cell is approximately spherical and the living cells have a diameter range of 0.00816 to 0.0102 mm. Their cytoplasm is packed with refractile globules which presumably represent the shell forming substance and these give a faint acidophilic reaction to neutral red. Interspersed between the differently sized globules are strongly basophilic droplets of unknown function which are sporadic in appearance and only a few ever occupy any one cell. Some of the vitelline cells begin to release their inclusions into the vitelline ducts so that intact cells and free, floating globules enter the oötypic chamber.

Associated with this chamber is a small cluster of dorsally situated, pale-staining, unicellular gland cells, collectively termed Mehlis' gland. Each of these cells produces minute quantities of a transparent fluid which passes into the oötype. It is under the influence of this secretion that initial shell formation takes place. This occurs normally around a fertilised (or occasionally unfertilised) ovum, together with an associated cluster of vitelline cells. After a short interval, following considerable cell movement in the oötype, a smooth transparent, slightly yellow, shell membrane is laid down around the cell group. Occasionally, however, in the absence of ova and free globules, vitelline cells were seen to pass intact through the oötype. If the function of Mehlis' gland secretion included that of stimulating the further release of shell material, then this latter occurrence would indicate that Mehlis' secretion is intermittent and regulated to coincide only with the variable ovarian production rhythms (which are discussed further on page 270-). The fact that no free, unenclosed ova were ever found in the uterus would indicate that some ovarian-glandular regulatory mechanism does exist. However, shell formation frequently takes place in the absence of ova and membranes are produced surrounding various numbers of vitelline cells, both during periods of ovarian activity and inactivity. This suggests that Mehlis' secretion may be of longer duration and that its function is merely confined to the conversion of released globules of shell material in the oötype and not to stimulate the releasing mechanisms of the vitelline cells. A probable explanation for these phenomena lies in the fact that the actual stimulus for Mehlis' secretion is

likely to be of both a chemical and mechanical nature, the latter involving the muscular activity of the oötype in particular. Exact co-ordination is thus achieved with the ovary, but the secretion is less closely associated with vitelline production. The very few vitelline cells which enter the uterus unenclosed remain in this state and their remnants are eventually passed out via the genital pore. Most of the globules found floating in the uterine fluid are a product of these cells.

Willey and Koulish (1950), when working upon Gorgoderina attenuata Stafford, found that acidophilic globules observed within the vitelline cells disappeared following shell formation. No similar visible disappearance of content could be noted in this case since large quantities of refractile material remain in the vitelline cells after their encapsulation. As miracidial development proceeds, the quantity of this material gradually decreases. Approximately at the time of the formation of a recognisable larval structure, the remaining vitelline cells lose their identity and fragments of cytoplasm containing globules of secretion are liberated to float freely in the fluid-filled space between the larva and the shell. The cytoplasm and the nuclei of the vitelline cells eventually disappear but some of the globules remain failing to coalesce. At the time of hatching, therefore, the only recognisable remains of the original vitelline material are represented by a minute cluster of hyaline droplets which are left behind in the shell following miracidial release. The cytoplasmic remains are probably absorbed by the developing larva and used as an additional source of protein and perhaps some glycogen. It is unlikely that

the refractile material in the vitelline cells merely represents a form of 'yolk' and that the cells secrete an undetected, colourless shell precursor. The fact that a small amount of vitelline material is always discarded upon the larva's release suggests that it has no nutritive value. Also, the globules are never taken into the gut of the fully formed miracidium, an action which would be expected to have taken place if either structures performed a nutritive function. The retention of such large quantities of shell-forming material apparently allows for continual addition to the shell from the vitelline residue so that, throughout the considerable growth changes that take place during development, approximately the same thickness of shell is maintained. The shell lacks an obvious laminate structure and the method of addition is unknown. The presence of an undetected shell precursor, released immediately upon its formation from the vitelline cytoplasm, is rendered unlikely by the death of the cells prior to the completion of shell growth. Gradually disintegrating cytoplasmic fragments surrounding the remaining acidophilic globules would be sufficient to prevent their immediate conversion into shell material and its addition would continue as a gradual process.

The egg shell does not appear to be a quinone-tanned protein. Application of the Catechol technique, described by Johri and Smyth (1956) as a test for the presence of the enzyme polyphenol oxidase, proved negative. This result is identical to that obtained by the latter authors for a species of the related genus Gorgoderina.

* Smyth and Clegg (1959) suggested that the absence of phenolase in Gorgoderita vitelliloba indicated that tanning might take place by a

(* Expt. Parasit. 8, 286.)

non-enzymic process. Llewellyn (1965) proposed that tanning did not occur at all and supported his view by the observation that the shell was stretched during the intra-uterine development phases of Gorgoderia eggs. It has been more recently stated that the enzyme phenolase can be lost during experimental procedure and that the only satisfactory test is the chemical test for phenol. This substance is present in the shell of Gorgoderia eggs. More detailed chemical analysis of eggs from various genera and species of Gorgoderids is required before conclusions as to the true structure of the shell can be made.

The shell is moulded into a characteristic ovoid shape within the oötype. The contractile nature of this chamber is illustrated by its ability to endow a similar shape to the variously sized vitelline 'eggs'. These are frequently much smaller than newly formed fertile ones and measure only 0.0195 x 0.0146 mm. They vary in size, however, depending upon the number of vitelline cells enclosed, and reach a maximum equal to that of the normal egg, namely 0.03061 x 0.02041 mm. when measured live. The formation of these vitelline 'eggs' is a common happening and leads to considerable waste. This can only be explained by assuming that once the fluke has become sexually mature either the vitelline glands continuously release cells or their production rhythms are not closely co-ordinated with those of the ovary. The fact that the two processes are not regulated to coincide more exactly is surprising since it results in a fluctuating protein and glycogen loss, which at times can be considerable. A certain amount of vitelline waste is not uncommon in trematodes, however, and was noted to occur in Gorgoderina attenuata by Willey and

Koulish (1950).

The vitelline 'eggs', in contrast to fertile ones, do not increase in size. The enclosed vitelline cells continue to release shell-forming fluid which coalesces to form large refractile droplets. The shell, measuring 0.000937 mm. across, does not therefore increase appreciably in thickness. This indicates that, at most, only a minute trace of Mehlis' gland secretion can be included within any egg following its passage through the oötype and that this secretion cannot be responsible for the continued conversion of shell material throughout growth. It also points to the fact that successful conversion of shell substance, subsequently released by the vitelline cells within the egg, is dependent upon the presence of the actively dividing ovum. Throughout development the eggs remain permeable to intra-vital dyes and therefore it can be assumed that the egg shell is either completely or selectively permeable. In either case there would be little osmotic stress placed upon the developing larva under natural conditions. Experimental evidence appears to suggest that the shell is not completely permeable. For example, eggs in their early stages of development were further advanced and exhibited no adverse osmotic changes following a period of 26 days spent in tap-water. Similarly, eggs of various developmental stages remained unaffected by 24 hours immersed in differing dilutions of horse serum. Therefore, assuming that the shell is selectively permeable, it is possible that during development a change in ionic composition occurs within the egg fluid as a result of the metabolism of the actively dividing larva and that this change does not occur in vitelline 'eggs'. Protein and carbohydrate metabolic activity will vary and perhaps the

nature of the waste products formed in the early stages differ from those produced by the more advanced larva. The actual conversion of the proteinaceous sol into a membranous structure may be dependent upon a certain pH range which is exceeded during the last stages of miracidial development thus resulting in a residue of vitelline material being left in the shell at hatching. If the shell is completely permeable, it is difficult to see how the existence of this residue can be explained.

The shelled ova pass from the median oötype dorsally into the descending limb of the uterus. Their passage is assisted by contractions of the uterine wall and, on occasions, the general body musculature is also involved. A sparse transparent secretion acts both as a lubricant for the easier passage of eggs and as a fluid medium for the sperm. The permeability of the egg shells indicates that the uterine fluid may also be of some nutritive value, and that not all nutritional requirements are contained within the embryonic and vitelline cells. It also implies that development may be at least partially aerobic and this is substantiated by the relatively low glycogen content of the eggs.

The shell, whilst in contact with the uterine fluid, remains pliable, and it is only under unusual mechanical stress that it can be induced to tear. This occurs when small vitelline 'eggs' are caught between larger fertile ones during uterine contraction and subsequent egg movement. The shell is either misshapen or torn and the contents spilled into the uterus thus adding to the vitelline debris. Normal eggs are not misshapen in this way despite the fact

that the larva never completely fills the shell cavity. The cell group is protected by fluid which transmits an equal pressure onto the shell preventing its distortion and accommodating for the growth of the larva by stretching the membrane. Muscular contraction of the fully developed miracidium can cause slight shape changes to occur in the shell, however, but in utero it is resilient and always returns to its original shape. Miracidia are never released whilst within the uterus. When once in contact with water, the same degree of miracidial activity and identical movements eventually, following a short delay, succeed in tearing the inoperculate membrane. It is possible that the progressive loss of the coating of uterine secretion, which surrounds each egg when it is laid, plays some part in the hatching process. Eggs are often released in small groups which are temporarily held together by uterine secretion, but they never hatch until they have separated and the coating is lost. Other factors involved in hatching are discussed later on page 54.

The shelled eggs, when first formed, measure on average 0.03061 x 0.02041 mm. in the live state. According to Looss (1894) the eggs of D. folium measured 0.035 x 0.018 mm. in the live state following fertilisation and these dimensions lie within the variation range for this species. Smaller sizes were recorded for the newly formed eggs of P. linguale and P. trinectes by Odhner (1911) and Corkum (1961) respectively. It is not clear however whether or not these latter measurements were obtained from mounted material. Both species possessed newly formed eggs of a similar size - 0.026 - 0.028 mm. in length (P. linguale) and 0.029 x 0.018 mm. (P. trinectes).

The miracidial developmental stages traced in this investigation are illustrated on page 30 diagram 2. The first cleavage is unequal and the second equal but involving only the larger cell. This three-cell stage measures 0.03571×0.02041 mm. Continued equal divisions involving products of only one of the cells formed as a result of the first cleavage continue up to the six-cell stage. The other main initial cell divides for the first time and the resulting eight-celled eggs measure from 0.0367×0.02945 mm. to 0.04082×0.0255 mm. depending upon the individual. Continued divisions steadily reduce the size differences of the products so that in eggs measuring 0.04592×0.03061 mm. the embryo possesses an appearance similar to that of a mulberry.

It is in the terminal stages of growth that a marked size difference in the eggs produced by different individuals becomes more apparent. For descriptive purposes, subsequent measurements will relate to one fluke only and the size range of the eggs of this species will be discussed separately in the next subsection on page Continuing division accompanied by growth eventually results in structures characteristic of the miracidium becoming visible as the eggs approach the terminal uterine coils. Firstly, two large, laterally placed gland cells appear, becoming increasingly basophilic as their development proceeds. In eggs measuring 0.05714×0.03673 mm. these cells and their ducts reach a total size of 0.02041×0.01020 mm. and 0.1531×0.01020 mm., one gland being already notably larger than the other. Also, at this stage, the conical shaped gut is formed, measuring 0.01020×0.01531 mm. Active flame cells are

A DIAGRAMMATIC REPRESENTATION OF VARIOUS STAGES OF MIRACIDIAL DEVELOPMENT OCCURRING IN UTERO

(The observations were based upon living material and were aided by the use of neutral red staining).

Diagram A represents - The structure of the shelled egg upon its entry into the descending limb of the uterus.

Diagram B represents - The result of the second cleavage - the three-celled stage.

Diagram C represents - The eight cell stage.

Diagram D represents - An early multicellular stage; note the large space existing between the shell and the developing larva.

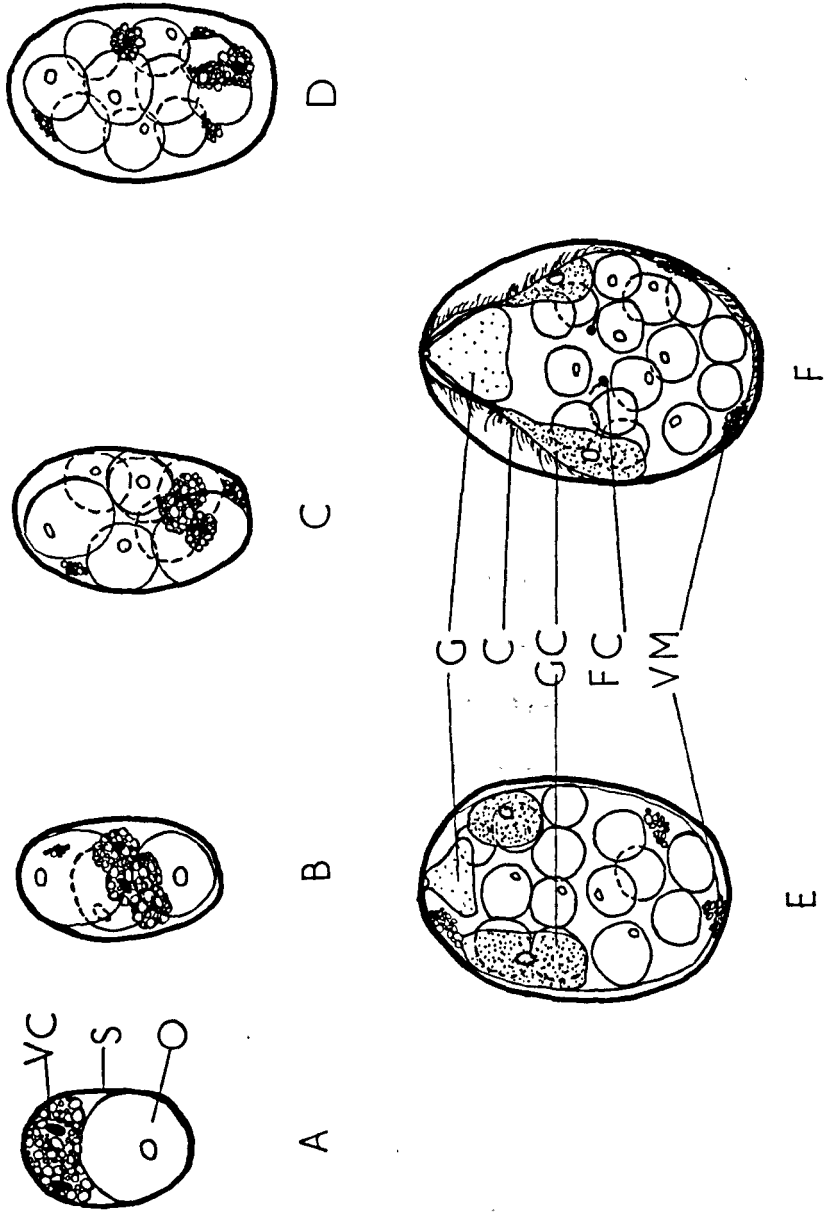
Diagram E represents - A late stage in development; the embryo remains static; the gland cells are developing and the gut is visible.

Diagram F represents - The fully developed contractile larva still surrounded by the egg shell. This stage is located in the terminal coils of the uterus; ciliary and flame cell action has begun; the gland cells and the gut are fully developed; the vitelline cells have completely disintegrated leaving a small quantity of secretory debris within the shell.

Abbreviations

- | | | |
|---------------------------|---|-------------------------|
| <u>Diagram A</u> | { | VC = Vitelline cell |
| | { | S = Shell |
| | { | O = Ovum |
| <u>Diagrams E & F</u> | { | G = Gut |
| | { | C = Cilia |
| | { | GC = Gland cells |
| | { | FC = Flame cells |
| | { | VM = Vitelline material |

2



PHYLLODISTOMUM - MIRACIDIAL DEVELOPMENT.

visible only when well developed muscular movements have become an established feature and cannot be seen in the static embryo. The reason for their functioning at this time whilst the larva remains within the osmotically controlled environment of the adult may reflect the selective permeability of the shell and a change in the ionic content of the egg fluid as a result of muscular activity. A gradual increase in the activity of the embryo occurs until, finally, shortly before laying, ciliary movement commences. Eggs reaching these stages of development are found in the terminal coils of the ascending limb of the uterus. They are continuously washed backwards and forwards entering and re-entering the metraterm as much as a day before they are due to be laid. The terminal coils of the uterus of older flukes and the insignificantly muscular metraterm, when once large numbers of mature eggs have passed through them, fail to return to their former narrow diameter and remain as clear expanded channels. In such cases the movement of eggs is accomplished by four means: continuing egg production pushes older specimens forward; general muscular movements of the body aid the process; contractions of the narrow-bored regions of the uterus and the resulting forward flow of uterine fluid washes eggs through the expanded regions. Fully developed eggs obtained from the fluke, from which the later measurements were taken, are in the upper limits of the size range for this species and measured 0.07142×0.04082 mm. It is in this state that the eggs containing active miracidia are eventually passed out of the uterus via the genital atrium to the exterior. The egg laying habits of the fluke are discussed in Section 7C on page 270.

c) The fully developed egg.

cl) Living material

When the living eggs laid by one or several individuals were compared, it was found that they varied considerably in size.

Similar variations had been noted by Stafford in 1902 for the eggs laid by Gorgoderina translucida. An attempt was therefore made to establish the total range for this species; to examine the extent of the individual variation and investigate the relationship, if any, between the size of the trematode and the dimensions of its eggs. Due to the fact that there are regular periods of minimal production and even complete rest within the ovarian cycle, (a topic discussed further on page 238), it proved difficult to obtain large numbers of differently sized flukes which were laying at the maximum rate. Ten fully developed eggs was the highest number which could be guaranteed from the smaller trematodes, but in many cases only five or a single measurement was obtained despite attempts to artificially induce laying over a longer experimental period (see page 234). Thus, out of a total of 200 live eggs obtained from 38 flukes, only 140 involved averages of 10 eggs laid by 14 trematodes, 45 represented averages of 5 from 9 flukes and 15 were individual records.

The total and average size range for eggs laid by this species is illustrated in Table 1 and graph (1) page 38 and extends from 0.0541 - 0.0724 mm. in length by 0.0330 - 0.0459 mm. in width. The total variation in both dimensions is under 0.02 mm., and the greatest average difference occurs in the length of the egg.

Variation in the size of the eggs produced by one individual

differ according to the dimension under consideration. Eggs from one fluke may vary by differing amounts in both measurements, some possessing a wider range as regards length and others in respect to the width. (See examples, 1, 2 and 3 on Table 3, page 35). Over a range of 10 eggs laid consecutively, some trematodes show a variation in one dimension only (examples 4 and 6, Table 3) whilst, in 3 out of 14 flukes, no alteration in either measurement under these conditions was recorded, (example 5, Table 3). The maximum difference between either the length or the breadth of eggs laid by one fluke was 0.0055 mm. The majority of trematodes, however, produce eggs which do not vary by more than 0.0025 - 0.0035 mm. in either dimension.

Table 1 and graph 2 (page 40) illustrate that the total length of the fluke and the dimensions of the eggs show no clear relationship. Similar results are shown on Table 2 and graph 4 (page 43), where the area of the posterior region was compared with the size of the egg laid. Such a measurement may possibly give a better indication of the reproductive age of the individual since the region's size is proportional to uterine development. The only conclusion that can be drawn from the data concerning living material, outlined in Tables 1-3 and graphs 1-4 is that above the length of 0.896 mm. flukes tend to produce either large or small eggs independent of their size.

TABLE I

| Total length of relaxed laying fluke (live) | Average size of eggs laid (live) | Total number of eggs measured |
|---|----------------------------------|-------------------------------|
| 1. 1.823 mm. | Max.- 0.0661 x 0.0451 mm. | 5 |
| 2. 1.118 mm. | 0.0663 x 0.0435 mm. | 5 |
| 3. 1.245 mm. | 0.0686 x 0.0412 mm. | 5 |
| 4. 1.489 mm. | 0.0663 x 0.0404 mm. | 5 |
| 5. 1.304 mm. | 0.0622 x 0.0403 mm. | 10 |
| 6. 0.896 mm. | 0.0625 x 0.0396 mm. | 10 |
| 7. 0.910 mm. | 0.0616 x 0.0403 mm. | 10 |
| 8. 0.956 mm. | 0.0582 x 0.0408 mm. | 5 |
| 9. 1.014 mm. | 0.0602 x 0.0369 mm. | 5 |
| 10. 0.934 mm. | 0.0604 x 0.0361 mm. | 5 |
| 11. 0.962 mm. | 0.0592 x 0.0367 mm. | 10 |
| 12. 1.312 mm. | 0.0587 x 0.0371 mm. | 10 |
| 13. 2.450 mm. | 0.0588 x 0.0367 mm. | 10 |
| 14. 1.245 mm. | 0.0571 x 0.0357 mm. | 5 |
| 15. 1.267 mm. | 0.0561 x 0.0367 mm. | 10 |
| 16. 1.148 mm. | 0.0557 x 0.0367 mm. | 10 |
| 17. 1.575 mm. | 0.0550 x 0.0367 mm. | 5 |
| 18. 1.750 mm. | 0.0550 x 0.0367 mm. | 10 |
| 19. 0.896 mm. | 0.0552 x 0.0363 mm. | 10 |
| 20. 1.141 mm. | Min.- 0.0545 x 0.0364 mm. | 10 |
| <u>AV. 1.272 mm.</u> | <u>AV. 0.0593 x 0.0382 mm.</u> | <u>155 eggs</u> |

Fluke size range: 0.896 - 2.450 mm. Egg size range: 0.0545 - 0.0686 mm.
(length) (based on average measurements)
0.0361 - 0.0451 mm.

Actual size range of eggs: 0.0541 - 0.0724 x 0.0330 - 0.0459 mm.

TABLE 2

| Area of the posterior region - relaxed laying fluke (live) | Average size of eggs laid (live) | Total number of eggs measured |
|--|----------------------------------|-------------------------------|
| 1. 0.864 sq. mm. | Max.-0.0661 x 0.0451 mm. | 5 |
| 2. 0.609 sq. mm. | 0.0663 x 0.0435 mm. | 5 |
| 3. 0.491 sq. mm. | 0.0686 x 0.0412 mm. | 5 |
| 4. 0.578 sq. mm. | 0.0663 x 0.0404 mm. | 5 |
| 5. 0.240 sq. mm. | 0.0582 x 0.0408 mm. | 5 |
| 6. 0.176 sq. mm. | 0.0595 x 0.0391 mm. | 5 |
| 7. 0.298 sq. mm. | 0.0602 x 0.0369 mm. | 5 |
| 8. 0.213 sq. mm. | 0.0604 x 0.0361 mm. | 5 |
| 9. 0.516 sq. mm. | 0.0571 x 0.0357 mm. | 5 |
| 10. 0.328 sq. mm. | Min.-0.0557 x 0.0367 mm. | 10 |

TABLE 3

Selected examples to illustrate individual variation in the size of eggs laid.

| Total length of relaxed laying fluke (live) | Total range in size of eggs | Total number of eggs measured |
|---|-----------------------------------|-------------------------------|
| 1. 0.896 mm.-min. | 0.0550-0.0568 x 0.0330-0.0367 mm. | 10 |
| 2. 0.896 mm. | 0.0623-0.0642 x 0.0367-0.0403 mm. | 10 |
| 3. 0.934 mm. | 0.0571-0.0612 x 0.0367-0.0388 mm. | 5 |
| 4. 1.312 mm. | 0.0587 x 0.0367-0.0403 mm. | 10 |
| 5. 1.750 mm. | 0.0550 x 0.0367 | 10 |
| 6. 2.450 mm.-max. | 0.0587-0.0623 x 0.0367 | 10 |

TABLE 4

Mounted material - (results obtained from various fixation techniques).
(Trematodes differ from specimens utilised in Table 1).

| Total length of mounted fluke | Average size of fully dev. mounted eggs (measured in utero). | Total number of eggs measured. |
|-------------------------------|--|--------------------------------|
| 1. 0.861 mm. | Max. - 0.0413 x 0.0309 mm. | 5 |
| 2. 0.700 mm. | 0.0402 x 0.0253 mm. | 5 |
| 3. 0.938 mm. | 0.0375 x 0.0257 mm. | 5 |
| 4. 0.581 mm. | 0.0375 x 0.0253 mm. | 5 |
| 5. 1.232 mm. | 0.0385 x 0.0246 mm. | 10 |
| 6. 0.735 mm. | 0.0388 x 0.0241 mm. | 10 |
| 7. 0.658 mm. | 0.0363 x 0.0265 mm. | 5 |
| 8. 0.945 mm. | 0.0365 x 0.0259 mm. | 5 |
| 9. 0.777 mm. | 0.0357 x 0.0250 mm. | 5 |
| 10. 1.232 mm. | 0.0369 x 0.0221 mm. | 10 |
| 11. 1.267 mm. | 0.0360 x 0.0225 mm. | 10 |
| 12. 0.784 mm. | 0.0360 x 0.0219 mm. | 10 |
| 13. 0.777 mm. | 0.0358 x 0.0212 mm. | 10 |
| 14. 0.966 mm. | 0.0355 x 0.0214 mm. | 10 |
| 15. 0.931 mm. | 0.0345 x 0.0222 mm. | 5 |
| 16. 0.672 mm. | 0.0355 x 0.0207 mm. | 10 |
| 17. 0.861 mm. | 0.0335 x 0.0220 mm. | 5 |
| 18. 0.651 mm. | 0.0333 x 0.0190 mm. | 10 |
| 19. 0.742 mm. | 0.0323 x 0.0194 mm. | 5 |
| 20. 0.805 mm. | Min. 0.0335 x 0.0168 mm. | 10 |
| AV. 0.856 mm. | AV. 0.0362 x 0.0229 mm. | 150 |

Fluke size range (length)

0.581 - 1.267 mm.

Size range of eggs based on average measurements:

0.0323-0.0413 x 0.0168-0.0309 mm.

Actual size range of eggs:

0.0306-0.0469 x 0.0163-0.0316 mm.

37 E

EGG SIZES - 1

Graph 1 illustrates the total size range for the eggs of this species

Section A: The size range for living material based upon a total of 200 eggs measured following their release.

Symbols utilised in the graph:

- . = the average size of 10 eggs laid consecutively by one fluke
- x = the average size of 5 eggs laid consecutively by one fluke
- o = individual records

Total trematodes involved = 38

(Reference Table 1 (in part))

Section B: The size range for mounted material based upon a total of 150 eggs. The eggs were examined and measured in utero. Before fixation they were observed to contain active miracidia; various fixatives were utilised in order to study their effects and to give as wide a size range as possible.

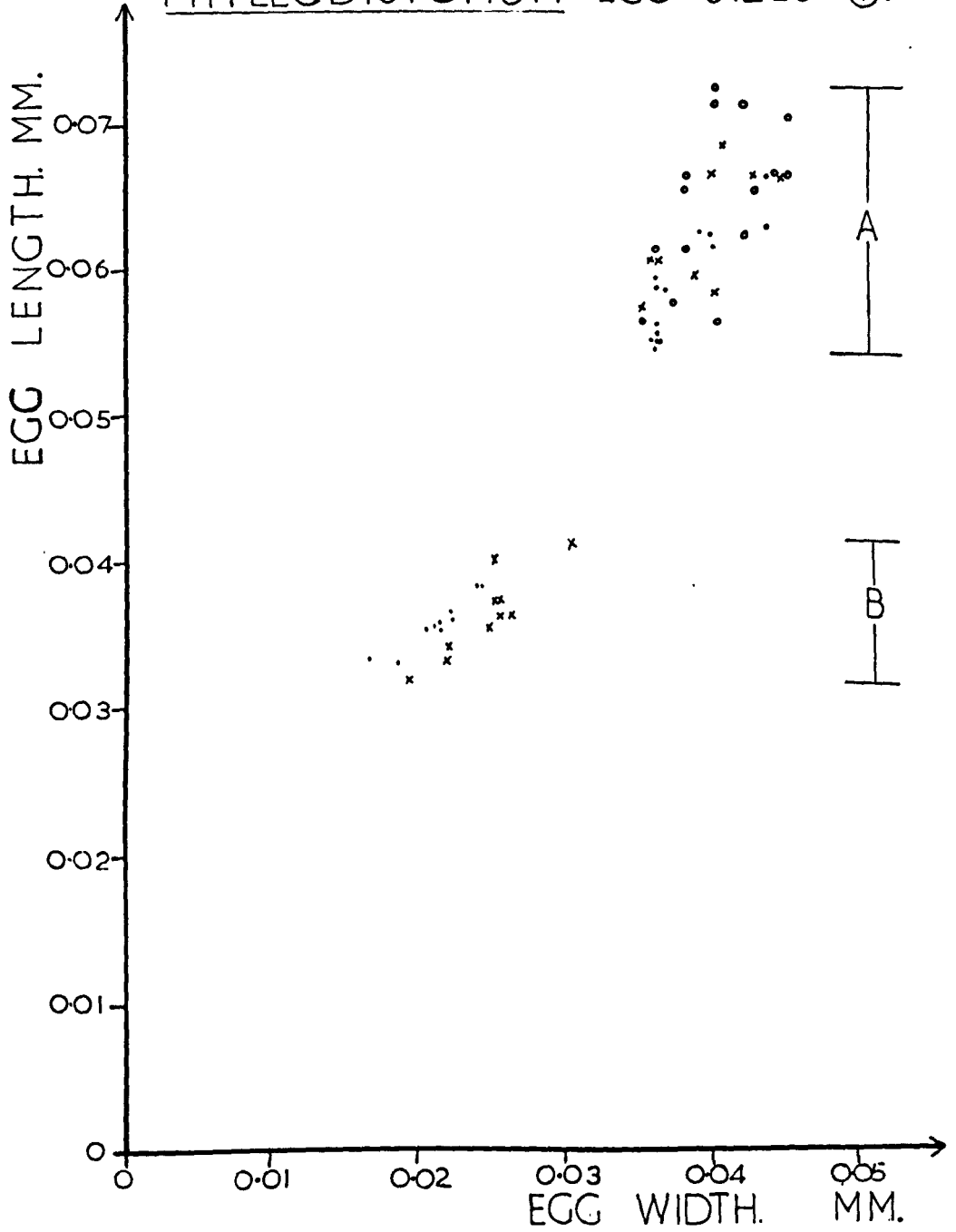
Symbols utilised in the graph:

- . = the average size of 10 eggs in the terminal coils of the uterus of one fluke
- x = the average size of 5 eggs in the terminal coils of the uterus of one fluke

Total trematodes involved = 15

(Reference Table 4).

PHYLLODISTOMUM~EGG SIZES~①.



32

EGG SIZES 2 and 3

Graphs 2 and 3 illustrate a relationship between the sizes of the eggs produced and the overall size (i.e. the length) of the trematodes

In graph 2 the average length of the eggs is plotted against the length of the trematode; in graph 3 the average width of the eggs is plotted against the body length.

Section A: (Graphs 2 and 3)

The measurements involve a total of 155 eggs measured live following their release.

Total trematodes involved = 20

(Reference Table 1).

Section B: (Graphs 2 and 3)

The measurements involve a total of 150 eggs measured in utero in the mounted state. The eggs were examined prior to fixation and observed to contain active miracidia.

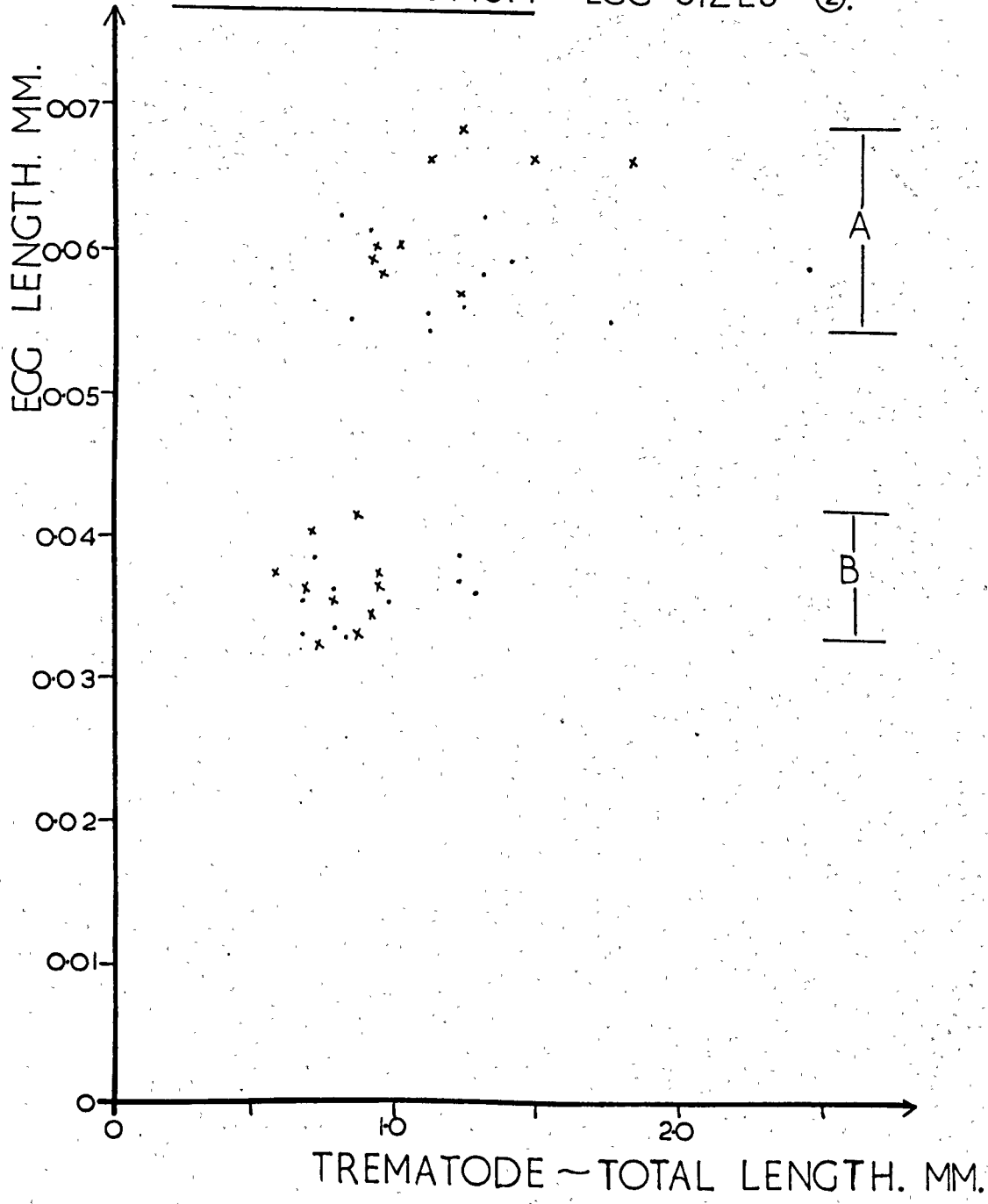
Total trematodes involved = 20.

(Reference Table 4).

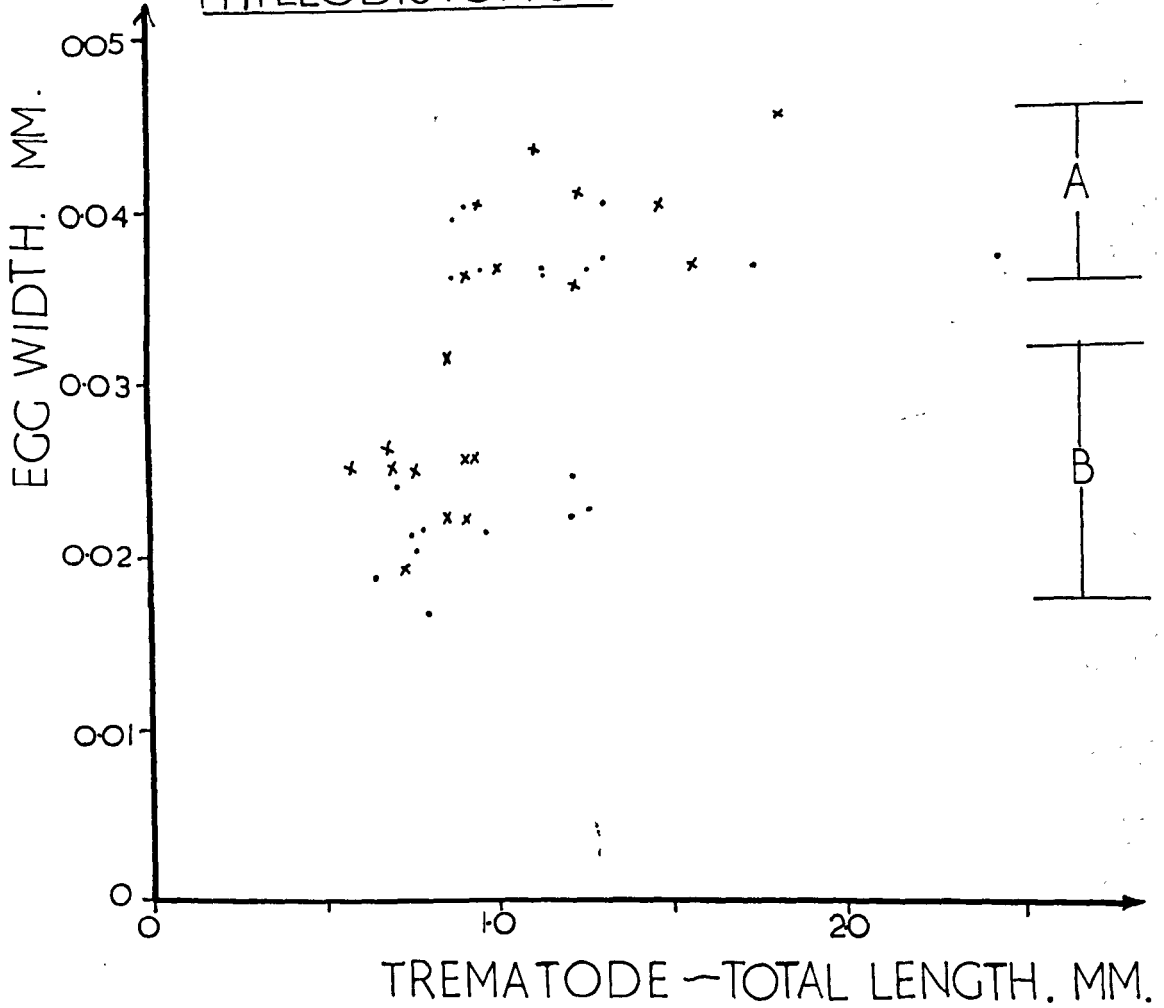
Symbols utilised in the two graphs:

- . = the average size of 10 eggs produced by one fluke
- x = the average size of 5 eggs produced by one fluke

PHYLLODISTOMUM ~ EGG SIZES ~ ②.



PHYLLODISTOMUM ~ EGG SIZES ~ ③



02

EGG SIZE - 4

Graph 4 illustrates the relationship between the area of the posterior region of relaxed living flukes and the average size of the eggs laid; The latter were measured in the live state upon release and an average measurement of 5 eggs was recorded in all cases except one where a mean of 10 eggs was taken.

Total trematodes involved = 10.

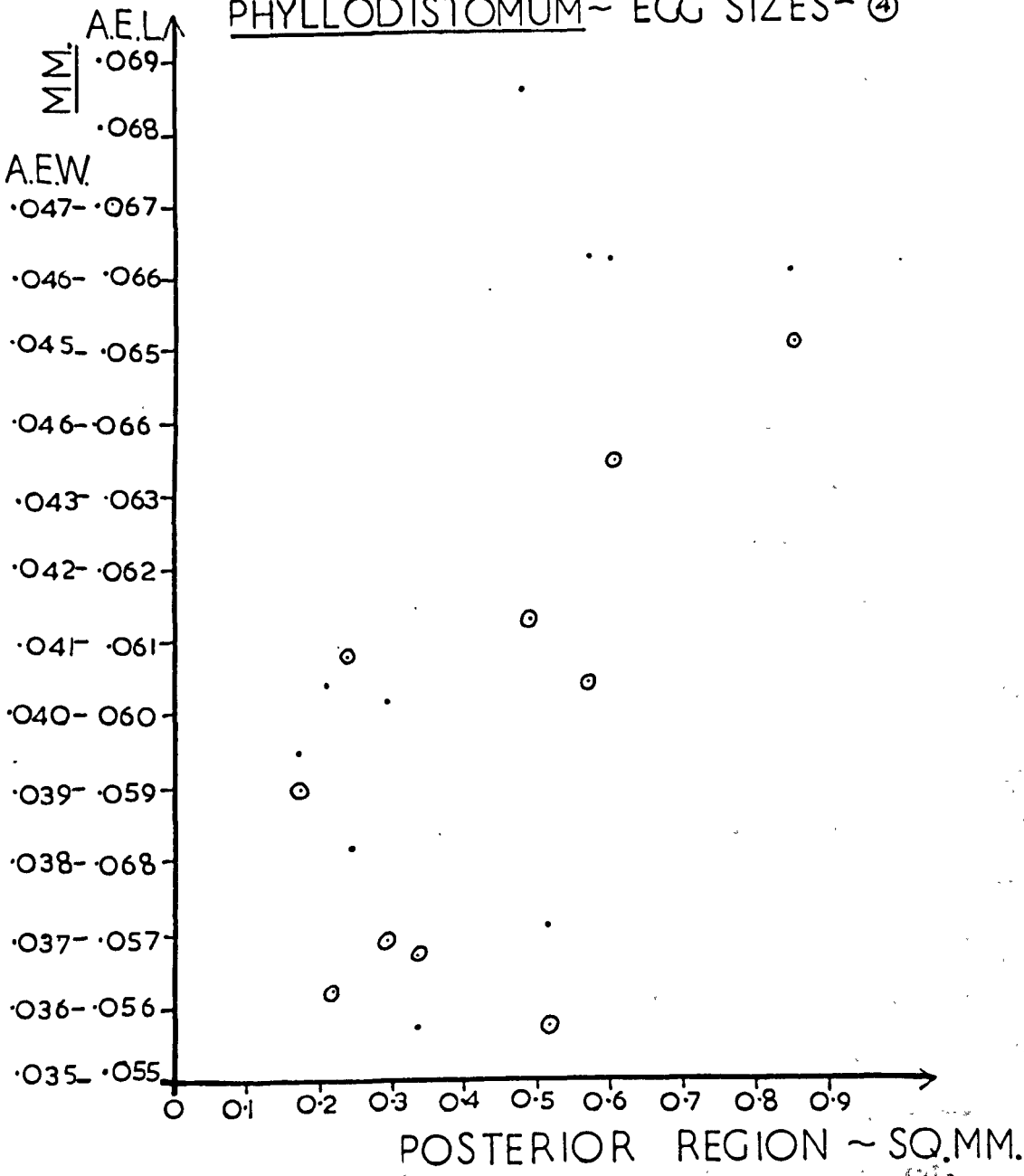
(Reference Table 2).

Abbreviations and symbols utilised in the graph:

AEL = Average length of the eggs = •

AEW = Average width of the eggs = (0

PHYLLODISTOMUM ~ EGG SIZES ~ ④



c2) Mounted Material

The size of the trematode egg has long been utilised as a useful diagnostic criterion. Unfortunately its value can be considerably reduced to the taxonomist by failure to record (1) the number and size of the flukes from which the eggs were taken, (2) the number of eggs actually measured, (3) the size of the living eggs, (4) the degree of individual variation in egg size and (5) the state of development of the embryo. Within the literature concerning this genus the points enumerated above are frequently omitted in diagnostic descriptions of new species and although ideally large numbers of measurements should be made the position is often complicated by an initial lack of material. At least eight authors for example erected new *Phyllodistome* species from a description based upon 1-2 specimens and four others collected only 4-5. In other cases the diagnosis is restricted to a type or a few paratype specimens regardless of the numbers of flukes recovered.

Although the considerable effects of fixation and dehydration upon the thin-shelled eggs of the Gorgoderidae have been known for some time the practice of measuring them while situated in the terminal coils of the uterus of mounted specimens without reference to the living structures has persisted. As long ago as 1902 Stafford reported shrinkage in the preserved specimens of *Gorgoderina translucida* but gave no data. Since this period three authors have referred to this phenomenon and their results are tabulated overleaf.

| Trematode | Average size of living eggs | Author | Possible degree of shrinkage * |
|----------------------------------|-----------------------------|-------------|--------------------------------|
| <u>Phyllodistomum brevicecum</u> | 0.0561 x 0.0373 mm. | Steen(1938) | 0.0263 x 0.0173 mm. |
| <u>Gorgoderina attenuata</u> | 0.0530 x 0.0340 mm. | Cort(1912) | 0.0210 x 0.0120 mm. |
| <u>P. undulans</u> | 0.0410 x 0.0290 mm. | Steen(1938) | 0.0143 x 0.0093 mm. |
| <u>P. patellare</u> | 0.0430 x 0.0270 mm. | Lynch(1936) | 0.0120 x 0.0060 mm. |

* N.B. The possible degree of shrinkage is calculated by subtracting the average measurements given for mounted material from the average size of the live structures. The number of eggs measured was not stated. Steen(1938) employed identical fixation and staining techniques for both P. undulans and P. brevicecum and his results illustrate the general principle that the larger the egg, the greater the shrinkage effects are likely to be.

In view of this chaotic position in the literature it was thought advisable to measure mounted material so that the effects of fixation and dehydration upon this species could be studied and a size range established.

Twelve of the most common fixation techniques used by previous workers in this field had the effect, in all cases, of shrinking the eggs. Attempts to fix eggs once they had been laid resulted in a variable percentage of the shells being ruptured or distorted according to the alcoholic content of the fixatives. Another reason for this probably lies in the increasing brittle nature of the shell following the egg's release. Insufficient eggs could be

obtained from a suitable number of variously sized flukes to obviate this loss, so it was made necessary to measure eggs seen to contain active miracidia whilst they remained in the terminal coils of the uterus. The percentage of damaged eggs was thus effectively reduced (although some methods of fixation were not as efficient as others in this respect) and the material was easier to handle. Very few fixatives distorted the eggs but, in two cases (F.A.A. and 70% Alcohol), this was associated with the use of heat and did not occur when the reagent was used cold. In cases of distortion, the egg shape was always altered in a similar way, the length being reduced, but the width increased by a maximum 0.001 mm. beyond that of the living structure. Apart from the varying degrees of shrinkage associated with the differing alcoholic or aquatic nature of the fixatives, the only other fixation effect was the occasional rupture of the shell which, in mature eggs, gave the impression that the miracidium had hatched in utero. This phenomenon may have been the basis for the report by Looss in 1901 that the eggs of P. acceptum had hatched within the uterus. It is significant that these observations were probably made upon preserved material. The main fixatives utilised were Susa (at 50°C.), Gilson's Fluid, Sanfalice (heated and shaken), Corrosive Sublimate (with and without acetic acid), Bouin (alcoholic and non-alcoholic at 56°C.), 70% Alcohol (cold), F.A.A. (cold), 10% Formalin (shaken), 70% alcohol (heated) and F.A.A. (heated). All these proved to be satisfactory, slight distortion occurring only in the latter three cases. Other variations occurred however in the effects upon the fluke and this is

discussed further on page 404.

Table 4 and graphs 1, 2 and 3 illustrate the overall effects of complete dehydration upon the size of fully developed eggs. The methods of staining and dehydration were identical in all cases. The stain used was acetoalum carmine.

From 150 eggs obtained from 20 flukes ranging in size from 0.581 - 1.267 mm. when mounted, the total size range is 0.0306 - 0.0469 mm. in length, and 0.0163 - 0.0316 mm. in breadth. Variation in either dimension, despite differences in fixation methods, remains below 0.02 mm. as in living material. The average measurement of 150 mounted eggs equals 0.0362 x 0.0229 mm. Comparison of the living and mounted total averages indicate that shrinkage is unequal, mainly effecting the longitudinal dimension, and reducing the egg to a size well below the living ranges. Dehydration also emphasises any distortions which may occur at fixation. The wide difference between living and mounted material (shown clearly in graph 1) illustrates the inadvisability of utilising only the latter in taxonomic studies, a warning originally given by Cort in 1912. The average shrinkage of 0.0231 mm. in length and 0.0153 mm. in width is proportionally less than would have been expected from the initial large size of the living egg especially when comparing Steen's results for P. brevicecum (page 45).

Thomas stated in the diagnostic description of P. simile in 1958 that preserved embryonate eggs ranged in size from 0.030 - 0.0325 mm. in length and 0.025 - 0.0275 mm. in width. In Table 3 of his article, however, the sizes are given as 0.030 - 0.037 mm. in

length and 0.020 - 0.022 mm. in breadth. Thomas does not state upon how many measurements these findings were based, and it seems possible that the range for P. simile could be extended to further coincide with the dimensions for this species.

SECTION 2 THE MIRACIDIUMa) Method of investigation

The majority of observations concerning miracidial structure were made from living material, using the intravital stain, Neutral Red. Considerable difficulty was experienced in tracing the boundaries of the ciliated epithelial cells in this species. The best results were obtained by allowing stained miracidia to rotate slowly under a coverslip. Their rate of movement was artificially reduced by the use of very dilute horse serum and the heavily staining gland cells acted as reference points. Results so obtained were then confirmed on fixed material. Several techniques were employed, including Bresslau's Aniline Blue and Horvath's Formal Silver method. The most satisfactory, however, was a modification of a silver nitrate impregnation technique utilised by Goodchild (1943) after Lynch (1933).

b) Size

Miracidia are highly contractile organisms and the body wall possesses fine layers of longitudinal and circular muscles. Ten miracidia hatching from eggs averaging 0.0545×0.0364 mm. in size possessed a total contractile range of $0.0763 - 0.0510$ mm. in length and $0.0405 - 0.0182$ mm. in the region of maximum width. In the pyriform position commonly assumed during locomotion, these miracidia were found to possess the average measurement of 0.0662×0.0247 mm.

Within the literature the structure of the miracidium has received scant attention.*

* The reference Wootton & Peters, L. (1957) - "A comparative morphology of miracidia in the Gorgoderidae" - given as Journal of Parasitology 43, 35, could not be traced.

Goodchild (1943) recorded a size range of 45-55 μ x 40-47 μ for P. solidum larvae whilst Groves (1945) reported that miracidia of what he considered to be the same species ranged from 43-56 μ in length x 24-28 μ in width; 10 specimens averaged 50 x 25 μ . Gorgodera amplicava miracidia reached a size of 55 x 28 μ according to Goodchild (1948). The contractile range recorded in this investigation appears to be far wider than in any case previously recorded for Phyllodistomum.

c) Internal structure (illustrated on page 53)

Anteriorly, the fully developed miracidium possesses a small ovoid opening which is flanked by epithelial cilia. This aperture, the mouth, opens into a flask-shaped gut measuring from 0.0130-0.020 mm. in length and 0.0153-0.0167 mm. in width according to the degree of miracidial contraction. Situated on either side of the mouth are two small openings which lead posteriorly via fine uncoiled ducts to two laterally placed, strongly basophilic gland cells. These differ in size, the larger together with its duct, measuring a maximum of 0.0408 mm. and the smaller 0.0306 mm. in extended larvae. Goodchild (1943) noted that the two similar gland cells found in the miracidium of P. solidum were inter-connected. Such a connection was not noted in this species. The rest of the miracidium is packed with small rounded cells, the cytoplasm of which gives a weak basophilic reaction to Neutral Red. The germinal material is not distinct.

The excretory system consists of two flame cells lying on either side of the mid-line in the middle third of the body which empty into two separate ducts. The latter follow a tortuous path passing

laterally, posteriorly and finally, anteriorly for a short distance before terminating at two lateral pores. These openings are situated on either side between the epidermal plates of the second and third rows from the anterior end. The possession of 2 flame cells in Gorgoderid miracidia has been reported in P. semotili (Fischthal, 1942), P. trinectes (Corkum, 1961), P. solidum (Goodchild, 1943 and Groves, 1945) and also in Gorgodera amplivava (Goodchild, 1948). They are all described as occupying the middle third of the body, functioning whilst in utero and as possessing an excretory pore opening immediately anterior to the last row of epithelial cells. They appear to differ only on one point - the exact path of the excretory ducts. This however may well vary within the species (and does so at least according to a comparison between the accounts given by Goodchild and Groves concerning P. solidum.)

Scattered throughout the larva studied in this investigation are variously sized refractile droplets. Similar structures were noted by Goodchild (1943) in the miracidium of P. solidum. These hyaline particles appear to be an oil deposit which may be formed from glycogen, under aerobic conditions, during the starvation period. An anterior concentration of nervous tissue, although undoubtedly present, was not seen. Eyes are absent.

d) Epithelial coat (illustrated on page 53)

The epithelial coat consists of 15 cells arranged in 4 rows. The cells, from the anterior to the posterior end, number 4, 4, 5 and 2. This arrangement appears to be a constant feature for the majority

PHYLLODISTOMUM MIRACIDIAL STRUCTURE

Diagram A: A diagrammatic representation of an extended living miracidium in the typical form assumed when swimming. (Observations were aided by the use of neutral red staining).

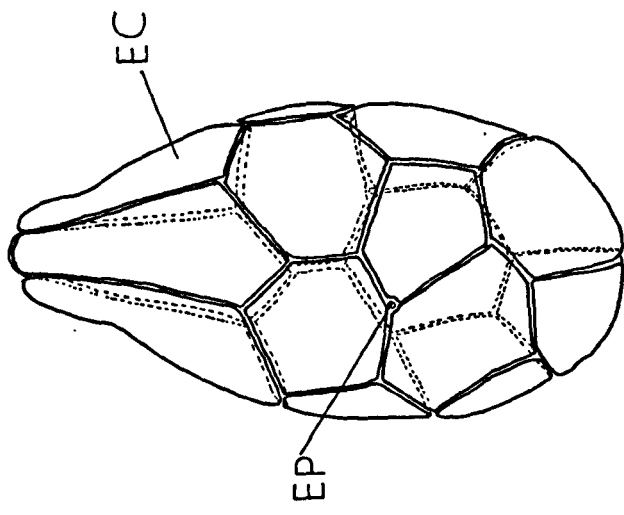
Abbreviations

- CC = ciliated coat
- EP = excretory pore
- FC = flame cell
- G = gut
- GC = gland cell
- GCD = gland cell duct
- M = mouth

Diagram B: A diagrammatic representation of the epithelial coat of the miracidium as revealed by silver staining techniques, etc.

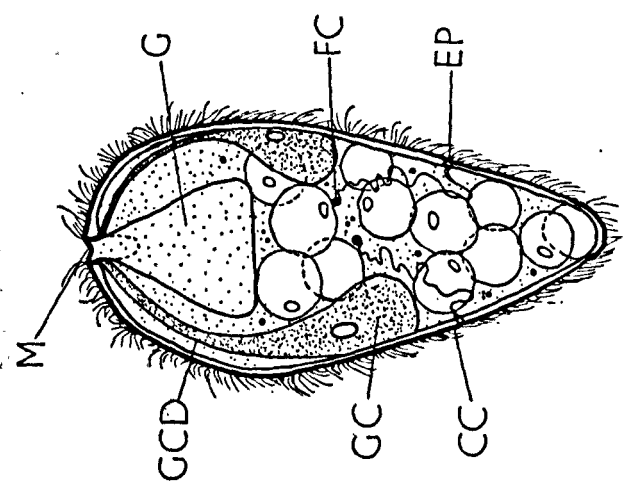
Abbreviations

- EC = epithelial cell
- EP = excretory pore



PHYLLODISTOMUM ~

MIRACIDIUM. ~ B.



PHYLLODISTOMUM ~

MIRACIDIUM. ~ A.

of the miracidia examined, but rare variations of 4, 4, 4, 2; 4, 5, 4, 2 and 4, 5, 5, 2 were noted. Occasionally, by the use of horse serum, the miracidium could be induced to shed its coat. It is lost initially from the anterior end and progressively peels off until the internal muscular organism, the mother sporocyst, is freed from it. The epithelial cells then curl over, their ciliated surfaces outermost, and the entire constricted coat swims away.

The only other occasion when a total of 15 plates was reported for a Gorgoderid miracidium was when Goodchild (1948) recorded an arrangement of 3 tiers of 6, 6, 3 plates for Gorgodera amplicava. A review of the literature suggests that the number of cell rows found within the genus Phyllodistomum would appear to vary from 3-4.

Goodchild's and Groves' accounts for P. solidum differ markedly over the question of both the total cells present and their arrangement.

Goodchild recorded a total of 16 plates arranged in 3 rows of 6, 6 and 4 while Groves found 4 rows possibly consisting of 6, 8, 8 and 4.

Similar confusion occurs in European flukes parasitising fish.

Ssinitzin (1905) indicated 3 rows of cells for P. folium; Pigulevsky (1953) indicated 4 rows for a species he called P. dogieli stating that the latter was in part synonymous with Ssinitzin's specimens.

e) Miracidial hatching

After the eggs are released from the trematode, the contained miracidia are continuously moving, periods of slow longitudinal rotation alternating with bursts of contractile activity. The larva secures its release by suddenly contracting and twisting round in a plane parallel

to the longitudinal axis of the egg so that the position of the anterior and posterior ends of the miracidium are reversed and in the process the inoperculate shell is ripped apart. Diagrams A and B (page 58) illustrate the range of miracidial movements within the shell and the two methods whereby the membrane can be opened.

Normally, eggs containing active miracidia are passed intact into the host's urine and are released upon urination into the surrounding water, sinking to the bottom of the pond or lake, where they hatch. The copious flow of hypotonic urine keeps the host system free from eggs so that under natural conditions hatching does not take place in the urine solution. If, however, the host is killed and chilled for a few hours pending examination, active miracidia can be recovered from the bladder. It follows, therefore, that the differing ionic concentrations between the urine and the pond water offer neither a barrier nor a stimulant to hatching but the actual transference from the uterine fluid to another medium of different ionic composition is of greater importance. The exact role played by dissolved gases in this process is unknown. Since shells enclosing inactivated or under developed miracidia do not burst when kept in water and miracidial activity within the uterus has no effect upon the shell, it can be assumed that successful hatching is dependent upon one or more of the following factors:

- 1) that the permeability of the shell is suddenly increased with an ensuing osmotic effect stretching the membrane and reducing its resilience;
- 2) that the shell is weakened as a result of enzymic digestion and

finally

3) that the loss of the uterine fluid coating renders the shell more brittle.

In points 1 and 2 it is presumed that the larva releases an enzyme following the stimulus of ionic variation. The presence or absence of such a secretion could not, however, be proven. Release of content from the lateral gland cells was caused artificially by excessive cover-slip pressure but was not observed to occur naturally. Similarly, no fluid was seen to issue from the gut although, if it is transparent and colourless, it could have been overlooked. In order to prevent undue loss, it is more likely that such enzymic secretions are stimulated only by contact with the tissues of the intermediate host and that their function is purely penetrative.

Any osmotic effects which might be caused must be relatively insignificant since the eggs were not observed to swell prior to hatching; the rate of the flame cell beat was not noticeably increased in free larvae and miracidial activity remains an essential constituent of the hatching process. This latter is emphasised by the fact that in eggs in which the larva was eventually completely inactivated by extreme low temperature treatment, following a period in which hatching was only just prevented, the shell remained unbroken after 7 hours in water. Therefore, if enzymic action does occur it must render the shell completely permeable (which is of questionable value) and structurally weaken it.

Whether the loss of the uterine fluid coating does effect the shell's resilience is not clear since the essential delay between the

PHYLLODISTOMUM MIRACIDIUM

Diagram Series A:

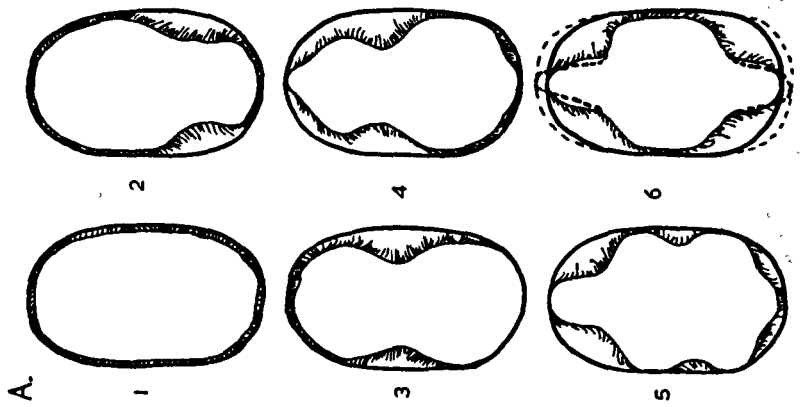
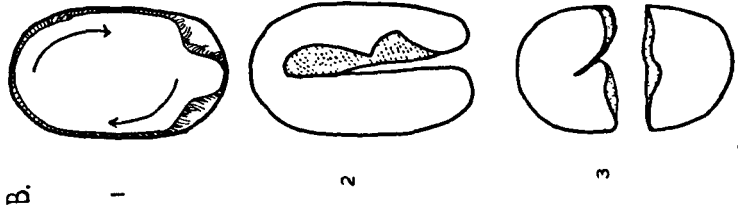
A diagrammatic representation of the series of miracidial movements which take place within the egg shell. Contractions do not necessarily follow the sequence 1-6 illustrated. In stage 6 the larva exerts an extensile force upon the shell and is capable of stretching it slightly; the maximum extended position thus achieved is indicated by dotted outlines.

Diagram Series B:

A diagrammatic representation of the hatching process.

- B1 - The arrows indicate the direction in which the larva will turn within the shell in order to release itself.

- B2 & 3 - Illustrate the two ways in which the shell is ruptured.



acts of laying and hatching, when the coating is dissolving, could also be a consequence of a general enzymic action digesting the membrane.

Whilst most observers state that hatching is practically instantaneous following release into water, Crawford (1940) noted that there was a 2-day delay in the hatching of an unidentified Phyllodistomum species he studied. In contrast Goodchild (1948) reported that Gorgodera amplicava miracidia hatched by means of an explosive process following urine dilution and that the gut of the miracidium absorbed water on hatching. This suggests that the shell was initially semi-permeable and that an osmotic force may have been involved in the hatching process (a supposition supported by Cort (1912) for Gorgodera attenuata). However, the two Gorgodera species parasitise Amphibia where the urine is concentrated to some degree within the bladder. This does not occur in fish and consequently osmosis may not be such an effective method of release. Lees & Mitchell found, for example, that the eggs of Gorgoderina vitelliloba swelled visibly following their release into water from the Amphibian host's urine and obviously hatching is an osmotic process in this instance. (Private communication 1964).

A series of experiments were performed to investigate the conditions necessary for successful hatching in the species under investigation. Some trematodes were placed into a regularly changed, dilute horse serum and host-urine mixture and placed into a solid watch-glass. Each watch glass was put into a container and surrounded with ice cubes or fluids of varying temperatures, covered and kept at a required experimental temperature for several hours. For experiments involving the

lower temperature ranges, trematodes were taken from dead hosts which had been kept for various periods within a refrigerator and for the higher temperatures flukes from freshly killed fish kept in warm-water tanks were employed. Thus, the flukes and their contained miracidia were to some extent acclimatised before the experiment began. A further period of adjustment at the required experimental temperature was allowed before the flukes' ability to lay (which is discussed further on page 284) and the miracidia to hatch under the various conditions was tested. Using this procedure, it was found that successful hatching occurred throughout the experimental range of $-1^{\circ}\text{C}.$ to $+21^{\circ}\text{C}.$ This indicates that eggs are capable of hatching throughout the year in Great Britain except in extremes of climatic variation in shallower stretches of water.

Temperature affects not only the length of the pre-hatching period but also the rate of the hatching process itself. At $-1^{\circ}\text{C}.$ the pre-hatching period is extended to as much as 30 minutes. Under these conditions, the process takes place as a series of slow contractions; the speed with which the larva leaves the torn shell is so reduced that the slightly glutinous fluid within the egg is not immediately washed from the epithelial cilia. This serves as a temporary connection between the shell and the miracidium, the latter swimming for a short distance before completely separating from its shell. At higher temperatures, hatching takes place within one minute of laying. For each individual miracidium, even those from the same fluke, the exact time for this process varies, but the optimum temperature for the most rapid hatching rate generally lies between $17^{\circ}\text{C}.$ and $19^{\circ}\text{C}.$

f) Miracidial behaviour

The effective stroke in ciliary action is inclined, with the result that the miracidium is rotated about its longitudinal axis during progression. The direction of rotation is frequently changed both when the larva is avoiding obstacles and when slowly following a straight course. There is also considerable variation in speed at normal room temperatures and several shape changes, (identical with those executed within the shell) occur at irregular intervals. The miracidia avoid any obstacle by temporarily arresting the ciliary beat and altering the angle of their approach. It is this behaviour which prevents them from escaping unaided from the living or dead host. If the entire urinary system of an infected fish is removed and placed in water, the contained flukes will continue to lay normally for some time. Active miracidia were found swimming around the bladder some 27 hours after they had been laid and none had escaped to the exterior. The trematodes themselves rarely succeed in passing down the urethra so that their larvae can escape. It would appear that the flukes' survival and laying habits under these circumstances offer little to no advantage as regards the continuation of the species.

Throughout the temperature range of -1°C . to $+21^{\circ}\text{C}$. miracidia are capable of remaining active and they are not adversely affected by long exposure to low or high temperatures. An average speed of 1.2 mm. per 20 secs. at -1°C . is increased to a maximum 1.2 mm. in 1 sec. at 18°C . Miracidia are therefore capable of hatching, swimming and presumably infecting molluscs throughout all seasons in the British Isles.

Miracidia are totally insensitive to light. When placed for short periods or several hours in a horizontal, water-filled tube at constant temperature, they were found uniformly distributed throughout both the illuminated section and the area in shadow. However, when placed in a uniformly illuminated vertical tube at constant temperature, miracidia inserted at the top were always found at its base. This response was rapid and occurred in under 5 minutes in a length of tubing 10 inches long and half an inch internal diameter. Upon leaving the tube for an hour, the area occupied by the larvae was extended slightly upwards and this effect could be increased by using tubes of narrower bore. This is a direct result of the avoiding behaviour of the larvae during which process they repeatedly strike against the side and base of the tube. In a confined space they inevitably rise on assuming a differently angled path. This was easily demonstrated by using tubes of markedly differing diameters and measuring the length of the tube occupied by the larvae after 5 minutes and after 1 hour in each. In all cases, after the shorter period the miracidia occupied a small basal area but, after 1 hour, although still remaining in the lower end of the tubes, they were more widely distributed, the maximum distribution being found in the tube with a narrower bore. This slight tendency to rise would not occur in nature where the marked gravitational response keeps the miracidia in close proximity to its bottom-dwelling intermediate host.

In nature, miracidia probably survive up to a maximum of three days and have been kept in tap water for more than two and a half days in the laboratory. In 0.25% sodium chloride solution, however, mira-

cial movement and reactions are slow and they die within the hour. True colonisation of estuarine conditions would therefore appear to be impossible even though the definitive host and Sphaerium corneum, the intermediate host, are both capable of tolerating brackish waters. Although the adult fluke might be recovered from Gasterosteus which had migrated into brackish areas, it is extremely doubtful whether the trematode would be able to complete its life cycle under such conditions.

The genus characteristically parasitises Lamellibranchs. The miracidium enters the molluscan host passively and is sucked in by the inhalent current together with other microorganisms. When freshly released and two-day-old miracidia were placed with either living gill tissue or intact molluscs of various genera, they exhibited no attractive response whatsoever. The older larvae were presumably in greater need of nutrients than freshly released specimens but they were unattracted even to the tissues of their true intermediate host. On colliding with the latter, they reacted as they would to any other obstacle. It was only when the ciliary beat of the gills disorganised those of the miracidium that the larva was forced to stop. Even then, if the larva managed to disentangle itself it would swim away. The entry of the miracidium into molluscan tissue was not seen.

The lack of attraction as regards the primary intermediate host may be a characteristic of most Gorgoderid miracidia. Goodshild (1948) noted such behaviour in Gorgodera amplicava miracidia but he was successful when utilising portions of gill tissue in attaining eventual penetration and recorded that it was accomplished in approximately 10 minutes. The behaviour of the miracidium is somewhat surprising if it

is related to the marked specificity shown by a number of Gorgoderids for their molluscan hosts. (at least according to present information).

Under natural conditions any mollusc with sufficient inhalent force to divert the path of passing miracidia can cause them to enter into the gill chamber. Young Sphaerium corneum up to 3 mm. across and many Pisidium species of most ages are thus excluded. Active miracidia, under stress from a weak divergent stream, even from an acceptable host, have a marked tendency to resist and pull away. In the laboratory, however, larvae were seen to enter Sphaerium corneum (from 5 mm. across and upwards); S. lacustre (4 mm. across); all stages of Dreissena polymorpha (except the juveniles under 5 mm. long) and large Anodonta cygnea specimens.

Miracidia, in the second and third days following their release, become slower and tend to adhere to objects and rest. Possibly in this state smaller molluscs, particularly Pisidium species, would be able to draw them into the gill chamber, but the chances of successful infection by such larvae are few.

P. solidum was reported by Goodchild in 1943 to be capable of successfully utilising specimens of Pisidium abditum reaching only 2 mm. in length. The size range of the P. solidum miracidia and those studied in this investigation overlaps to some degree but there is the possibility that the latter may be a stronger swimmer. Joyeux and Baer (1947) detected discrimination in the sizes of parasitised and uninfected hosts. A trematode which they believed to be Gorgoderina was only recovered from S. corneum above 5 mm. in length in the cercarial state suggesting miracidial entrance of molluscs in the 4.5-5mm. size range.

g) Infection experiments

Attempts to infect various Lamellibranch genera and species with Phyllodistomum larvae followed two main procedures: firstly, direct feeding of the miracidia to the molluscs and secondly the simulation of natural conditions by keeping infected fish in tanks containing uninfected molluscs.

Throughout all the experiments, the temperature of the water did not fall below 56°F. (13.34°C.), in winter or rise above 70°F. (21.12°C.) in summer and the pH remained approximately neutral. The size of the container was varied, particularly where fish were not involved, in order to concentrate the area and so increase the chance of infection. Aerators were placed and adjusted so that their presence could not interfere and cause turbulence in the miracidial swimming zone.

The molluscs were fed upon an algal solution derived from culture and provided the water was continually aerated and occasionally renewed, this method was found to be satisfactory.

No difficulty was experienced in keeping small molluscs and large specimens of Gasterosteus in the same tank and special enclosed protective chambers were not found to be necessary. Initial interest in the molluscs placed in gravel at the bottom of the tank consisted in snapping at the syphons but, with regular feeding, the fish soon became disinterested.

The results of the experiments are summarised in Tables 5 and 6 (pages 7-8). Successful infection, although occurring only in the case of Sphaerium corneum, was of sporadic occurrence and could not be predicted. Due to such variable success, little weight can be given to

the apparent host specificity shown in the results. However, failure to find alternative hosts in nature (a point which is illustrated further in pages 129) indicates that specificity is a characteristic of this species. S. corneum utilised in infection experiments were carefully chosen so that all sizes, except the smallest, were exposed to infection in order to test whether age resistance was involved. The results were inconclusive, but examination of molluscs from the 5 main areas investigated indicates that size and not age is the crucial and only initial factor and this has no physiological significance.

Goodchild (1943) listed 13 Gorgoderids which were then known to utilise the family Sphaeriidae in their larval phases but omitted the European Cercaria Gorgodera pagenstecheri reported by Wesenberg Lund (1934). Since Goodchild's article other Gorgoderids have been found which behave similarly, for example the American C. steelmani (Baker, 1943), C. rabbi (Dunagan, 1957) and the larvae of P. caudatum and P. lohrenzi according to Beilfuss, (1954). In addition a few have been reported associating with S. corneum including, in Europe, several Gorgodera species and P. simile, a parasite of Salmo trutta in West Wales, recorded by Thomas (1958).

TABLE 5

| Mollusc | Number of molluscs utilised | Miracidial dosage | Period of exposure | Total duration of experiment | Result of examination of molluscs |
|-------------------|-----------------------------|-------------------|--------------------|------------------------------|--|
| <u>Sphaerium</u> | 12 | 24 | 2 days | 10 weeks | - |
| <u>corneum</u> | 6 | 57 | 5 days | 4 weeks | - |
| | 16 | 1026 | 3 days | 12 weeks | - |
| | 12 | 35 | 11 weeks | 12 weeks | 1 infected (no fully developed cercariae present) |
| <u>Dreissena</u> | 24 | 190 | 9 days | 7 weeks | - |
| <u>polymorpha</u> | 24 | 140 | 5 weeks | 7 weeks | - |
| <u>Anodonta</u> | 2 | 160 | 7 weeks | 8 weeks | - |
| <u>cygnea</u> | | | | | |
| <u>Pisidium</u> | 30 | 50 | 3 weeks | 4 weeks | - |
| <u>pusillum</u> | | | | | |

TABLE 6

| Number of <u>Gasterosteus aculeatus</u> used. <u>Phyllodistomum</u> load in parentheses: post- mortem examination | Number of <u>Sphaerium</u> <u>corneum</u> used (1) | Period of exposure | Extra mira- cidial dosage | Result of examination of molluscs |
|--|---|-----------------------|---------------------------------|--|
| 2 (2) | 24 | 4 weeks | - | - |
| 6 (11) | 11 | 4 weeks | - | - |
| 1 (6) | 33 | 8 weeks | - | - |
| 9 (40) | 13 | 20 weeks | - | - |
| 1 (6) | 18 | 4 weeks | - | 1 infected (no fully- developed cercariae present) |
| 7 (58) | 30 | 8 weeks | - | 1 infected (fully devel- oped cercariae present) |
| 3 (42) | 21 | 12 weeks | 95 | - |
| 1 (6) | 18 | 16 weeks | 50 | 1 infected (fully devel- oped cercariae present) |
| | Number of <u>S. lacustre</u> used (2) | | | |
| 4 (8) | 6 | 8 weeks | - | - |
| | Number of <u>Anodonta</u> <u>cygnea</u> used (3) | | | |
| 5 (12) | 1 | 16 weeks | - | - |

SECTION 3 THE SPORO CYST GENERATIONSa) Method of investigation

Living specimens were studied with the aid of neutral red and dilute horse serum. Infected specimens of Sphaerium corneum were fixed in FAA, sectioned, stained in Mallory's Triple or Mayer's Haemalum and Eosin counterstain and mounted in Euparal or Canada balsam.

b) Mother sporocysts

The mother sporocysts lie within the interlamellar spaces protruding into the epibranchial cavity of specimens of Sphaerium corneum. Small specimens measuring 0.35 x 0.105 mm. contain approximately 6 developing sporocysts and are white, unbranched, elongate structures. The largest recovered measured 0.77 mm. in length x 0.21 mm. at maximum width. The anterior end is commonly, but not always, constricted and buried below the host's epithelium. Younger sporocysts are capable of actively probing and bending the extensile unenclosed region, but in specimens as small as 0.441 x 0.161 mm. this mobility is reduced to a few contractile waves passing along the walls. The greatest number of daughter sporocysts produced at any one time within the largest mother structures appears to be 12. The likelihood of a successful simultaneous invasion of the intermediate host by large numbers of miracidia in nature seems too small for this to account for the common severity of infection or the percentage of molluscs recovered harbouring numerous daughter sporocysts which had all reached a similar size and stage of development. The mother sporocysts therefore appear capable of either giving rise to several batches of daughter structures which release

cercariae, or of releasing a further generation of sporocysts producing daughter forms.

The thick muscular wall of the parasite possesses an inner layer of cells laden with dense refractile material which prevented the excretory system being completely traced. The arrangement of the ducts, however, was seen to be identical to that of the daughter generation. A sub-terminal birth pore is situated at the unattached end of the sac.

The density of infection in these early stages ranged from 237 to 1. When infected with 53 sporocysts and above, molluscs underwent a high mortality following transportation to the laboratory. Their sensitivity depended largely upon the size of the mollusc relative to the density of infection.

c) Daughter sporocysts

These are straight or strongly curved unbranched structures similar to the previous generation. The primary point of attachment is commonly narrowed and regarded by Goodchild (1943) and Thomas (1958) as the anterior end. The extent to which this region is buried beneath the host's epithelium varies and very often a single layered epithelial sheath suspends and supports the sporocyst for more than half its length. It seems likely that in addition to the host adding tissue to this sheath after it is initially formed, the sporocyst in the earlier mobile phases continues to burrow into the host, thus anchoring itself firmly and increasing the area for food absorption.

There appears to be no regular orientation or favoured region of the gills to which the sporocysts of either generation adhere. They

are found arranged at all angles across the lamellae from the gills' point of attachment with the molluscan body to the lamellar terminations. Sections of infected molluscs are basically identical to the illustration (Fig. 8) given by Thomas (1958) for P. simile.

The structure of the sporocyst wall is identical to the description given by Thomas (1958) in that it consists of an outer cuticle, narrow muscular layers and an inner single layer of parenchymatous cells.

Situated at the anterior end of the sporocyst is a denser region formed by the enlarged cells of the innermost layer. Thomas noted that this was the main site for the release of germ cells. Growth of the daughter sporocysts is accompanied with a progressive loss of mobility, finally resulting in the motionless sacs being deformed only by the contractions of their cercarial contents. Within the walls of many large, older sporocysts, there is a noticeable increase in the amount of refractile granular material present.

The excretory system, although difficult to determine, appears identical to that described by Thomas (1958) in consisting of two lateral dilated bladders into which both anterior and posterior canals open. The flame cell structure, their number $2\{4 + 4\} + (4)$ and main arrangement of the ducts appear to agree with Thomas's description and illustration (Fig. 11) although there is variation in the exact positioning of the flame cells and the finer tubules.

Thomas (1958) stated that the birth pore lay at the narrow end of the sporocyst. This presumably refers to the anterior attached region. A similar position was recorded for a number of Gorgoderids including P. solidum (Goodchild, 1943); Gorgodera amplicava (Goodchild, 1948);

C. conica (Goodchild, 1939); the rhopalocercous cercariae C. micromyae (Fischthal, 1951) and C. duplicata (Reuss, 1902, 1903). This is not the case in this species however. In the mother and daughter sporocysts the birth pore is an inconspicuous sub-terminal opening situated at the free posterior end of the cyst, an identical position to that recorded by Groves (1945) in contrast to Goodchild (1943) for P. solidum. Frequently, the sporocyst is enveloped by the monolayered host epithelium both at the anterior and posterior end so that Thomas's observations that the cercariae perforate this supporting envelope is also applicable here under these circumstances.

Another point of apparent difference between the observations made by Thomas (1958) for P. simile and this species lies in the mode of release of the cercariae. Thomas observed the larvae being released whilst enclosed within the cercarial chamber and passed in this state to the exterior. In this investigation, however, cercariae were observed to pass out of the birth pore unenclosed, oral sucker first, and either remained free until they reached the exterior or entered the chamber whilst within the epi-branchial cavity. Their ability to release themselves and re-enter the chamber (an occurrence readily observed in C. vitelliloba according to Wesenberg-Lund (1934)) may explain the discrepancies but two points make this doubtful. Firstly, cercariae were not observed to enter the chamber whilst within the sporocyst and secondly, release from the chamber is most commonly associated with older cercariae which have failed to contact an intermediary host and are about to cast their tails.

During this investigation sporocysts of varying sizes were found to contain fully developed cercariae. The smallest measured 0.588 x 0.448 mm. and contained 2 mature cercariae, 1 encysted metacercaria and a few germinal balls. The largest recorded measured 3.5 x 0.525 mm. and contained 22 cercariae which were nearing complete development, 1 encysted metacercaria and practically no germ balls. In the latter case termination of production appeared imminent. The average numbers of maturing cercariae recovered from sporocysts ranged between 5 and 11 in addition to 1-3 metacercariae being present. This data indicates that daughter sporocysts continue to grow for some time. They are all able therefore to release cercariae whilst still comparatively small and to continue production over a considerable period allowing the maximum chance for secondary host infection. The sporocysts whilst approximating the average size recorded for P. simile by Thomas (1958) are considerably larger than equivalent structures in P. solidum (Groves 1945, Goodchild 1943). It is perhaps significant that the latter fluke utilises the smaller intermediary host - Pisidium.

The length of time during which a host would be able to release cercariae and survive could not be established in this investigation due to the high mortality of heavily infected molluscs under laboratory conditions. The early release of cercariae from small sporocysts is of obvious value considering the susceptibility of parasitised molluscs to adverse conditions and fungal infections. Whilst a density of 30 sporocysts per host was well tolerated under laboratory conditions casualties increased with an infection density of 50 and above. The maximum number of daughter sporocysts found in one host was 88, a

marked reduction upon the maximum figures recorded for mother cysts. This would seem to indicate that mortality is also high in heavily infected host under natural conditions. From the time of miracidial entry to the release of the first cercaria required 2 months in the laboratory where the average temperature of 18.34°C (64°F) was maintained. In nature this developmental phase would probably be extended to an average 3 months in summer, as recorded for P. solidum by Goodchild (1940), and for a longer period in the cooler seasons.

d) Effects upon the molluscan host

d1) Sporocyst damage: In dense infections the ciliated food tracts along the lamellae are deformed, disrupted and discontinuous. The main cause of this dislocation is the burrowing action of the sporocysts, their irregular arrangement and pressure effects. Host cells overlying the sacs are only sporadically ciliated and this results in further ciliary loss. As previously stated, heavily infected molluscs are highly susceptible to changes in environment and, as a result, do not travel well nor survive for long periods under laboratory conditions. They also are readily attacked by fungal disorders. Such susceptibility was noted for C. conica by Goodchild in 1939 and Thomas (1958) suggested for a similar situation occurring in P. simile that the greatest harm to the host was probably caused by the interruption of feeding currents and oxygen supply due to the presence of large numbers of sporocysts within the epibranchial cavity and between the gill lamellae. Reduction in the effective epithelial surface definitely decreases the host's chances of survival but, under laboratory conditions,

these effects can be largely reduced by adequate aeration and a good food supply. This latter condition also offsets, to some extent, the steady drain of metabolites from the host's tissues. The continual release of waste products, possibly of a toxic nature, over and between the gills, is also likely to have some effect, particularly during periods when the gill chamber fluid is not being constantly renewed as occurs during transportation. It is even possible that in heavy infections the host is forced to continually flush the chamber at a rapid rate and forego the normal periods involving less expenditure of energy. It therefore seems probable that it is the steadily increasing release of waste products in addition to structural damage caused by the parasite that has the greatest final adverse effect upon the host.

d2) Cercarial damage

Although in contrast to the observations made by Thomas (1958) for P. simile where cercariae were seen to enter the epibranchial chamber in an enclosed state, the stylet, in this case borne on the free cercarial body, is also prevented from damaging the molluscan tissues. The cercaria is initially inert but when it does move within the epibranchial chamber the body is flexed in such a way that the stylet is not held in the normal operational position. Torn host epithelium was noted only when it had covered the terminal birth pore of the sporocyst and isolated patches of damaged tissue, conducive with stylet or sucker damage, were never discovered. The fact that the tissue overlying the sporocyst was distorted at an early stage and remained of little use to the host results in minimal damage

being caused by cercarial release. This observation contrasts with Vickers (1941) report that the gills of the mollusc S. corneum were ruptured upon the release of Cercaria macrocerca and that continual emergence of, for example, 1,276 cercariae, from one individual over a period of 23 days resulted in large areas of the gill being put out of action.

Reproduction in the intermediary host is not prevented even in the severest cases of Phyllodistomum infection. Further more, there is no reduction in the number of young produced by infected stock regardless of the density of infection (as can be seen from Table 7 below) and all the offspring are healthy, normal and easily reared. This suggests that the drain upon the hosts' food reserves is well tolerated. The most serious consequence of this infection lies in the long term effect upon the mollusc population as a whole since, by increasing the hosts's susceptibility to disease and adverse environmental changes, the chances of the molluscs surviving long enough to release their normal quota of young are proportionally reduced according to the level of infection. Thus, in shallower waters, where environmental fluctuations are more marked, the mollusc population could be seriously reduced in numbers as a result of this parasite.

TABLE 7

| Mollusc: maximum dimension (cm.) | Number of sporo- cysts (mother/ daughter gener- ations) | Number of young carried by infected molluscs | Uninfected mol- luscs. Number of young carried |
|---|--|---|---|
| 0.9 | 53 - 97 | 5 - 19 | 12 |
| 1.0 | 69 | 14 | 6 - 21 |
| 1.1 | 60 - 237 | 18 - 23 | 11 |
| 1.3 | 1 - 76 | 9 - 24 | 8 - 12 |
| 1.5 | 51 | 10 - 38 | 15 |

In contrast to these findings concerning reproduction Vickers (1941) reported that the pressure from over 100 sporocysts of C. macrocerca prevented the development of S. corneum embryos. Reproduction was not entirely prevented however by lighter infections although this suggests that an effect could still be traced. The sporocysts, measuring a maximum 2.0 x 0.7 mm. and containing 12 cercariae, were within the size range of the Phyllodistimum larvae being studied and a lack of comparable effects in the latter appears at first sight to be anomalous. It is probable that the lesions in the gills caused by cercarial release in C. macrocerca however provide an additional depressent to reproduction by interfering with the quantity of food available to the host.

SECTION 4 THE CERCARIAEa) Cercarial development

The developmental phases of cercarial formation are basically identical to those indicated by Thomas (1958) for P. simile. The ventral sucker primordium is the first structure to make its appearance in larvae reaching a size of approximately 0.16 x 0.116 mm. This is followed by the development of an inconspicuous oral sucker and small rounded tail bud at an overall larval size of 0.28 x 0.13 mm. The point at which the tail is attached progressively narrows and for some time this rounded tail bud and the cercarial body are approximately equal in length. Rudiments of the excretory bladder cells first become visible at this time. Increase in the rate of growth in length of the tail eventually exceeds that of the body whilst anteriorly, immediately posterior to its point of attachment, it gradually expands to form the initial rudiment of the cercarial chamber. The developing stylet, arising dorsally, is also visible in these larvae which measure a total of 0.523 mm. (which includes a tail length of 0.278 mm.) x 0.112 mm. at maximum body width. In larger cercariae reaching a total length of 0.634 mm. the thick walled excretory bladder, extending from immediately behind the ventral sucker is well developed and the cercarial chamber is noticeably enlarged. Both the gut and the penetration glands are apparently fully formed prior to the completion of the tail which is now weakly motile. Expansion of the cercarial chamber continues, the cuticle separates from the underlying globe of vacuolated cells,

particularly anteriorly, where a second, smaller, cuticular swelling is formed around the attachment point of the cercarial body.

Extended specimens at this stage reach a maximum length of 1.012 mm., the body measuring 0.425 x 0.087 mm. and the total length of the tail 0.587 mm.

The time taken to complete cercarial development is unknown.

A comparison between measurements of the developmental stages of P. simile obtained from Fig. 12 in Thomas's (1958) article and the measurements given here reveals slight size differences in the later stages. This can be related either to the contractile state of the specimens or to the fact that the fully formed cercariae in this investigation are essentially smaller than those described by Thomas (as can be seen on page 102), and that this difference is reflected relatively early in their formation.

b) The fully Developed Cercariae

b1) Method of investigation

The fully developed cercariae were examined in tap or pond water and measured live. Neutral red was found to be a useful intra-vital stain. The tissues were cleared and the flame cells stimulated by the use of dilute horse serum. Permanent whole mounts of the cercarial bodies only were made because the tail chamber was generally badly distorted when dehydrated. Similar distortion and collapse was reported by Goodchild (1948) for the cercarial tails of Gorgoderia amplicava. Cercariae were fixed in 70% Alcohol and stained in Acetoalum Carmine, mounted in Canada

balsam and measured. Sections of infected molluscs were fixed in F.A.A., stained with Mallory's triple or Mayer's Haemalum and Eosin.

The release from a host of only a few cercariae at any one time, the difficulty in keeping infected molluscs alive for long periods in the laboratory and the restricted nature and low density of the infections in the areas examined severely limited the number of fully developed cercariae available, both for infection experiments and measurement. As a result, many of the cercariae had to be obtained directly by dissection from the molluscan host and these were carefully compared with known mature forms before being measured. The results of the investigation are summarised on page 130, and cercarial structure is illustrated on pages 82, 87 and 93.

b2) Structure

The cercariae may be classified as macrocercous. They belong to the so-called "Gorgoderina" group erected by Sewell in 1922 and are similar in many respects to the description given by Thomas (1958) concerning P. simile. The differences between these cercariae are discussed on page 102.

The Cercarial Body

The cercarial body is muscular and highly contractile, and its regional proportions are consequently variable. It is identical to the adult fluke as regards its ridged cuticle, papillary patterns, range of contractile movements, the extent to which the gut is developed and the arrangement of its excretory and nervous systems. Detailed descriptions of these are given on pages 234 to 269.

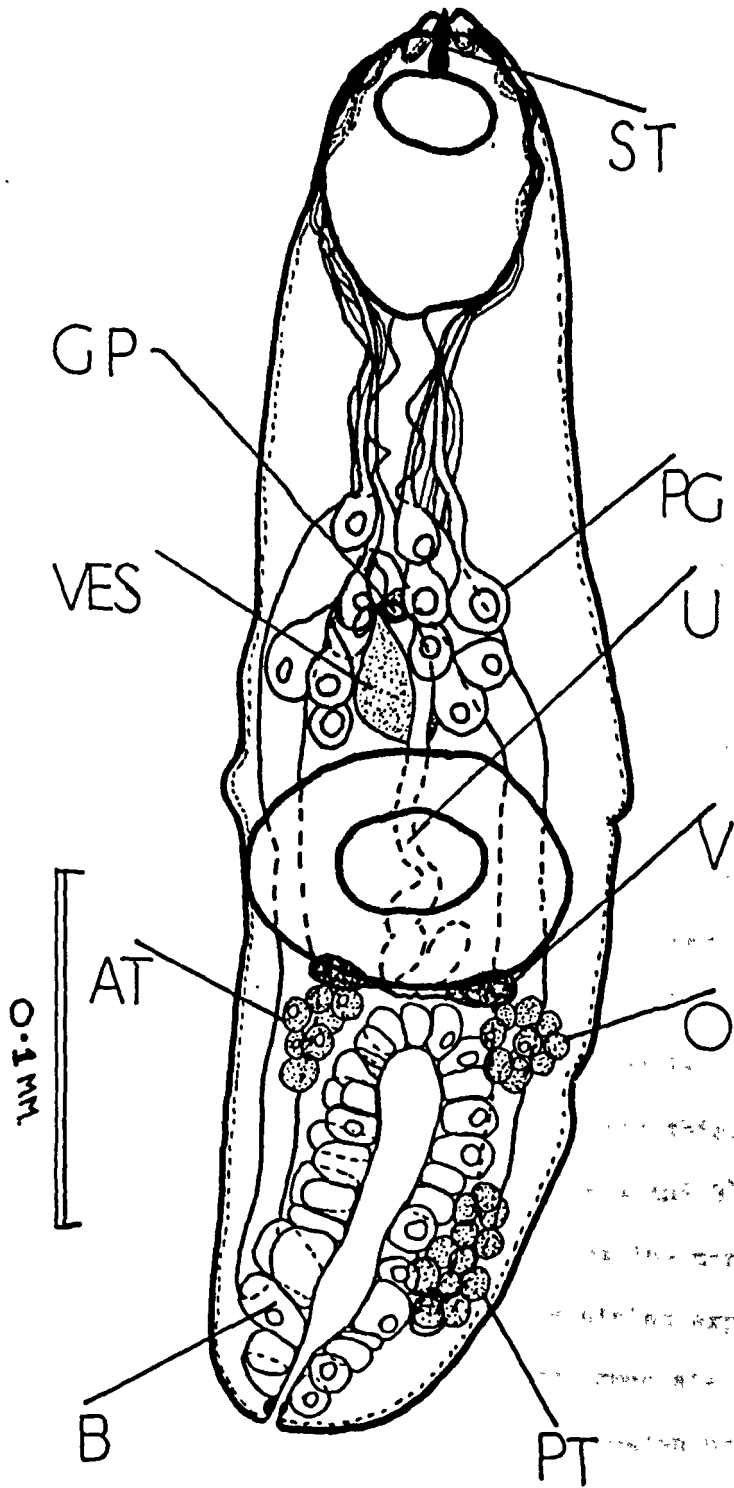
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THE CERCARIAL BODY - VENTRAL VIEW OF A LIVE SPECIMEN

Abbreviations utilised in the diagram:

- AT = Anterior testis
- B = Excretory bladder cell
- O = Ovary
- PG = Penetration gland
- PT = Posterior testis
- ST = Stylet
- U = Uterus
- V = Vitellaria
- VES = Seminal vesicle

(drawn with the aid of the camera lucida and the assistance of neutral red staining).



PHYLLODISTOMUM - CERCARIA.

There are, however, a few points which differ from the adult state. The cuticular papillae, for example, are more noticeable in the cercaria, metacercaria and extremely young fluke than they are in the larger adult phases. These papillae, presumably tactile in function, apparently possess a different proportional growth rate than the body and, particularly in the case of the marginal papillae, may lose some of their extensile qualities in older flukes. A possible reason for this may lie in the fact that these structures are of greater importance to the migrating and early urinary wandering stages than to the established adult and are correspondingly comparatively larger during the juvenile period.

The oral sucker bears a stylet upon its dorsal surface. This structure is illustrated on page 87 diagrams A-C. It lies within a well-defined, elongate cavity in the median antero-dorsal region of the oral sucker with the ovoid opening to the chamber lying immediately above the bifid muscular lip. This eosinophilic structure, colourless in life, bears a main cutting spine which is directed anteriorly when held in the erect position. Behind this, lying dorsally, is a tilted longitudinal keel (diagrams A and B) bearing two prominent points, the larger of which occupies the more posterior position. Posterio-laterally to the spine the stylet expands on either side to form rounded wings. It narrows immediately posterior to the median point along its length, gradually broadening again forming the rounded base (diagram C). The stylet is erected for use by means of muscles attached to its base and below the keel. In this position, illustrated in diagram A, it is held at right angles to the longitudinal

body axis so that it protrudes well beyond the aperture of the stylet chamber. Thus when the anterior region of the fluke is depressed and the oral sucker drawn back, the erect stylet is brought into a prominent anterior position. The distal spine is then scooped forwards and performs the initial cut which is gradually widened as the beginnings of the wings are driven into the wound; a prowess which is continued in the vertical plane by the keel. Repeated slashing cuts of this type in conjunction with the probable release of penetration fluid, form an effective and rapid cutting mechanism. When not in use, the stylet is returned to a recumbant position (indicated by a broken outline in diagram A), by relaxation of the stylet muscles and lies approximately at a 45° angle to the longitudinal body axis.

This description differs mainly from that given by Thomas for the stylet of P. simile (1958) in the rounded shape of the base. This is quite distinct from the truncated form recorded by that author. In shape the stylet tends to resemble more closely several structures described for the Gorgoderina genus such as G. varsoviensis Ssinitzin (1905) or G. amplicava Krull (1935). The stylet also appears similar to that recorded for Gorgoderina vitelliloba by Ssinitzin (1905) and in addition overlaps its size both in the length and the breadth of the various regions.

Emptying into the stylet chamber are the ducts of 12 penetration glands. Six apertures, arranged in a cluster on either side of the stylet, open slightly posteriorly to the stylet wings. The ducts to these glands curve obliquely away and then, by a tortuous path, pass posteriorly, around the oral sucker dorsally on either side of

the oesophagus, finally expanding into large gland cells upon reaching the level of the developing vesicular seminalis. The glands are never found anterior to the gut bifurcation in the transverse plane. They range from a completely intercaecal position posterior to the bifurcation and anterior to the acetabulum, to one overlapping the caecae on either side. The entire gland cell cluster was never completely extra-caecal in any of the cercariae examined. The glands, from the cells to their openings, are almost as long as the anterior region of the fluke and, on average, the two differ by as little as 0.0033 mm. in mounted material. The total length of the system, discounting the sinuous nature of the ducts which allows for their considerable extension in the active larva, measured an average 0.194 mm. in 5 mounted extended specimens. The gland cells lie ventral to the gut caecae but slightly dorsal to the vesicle anlage. They possess a large nucleus, an eccentric nucleolus and contain a transparent fluid which occasionally has extremely fine granules suspended within it. The fluid contents tend to aggregate where the ducts bend forming swellings similar to those noted by Fischthal (1951) for C. micromyae.

The position and number of penetration glands described for this cercaria is a feature which it shares in common with several other Gorgoderid cercariae in Britain, America and the Continent. For example, P. simile (Thomas, 1958); C. conica, C. donecercia, (Goodchild, 1939); C. macrocerca (Vickers, 1941) all possess 6 pairs of penetration glands and at least in P. simile and C. macrocerca the glands occupy an identical intercaecal position as described in this species.

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A DIAGRAMMATIC REPRESENTATION OF THE CERCARIAL STYLET

Diagram A:

Lateral view of the dorsal region of the oral sucker showing the stylet in the erect and recumbent positions.

(The latter state is indicated by a broken outline). The arrows represent the results of fibrillar contraction and relaxation.

Abbreviations utilised in diagram A:

- C - Stylet chamber
- DL - Dorsal lip
- EM - Erector muscle
- K - Keel
- M - Mouth
- P - Papilla
- PD - Penetration ducts
- PM - Protractor muscle

Diagram B:

'Dorsal' view of the stylet - Keel uppermost

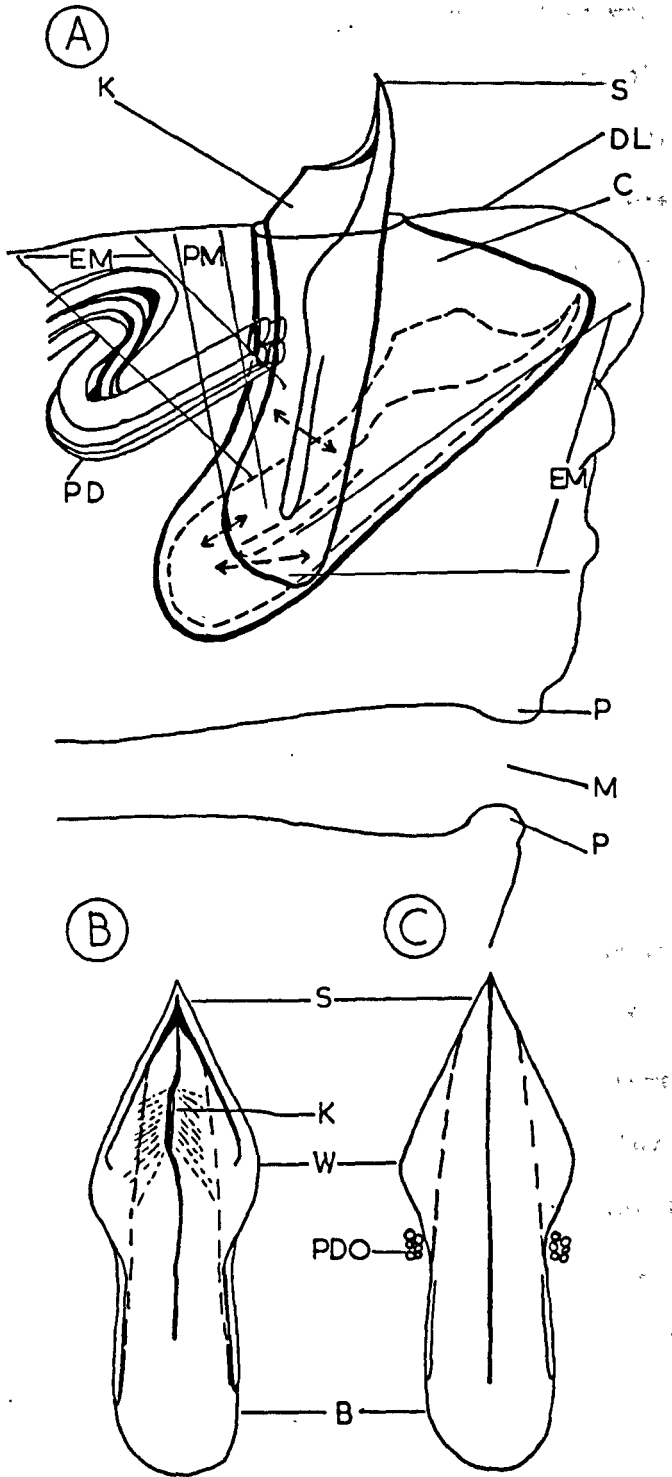
Diagram C:

'Ventral' view of the stylet - Wings uppermost

Abbreviations utilised in diagrams B and C:

- B - Base
- K - Keel
- PDO - Penetration duct openings
- W - Wings

(Information for these diagrams was obtained from both living and mounted, sectioned material with the aid of an oil immersion lens).



PHYLLODISTOMUM ~STYLET.

The close association of the stylet and the duct apertures indicates that the glands are penetrative in function. This would explain why metacercariae obtained from sporocysts within molluscan hosts retain fluid within the penetration glands and ducts. However, this latter condition also applies to larvae recovered from secondary intermediate hosts, whilst the penetration glands found within adult flukes are always empty. The explanation for this apparent anomaly would appear to lie either in overproduction which is made necessary by the variation in cercarial behaviour at this time and/or in the possibility that the secretion also functions in the excystment.

Prior to encystment, there are three main situations in which penetrative secretion could be involved. Apart from (a) the actual penetration of the host's gut, cercariae have been seen (b) attacking the cuticular ring on failure to enter the chamber and (c) releasing themselves from the chamber by cutting directly through it. This latter behaviour occurred occasionally when the movement of the cercaria, with the body enclosed in its chamber, was artificially restricted by means of a cover slip. The usual scooping movement of the stylet-bearing anterior end was accompanied by a sucking action of the oral sucker which drew the chamber wall tightly over the stylet and thus increased its cutting power. It was also, by this means, that large numbers of pre- and circum-oral papillae, particularly those on the under surface of the lip, were strongly stimulated. It seems likely that this latter action may lead to the automatic release of penetrative fluid whenever the stylet is used in addition

to the glands secreting at a later stage in response to pH and other chemical changes. Presumably, the mechanical stimulatory threshold for the glands is relatively high so that release of content does not occur during commonly executed actions such as the gentle plucking movements of the oral sucker upon the cercarial and metacercarial body. It has been shown above that the cercaria can be induced to use the stylet and possibly release penetrative fluid (although this latter was not observed) when artificially confined. In nature perhaps either the continued restriction of movement within the confined space of a crowded sporocyst or the oesophagus of the intermediate host, (or, alternatively, damage to the tail during the host's masticatory processes) would be sufficient to obtain a similar result. The sporocyst walls would therefore be largely resistant to small quantities of these substances. As a result of such free and variable use of the stylet, it is necessary that the glands produce sufficient secretion to ensure penetration following the possibility of considerable fluid loss. Utilisation of this secretion during excystment may occur but host enzyme action is likely to be of greater importance.

The cells surrounding the excretory bladder, in marked contrast to the penetration glands, contain coarse granules which show great affinity for eosin and neutral red. Throughout the development of the cercaria there is a steady increase in the number present in each cell. The nucleus is situated farthest away from the bladder cavity whilst the granules are packed on the opposite side of the cell nearer to its opening. They are completely lacking in the

equivalent, but smaller, excretory cells of the metacercaria. Any fine granulation present in the cells of the older larva differs markedly from that found in the cercaria and is sporadic in occurrence although the density is not affected by age. Thomas (1958) observed encystment in P. simile and stated that the source of the primary secretion lay in the excretory cells. This would also appear to be true for this cercaria and for most Gorgoderids (Fischthal, 1951, Coil, 1954). The bladder occupies a position equivalent to that in the adult and is of identical basic structure. The transparent, non-staining fluid contents are released at regular intervals upon relaxation of the sub-terminal muscular sphincter and pass out via a dorsal excretory pore. The flame cell pattern is identical to the adult system and does not extend into the tail.

Large eosinophilic gland cells are scattered throughout the cercarial body. They are situated more particularly in areas immediately posterior to the ventral sucker and anterior to the genital pore on both the dorsal and ventral surfaces. The granulation is noticeably finer than that occupying the bladder cells. The slender protoplasmic extensions of these multibranched cells can easily be traced through the cuticle to the exterior. Similar cells were never found in the adult and could not be traced within the metacercaria. Their function, as suggested by Thomas for P. simile would appear to be cystogenous. Unicellular glands presumably cystogenous in function and located throughout the body of cercariae have been described for C. eriensis, C. lamsilae, C. anodontae and C. pyriformoides by Coil (1953, 1954), and were possibly found anteriorly by

Vickers (1941) in C. macrocerca (according to Goodchild (1943)). Although Fischthal (1951) suggests that these may have been penetrative. Dunagan (1957) reported that cystogenous material obscured the arrangements of the penetration glands in C. rabbi and excretory system of C. ruddi but did not describe the cells in detail. Specialised cells scattered throughout the body of Gorgoderid cercariae however are generally assumed to possess a secondary systogenous function.

The genital primordia are differentiated into a hermaphrodite system, which is arranged in a fashion comparable to that of the adult. The uterus, however, consists merely of an ascending limb and, in some specimens, the primordia remain/^{more} closely apposed to one another than in the succeeding metacercarial stages. The description given by Thomas (1958) agrees completely with the condition found in this cercaria.

The Cercarial Tail

The form of the tail is distinctive. It is similar in form to C. macrocerca (Vickers, 1941), C. Gorgoderina vitelliloba (Ssnitizin, 1905) and C. steelmani (Baker, 1943) but is much smaller and resembles that of C.P. simile (Thomas, 1958) more closely. The tail is divided into three main regions:

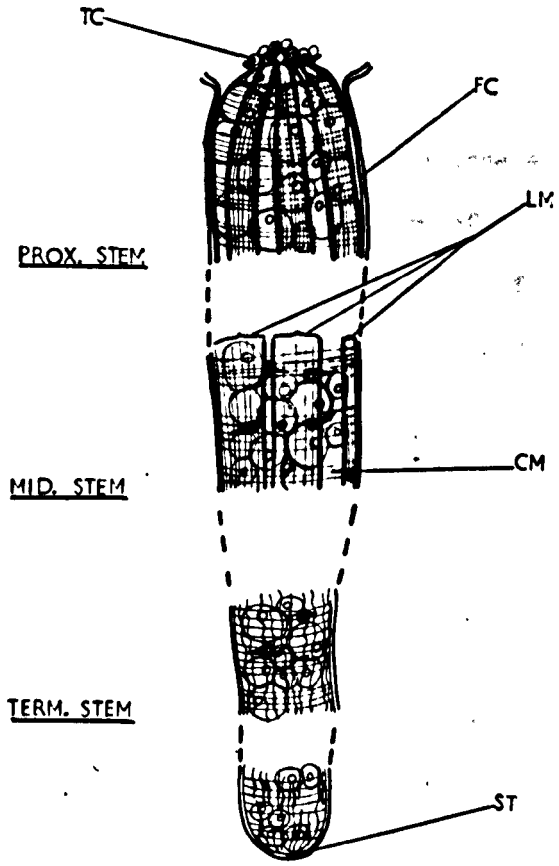
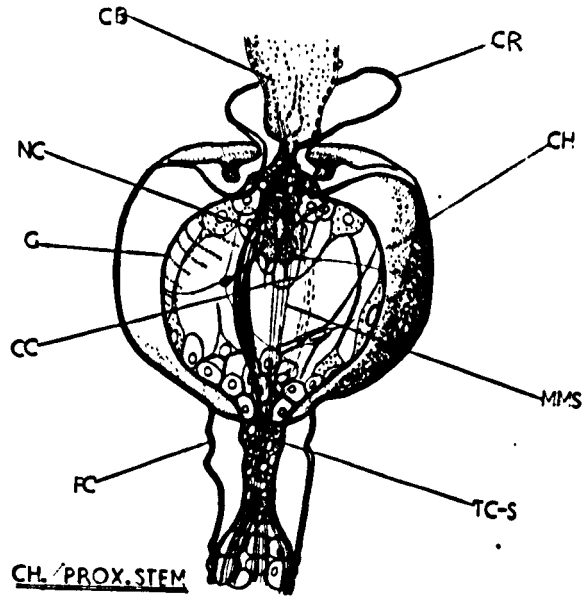
- (1) a proximal cuticular expansion or ring surrounding the attachment point of the cercarium,
- (2) a globular chamber into which the cercarial body can be withdrawn, and
- (3) a narrow distal portion, the stem, which provides the locomotive power during swimming. Tail structure is illustrated in detail on page 93.

PHYLLODISTOMUM - CERCARIAL TAIL

A diagrammatic representation of the regions of the cercarial tail of Phyllodistomum as it appears in the living state.

ABBREVIATIONS

- CH/PROX.STEM = Diagram of the chamber and proximal region of the tail stem during mild longitudinal muscular contraction.
- CB = Cercarial body - (extreme posterior end)
- CR = Cuticular ring
- NC = Nerve cell
- CH = Cuticular chamber
- G = Cellular globe
- CC = Central column of cells in globe
- MMS = Median muscle strand
- FC = Cuticle of stem folded slightly as a result of muscular contraction and free from underlying tissues.
- TC-S = Terminal (distal) cell group elongated and pulled into the stem by muscular contraction.
- PROX.STEM = Diagram of the proximal region of the stem in a relaxed specimen.
- TC = Terminal (distal) cell group protruding into the chamber
- FC = Stem cuticle free from underlying tissues at this point
- MID STEM = Diagram of the median section of the stem.
- LM = Longitudinal muscle bands - now wider and with fibres spaced further apart than in the proximal section
- CM = Circular muscles
- TERM.STEM = Two diagrams representing the terminal (distal) regions of the stem
- ST = Smooth stem tip



PHYLLODISTOMUM - CERCARIAL TAIL.

The unenclosed cercarial body is attached to the tail by a narrow stalk which lies in a ventral position to the excretory pore. Longitudinal muscle bands pass along this stalk and traverse a compact mass of small cells situated at the proximal end of the globe which is strengthened at this point by a localised increase in the thickness of the cuticle. The longitudinal muscle strands, divided into three main blocks, then pass medially and on either side of the mid-line through a spherical mass of large cells which are surrounded and completely separated from an expanded cuticle. In the distal portion of the sphere, at the stem base, the longitudinal muscles traverse a group of small, closely-packed cells, similar to those found immediately below the stalk.

The majority of large cells occupying the sphere are arranged peripherally with only two triangular clusters of cells protruding into the centre of the cellular ball. The cells of the sphere are bound together by a fine outer cytoplasmic layer containing circular muscles, and they are inter-connected by means of narrow, protoplasmic strands. A few large multibranched nerve cells are also present.

In the narrow tail stem, the longitudinal muscles are arranged in several distinct bands which only lose their precise arrangement at the extreme stem tip. By their rapid contraction, they provide the main motile force of the tail and are antagonised by a fine layer of circular muscles and a resilient cuticle. The circular muscles lie externally to the longitudinals and form a regular series of transverse blocks along the length of the tail.

The stem is also packed with cells of various sizes. Large,

rounded, pale-staining cells are interspersed with small interconnected ones, possessing finely granular contents. Many of the unbranched processes arising from the latter pass peripherally but, because they do not perforate the cuticle, they are not considered to represent the site of production of the adhesive substances which are subsequently exuded by the tail. They appear to supply the muscle layers and they may therefore act as nerve cells. They are, however, unlike the larger nerve cells found within the chamber. Thomas (1958) noted a central strand of small, elongated cells running medially through the tail stem of P. simile cercariae and a similar chain was found in C. macrocerca by Vickers (1941). Such a structure was not noted in the living, preserved or sectioned specimens of this cercaria.

c) Cercarial Behaviour

cl) Enclosure within the chamber

Slight longitudinal muscular contraction causes tail flexure and depresses the proximal region of the cuticular expansion of the globe, pulling the cercarial body downwards towards the chamber. The tail is repeatedly flexed and coiled and the cellular sphere is twisted freely around within the comparatively static cuticular globe. Immediately below the chamber there is an area of separation between the cuticle and the underlying muscle. Posterior to this region, the cuticle becomes progressively more closely applied to the muscle layers so that the two are firmly attached throughout most of the tail stem. Upon more pronounced muscular contraction, the total length of the cercarial tail can be shortened by the withdrawal of

the more distal cellular regions of the sphere into the stem; the subsequent compensatory folding of this detached area of cuticle and further depression of the globe. The contraction may be so great that the cluster of small globe cells following their elongation into the stem are compressed and a swelling forms within the tail at the junction of the moveable tissues and those firmly attached to the cuticle.

Eventually, such contractions successfully draw the cercarial body into the terminal globe. The proximal cuticular ring is pulled inwards upon the entry of the cercarium into the chamber and no trace of this structure remains in the enclosed larva. The cercarial body occupies only the anterior portion of the cuticular sphere, while the original circular cluster of cells is compressed into the more distal area. The body, remaining attached to the tail by means of the muscular stalk, curves round upon itself within the confines of the chamber and lies at right angles to the longitudinal tail axis. The narrow opening to the chamber through which the cercaria passed is closed, leaving only a slight prominence in this region. If the tail tip is removed the cercaria cannot become encapsulated and activity on behalf of the cercaria, seemingly towards this end, merely results in the stylet cutting the cuticular ring and is always unsuccessful.

c2) General cercarial behaviour

Cercariae appear to be released at any time of the day or night as recorded for C. raiacauda Steelman, 1938, C. macrocerca (Vickers

1941) and for P. simile by Thomas in 1958. If the body is unenclosed upon its release, it remains passively contracted during periods of vigorous tail movement. Following encapsulation however, the body actively moves around in the chamber and rest periods are short and infrequent. Unlike P. simile, the cercariae move a short distance away from the host and do not attach to the latter. Locomotory progression of the tailed cercariae in any one direction is, however, restricted. The erratic muscular contractions succeed in raising the cercariae from the substratum only in periods of vigorous activity. During such movement, the chamber is lashed back and forth in the water forming a conspicuous white moving sphere. Eventually they adhere to the substratum or bottom vegetation where the tail movements continue. The primary point of attachment in the majority of cases is the tail tip and transparent droplets entangled in sticky threads bind the tail to its attachment surface. A sucker-like attachment as found in C. macrocerca (Vickers, 1941) and C. pagensteeheri (Wesenberg-Lund, 1934) is not involved. The terminal area was examined for minute spines similar to those recorded for C. macrocerca by Vickers (1941) and P. simile by Thomas (1958), but structures of this nature were only recorded on one occasion. The minute spines perforating the cuticle were visible when using an oil immersion lens and they occupied a very small terminal area. Either the spines are easily lost, as is the case of the sucker spines of the Gorgodera cercariae examined in this investigation (page 356), or they are of sporadic occurrence and may be a relic of an ancestral state where spines played a more prominent role. This topic is discussed further

on page 357-8. Secondary points of attachment occur throughout the length of the tail, eventually even involving the chamber. This serves only to restrict the tail movement still further and to reduce the conspicuous nature of the larva.

Towards the end of a 24 hour period tail movements weaken. Under such conditions the cercaria generally releases itself by passing through the chamber entrance, breaking the tail connection and crawls away exhibiting an identical series of contractile movements as are found in the adult fluke. Similar behaviour was noted in C. raia-cauda by Steelman(1938) but the same author recorded in 1939 that C. coelocerca broke out of the posterior wall of the chamber following its partial decomposition. This latter occurrence parallels behaviour noted occasionally (page 88) in this cercaria when artificially confined. Released cercarial bodies are incapable of forming a metacercarial cyst and eventually die some 12 to 24 hours later. No matter how long the cercarial body remains unenclosed it does not display any adhesive qualities similar to those of the tail. No specific glands or cell groups could be found within the tail to which this function of adhesive secretion could be ascribed. Similar terminal adhesion occurs in C. coelocerca according to Steelman(1939).

Throughout their active period, the cercariae exhibit no reaction to light, darkness, moving objects or potential invertebrate or vertebrate hosts. Identical lack of response was recorded for C. eriensis by Coil(1954).

Detailed measurements of Phyllodistomum cercariae

A The cercarial body

1) Total length (a) Living material

| | <u>Average</u> | <u>Range</u> |
|--|----------------|------------------|
| Average length of 10 specimens (slightly contracted - relaxed state) | 0.418 mm. | 0.350 - 0.462 mm |
| Maximum extensile range recorded for one individual from the fully contracted to the extended position | 0.238 mm. | |
| Minimum length recorded for a contracted specimen | 0.268 mm. | |
| Maximum length recorded for an extended specimen | 0.642 mm. | |

(b) Mounted material

| | | |
|--|-----------|-------------------|
| Average length of 10 specimens (slightly extended) | 0.391 mm. | 0.330 - 0.425 mm. |
|--|-----------|-------------------|

2) Regional proportions

(a) Living material

| | | |
|---|------------------|---------------------------------|
| Measurement of anterior region (excluding V/S) of 5 specimens (slightly contracted/relaxed state) | 0.179 x 0.091 | (0.128-0.240 x 0.070-0.144) mm. |
| Measurement of posterior region (V/S included) of 5 specimens (slightly contracted/relaxed state) | 0.193 x 0.086 | (0.139-0.227 x 0.063-0.140) mm. |
| Ratio: average anterior:posterior region | length = 1:1.078 | |
| Ratio: average anterior:posterior region | width = 1:0.941 | |
| Maximum extensile range recorded (each region measured separately) in one individual:- | | |
| anterior region = | 0.220 | |
| posterior region = | 0.128 | |

(b) Mounted material

| | | |
|--|------------------|---------------------------------|
| Measurement of anterior region (excluding V/S) of extended specimens | 0.184 x 0.084 | (0.147-0.205 x 0.073-0.103)mm. |
| Measurement of posterior region (V/S included) of 10 slightly extended specimens | 0.207 x 0.081 | (0.179-0.227 x 0.070-0.099) mm. |
| Ratio: average anterior:posterior region | length = 1:1.121 | |
| Ratio: average anterior:posterior region | width = 1:0.967 | |

3) Sucker sizes (a) Living material

| | | |
|--|----------------|---------------------------------|
| Measurements of O/S from 5 specimens (relative to body axes/& organ) | 0.071 x 0.058 | (0.063-0.074 x 0.051-0.063) mm. |
| Measurements of V/S from 5 specimens (relative to the body axes/& organ) | 0.073 x 0.072 | (0.063-0.086 x 0.061-0.077) mm. |
| Ratio; O/S:V/S (relative to body axes) | length 1:1.031 | (1:1.0 - 1:1.199) |
| | width 1:1.231 | (1:1.131 - 1:1.312) |
| Greatest diameter (relative to organ). | 1:1.031 | (1:1.0 - 1:1.199) |

(b) Mounted material

| | | |
|---|---------------------------------------|---------------------------------|
| Measurements of O/S from 10 specimens (slightly extended) (relative to body axes and organ) | 0.070 x 0.057 | (0.061-0.082 x 0.049-0.061) mm. |
| Measurements of V/S from 10 specimens (slightly extended) (relative to organ) | 0.073 x 0.069 | (0.061-0.085 x 0.057-0.079) mm. |
| Ratio; O/S:V/S (relative to body axes) | length 1:1.007 | (1:0.882 - 1:1.164) |
| | (relative to body axes) width 1:1.244 | (1:0.965 - 1:1.383) |
| relative to the organ (greatest diameter) | 1:1.035 | (1:0.950 - 1:1.375) |

4) Excretory bladder size

(a) Living material

| | | |
|--|---------------|---------------------------------|
| Average measurement of bladder from 5 specimens | 0.124 x 0.046 | (0.092-0.147 x 0.029-0.080)mm. |
| Average measurement of the posterior region of the 5 specimens above | 0.191 x 0.091 | (0.139-0.227 x 0.066-0.140) mm. |
| Average % of the posterior width occupied by the bladder | 53.11% | |
| Average % of the posterior length occupied by the bladder | 64.93% | |

(b) Mounted material

| | | |
|--|---------------|---------------------------------|
| Average measurement of bladder from 5 specimens | 0.133 x 0.048 | (0.125-0.147 x 0.037-0.055) mm. |
| Average measurement of the posterior region of the 5 specimens above | 0.216 x 0.083 | (0.205-0.227 x 0.070-0.099) mm. |
| Average % of the posterior width occupied by the bladder | 57.29% | |
| Average % of the posterior length occupied by the bladder | 61.34% | |

5) Cercarial stylet

(a) Living material

| | | |
|---|----------------------|--|
| Total size range from 5 specimens: | | |
| Total length | 0.0208 - 0.0235 mm. | |
| Depth at base (side view) | 0.0041 - 0.0051 mm. | |
| Depth of keel at wing level (side view) | 0.0041 - 0.0061 mm. | |
| Width - dorsal view across the wings | 0.00612 - 0.0071 mm. | |

B The cercarial tail

1) The cercarial ring (living material)

| | | |
|---|----------------|---------------------------------|
| Average size - lxb parallel to the body axes (5 specimens measured) | 0.0501 x 0.121 | (0.035-0.070 x 0.105-0.168) mm. |
|---|----------------|---------------------------------|

2) The cercarial chamber (living material - cercarial body free)

| | | |
|---|---------------|---------------------------------|
| Average size l x b parallel tail axes (10 specimens measured) | 0.263 x 0.214 | (0.210-0.354 x 0.182-0.301) mm. |
|---|---------------|---------------------------------|

3) Tail stem (living material)

| | | |
|------------------------------|----------------|-----------------------------|
| Average size of 10 specimens | 0.472 x 0.0792 | (0.385-0.560 x 0.070-0.091) |
|------------------------------|----------------|-----------------------------|

Abbreviations: O/S - oral sucker; V/S - ventral sucker.

All measurements are taken relative to the organ or area's axes except where stated otherwise.

The living and mounted specimens are not necessarily identical.

d). Discussion

Table 8 shows a detailed comparison between the cercarial measurements obtained during this investigation and those of the only closely similar description given by Thomas (1958) for P. simile. It is not clear whether Thomas based his measurements upon living relaxed specimens or mounted material. Although the living larvae were observed, measurements of the adult fluke were made upon mounted material and it can be assumed that this may also be the case for the cercarial stages. It is unfortunate that Thomas, working upon abundant material, did not state upon how many cercariae his measurements were based so that the extent to which his records represented the true range in size for the species could be judged more accurately. The figures 14 and 15 in Thomas's (1958) article are scale diagrams of cercariae. The measurements of these larvae exceed those given in the text and are included in Table 8 in parentheses.

The cercarial body, suckers and chamber tend to be smaller than the average sizes recorded by Thomas, although the regional proportions of the body, and the range of the sucker ratios, overlap. The stylet sizes generally coincide, but smaller structures were recorded occasionally in this investigation. The excretory bladder width appears proportionally narrower in relation to the posterior region in P. simile (at least from measurements obtained from the diagram).

The differences illustrated in Table 8 between P. simile and this cercarium do not appear to be very great. Structural differences do occur, however, such as the absence of spines within the sucker cavities and a marked contrast in the shape of the stylet bases. Also, the

papillary patterns and number of flame cells do not agree in the two cercariae. The significance of these differences will be discussed on page 427, following a more detailed description of additional features which may be used as taxonomic criteria and which remain constant throughout larval and adult phases.

Table 8: The Cercaria

| Region | Living material | Mounted material | Thomas 1958 |
|---|--|--|--|
| Body length | 0.418 (0.350 - 0.462 mm.) | 0.391 (0.330 - 0.425 mm.) | 0.45 - 0.65 (0.92)d |
| Contractile range | 0.238 mm. | - | 0.22 mm. |
| Anterior region minus V/S | 0.179 x 0.091 mm (av) (0.128-0.240 x 0.070-0.144 mm)(r) | 0.184 x 0.084 mm (av) (0.147-0.205 x 0.073-0.103 mm)(r) | - 0.20-0.30 x 0.11-0.16 mm (0.43x0.29)d |
| Posterior region plus V/S | 0.193 x 0.086 mm (av) (0.139-0.227 x 0.063-0.140 mm) (r) | 0.207 x 0.081 mm (av) (0.179-0.227 x 0.070-0.099 mm) (r) | - *0.23-0.34 x 0.11-0.16 mm (0.49x0.28)d |
| Anterior region: posterior region ratio | 1:1.078 (1:0.867 - 1:1.299) (lr) 1:0.941 (1:0.747 - 1:1.182) (wr) | 1:1.121 (1:1.058 - 1:1.258) (lr) 1:0.967 (1:0.888 - 1:1.008) (wr) | - *M 1:1.13-1:1.15 (lr) d(1:1.15(l)) d(1:0.98(w)) |
| Oral sucker | 0.071 x 0.058 mm (av) (0.063-0.074 x 0.051-0.063) (r) | 0.070 x 0.057 mm (av) (0.061-0.082 x 0.049-0.061 mm) (r) | - 0.074-0.082 x 0.064-0.070 mm (0.148x0.135) d |
| Ventral sucker | 0.073 x 0.072 mm (av) (0.063-0.086 x 0.061-0.077 mm) (r) | 0.073 x 0.069 mm (av) (0.061-0.085 x 0.057-0.079 mm) (r) | - 0.076-0.094 x 0.074-0.092 mm (0.181 x 0.177) d |
| O/S:V/S; length R/B/A | 1:1.031 (1:1.000 - 1:1.199) (r) | 1:1.007 (1:0.862 - 1:1.164) (r) | *M 1:1.03 - 1:1.14 (1:1.22)d |
| O/S:V/S; width R/B/A | 1:1.231 (1:1.131 - 1:1.312) (r) | 1:1.244 (1:0.965 - 1:1.383) (r) | *M 1:1.16 - 1:1.31 (1:1.31)d |
| Greatest diameter | 1:1.031 (1:1.000 - 1:1.199) (r) | 1:1.035 (1:0.950 - 1:1.375) (r) | - (1:1.22)d |
| Excretory bladder (av) | 0.124 x 0.046 mm. | 0.133 x 0.048 mm. | (0.290 x 0.120)d |
| Excretory bladder as a % of posterior region - width | 53.11% | 57.29% | (39.57%) (d) |
| Excretory bladder as a % of posterior region - length | 64.93% | 61.34% | (59.04%) (d) |
| Cercarial stylet length | 0.0208 - 0.0235 mm. | - | 0.0225 - 0.0235 mm. |
| Chamber of tail | 0.263 x 0.214 mm. (av) (0.210-0.354 x 0.182-0.301 mm) (r) | - | 0.32 - 0.41 x 0.28 - 0.41 mm. |
| Tail stem | 0.472 x 0.079 mm (av) (0.385-0.560 x 0.070-0.091) (r) | - | 0.37 - 0.59 x 0.11 - 0.12 mm. |
| Cercarial ring | 0.050 x 0.121 mm (av) (0.035-0.070 x 0.105-0.168) (r) | - | - |

Note:

(.)d signifies that measurement was obtained from diagrams given in 1958 article.

O/S and V/S are abbreviations for oral sucker and ventral sucker respectively.

(av) = average measurements

(r) = range. [(lr) = length. (wr) = width]

R/B/A = measured relative to body axes.

R/O = measured relative to organ.

* signifies that the measurements have been calculated from the figures provided.

*M signifies that the ratios have been calculated from the maxima and minima recorded and may only approximate the true condition.

Thomas recorded the sucker ratio as 1:1.03 - 1:1.19 but did not state the diameter to which these measurements referred.

a) General considerations concerning the Life Cycle

A review of the literature concerning this trematode genus reveals that Phyllodistomum not only characteristically parasitises Lamellibranchs but is capable of completing its life cycle by two methods involving a total of two or three hosts. The former cycle has been recorded from both Europe and America and is associated with the phenomenon of metacercarial encystment within the sporocyst. The latter cycle, involving the use of a secondary intermediate host, is of wider occurrence and has been reported from Europe, America, China, India and Japan. Goodchild (1943), Coil (1954), Thomas (1958) and Rai (1964) have summarised the life cycles of the Gorgoderidae and the topic has also been discussed by Goodchild (1948), Fischthal (1951), Pigulevsky (1953) and various other authors. In order to avoid undue repetition in the following discussion only a few relevant points are dealt with and life cycles which have been attributed to this genus (which have been both partially and completely experimentally established and where the definitive host is known) are briefly summarised in Table 9 on pages 104-5.

Ssinitzin in 1901 and 1905 described a unique life cycle involving motile, buoyant sporocysts which escaped from the mollusc Dreissena polymorpha containing encysted metacercariae to be eventually eaten by cyprinids. This life cycle was ascribed to P. folium by Ssinitzin but various authors have since concluded that the specimens he described belonged to Phyllodistomum macrocotyle (Luhe, 1909)

Table 9

A summary of the life cycles attributed to the genus Phyllodistomum

1) Definitive host - a Fish

| <u>Definitive host</u> | <u>1st intermediate host</u> | <u>2nd intermediate host</u> | <u>Author/ Country</u> | <u>Species according to author</u> |
|---|--------------------------------|--|----------------------------------|------------------------------------|
| Cyprinids: <u>Carassius vulgaris</u> <u>Abramis brama</u> (according to Yamaguti 1958) ("Karausche & Brachsen") of Ssnitzin) | * <u>Dreissena polymorpha</u> | - | Ssnitzin Poland (1901) | <u>P. folium</u> (?) |
| <u>Salmo trutta</u> | * <u>Sphaerium corneum</u> | - (not recovered but probably present) | Thomas (1958) Wales | <u>P. simile</u> |
| <u>Ameirus melas</u> | * <u>Musculium elevatum</u> | - (not recovered) | Beilfuss (1954) N. America | <u>P. caudatum</u> |
| <u>Lepomis cyanellus</u> | * <u>Musculium transversum</u> | <u>Oecetis cinerascens</u> <u>O. inconspicua</u> <u>Leptocella sp.</u> | Beilfuss (1954) N. America | <u>P. lohrenzi</u> |
| <u>Esox lucius</u> <u>Salvelinus sp.</u> <u>Perca fluviatilis</u> | <u>Anodonta sp.</u> | <u>Libellula quadrimaculata</u> | Scheer (1951) Germany | <u>P. folium(?)</u> |
| <u>Odontobutis</u> (= <u>Mogurnda</u>) <u>obscura</u> <u>Parasilurus asotus</u> | ? | <u>Palaemon (=Macrobrachium) nipponensis</u> | Yamaguti (1934) Japan | <u>P. macrobrachicola</u> |
| <u>Odontobutis obscura</u> | ? | <u>Neocaridina denticulata</u> (possibly <u>Leander naucidens</u>) | Shibue (1954) Japan | <u>P. macrobrachicola</u> |

| | | | | |
|---|------------------------------------|--|--|----------------------------------|
| <u>Heteropneustes</u> <u>fossilis</u> | ? | <u>Macrobrachium</u> <u>davanus</u> | Rai (1964) India | <u>P.sriv-</u> <u>astavai</u> |
| <u>Parasilurus asotus</u> <u>Pseudobagrus</u> <u>aurantiacus</u> | ? | <u>Macrobrachium</u> <u>nipponensis</u> | Kurokawa (1934) Japan | <u>P.folium</u> (?) |
| 2) <u>Definitive host - a Frog</u> | | | | |
| <u>Megalobatrachus</u> <u>japonicus</u> <u>Diemyctylus</u> <u>pyrrhogaster</u> | ? | <u>Macrobrachium</u> <u>nipponensis</u> | Kurokawa (1934) Japan | <u>P.folium</u> (?) |
| <u>Megalobatrachus</u> <u>japonicus</u> | ? | <u>Palaemon</u> <u>longipes</u> | Kurokawa (1935) (from Yamaguti (1958)) Japan | <u>P.folium</u> (?) |
| <u>Bufo boreas</u> <u>boreas</u> | <u>Pisidium sp.</u> | Damsel fly naiads Caddis fly larvae | Crawford (1939) N.America | <u>P.</u> <u>americanum</u> |
| <u>Bufo boreas</u> <u>boreas</u> <u>Ambystoma</u> <u>tigrinum</u> | <u>Pisidium sp.</u> | Damsel fly larvae Caddis fly larvae Diving beetle larvae | Crawford (1940) N.America | <u>P. (?)</u> |
| <u>Desmognathus</u> <u>fuscus fuscus</u> | <u>Pisidium</u> <u>abditum</u> | <u>Ischnura</u> <u>varticalis</u> <u>Argia sp.</u> <u>Enallagma sp.</u> <u>Libellula sp.</u> | Goodchild (1940) (1943) N.America | <u>P.solidum</u> |
| <u>Desmognathus</u> <u>fuscus fuscus</u> <u>Eurycea bislineata</u> <u>bislineata</u> | <u>Pisidium</u> <u>nusillum</u> | <u>Ischnura</u> <u>posita</u> <u>D.fuscus</u> <u>fuscus</u> larvae | Groves (1945) N.America | <u>P.solidum</u> |
| <u>(Rana clamitans</u> | <u>Sphaerium simile</u> | <u>Sialis sp.</u> <u>Cambarus sp.</u> | Hunt (1952) N.America | ?)) |

N.B. * signifies metacercarial encystment occurs in the molluscan host.

Odhner, 1911 and later in addition to Phyllodistomum (Phyllodistomum) dogieli according to Pigulevsky (1953).

In Britain in 1954 and 1958, a more conventional life cycle was completed experimentally by Thomas for P. simile by feeding meat balls each containing approximately 100 encysted metacercariae obtained from Sphaerium corneum to 12 two-year old trout (Salmo trutta). Thomas recorded that S. corneum formed a common constituent of the fish population's diet and proved that a two host cycle was in existence. However, he concluded that an alternative three host cycle might be utilised by the parasite, although a secondary intermediate host was never found, and based his hypothesis upon a comparison between the percentage frequency of Sphaerium in the diet of fish from two differing stations and their respective percentage infection with Phyllodistomum over a period of upwards to one year. The existence of a secondary intermediate host in this case, however, may be supported by other considerations. Thomas did not record the minimum size attained by the fish host at which it was capable of ingesting molluscs large enough to carry a mature Phyllodistomum infection. He recorded that from two to five parasites were recovered from one-year old trout and that S. corneum was found in the stomachs of fish of 1+ years of age. According to his 1954 and 1964 articles, the smallest average size of fish from the infected stations was 8.4 cms. in length. In this investigation, Sticklebacks 7 cms. long (including tail fin) were incapable of ingesting infective Sphaerium although smaller pieces of such flesh were accept-

able as food. Unless the fish were capable of extracting the mollusc from its shell by "worrying techniques" there is a possibility that a secondary host was necessarily involved in the infection of the smaller one-year old Trout.

In America in 1954, Beilfuss indicated that P. caudatum might be able to complete a two host cycle. He based his conclusions on the fact that after an intensive search he was unable to locate any secondary intermediate hosts but he did not record actually infecting the definitive host with metacercariae from infected clams.

In Europe the existence of a secondary intermediate host in cycles involving a definitive fish host has been reported only on one occasion. This was by Scheer in 1951. In America Beilfuss (1954) reported that P. lohrenzi was capable of completing both a two and three host cycle similar to the situation suggested for the British P. simile by Thomas in 1958. In Japan and India, in contrast to the countries in the western hemisphere, the life cycle includes a crustacean host where apparently fresh water shrimps are utilised exclusively. Kurokawa in 1934 reported that a trematode, which he referred to as P. folium from Japan (a diagnosis doubted by Yamaguti, (1958) was capable of utilising both fish and amphibian hosts in addition to possessing a three host life cycle. This is the only occasion when a single species of this genus has been recorded as lacking specificity as regards the definitive host at the phylum level.

Where amphibian definitive hosts are involved, the life cycle falls into the pattern: Lamellibranch-to-Insect larvae or nymphs-to-

Amphibian and in this respect is closely similar to other Gorgoderid genera, namely Gorgodera (according to Ssinitzin (1905) and Scheer (1951)) and Gorgoderina (according to Ssinitzin (1905) and Lutz (1926)). The secondary intermediate hosts most commonly recorded for Phyllodistomum in the western hemisphere are carnivorous Trichopteran and Coleopteran larvae and Odonatan nymphs. Within the Gorgoderidae as a whole, however, there are several variations. Krull (1933, 1935 and 1936) recorded the passive ingestion of the cercariae of Gorgodera amplicava by various gastropods and, similarly, Gorgoderina attenuata was noted utilising Pseudosuccinea columella by Rankin (1938 and 1939). The latter trematode and Gorgoderina vitelliloba were reported infecting Amphibian tadpoles by Rankin (1938 and 1939) and Lees (1953) respectively. The life cycle of Gorgoderina vitelliloba as elucidated by Rankin (1939) and Lees and Mitchell (1964) and Gorgodera amplicava established by Goodchild (1945 and 1948) is of interest as regards the marked lack of specificity shown at both the intermediate and definitive host levels. Secondary intermediate hosts of the two genera ranged from Urodele and Anuran larvae to Gastropods, Megaloptera and Odonatan nymphs. In addition, Martin reported from Indiana in 1937 that even Crayfish of the genus Cambarus carried G. amplicava metecercariae. The definitive host range was more restricted but still included various species of Rana and Triturus v. viridescens in G. vitelliloba; five Ranid spp., and a simple species of both Bufo and Ambystoma for G. amplicava. In contrast, marked specificity occurred at the molluscan phase in both

cases.

The present investigation into the life cycle of a species of Phyllodistomum recovered from Sticklebacks in various water systems in the counties of Essex, Surrey and Middlesex during the year 1961-1963, in view of the above evidence, was based upon the following broad concepts. Although in Britain the only record of a Lamellibranch host for this genus involved Sphaerium corneum, the association of this parasite with numerous species of Anodonta, Pisidium and Dreissena (in addition to other species of Sphaerium (Cylas) in the palearctic zone as a whole) made it necessary to examine in detail all available Lamellibranch fauna recovered from the collecting areas. Smaller samples of the frequently numerically dominant Gasteropods were examined purely to check the possibility that either the attached larva or the crawling older cercarial stages (the latter having cast their tails) might survive ingestion and encyst in these hosts. The successful utilisation of a gasteropod host in the completion of the life cycle, however, was rendered unlikely because these molluscs did not feature as a regular constituent of the Stickleback's diet. Small Limnaeids, but recently released from the egg, were the only gasteropods ever recovered from the gut of the 401 Gasterosteus examined. These were far too small to play any part in the life cycle of this parasite especially on consideration that it is doubtful if stylet-bearing macrocerous cercariae at any stage are capable of the initial active penetration through the external tissues of an intermediate host. According to Coil (1954) these cercariae require the stimulation of ingestion before the host's tissues can be traversed. It

was soon established that the primary intermediate host in this investigation was S. corneum and that encystment of the metacercaria within this mollusc was a common feature. Study of the gut contents and feeding behaviour of the definitive host however, illustrated that the regular ingestion of Lamellibranchs did not occur (see Section 7B page 201). When other food was available they showed no interest in the molluscs and syphon-snapping and general worrying behaviour never resulted in their ingestion or death. From a total of 401 Gasterosteus examined, only three contained Lamellibranch remains and these consisted of one to four specimens of S. corneum all of which were too small to be parasitised. Limitations to the omnivorous diet are based mainly upon availability and the size of the prey in comparison to that of the fish although, strangely, even Pisidium spp., accessible and plentiful in some areas, were not utilised. As the size of infected Sticklebacks ranged from 2 - 7 cms. (a measurement which includes the tailfin) it was obvious that the primary intermediate host was not being ingested in an entire state. There appeared therefore, three possible ways in which the cycle could be completed. Firstly, that the young fish were sufficiently attracted to the cercariae to attack and ingest them and consequently serve them as a metacercarial host; secondly that the infected soft tissues of a dying mollusc could be ingested during the gaping period prior to the death of the parasite and, thirdly, that an invertebrate intermediate host was required for the regular completion of this parasite's life cycle. The first alternative appeared to be a possibility

in view of Nybelin's report (1926) concerning the encystment of P. megalorchis in the minnow and was checked further when it was found that very young Sticklebacks did in fact ingest cercariae. The fish were too large at this stage to be eaten by the adult Sticklebacks despite cannibalism but there was the further possibility that if successfully infected they might act as intermediate hosts for larger fish of other genera. The second alternative, whilst a definite possibility, appeared too haphazard a method to account for the percentage of infected fish recovered from the various collection areas. (Section 78 page 182). A secondary intermediate host was therefore obviously essential for the completion of a life cycle involving such a small definitive host as the Stickleback.

The search for a second intermediate host was concentrated upon Odonatan nymphs and Trichopteran and Coleopteran larvae and all carnivorous forms with mouth parts large enough to cope with these fairly bulky cercariae. Large specimens of Isopods and Amphipods were also examined but they were only sampled in small numbers. It was considered, following the examination of their feeding methods and gut contents that the shredding action of their mouth parts reduced all food particles to too fine a state to allow successful infection by this particular trematode. This conclusion was ratified on finding all specimens of Asellus and Gammarus to be completely free from any trematode infection whatsoever. Later attempts to experimentally infect these Crustacea with Gorgoderids proved unsuccessful (page 143). Collections were therefore selective and were not proportionally representative of the animal populations sampled

at any one time.

b) Source of material and collection areas

During this investigation fish from five water systems were found to carry Phyllodistomum infections. A summary of the invertebrate fauna examined and the parasites recovered is given on pages 130 & 134. Maps 1 - 3 on pages 114, 118, 121 show the main collective areas involved.

The first system to be investigated was in Bushy Park, Middlesex. This Park contains a stretch of the Longford River which, as can be seen from Map 1, page 114, arises from the Duke of Northumberland Waterway. The latter joins both the Rivers Crane and Colne. By means of piped underground passages the Longford River also flows into the Thames from the Park at Hampton. There are two main stretches of water within the Park and the water levels in both are regulated by an overflow system. System A, a direct continuation of Longford River, was the one originally chosen as a collecting site since it was not used by the public to any great extent and supported a higher Stickleback population. Tributaries of this System pass through cultivated plantations and could not be sampled. System B included two boating lakes which were less suitable especially for hand-net collection.

System A is largely an artificial river with boarded sides and little marginal vegetation carrying mainly filamentous plants such as Spirogyra. It varied in depth from approximately 18 to 2 inches during the investigation, and the rate of flow throughout the seasons was extremely slow. The river bed in the Park consists of soft, fine mud although in places outside this area it sometimes

COLLECTION AREAS -

Maps 1 - 3.

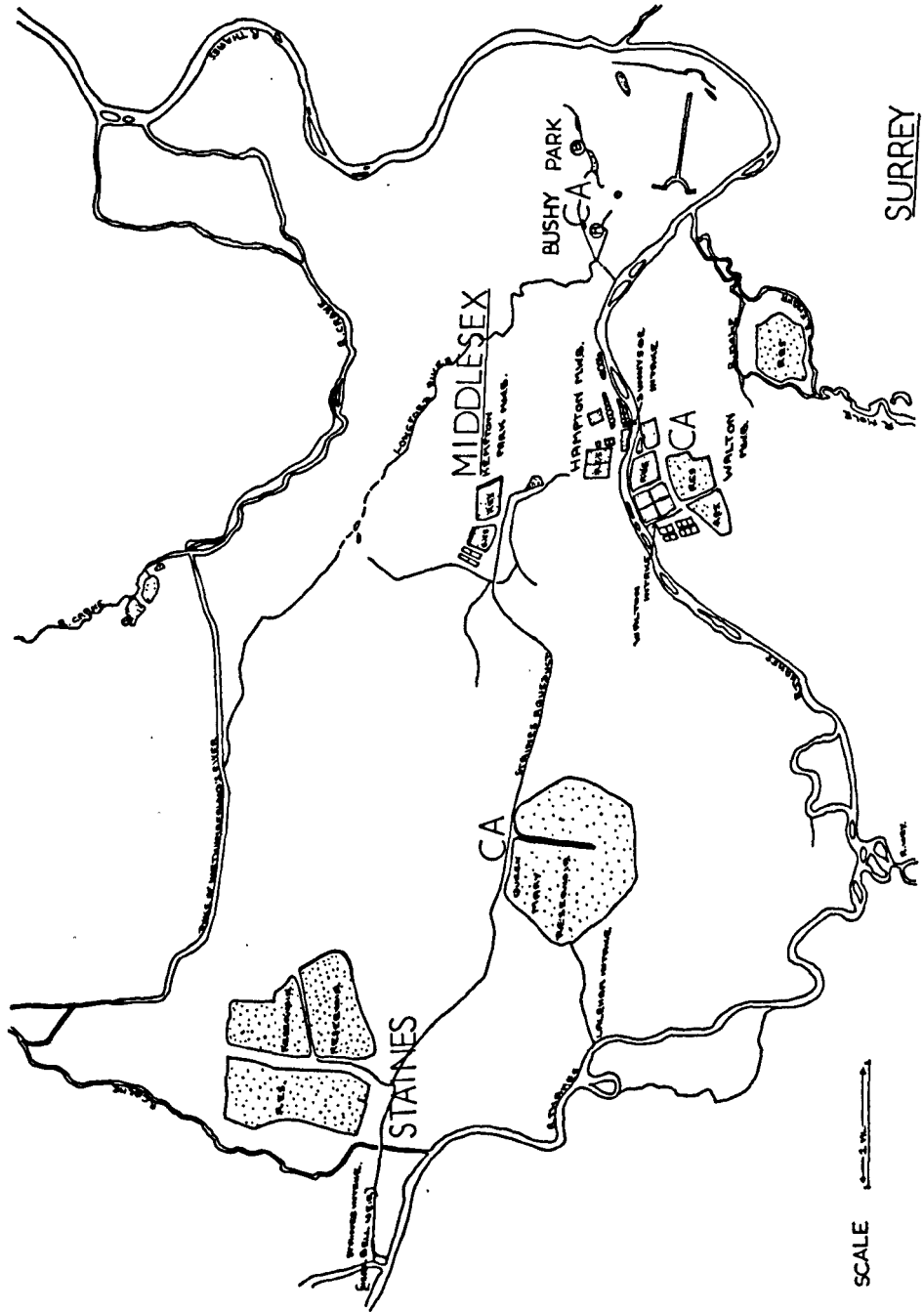
Abbreviations utilised in the three maps, pages 114, 118, 121.

CA = Collection area

RES = Reservoir

A & B = The river systems in Bushy Park
referred to in the text.

(Only relevant water systems are depicted).



COLLECTION AREAS. MAP I.

changes to a loose gravel. The water is hard and undoubtedly polluted but in the Park area this is increased to a marked extent by the presence of cattle, deer, horses and dogs. The water supported a Stickleback population feeding mainly upon Annelids, plankton and other Crustacea together with a few insect and beetle larvae and their adults. The fish were hosts to a small Phyllodistomum population but were heavily infected by both ecto- and endoparasites which considerably stunted their growth. (The Parasites found in this area are summarised on pages 227-229).

It was originally planned in 1961 that Bushy Park should be examined monthly for at least two years in order that the annual cycle of the parasite, together with its longevity, density of population throughout the year and other factors could be studied. However, upon the fourth monthly collection it was found that the river system had been dammed and the water level considerably lowered. This had the effect of reducing the density of the invertebrate fauna when followed by weed clearance in April and it was not until May that there was a sign of recovery. During this period the alternative river System B was utilised but was found to be poorer in both invertebrate fauna and parasites. During May, June and July fish were breeding successfully despite further temporary weed clearance in July. The invertebrate population decreased in numbers throughout July until August, when the river system was dammed, the water level lowered and System A thoroughly cleaned. Simultaneously, there were epizootics of fish ectoparasites and molluscan endoparasites and

this latter factor also considerably effected the alternative inferior System B. Throughout August, September and October of the year 1962, the area underwent a high mortality of all aquatic fauna which rendered it useless for further monthly collections. Although Sticklebacks began to re-invade this river stretch some months afterwards, Lamellibranchs did not return so readily and the early migrant fish were not infected with Phyllodistomum. Examination of this area was therefore terminated.

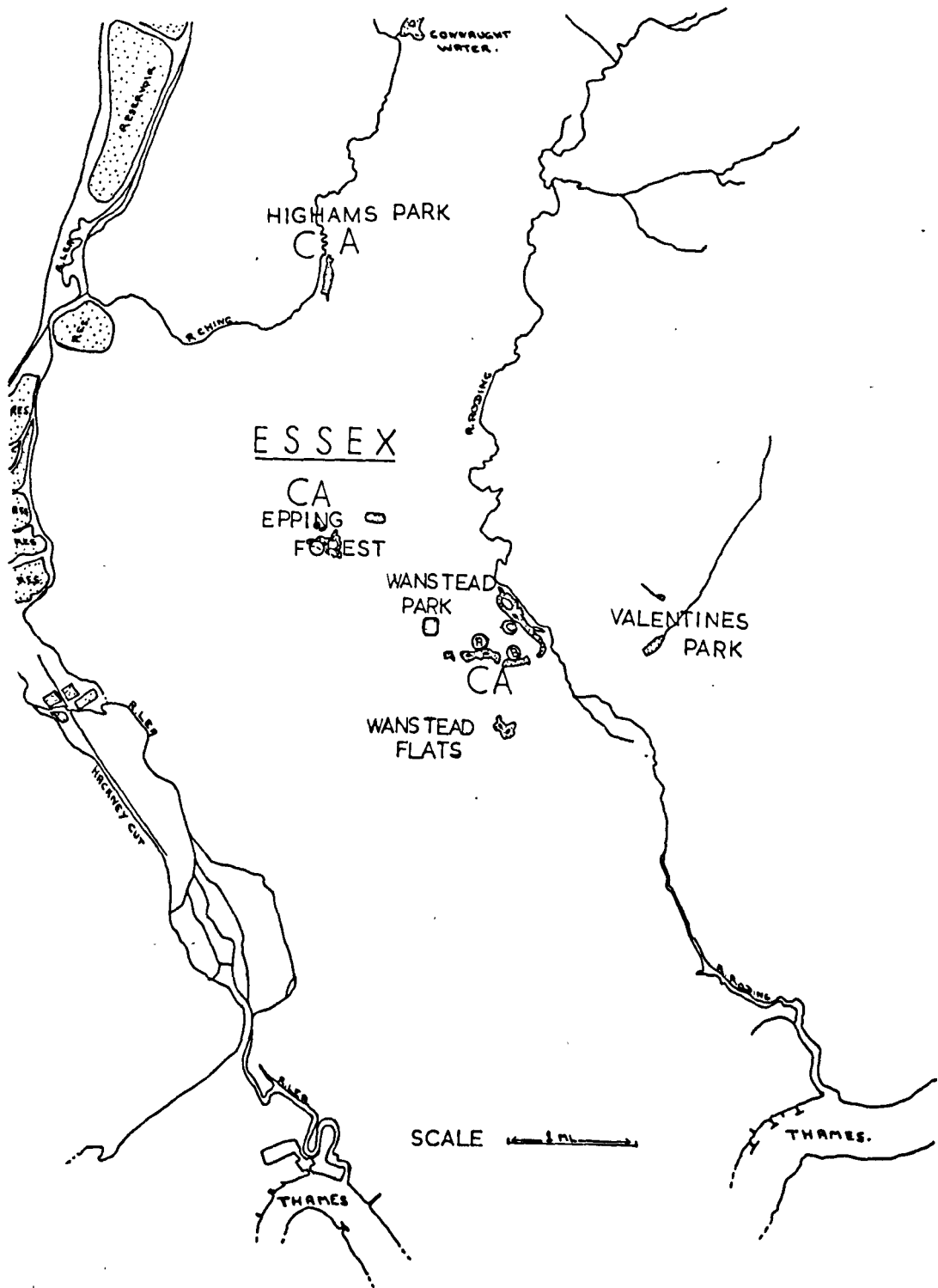
Whilst collections were being made in Bushy Park, a freelance collector offered to supply Sticklebacks from a site in Essex but declined to disclose the sampling area. The fish were heavily infected with Phyllodistomum but remained free from most of the other parasites which had severely stunted the Bushy Park population. Requests for additional vertebrate and invertebrate fauna were disappointingly fulfilled and a few months later supplies ceased completely. During this period, the firm of Haig's in Newdigate, Surrey supplied 88 Sticklebacks together with invertebrate fauna from ponds in their estate but these proved to be uninfected as regards Phyllodistomum.

In June, 1962, By kind permission of Dr. Ridley, Perch, Roach and Bream were obtained from the Metropolitan Water Board Staines Aqueduct. This channel connects up with the Thames and the Staines and Queen Mary Reservoirs by means of piped waterways. (Reference Map 1, page "4"). Insect larval fauna of the reservoir and channel was relatively poor and was mainly represented by Chironomids. The

molluscan fauna was composed of species from two genera, Dreissena and Limnaea. Samples of the former obtained from the Walton Works were free from any infection and were used in laboratory experiments. The fish from the aqueduct were uninfected by Phyllodistomum. The reason for this probably lay in the regular clearance of the channel and the frequent use of copper sulphate and chlorine in these waters preventing the establishment of suitable hosts. Further examination of the fauna in the Metropolitan Water Board's systems was therefore thought to be unprofitable and was terminated.

Various areas in Essex were examined including the water drainages of Valentines', Wanstead and Highams' Parks. (See Map 2, page 118). The commercialised waterways of Valentines' Park proved to be unsuitable for collection purposes. The lakes A, B and C in Wanstead Park, on the other hand, although concrete or wood-sided, represented a series of relatively untroubled lakes. Lake A was chosen as the principal collection site. The gravel and mud bed sloped gently away from the side to a depth not exceeding two feet so that the entire lake could be sampled. The boating lakes B and C were deeper, immediately reaching a depth of two feet or more along their reedless, steep-sided banks. All the lakes are situated on high ground above the River Roding and are allowed to overflow when necessary through subterranean pipes into the river. Water is occasionally pumped up to the lakes from the Roding in order to maintain the level of lakes B and C in particular. During this investigation there were no additions made to the waters of Lake A and the connect-

RECEIVED 1945 MAR 2



COLLECTION AREAS. MAP 2.


ing pipes to the boating Lake B, were permanently closed. Samples however taken from A were likely to be largely representative of both lakes at least in respect of fish parasites.

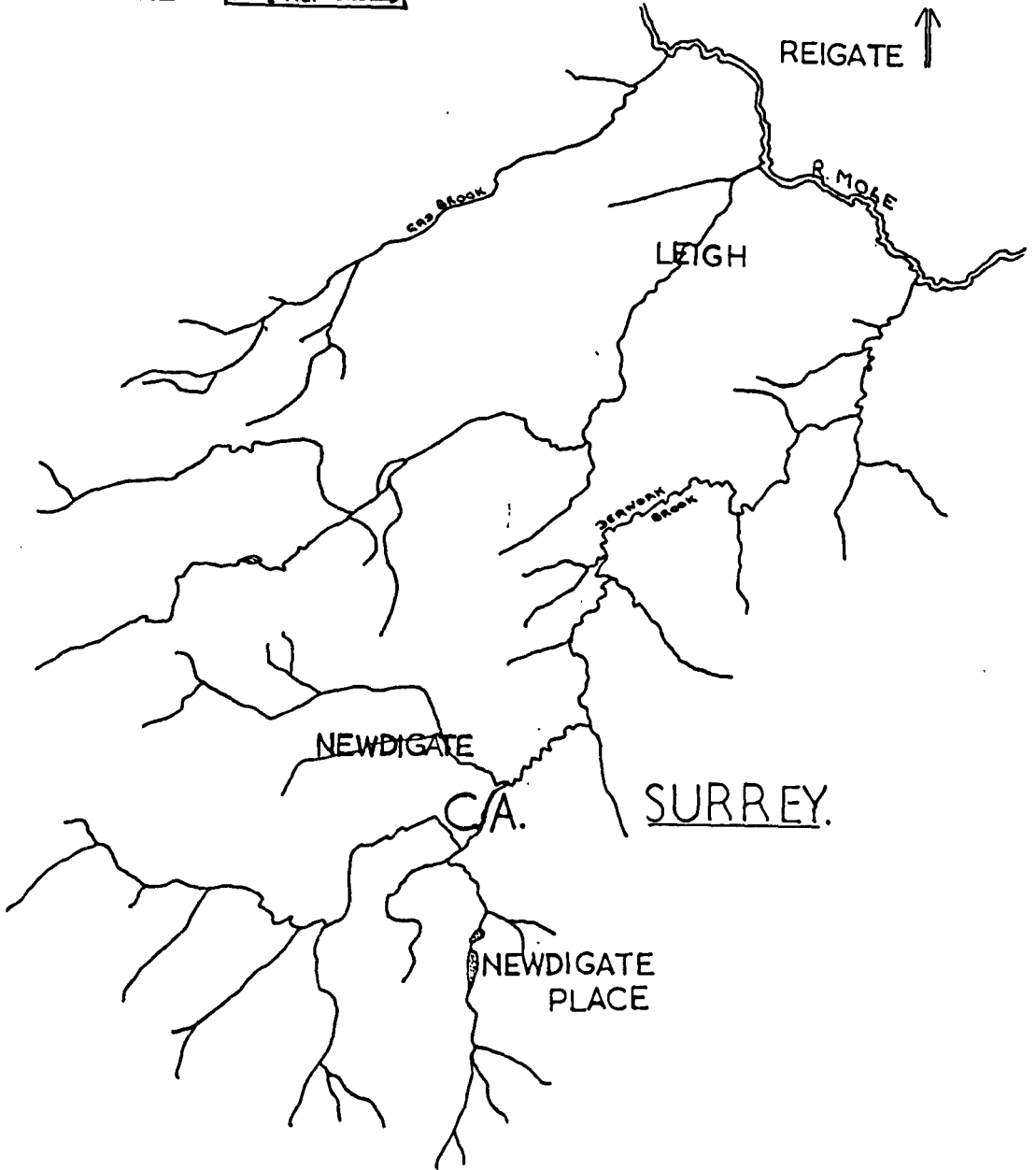
The fish population of Wanstead carried a small Phyllodistomum infection but were heavily parasitised with other Protozoa and Helminths and were not suitable for laboratory experiments. The first collection was made in July 1962 following a long dry spell. The water level was low (reaching a maximum of eighteen inches) and the invertebrate population depleted consisting mainly of Gasteropods, Planktonic forms and Annelids. The only insect larvae to be recovered were a few Chironomids and a single Dytiscus larva. During the following dry months, a return to this area was ^{not} considered profitable until after steady rain had begun to fall in late September. Examination of the area in October revealed that the water level had risen only slightly (reaching a maximum of two feet) and that there had been a considerable reduction in the fish population. Sphaerium corneum shells were collected from a restricted area close to what appeared to be a small spring near the centre of the lake and around the margins amongst the reeds. Living specimens, however, were not recovered.

An area in Highams Park (illustrated on Map 2, page 118) was also investigated during July and October of 1962. The River Ching in this region constitutes a steep-sided, narrow, meandering stream subject to considerable and rapid fluctuation in both speed and depth and was observed to rise from one to three feet in fifteen minutes following a storm. Meagre shelter is available and the invertebrate

fauna was limited. Chironomids, Limnaea spp. and Pisidium sp. were restricted to localised areas of soft mud and silted regions where there was a reduced rate of flow. The fish were healthy and mainly troubled by external parasites and were not supporting a Phyllodistomum infection. Even large fish of well over one year in age were free of this trematode and S. corneum was not recovered from these waters.

In August 1962 a batch of fish obtained from the Newdigate area which was previously considered to be Phyllodistomum-free was found to be carrying this parasite. Enquiries made to the distributors revealed that the collection site had been changed from their series of one hundred small ponds to a section of the Deanoak Brook which traversed their property. This brook, a tributary of the River Mole (see Map 3, page 121) was utilised in maintaining the level of the ponds throughout the year. The infection could only spread sporadically to the artificial clay-banked ponds through the small-bore pumping mechanism and by means of collectors' equipment transferring unwanted or unnoticed fauna from pond to pond and also from the brook itself. Since a large quantity of invertebrate fauna could be guaranteed from this supplier it was decided that all the ponds harbouring a) Gasterosteus and Sphaerium corneum and/or Pisidium sp. and, if possible, in addition, b) Trichoptera, c) Coleoptera and/or d) Odonata should be investigated. Unfortunately the stream itself yielded little invertebrate fauna, as a result of being dammed and cleaned at regular intervals. A large percentage of all the Brook

SCALE 



NEWDIGATE COLLECTION AREA.
MAP 3.

fauna, according to the collector, originated from two well-stocked private lakes at Newdigate Place which seemed a likely origin for the infection but from which they were not allowed to obtain samples. The migrant fauna collected from the Brook consisted mainly of Sticklebacks and a few Sphaerium, both of which were infected by Phyllodistomum. Anodonta was reported to occur in these waters but was not recovered during the investigation period. Due to the sporadic origin of the Phyllodistomum infection in the Newdigate area a concentrated examination of both the vertebrate and invertebrate fauna caused a temporary termination of the parasitic population in May 1963, when investigation of freshly collected stock from this area was terminated.

As the productivity of the Newdigate fauna began to decrease a return visit to Wanstead and Highams Park was made in March, 1963. Following the hard-winter and general lack of rain the maximum water level in lake A had been reduced to eight inches. The level of the boating lake had dropped to about a foot and, even in its deeper regions, an inch-thick layer of ice still covered the surface. The Gasteropod population was practically eradicated leaving masses of rotting Limnaeids, Planorbids and a few Valvata piscinalis shells only. The remains of the small S. corneum colony was traced to their principal habitat in the centre of the lake but no living specimens were recovered. Stickleback carcasses were strewn about the lake bed and were too rotten to warrant postmortem examination. Also present were the remains of twelve small Roach and one small Pike, all of which were in bad condition. In lake B larger Roach and several Pike, reaching a length of fifteen inches, were floating

about in the water. The reason for this devastation appeared to be basically due to the low water level which had allowed the main body of the water to freeze. Depletion of invertebrate stock, plus parasitic and predator toll, reduced the vertebrate stock of both the Wanstead and Highams areas in addition to many ponds in the Epping Forest examined during the dry summer and severe winter of 1962/1963.

Small numbers of fish and molluscs were obtained from other localities at various times during this investigation. Large Pike, measuring an average 2 ft. 6 ins long and numerous large specimens of Anodonta cygnea were examined from Lake Windermere. They did not carry a Phyllodistomum infection however. An assortment of Phoxinus, Gasterosteus, Rutilus and Gobio collected from ponds in Surrey and Epping Forest by King's College Zoology technicians and kept in an aquarium for several months were made available and the Gasterosteus, from the latter area, yielded a few large Phyllodistomum.

c) Results of the examination of Invertebrate Fauna from the Collection

Areas

(A detailed record is given on pp. 125-135. Summaries can be found on pp. 130, 136).

Phyllodistomum infected lamellibranchs were recovered from three main regions - Bushy Park, Newdigate, and a collector's area in Essex. In each case the percentage of the population carrying this parasite was usually low (see p. 130) and few specimens contained fully developed cercariae. A specificity, however, appeared to exist as regards Sphaerium corneum and other lamellibranchs were entirely free from this trematode at all times.

With the exception of the Newdigate area, all the invertebrate fauna examined was free from alternative Gorgoderid infections. A small population of Sphaerium lacustre recovered from only four ponds at Newdigate, was the specific host to a single infection of large macrocercous cercariae of Sewell's "Gorgodera" group. Only a small percentage (14.8%) of the S. lacustre population was effected and few of these contained fully developed cercariae. It was concluded from the cercarial type and later experiments that this Gorgoderid possessed an Amphibian definitive host. This localised infection may not have been indigenous to the region of the country in which it was discovered and may have arisen from the supplier's habit of discarding ailing Amphibians imported from the Continent, in addition to Rana temporaria obtained from other parts of England, into the grounds surrounding the ponds. Following a few collections, the adult S. lacustre population was exhausted.

Examination of potential secondary intermediate hosts revealed that Phyllodistomum was capable of utilising Ischnura elegans nymphs and the larvae of Phryganea grandis as encystment hosts.

The other Gorgoderid, later found to be a Gorgodera species, was also capable of using these hosts in addition to five more, these latter being Coenagrion puella, Aeschna cyanea, Enallagma cyathigerum nymphs, Chaoborus sp. and Phryganea striata larvae.

c 1/ Examination of Molluscan fauna - (specimens dissected immediately following collection)

1. Bushy Park

| <u>Genus</u> | <u>Numbers examined</u> | <u>Numbers recovered with Gorgoderid infections</u> | <u>Comment</u> | <u>Other infections present</u> |
|-----------------------------|-------------------------|---|----------------|--|
| <u>Limnaea stagnalis</u> | 14 | 0 | - | Sporocysts + xiphidiocercariae (Plagiorchiidae). Sporocysts + furcocercariae (= <u>Diplostomulum</u>). |
| <u>Limnaea pereger</u> | 50 | 0 | - | Echinostomatidae metacercariae Strigeidae metacercariae. Sporocysts + furcocercariae (Strigeidae). Echinostome cercariae + Rediae (yellow & white). |
| <u>Valvata piscinalis</u> | 84 | 0 | - | Echinostome metacercariae. |
| <u>Bythinia tentaculata</u> | 1 | 0 | - | - |
| <u>Planorbis carinatus</u> | 6 | 0 | - | Echinostome cercariae + Rediae (orange). |
| <u>Sphaerium corneum</u> | 31 | 3 | all immature | Echinostome metacercariae. <u>Phyllodistomum</u> |
| <u>Pisidium pusillum</u> | 227 | 0 | - | Echinostome metacercariae. |
| <u>TOTAL</u> | <u>413</u> | <u>3</u> | | |

2. Windermere

| | | | | |
|----------------------------------|----|---|---|--|
| <u>Anodonta</u> <u>cygnea</u> | 90 | 0 | - | <u>Pentatoc</u> sp.~ Mite infection |
|----------------------------------|----|---|---|--|

3. Metropolitan Water Board - Walton Works

| | | | | |
|---------------------------------------|----|---|---|---|
| <u>Dreissena</u> <u>polymorpha</u> | 84 | 0 | - | - |
|---------------------------------------|----|---|---|---|

4. Collector's area - Essex

| | | | | |
|------------------------------------|---|---|---|---|
| <u>Limnaea</u> <u>stagnalis</u> | 2 | 0 | - | - |
|------------------------------------|---|---|---|---|

| | | | | |
|----------------------------------|---|---|---|---|
| <u>Limnaea</u> <u>pereger</u> | 1 | 0 | - | - |
|----------------------------------|---|---|---|---|

| | | | | |
|------------------------------------|---|---|---|---|
| <u>Planorbis</u> <u>corneus</u> | 1 | 0 | - | - |
|------------------------------------|---|---|---|---|

| | | | | |
|------------------------------------|----|---|---|---|
| <u>Sphaerium</u> <u>corneum</u> | 31 | 7 | - | - |
|------------------------------------|----|---|---|---|

Only two fully developed infections present. Rest immature.
(Phyllodistomum)

Echinostome metacercariae

| | | | | |
|-------|----|---|--|--|
| TOTAL | 35 | 7 | | |
|-------|----|---|--|--|

5. Highams Park

| | | | | |
|----------------------------------|----|---|---|---|
| <u>Limnaea</u> <u>pereger</u> | 20 | 0 | - | - |
|----------------------------------|----|---|---|---|

| | | | | |
|---------------------|----|---|---|---|
| <u>Pisidium</u> sp. | 43 | 0 | - | - |
|---------------------|----|---|---|---|

| | | | | |
|-------|----|---|--|--|
| TOTAL | 63 | 0 | | |
|-------|----|---|--|--|

6. Wanstead Park

| | | | | |
|------------------------------------|---|---|---|---|
| <u>Limnaea</u> <u>stagnalis</u> | 6 | 0 | - | - |
|------------------------------------|---|---|---|---|

| | | | | |
|--------------------------------------|---|---|---|---|
| <u>Planorbis</u> <u>carinatus</u> | 1 | 0 | - | - |
|--------------------------------------|---|---|---|---|

| | | | | |
|-------|---|---|--|--|
| TOTAL | 7 | 0 | | |
|-------|---|---|--|--|

7. Newdigate, Surrey. Initial survey - various ponds

| | | | | |
|---|-----|----|---|---|
| <u>Limnaea</u> <u>nereger</u> | 2 | 0 | - | - |
| <u>Pisidium</u> spp. | 52 | 0 | - | Echinostome meta- cercariae. |
| <u>Bithynia</u> (<u>tentaculata</u>) | 3 | 0 | - | Unidentified young sporocysts present. |
| <u>Sphaerium</u> <u>corneum</u> | 381 | 2 | one mature in- fection only. (<u>Phyllodistomum</u>) | Echinostome meta- cercariae Type 1 * metacercar- iae. |
| <u>Sphaerium</u> <u>lacustre</u> | 44 | 3 | <u>Gorgodera</u> - only 2 mature infections present | - |
| TOTAL INITIAL SURVEY | 482 | 5 | | |
| <u>Deanoak Brook, Newdigate</u> | | | | |
| <u>Sphaerium</u> <u>corneum</u> | 8 | 7 | 1 mature infection only - rest immature (<u>Phyllodistomum</u>) | - |
| <u>Selected Ponds, Newdigate</u> | | | | |
| <u>Ponds 1 and 2</u> | | | | |
| <u>Sphaerium</u> <u>corneum</u> | 146 | 10 | 2 mature <u>Phyllodistomum</u> infections only out of 10 | Type 1* metacercar- iae. * |
| <u>Sphaerium</u> <u>lacustre</u> | 32 | 7 | 2 <u>Gorgodera</u> mature infections out of 7. | Type 1* metacercar- iae. |
| <u>Limnaea</u> <u>stagnalis</u> | 17 | 0 | - | 2 carried unidenti- fied young Sporocysts Type 1* metacercar- ium. |
| <u>Bithynia</u> (<u>tentaculata</u>) | 4 | 0 | - | Unidentified young Sporocysts |
| <u>Planorbis</u> <u>carinatus</u> | 3 | 0 | - | Unidentified young sporocysts |

| | | | | |
|--|----|---|--|---|
| <u>Planorbis</u> <u>complanatus</u> | 1 | 0 | - | - |
| <u>Planorbis</u> <u>corneus</u> | 1 | 0 | - | - |
| <hr/> | | | | |
| <u>Pond 3</u> | | | | |
| <u>Sphaerium</u> <u>corneum</u> | 36 | 0 | - | - |
| <u>Sphaerium</u> <u>lacustre</u> | 3 | 2 | one <u>Gorgodera</u> mature infection out of two | - |
| <u>Planorbis</u> sp. | 5 | 0 | - | - |
| <hr/> | | | | |
| <u>Pond 4</u> | | | | |
| <u>Sphaerium</u> <u>corneum</u> | 32 | 0 | - | - |
| <u>Planorbis</u> spp. | 7 | 0 | - | - |
| <u>Physa fontinalis</u> | 18 | 0 | - | - |
| <hr/> | | | | |
| <u>Pond 5</u> | | | | |
| <u>Sphaerium</u> <u>corneum</u> | 64 | 1 | immature <u>Phyllodistomum</u> infection | Type 1* metacercar- iae. |
| <u>Sphaerium</u> <u>lacustre</u> | 3 | 0 | - | - |
| <u>Limnaea</u> <u>stagnalis</u> | 5 | 0 | - | - |
| <u>Viviparus</u> <u>viviparus</u> | 4 | 0 | - | - |
| <u>Planorbis</u> sp. | 1 | 0 | - | Type 1* Sporocysts + xiphidiocercariae |
| <hr/> | | | | |
| <u>Pond 6</u> | | | | |
| <u>Sphaerium</u> <u>corneum</u> | 54 | 2 | 1 mature <u>Phyll-</u> <u>odistomum</u> in- fection out of 2 | - |

Pond 7Sphaerium corneum

76

0

-

-

Sphaerium lacustre

6

1

immature Gorgodera
infection

-

Pond 8Sphaerium corneum

107

0

-

-

Limnaea pereger

8

0

-

-

Viviparus viviparus

3

0

-

-

Planorbis carinatus

1

0

-

-

Pond 9Sphaerium corneum

28

0

-

-

Pond 10Sphaerium corneum

6

0

-

-

OVERALL

TOTAL

1,161

35

10 mature Gorgoderid infections

Newdigate

TOTAL MOLLUSCS EXAMINED IMMEDIATELY FOLLOWING COLLECTION = 1,853.

N.B. Newdigate:- Unidentified trematode infection referred to as Type 1 - Pharyngeate xiphidiocercariae armed with a hollow stylet develop in sporocysts in Planorbis sp. Encyst as metacercariae in Gastropods, Lamellibranchs, Beetle-, Gaddis and Alderfly-larvae.

Summarya) Results from all areas

1. Total number of S. corneum examined = 1,000)
Total number of S. lacustre examined = 88) 1,088 Sphaeriids
2. Total Sphaeriids infected by Gorgoderids = 45 = 4.1%
3. Total number of Phyllodistomum infected molluscs recovered = 32 = 3.2% of total S. corneum examined
- Total number of mature Phyllodistomum infections recovered (i.e. cercariae fully developed) = 7 = 0.7% of total S. corneum examined
- Total % of infected molluscs carrying fully developed Phyllodistomum cercariae = 21.9%
4. Total number of Gorgodera infected molluscs recovered = 13 = 14.8% of total S. lacustre examined
- Total number of mature Gorgodera infections recovered (i.e. cercariae fully developed) = 5 = 5.7% of total S. lacustre
- Total % of infected molluscs carrying fully developed Gorgodera cercariae = 38.5%

b) Area Results1. Phyllodistomum - S. corneum

| <u>Area</u> | <u>% of Molluscs infected</u> | <u>Total % carrying mature infections</u> | <u>% of infected Molluscs carrying mature infections</u> |
|-------------|-------------------------------|---|--|
|-------------|-------------------------------|---|--|

| | | | |
|------------|--------------|--------------|--------------|
| Bushy Park | 9.7% (3/31) | 0 | 0 |
| Essex | 22.6% (7/31) | 6.4% (2/31) | 28.6% (2/7) |
| Newdigate | 2.3% (2/938) | 0.5% (5/938) | 22.7% (5/22) |

2. Gorgodera - S. lacustre

| | | | |
|-----------|---------------|-------------|--------------|
| Newdigate | 14.8% (13/88) | 5.7% (5/88) | 38.5% (5/13) |
|-----------|---------------|-------------|--------------|

c2/ Investigation of invertebrate fauna - potential secondary hosts
(Specimens dissected immediately upon collection).

Area 1 - Bushy Park

| <u>Chief nymphs & larvae examined</u> | <u>Total numbers examined</u> | <u>Total numbers infected with Gorgoderids</u> | <u>Type and density of infection</u> | <u>Comments upon other infections</u> |
|---|-------------------------------|--|--------------------------------------|--|
| <u>Ischnura elegans</u> | 35 | 1 | 1 cyst - (Phyllo-distomum) | Transport host for <u>Gyrodactylus</u> sp. |
| <u>Cloeon dinterum</u> | 140 | 0 | - | Nematodes, <u>Trichodina</u> sp. & uncysted metacercariae <u>Strigeidae</u> |
| <u>Anabolia nervosa</u> | 1 | 0 | - | None |
| <u>Sialis lutaria</u> | 11 | 0 | - | Numerous Echinostome metacercariae; 57 metacercarial cysts - (<u>Plagiorchidae</u>). |
| <u>Agabus</u> sp. (adults and larvae) | 7 | 0 | - | None |
| <u>Dytiscus marginalis</u> | 8 | 0 | - | None |
| <u>Hygrobia hermanni</u> | 4 | 0 | - | None |
| <u>Gammarus</u> sp. (adults) | 10 | 0 | - | None |
| <u>Asellus</u> sp. (adults) | 14 | 0 | - | None |
| <u>Chironomids</u> | 40 | 0 | - | None |
| <u>Culex</u> sp. | 20 | 0 | - | None |
| TOTAL | 290 | 1 | | |

(In addition, various Dipteran larvae and adult beetles examined - no Gorgoderid infection recorded).

Area 2 - Collector's Area, Essex

| | | | | |
|---------------------|---|---|---|------|
| <u>Dytiscus</u> sp. | 1 | 0 | - | None |
| | | 3 | | |

Area 3 - Wanstead

| | | | | |
|---------------------|----|---|---|------|
| <u>Bytiscus sp.</u> | 1 | 0 | - | None |
| Chironomids | 24 | 0 | - | None |

Area 4 - Highams Park

| | | | | |
|---------------------|----|---|---|------|
| Chironomids | 12 | 0 | - | None |
| <u>Gammarus sp.</u> | 12 | 0 | - | None |

Area 5 - Collectors' Area Newdigate, Surrey

Total of 18 selected ponds divided into 8 main areas:

A)

| | | | | | |
|--------------------------|----|---|--|------|--|
| <u>Ischnura elegans</u> | 31 | 2 | 1 cyst per larva (<u>Gorgodera</u>) | 25 | } infected - total 107 <u>Haematoloech-</u> <u>us.</u> Range 1 - 10 per larva |
| <u>Coenagrion puella</u> | 6 | 0 | | 2 | |
| <u>Phryganea grandis</u> | 1 | 1 | 1 cyst (<u>Gorgodera</u>) | None | |

B)

| | | | | | |
|---|----|---|--|----------------------|--|
| <u>Ischnura elegans</u> | 17 | 4 | 1-2 cysts) (<u>Gorgodera</u>) | } Total 9 cysts 8 | } 14) carrying a total of 105 <u>Haematoloe-</u> <u>chus.</u> Range 1 - 14 |
| <u>Coenagrion puella</u> | 10 | 3 | 1-2 cysts) (<u>Gorgodera</u>) | | |
| <u>Enallagma cyathigerum</u> | 4 | 1 | 1 cyst (<u>Gorgodera</u>) | | 2 infected with a total of 13 <u>Haematoloechus</u> Range 5 - 8. |
| <u>Aeschna juncea</u> | 3 | 0 | | - | None |
| <u>Aeschna cynea</u> | 1 | 0 | | - | None |
| <u>Libellula quadri-</u> <u>maculata</u> | 1 | 0 | | - | None |
| <u>Phryganea grandis</u> | 17 | 3 | 1-4 cysts (<u>Gorgodera</u>) | | None |
| | | 1 | Range 1-2 1 cyst (<u>Phy-</u> <u>llocladostomum</u>) | | |

| | | | | |
|--------------------------------|----|---|---------------------------------|---|
| <u>Phryganea striata</u> | 10 | 2 | 2 cysts (<u>Gorgodera</u>) | Gregarines. Type 1* metacercariae. |
| <u>Limnephilus flavicornis</u> | 20 | 0 | - | None |
| <u>Limnephilus rhombicus</u> | 5 | 0 | - | None |
| <u>Agabus sp. larvae</u> | 23 | 0 | - | Numerous Echinostome larvae Type 1* metacercariae. |
| <u>Agabus sp. adult</u> | 3 | 0 | - | None |
| <u>Sialis lutaria</u> | 7 | 0 | - | None |
| <u>Asellus sp.</u> | 24 | 0 | - | None |
| <u>Gammarus sp.</u> | 10 | 0 | - | None |
| <u>Tipula sp.</u> | 1 | 0 | - | None |
| Chironomids | 20 | 0 | - | None |
| <u>Chaoborus sp.</u> | 3 | 0 | 1 cyst (<u>Gorgodera</u>) | None |
| <hr/> | | | | |
| C) | | | | |
| <u>Ischnura elegans</u> | 8 | 1 | 2 cysts (<u>Gorgodera</u>) | 3 infected with 17 <u>Haematoloechus</u> Range 5 - 7. |
| <u>Phryganea grandis</u> | 9 | 1 | 2 cysts (<u>Gorgodera</u>) | None |
| <u>Limnephilus flavicornis</u> | 22 | 0 | - | None |
| <u>Limnephilus rhombicus</u> | 3 | 0 | - | None |
| <u>Glyptotaelius sp.</u> | 2 | 0 | - | None |
| <u>Asellus sp.</u> | 33 | 0 | - | None |
| Chironomids | 12 | 0 | - | None |
| <hr/> | | | | |

| D) | | | | |
|--------------------------|----|---|---|--|
| <u>Ischnura elegans</u> | 1 | 0 | - | 1 infected with 9 <u>Haematoloechus</u> |
| <u>Cloeon dinterum</u> | 2 | 0 | - | None |
| <u>Phryganea grandis</u> | 9 | 0 | - | None |
| <u>Agabus sp.</u> | 3 | 0 | - | None |
| <u>Tinula sp.</u> | 2 | 0 | - | None |
| <u>Gammarus sp.</u> | 6 | 0 | - | None |
| Chironomids | 29 | 0 | - | None |

| E) | | | | |
|------------------------------------|----|---|------------------------|--|
| <u>Ischnura elegans</u> | 5 | 0 | - | 4 infected with 13 <u>Haematoloechus</u> Range 1 - 5 |
| <u>Aechna cyanea</u> | 1 | 0 | - | None |
| <u>Phryganea grandis</u> | 16 | 2 | 2 cysts (Gorrodera) | None |
| <u>Limnephilus flavicornis</u> | 57 | 0 | - | None |
| <u>Agabus sp.</u> | 8 | 0 | - | None |
| <u>Sialis lutaria</u> | 2 | 0 | - | Type 1* metacercar- iae present. |
| <u>Asellus sp.</u> | 5 | 0 | - | None |
| <u>Gammarus sp.</u> | 7 | 0 | - | None |

| F) | | | | |
|------------------------------------|----|---|---|-------------------------------------|
| <u>Limnephilus flavicornis</u> | 8 | 0 | - | Type 1* metacercar- iae present. |
| <u>Triaenodes bicolor</u> | 3 | 0 | - | None |
| <u>Limnephilus vittatus</u> | 11 | 0 | - | None |
| <u>Cloeon dinterum</u> | 2 | 0 | - | None |
| <u>Agabus sp.</u> | 1 | 0 | - | None |
| <u>Asellus sp.</u> | 8 | 0 | - | None |

| | | | | |
|---------------------|----|---|---|------|
| <u>Gammarus sp.</u> | 6 | 0 | - | None |
| <u>Chironomids</u> | 10 | 0 | - | None |

G)

| | | | | |
|--------------------------|----|---|-----------------------------------|---------------------|
| <u>Ischnura elegans</u> | 10 | 0 | - | Gregarine infection |
| <u>Coenagrion puella</u> | 13 | 1 | 1 cyst (<u>Gor- godera</u>) | Gregarine infection |

H)

| | | | | |
|------------------------------------|----|---|---|------|
| <u>Limnephilus flavicornis</u> | 18 | 0 | - | None |
| <u>Limnephilus rhom- bicus</u> | 29 | 0 | - | None |
| <u>Phryganea striata</u> | 1 | 0 | - | None |

Termination of infections

I) Total area collection - various ponds

| | | | | |
|------------------------------|-----|----|---------------------------------|-------------------------|
| <u>Aeschna cyanea</u> | 4 | 1 | 3 cysts (<u>Gorgodera</u>) | None |
| <u>Anabolia nervosa</u> | 5 | 0 | - | None |
| <u>Aeabus adult</u> | 3 | 0 | - | None |
| <u>Sialis lutaria</u> | 6 | 0 | - | 5 Type 1* metacercariae |
| <u>Ischnura elegans</u> | 1 | 0 | - | 3 <u>Haematoloechus</u> |
| <u>Limnephilus rhombicus</u> | 2 | 0 | - | 1 Type 1* metacercariae |
| <u>Asellus</u> | 5 | 0 | - | None |
| <u>Gammarus</u> | 4 | 0 | - | None |
| TOTAL FOR NEWDIGATE | 609 | 24 | | |

N.B. Newdigate Area

Unidentified trematode referred to as Type 1* recovered from Phryganea striata, Limnephilus flavicornis, L. rhombicus, Sialis lutaria and Agabus sp. as the metacercarial encysted stage. For brief description see page

Summary

Total number of invertebrates examined = 949

Total number of invertebrates infected = 25

by Gorgoderids

Phyllodistomum - Percentage of Invertebrate Fauna to be infected (Detail)

Bushy Park: Ischnura elegans 2.8% (¹/35)

Newdigate: Ischnura elegans 0.0% (⁰/73)

Phryganea grandis 1.5% (¹/68)

Gorgodera - Percentage of Invertebrate Fauna to be infected (Detail)

Newdigate: Ischnura elegans 9.6% (⁷/73))

Coenagrion puella 17.4% (⁴/23)*) Odonata
) (13/106)

Enallagma cyathigerum 25.0% (¹/4)*)

Aeschna cyanea 16.7% (¹/6)*)

Phryganea grandis 13.2% (⁹/68)) Trichoptera
) 13.9% (¹¹/79)

Phryganea striata 18.2% (²/11)*)

Chaoborus sp. 33.4% (¹/3)*

* signifies that the percentage is abnormally high due to the low numbers of larvae or nymphs examined.

d) Infection Experiments Involving the Second Intermediate Host.

d 1) General considerations

The only other Gorgoderid cercariae to be recovered during this investigation were easily distinguishable from the equivalent stages of Phyllodistomum particularly as regards the structure of the tail. Both genera remained host specific to two different Sphaerium species. Preliminary collections had shown however that they overlapped as regard to their secondary intermediate hosts and examination of the Gorgodera metacercariae revealed several features in common with Phyllodistomum including identical sucker papillary patterns. This obviously depreciated the value of such a feature as a specific taxonomic criterion and indicated that a closer comparison of the two genera might yield further useful information along these lines. It was decided that such a comparison should be extended to include all the stages in the life cycles and thus infection experiments involving Gorgodera were included in this investigation. A brief description of this latter trematode is given in Section 9.

A comparison of the sizes of the Phyllodistomum metacercariae obtained from Sphaerium corneum with those recovered from insect larvae during preliminary examinations indicated that the trematode larvae did not appreciably increase in size whilst within the secondary intermediate host and that the latter was probably serving as little more than a vector. The Gorgodera metacercariae, on the other hand, obviously underwent considerable growth changes during this period and yet were capable of utilising a far wider range of hosts. There appeared to be no reason why Phyllodistomum should differ in this

respect and it seemed possible that the number of hosts could be extended by means of infection experiments.

d 2) Methods and materials

Throughout the series of experiments performed there was a continual lack of infective material. In the preliminary survey of all the collection areas only 0.7% of the Sphaerium corneum examined bore mature infections whilst 5.7% of the S. lacustre were similarly effected by Gorgodera. Numerous attempts to increase by laboratory infection the number of Phyllodistomum-parasitised molluscs available and so produce cercariae when required had already met with little success (page 67-8). Infected molluscs could not be detected except upon the release of cercariae because they were too large and opaque to reveal their contents prior to dissection. As a result infected stock could not be concentrated in a manner comparable to that described by Goodchild (1943) for P. solidum.

Two types of experimental infection were attempted. When cercariae became available as a result of dissection, a series of direct feeding experiments were carried out utilising Gorgoderid-free insect nymphs and larvae (page 142). The latter were obtained from ponds where there were no lamellibranchs and a sporadic infection of Haematoloechus was the only trematode recovered from these waters. Useful information was gained concerning the varying attraction that these cercariae had for widely differing larval forms and a study of ingestion methods gave some indication of the likelihood of the parasites' success.

A series of long term experiments were performed (pages 143) which involved keeping various arthropods with large numbers of potentially infected molluscs. The material for these experiments was obtained from Newdigate which was the only reliable long-term source of invertebrate fauna available at that time. The sparse population of S. corneum in Deanoak Brook made it necessary to use lamellibranchs which had mainly been collected from the few ponds in which Phyllodistomum infections had been traced in spite of the fact that it was known that only a small proportion of the population (2.3%) would be parasitised. In the case of Gorgodera the percentage infection of S. lacustre was higher (14.8%) but the number of molluscs available was low. In order to offset these disadvantages, the experiments were continued for as long as possible and generally in excess of the experimental period already determined for Phyllodistomum development. It was hoped that cercariae would eventually be released and that for a considerable time insect nymphs and larvae would be regularly associated with these infective organisms. The containers were examined daily for released cercariae and the dates of such occurrences noted so that the exact age of the metacercariae would be known.

Unfortunately, there was a marked tendency for the molluscs to die at various stages of the infection both before and following cercarial release. This could not be avoided since the susceptibility of the host was largely related to the density of infection (see page 74). In order to maintain the numbers of potentially infected

molluscs in the aerated experimental containers, it was necessary to replace the casualties by new stock collected from the same ponds. The date of such additions was noted and the experiment was continued for long enough to allow for the completion of the parasites' development. On occasions, however, a power cut or pupation of the majority of the larvae concerned in the experiment terminated it prematurely and this gave rise to the apparent anomaly of recovering Sphaerium carrying an immature Phyllodistomum infection at the end of an experiment lasting from 18 to 22 weeks (as shown on page 143).

The preliminary examination of the total Newdigate arthropod fauna revealed a 0.16% Phyllodistomum and 3.7% Gorgodera infection from 609 larvae examined (as shown on page 136) and indicated that the numbers of naturally infected larvae which would be available for use in future experiments involving the definitive host would be severely limited. The chances of successfully infecting insect larvae experimentally whilst utilising a mollusc population carrying only a 2.3% Phyllodistomum or 14.8% Gorgodera infection appeared to be minimal. It was therefore decided that all insect larvae utilised in the long-term experiments should be taken from areas known to carry natural infections of Phyllodistomum or Gorgodera. (The only other trematode involved being Haematoloechus). By this means it was hoped to increase the numbers of metacercariae per larva to ensure a more certain subsequent infection of the definitive host; to compare the sizes of metacercariae of known age with larvae resulting from natural infections occurring several months previously, so that the growth rate within differing hosts could be studied; to test whether

double infections could occur and to increase the number of intermediate hosts known to be involved in the parasites' life cycles.

In order to fulfil the above requirements it was necessary, upon the termination of many of the experiments, to dissect only a minimal number of the potential intermediate hosts and to feed the remainder to Amphibia and Fish. The number of transferences made are listed on page 143 . Due to the length of these experiments, it was not possible to starve the potential intermediate hosts, prior to infection, in order to increase transparency in a manner similar to that described by Goodchild in 1943 when working with P. solidum. None of the larvae used in the experiments were transparent enough to enable the contained metacercariae to be seen in vivo and regrettably infection had to be presumed in many cases (a fact indicated by ** on page 143) in order to provide a quantitative method of definitive host infection whilst simulating the natural cycle. This method was considered to be largely unsatisfactory and experiments involving direct feeding of fish and frogs with known numbers of metacercariae of Phyllodistomum and Gorgodera were also performed and discussed more fully on page 145

Table 10.

Infection Experiments Involving the Second Intermediate Host A/ Direct feeding experiments

Series 1 : Attempted Phyllodistomum infections

| <u>Total numbers of potential hosts utilised</u> | <u>Dosage</u> | <u>Reaction</u> | <u>Result upon examination</u> |
|--|-----------------|--|--|
| <u>Cloeon dipterum</u> | 3 | 3 cercariae each | Cercariae eaten readily Larvae fed to Fish directly. No examination. ** |
| <u>Aeschna cyanea</u> | 1 | 14 cercariae | Larva well fed - no interest shown. |
| | | 16 cercariae | Given one day later - no interest shown. |
| | | 15 cercariae | Given one day later. Larva hungry - cercarial movements amplified by use of water currents from a pipette. Cercariae eaten. Normal cercarial movements unattractive. |
| | | 8 sporocysts with the wall cut so that cercarial tails protrude and are actively lashing. | Readily captured and eaten. Insect <u>uninfected</u> . |
| <u>Aeschna cyanea</u> | 1 | 2 sporocysts prepared as above. | Readily captured and eaten. Insect <u>uninfected</u> . |
| <u>Agabus sp.</u> | 1 | 10 cercariae | No interest shown. Larva well fed. |
| | | 21 cercariae | One day later - no interest shown Cercariae left in bowl until tails detached - all recovered. Beetle larva <u>uninfected</u> . |
| | | 5 cercariae | One day later - no interest shown. Cercarial movements artificially amplified - no stimulus. |
| <u>Agabus sp.</u> | 1 | 5 cercariae | Larva hungry - no interest |
| | | 4 cercariae | As above - one day later. |
| | | 5 cercariae | As above - one day later. Amplification of cercarial movement has no effect. |
| | | 2 sporocysts cut so that cercarial tails protrude and lash actively. | No interest shown. All cercarial material recovered. Larva <u>uninfected</u> . |
| <u>Agabus sp.</u> | 8 | Repetition of similar experiments to those described above - each beetle larva treated separately. | No interest shown. All cercarial material recovered. Larva <u>uninfected</u> . |
| <u>Ischnura elegans</u> | 8 | 18 cercariae into single bowl. | Larvae show varying interest - all cercariae eaten eventually but method of ingestion causes damage. Larva which had ingested 3 cercariae examined, <u>uninfected</u> . 7 larvae fed to Frogs ** |
| <u>Coenagrion puella</u> | 4 | 7 cercariae into one bowl. | Reactions identical to <u>Ischnura elegans</u> . 1 larva examined - seen to ingest 2 cercariae - <u>uninfected</u> . Remaining 3 larvae fed to Frogs. ** |
| <u>Sialis lutaria</u> | 4 | Total of 12 cercariae | Each larva treated separately - no interest shown. All cercarial bodies recovered eventually - larvae <u>uninfected</u> . |
| <u>Sialis lutaria</u> | 4 | 1 - 5 cercariae supplied to each larva in separate bowl per week over a period of 4 weeks. | No interest shown. Amplification of cercarial movements has no effect. All cercarial bodies recovered - larvae <u>uninfected</u> . |
| <u>Limnephilus flavicornis</u> | 3 | 3 cercariae each | No interest shown. All cercarial bodies recovered. Larvae <u>uninfected</u> . |
| <u>Phryganea grandis</u> | 3 | 10 cercariae each | No specific interest. Scavenging results in cercariae being eaten. One cercarial body recovered only. Larvae <u>uninfected</u> . |
| TOTAL: | Potential hosts | 33 | |
| | Cercariae | 180+ | |

Series 2 : Attempted Gorgodera infections

| | | | |
|--------------------------------|-----------------|---------------------------|---|
| <u>Ischnura elegans</u> | 1 | 2 cercariae | Cercariae readily eaten Larva fed to Frogs ** |
| <u>Coenagrion puella</u> | 4 | 2 cercariae to each larva | Readily caught and ingested |
| | | 2 cercariae to each larva | One day later - eaten 2 examined - <u>uninfected</u> 2 fed to Frogs ** |
| <u>Limnephilus flavicornis</u> | 3 | 1 cercaria each | No specific interest |
| | | 2 cercariae each | One day later - no response Cercarial bodies eventually eaten. <u>Uninfected</u> . |
| <u>Phryganea grandis</u> | 2 | 1 cercaria each | No interest. |
| | | 2 cercariae each | One day later - scavenging feeding methods allowed ingestion of cercariae 1 <u>uninfected</u> 1 <u>experimental infection</u> by 2 <u>Gorgodera metacercariae</u> 1 cyst fed to Fish ** |
| <u>Phryganea grandis</u> | 1 | 4 cercariae | Eaten <u>Uninfected</u> |
| <u>Agabus sp.</u> | 1 | 3 cercariae | Eaten <u>Uninfected</u> |
| <u>Sialis lutaria</u> | 1 | 3 cercariae | Eaten <u>Uninfected</u> |
| TOTAL: | Potential hosts | 13 | |
| | Cercariae | 43 | |

PULLOUT

d 3) Results of the Infection Experiments concerning the Intermediate Host (pages 142-143)

Infection following direct cercarial feeding experiments was only successful in the case of Gorgodera and Phryganea grandis. Phyllodistomum successfully infected both Phryganea striata and P. grandis in the series of long-term experiments. In addition, the two latter larvae were found to be naturally parasitised by Gorgodera and this trematode was also recovered from Ischnura elegans and Coenagrion puella. In Experiment 1 the size of the Phyllodistomum metacercaria obtained from P. grandis, indicated that it was more likely the result of a natural rather than an experimental infection.

d 4) Phases in the Infection of the Invertebrate Secondary Host
Attraction and ingestion

Anisopteran nymphs such as Aeschna cyanea, when approaching pupation measured approximately 48 mm. in length, and at this stage could only be induced to capture Phyllodistomum cercariae by artificial means. It can be deduced from the feeding experiments that these cercariae are likely to attract nymphs of a maximum 24 mm. in length because they are readily ingested by Zygoptera of this size. Metacercarial infection of Anisoptera by Phyllodistomum seems a distinct possibility but was not achieved experimentally nor recovered from nature. The success of the Gorgoderia cercariae in this direction would appear to be related to their greater opacity, more efficient method of progression and larger size which renders them attractive to Anisopteran nymphs at practically all stages of the latter's development.

Sialis and Agabus larvae ranging from 15 - 20 mm. in length showed no interest in Phyllodistomum cercariae. Both the larvae were naturally infected with encysted Echinostomes. The latter developed from cercariae which were similar in size to the equivalent stage in the Gorgoderid but were capable of actively penetrating their secondary hosts although encystment also followed inhalation (by Lamellibranchs, Amphibian tadpoles and Sticklebacks). Noticeably successful, the Echinostome cercariae, in contrast to Phyllodistomum, were extremely active and released in large numbers from their

molluscan hosts.

Trichopteran larvae were not found to be heavily infected by either trematodes or protozoa in nature. However, Gorgoderids were recovered from both Phryganea grandis and P. striata. The manner in which Phyllodistomum cercariae readily attach themselves to the substratum has an obvious adaptive value in relation to the feeding habits of the Phryganids. These larvae continually turn over debris with their limbs and mouthparts while searching for food and are brought in contact with the attached cercariae and detached crawling bodies. Specific attraction for the former was never exhibited and for Trichoptera ranging in size from 20 mm - 35 mm cercarial ingestion occurred by chance. Successful infection by freed cercarial bodies may occur in cases where the parasite is ingested together with a quantity of vegetation which might provide the protection normally afforded by the chamber during masticatory processes. Within the experimental containers the caddis larvae frequently dislodged Sphaerium whilst hunting and undoubtedly would come in contact with both active and dying infected molluscs under natural conditions. A Trichopteran was seen to ingest the soft parts of a parasitised, dying Sphaerium corneum whilst in the laboratory. The various stages of the Phyllodistomum infection were alive at the time of the ingestion. In nature they may infect themselves in this way by eating unreleased but fully developed cercariae. The inability to infect other Caddis particularly Limnephilus species with Phyllodistomum may be associated only with slight differences in the larval behaviour, and need not involve

physiological factors. During the experimental period Limnephilus larvae were observed to spend a greater percentage of the time clinging to vegetation well above the substratum than was the case for the Phryganea species and, although carnivorous, they appeared to ingest preferentially a greater proportion of vegetational matter.

Although Phyllodistomum cercariae are capable of attracting Ephemeroptera such as Cloeon dipterum and Zygopteran nymphs Ischnura elegans and Coenagrion puella, successful invasion is by no means certain. The cercaria is frequently seized by the tail and the chamber containing the cercarial ^{body} is discarded. The ingestion method favours the parasite in many ways, however, for the amount of mastication is limited, the cercaria being mainly pulled, pushed and squeezed into the oesophagus in the first thoracic segment. If the chamber is the first region to be grasped by the labrum its plasticity is sufficient to protect the body from damage and there is considerably less risk of it being lost during the manipulations of the mouth parts. Similar losses occurred when Phyllodistomum cercariae were ingested by Phryganids. In the case of Gorgodera, a Phryganea striata specimen was found to contain several cercarial tails and one dead body in the gut, but remained uninfected by metacercarial cysts. In nature, such mastication injuries are probably common. Goodchild (1943) recorded that 15% of P. solidum cercariae failed to establish themselves in the insect host following ingestion, whilst Groves (1945) recorded an average 40.4% success for these cercariae. Several authors have recorded failure to infect insect larvae experimentally

with Gorgoderids (e.g. Thomas (1958), Joyeux and Baer (1947)) and masticatory damage may be partially to blame. Vickers (1941) obtained cysts of C. macrocerca from several Chironomus pedellus larvae following 14 days contact with cercariae, but was unable to repeat the experiment. Groves (1945) reported that P. solidum cercariae proved distasteful to damsel fly naiads when once the tails was ruptured and they were often regurgitated as a result. In this investigation the larvae did not appear to be abnoxious.

Penetration

The insect larvae used in infection experiments were opaque and the act of penetration was not observed but this was recorded by Goodchild (1943) for P. solidum to occur within one minute of ingestion. The metacercarial penetration paths in this investigation could be traced through the gut wall particularly in the Trichoptera. This was made possible by the fact that all carnivorous larvae, whilst kept in the laboratory, had been fed upon Tubifex and for some time following ingestion traces of congealed pigment from the worms were to be found within the alimentary tract. As metacercariae cut their way through the oesophagus and upper expansion of the crop they carried these pigments with them and left permanent brown scars in the wall which were quite distinct from the temporary streaks of haematin lying in the folds of the gut lining. The penetration paths were mainly straight, passing obliquely through the wall, the thin nature of which did not allow for lateral progression. In the case of a heavy Phyllodistomum infection, the larvae retained sufficient haematin around themselves, following penetration, to incorpor-

ate it in the normally transparent cyst walls. The cysts were blotched with dense pigment and the wall rendered opaque by a red-brown film. In Gorgodera cysts, only the extreme outer surface was coloured in this way.

The point of penetration occurred in the oesophagus or crop of both Trichoptera and Zygoptera always within the thoracic region. Groves (1945) recorded that when once P. solidum larvae had been pushed by oesophageal action into the stomach of Ischnura posita they were digested and lost. Both Groves (1945) and Goodchild (1943) reported thoracic penetration for P. solidum and the latter author (in 1948) recorded a similar regional restriction for Gorgodera amplicava in the experimental host Enallagma. Whilst Gorgoderid-infecting insect larvae in the West characteristically penetrate and encyst anteriorly, those recorded from India, China and Japan infecting Crustacea, are mainly restricted to the gonads and liver (e.g. P. macrobrachicola - Yamaguti, 1934; P. lesteri - Wu, 1938; P. folium (?) - Kurokawa (1934); P. sp. - Komiya and Tajimi (1943); P. macrobrachicola - Shibue (1954) and P. srivastavai - Rai, 1964). On one occasion however, in China, Phyllodistomum larvae were reported by Du (1930) to be feeding upon the stomach lining of a shrimp, to be progenetic and apparently unencysted. In this investigation Phyllodistomum and Gorgodera cysts were found with only two exceptions in the first, second or third thoracic segments of insect larvae. Only two Gorgodera metacercariae were able to successfully penetrate the first abdominal segment of Ischnura elegans and encyst but one of these flukes was moribund. Goodchild (1943) reported a similar

deviation from the norm for P. solidum when encystment occurred in the second abdominal segment and even as far forward as the dorsum of the head. (Groves (1945) did not find any similar variations however).

The advantages of anterior penetration in insect larvae are two-fold. Firstly, in Anisoptera such as Aeschna the crop occupying the metathoracic region is lined with sclerites, the action of which would be detrimental to soft-bodied cercariae. All Gorgodera specimens penetrated anterior to this point. In Trichoptera and Zygoptera sclerites do not occur, but the position of the cyst affords the maximum chance for successfully infecting the definitive host. Small fish such as Sticklebacks grasp the protruding sclerotised anterior region of scavenging Trichoptera and, by biting and shaking, often separate the thorax from the softer abdomen which is left firmly adhering to the case. Sticklebacks successfully attack larger Zygopteran larvae most frequently from the head since they receive an effective nip if they seize the posterior end leaving the mouth-parts free. They are often dissuaded from further attack by this treatment.

The site for encystment normally occurs immediately opposite to the point of entry. Movement within the haemocoel must be limited since the surrounding film of haematin in experimental infections was not lost before encystment took place and the cyst was found in some cases situated within the oesophageal wall, protruding into the gut cavity, surrounded and supported by a pouch of host tissue. Other Phyllodistomum cysts were found attached to the oesophageal wall by

host tissue lying completely in the haemocoel, whilst others were adhering to the undersurface of the dorsal tergum immediately above the penetration point. Similar variations in encystment sites were noted in the case of Gorgodera. Phyllodistomum were only reported wandering within the haemocoel prior to encystment on one occasion (Goodchild, 1943) and this was an unusual feature and of limited duration. Shibue recorded in 1954 finding P. macrobrachicola larvae in an unencysted state in the gonads and spermatid ducts of a Crustacean, but although there is the possibility that this was the result of recent penetration and that examination took place before encystment could occur, Shibue's findings parallel those of Du (1930) in that the larvae had been actively feeding. Cyst formation, when it did occur, was communal enclosing up to three or more larvae - which suggests that the wall was of host origin.

The act of penetration is often accompanied by a host reaction of apparent discomfort followed by an unwillingness to ingest further cercariae. Such reactions were recorded by Ssinitzin (1905), Krull (1935), Goodchild (1943) and Groves (1945). Insect hosts did not reveal these behaviour patterns in either Phyllodistomum or Gorgodera infections in this investigation and negative results were also recorded by Goodchild (1948) for Gorgodera amplicava.

The time taken to complete cyst formation after penetration, according to Goodchild (1943) was only 18 minutes for P. solidum, Thomas (1958) found 12 hours were sufficient for the cyst formation of P. simile within the molluscan host, and in this investigation Gorgodera cysts were complete when Phryganea were examined 24 hours

after infection (a similar situation to that reported by Coil (1954) for C. anodontae). The period required for both Phyllodistomum and Gorgodera is likely to be short and to approximate 12 hours or less depending upon the temperature.

SECTION 6 THE METACERCARIA

(a) Method of investigation

The metacercariae were examined live in the encysted and excysted state while mounted in diluted horse serum or tap water. Neutral red proved to be the most useful intra-vital stain. These extensile larval phases were fixed in 70% alcohol without coverslip pressure, stained in aceto-alum-carmin and mounted in Canada Balsam. Studies based on sections of the encysted phase situated in the sporocyst were disappointing and at all times the living material was found to be superior to mounted specimens. The living metacercariae was excysted by slitting the cyst wall with Iris scalpels. This method prevented the larva being damaged, whereas the application of steadily increasing coverslip pressure resulted in a high percentage of the larvae being lost.

(b) The cyst

The metacercarial cysts recovered from Sphaerium corneum varied considerably in size from host to host, from sporocyst to sporocyst and amongst individuals derived from a single larval sac. The extent of these variations is illustrated in Table 12, p. 154 and is probably the result of original variations in the species exaggerated by age differences.

A comparison of the minimal values given in the total ranges of cyst sizes in Tables 12 and 13 would appear to indicate that metacercariae, upon entering the insect host either produce larger cysts than their counterparts in the mollusc or are stimulated at an early stage of their development to increase their growth rate. A comparison of the

Table 12. *Phyllodistomum metacercariae* encysted within the molluscan host

| <u>Host 1 (exp.inf.)</u> | | <u>Host 2 (nat.inf.)</u> | | <u>Host 3 (nat.inf.)</u> | |
|--------------------------|------------------|--------------------------|--|--------------------------|--|
| 1. | 0.220 x 0.209 mm | 0.165 x 0.165 mm | | 0.205 x 0.187 mm | |
| 2. | 0.172 x 0.172 | 0.172 x 0.172 | | 0.191 x 0.187 | |
| 3. | 0.187 x 0.187 | 0.235 x 0.213 | | 0.213 x 0.201 | |
| 4. | 0.209 x 0.191 | 0.172 x 0.172 | | 0.198 x 0.187 | |
| 5. | 0.209 x 0.202 | 0.187 x 0.187 | | 0.213 x 0.187 | |
| 6. | 0.202 x 0.183 | 0.172 x 0.165 | | 0.220 x 0.201 | |
| 7. | 0.238 x 0.202 | 0.172 x 0.172 | | 0.209 x 0.191 | |
| 8. | 0.194 x 0.183 | 0.172 x 0.154 | | 0.180 x 0.158 | |
| 9. | 0.183 x 0.165 | 0.187 x 0.180 | | 0.201 x 0.191 | |
| 10. | 0.189 x 0.189 | 0.180 x 0.154 | | 0.209 x 0.183 | |
| Av. | 0.200 x 0.189 | Av. 0.181 x 0.173 | | Av. 0.204 x 0.187 | |

| <u>Host 4 (nat.inf.)</u> | | <u>Host 5 (nat.inf.)</u> | |
|--------------------------|------------------|--------------------------|--|
| 1. | 0.194 x 0.183 mm | 0.168 x 0.161 mm | |
| 2. | 0.183 x 0.165 | 0.202 x 0.183 | |
| 3. | 0.187 x 0.154 | 0.176 x 0.165 | |
| 4. | 0.172 x 0.150 | 0.187 x 0.165 | |
| 5. | 0.165 x 0.165 | 0.227 x 0.213 | |
| 6. | 0.172 x 0.165 | 0.191 x 0.183 | |
| 7. | 0.165 x 0.147 | 0.257 x 0.202 | |
| 8. | 0.169 x 0.165 | 0.220 x 0.205 | |
| 9. | 0.183 x 0.165 | 0.238 x 0.220 | |
| 10. | 0.169 x 0.165 | 0.183 x 0.183 | |
| Av. | 0.176 x 0.162 | Av. 0.205 x 0.188 | |

Range: Host 1 0.172 - 0.238 x 0.165 - 0.209 mm
 Host 2 0.165 - 0.235 x 0.154 - 0.213
 Host 3 0.189 - 0.220 x 0.157 - 0.201
 Host 4 0.165 - 0.194 x 0.147 - 0.183
 Host 5 0.168 - 0.257 x 0.161 - 0.220

Largest cyst to be recovered from a molluscan host: 0.268 x 0.205 mm

Mean: 0.193 x 0.180 mm

Total range: 0.165 - 0.257 x 0.147 - 0.220 mm

The above measurements concern living material; the figures are corrected to 3 decimal places
 Abbreviations signify: exp.inf., experimental infection; nat.inf., natural infection.

Table 13. *Phyllodistomum metacercariae* obtained from insect nymphs and larvae

| <u>Host</u> | <u>Cyst size</u> | |
|--------------------------|------------------|---|
| <u>Phryganea grandis</u> | 0.257 x 0.238 mm | Natural infection (minimum 18 weeks duration) |
| | 0.220 x 0.205 | Experimental infection (9 weeks duration) |
| | 0.209 x 0.191 | Natural infection |
| <u>Phryganea striata</u> | 0.180 x 0.176 | Natural infection |
| | 0.201 x 0.183 | Experimental infection (9 days) |
| | 0.205 x 0.198 | " |
| | 0.220 x 0.183 | " |
| | 0.201 x 0.180 | " |
| | 0.209 x 0.201 | " |
| | 0.220 x 0.183 | " |
| <u>Ischnura elegans</u> | 0.258 x 0.233 | Natural infection |

Range: in P.grandis 0.209 - 0.257 x 0.191 - 0.238 mm
P.striata 0.180 - 0.220 x 0.176 - 0.201 mm

Mean: in Trichoptera 0.194 x 0.176 mm

Total range: 0.180 - 0.258 x 0.176 - 0.238 mm

Total mean: 0.216 x 0.197 mm

All measurements are taken from living material; figures are corrected to 3 decimal places

maximum sizes shown illustrates that Phyllodistomum cysts increase in size with age and attain similar maxima in both hosts. The mechanisms involved in cyst growth are not clear. The wall consists of a single, non-laminated unit with slightly elastic properties. It is due to its resilience that the restricted fluke is able to undertake major changes in position. During growth the wall would be expected to be stretched, added to or remodelled in some manner. There appears to be no relationship between the cyst size and the width of the wall however, which varies between 0.0031-0.0061 mm. The wall, when cut, always peeled apart cleanly in a manner comparable to extremely fine rubber and no part could be separated into layers as in the case of Gorgoderia cysts (p.367). Although the larvae were observed closely additional secretions within the cyst were not noted and when once the wall was completed the fluke did not appear to have any further interest in it.

Thomas (1958) reported that the cysts of P.simile increased in size with age, newly formed cysts measuring 0.16-0.19 mm in diameter, enlarging to 0.242 mm in older specimens. The range is noticeably similar to that recorded for the fluke studied in this investigation. Increase in the size of the cyst appears to be a characteristic of the Gorgoderidae and has been reported on several occasions. A comparison of the measurements given by Goodchild (1943) and Groves (1945) for the growth rates of P.solidum metacercarial cysts in differing species of Ischnura would appear to indicate that, progress is more rapid in I.posita than I.verticilis. In view of the numerous other discrepancies between these two accounts, the value of this fact in relation to the effects of the host

upon growth rates is questionable. Joyeux and Baer (1947) gave a detailed description of the steady increase in size of the cysts of a Gorgoderina species. These authors also commented upon the marked shrinkage effects which took place on the dehydration and mounting of metacercarial cysts. The methods whereby cyst size changes occur, however, have not been elucidated by any author.

In accordance with this Phyllodistomum species the metacercarial cyst wall of C.lampsilae was recorded by Coil (1954) as an amorphous structure, but was much thicker, measuring 0.01 mm across. In contrast to these observations several authors have noted the presence of laminated membranes. Goodchild (1943) reported that the metacercariae of P.solidum added to the original cyst wall (measuring 1-4 μ in thickness [5 μ according to Groves, 1945]) after five days spent in the damselfly naiad host, and as a result trapped the stylet between the primary and secondary cystogenous deposits. Ssinitzin reported the same phenomenon occurring in the metacercariae he studied in 1905. Thomas (1958) described the inner region of the cyst wall of P.simile as probably being derived from the mesenchymatous cystogenous cells found scattered within the cercaria..

Joyeux and Baer (1947), when studying a trematode they believed to belong to the Gorgoderina genus, described the stylet as floating freely in the cystic fluid, indicating either that the cyst was observed before a secondary cystogenous deposit was laid down or that the stylet was cast after cyst formation had been completed. In the species of Phyllodistomum studied in this investigation the stylet, judging from

its position, was nearly always cast during or immediately following cyst wall formation. It was found either completely surrounded by cystogenous material or merely impaled terminally with the basal expansion jutting obliquely into the cystic fluid. Occasionally the stylet was retained in the oral sucker chamber following encystment. On three occasions the stylet tip had been broken during penetration of the gut wall.

The cysts are usually ovoid in shape and occasionally acquire a pronounced elongation, but are rarely spherical. The variation in shape may represent a response to the degree of compression occurring within the sporocyst or haemocoel. The faintly yellow, transparent cyst wall is at all times permeable to intra-vital dyes (a fact also recorded for C.lampsilae by Coil [1954]), and allows for the passage of dissolved gases and ions. The oxygenated haemocoel of an insect larva or nymph offers a more concentrated osmotic environment than Teleost urine. The main differences between the adult fluke and the metacercariae are based upon the degree of reproductive maturity and size and not on the excretory system which is structurally identical in both cases. Although when kept in the laboratory in water the average adult does not exhibit adverse osmotic changes until 24 hours or more after they have appeared in the larva, this can be explained by variation in the quantity of food reserves and other factors and need not relate to a marked difference in either osmotic concentration of the body fluids, reflecting discrepancies in the metabolism, or osmoregulatory powers. It can be concluded from these facts that in both organisms the power of osmoregulation and the extent by which the body fluids depress the freezing point will not differ

significantly. The long period of longevity in water suggests further that the body fluids of both the adult and larva possess a low osmotic concentration, and it is probable that they are either slightly hypertonic or isotonic to the dilute urine of the definitive host. Assuming this to be true, the presence of a completely permeable cyst wall would apparently involve too great an expenditure of energy in order to sustain life in the intermediate host for four months or more unless the larva was capable of utilising materials present in the haemocoelic fluid as an additional energy source. The haemocoel contains, in addition to quantities of inorganic ions, some free amino acids which may be utilised by the fluke. Bodily growth appears to be minimal during this period and any additional nutrients acquired at this time must be utilised eventually to provide energy for osmotic regulation. The species of Gorgodera studied in this investigation underwent considerable bodily growth while encysted. This metacercarian differs metabolically at least in one respect from Phyllodistomum, since the waste product is insoluble, but it may also either store more glycogen or utilise haemocoelic derivatives more efficiently under these conditions, being already adapted for a final habitat of greater concentration than fish urine.

When mounted in water encysted metacercariae remain alive for 5 days or more, depending on their age and the temperature, before showing signs of reduced mobility. Similarly, Kurokawa (1934) reported that Phyllo-distomum, which he referred to as P.folium, remained alive in water in the encysted and unencysted state from 7-10 or 14 days depending upon the temperature. Groves (1945), however, reported that P.solidum

metacercariae died in a few minutes following artificial excystment but neglected to state into what medium the larvae were placed. In this investigation the fluke ingests cystic fluid during the normal motile period which involves primarily the extension of the anterior end and, accompanying these movements, the occasional relaxation of the circular oesophageal muscles. Regular contractions of the gut flush the contained fluid back and forth within the caecae. The flame cell beat appears identical to the adult degree of activity and the bladder contents are released cyclically at intervals of 35-40 seconds. As the activity of the metacercaria decreases steadily from the period of cyst formation, movement in the fluid-filled space system in the cyst which surrounds (in contrast to C. eriensis [Coil, 1954]) even the largest larva is continued, but more particularly by regular pulsations from the bladder. The granular cytoplasmic reserves are gradually reduced in quantity as the larva ages and metacercariae with the lowest reserves were the least motile when excysted. Fat droplets do not increase in quantity during ageing, suggesting that the glycogen reserves are aerobically converted as opposed to anaerobic fermentation. The cytoplasm and the bladder do not store crystalline waste at any stage and it seems feasible that simple nitrogenous materials diffuse out of the cyst together with acidic products of any aerobic respiration which may take place. The survival period spent within the insect host is extensive and is known to last for longer than 4 months. The presence of the larvae does not interfere with pupation or the emergence of the imago and the fluke is therefore available for the infection of Amphibian adults. The release of waste

products into the haemocoel appears therefore to be well tolerated. It can be concluded that if the cyst wall is not completely permeable but selectively so, energy would be conserved. This would allow an additional point of control which otherwise is found only at the flame cell level or with protoplasmic organic buffers because the continual ingestion of cystic fluid prevents cuticular control from being effective.

(c) The Metacercariae - general structure

The metacercariae resemble the adult fluke in numerous features including their locomotory methods, papillary patterns, the development of the gut and the arrangement of the excretory, nervous and the essentials of the reproductive systems. Detailed descriptions of these will be included in section 7C d (p.234+269).

A record of the measurements taken from metacercariae obtained from molluscan and insect larval hosts is given on p.162. Some of the latter had been recovered following the experimental infection of Trichoptera nine days previously. These metacercariae differed little in length from the cercarial state (p.99) and as the degree of activity in the specimens examined was less than that exhibited by the cercariae, their recorded extensile range and body size appear smaller. The metacercariae obtained from the molluscan hosts were of unknown and undoubtedly mixed ages. The average length exceeded that recorded for the cercariae, indicating that some growth occurs as the larva ages but that the difference is so slight as to be masked on some occasions by contraction effects (a fact which is amply illustrated by the lengths recorded for mounted material). Thomas (1958) stated that the metacercariae of P.simile began

to grow only after 14 days of encystment. After 21 days within the mollusc host the metacercariae had increased in length by a maximum 0.09 mm. Goodchild recorded (1943) a closely similar size change but a much slower rate of growth for P.solidum which increased by 0.08 mm in 60 days. Groves (1945) reported a more rapid rate, however, for the same species, stating that it exceeded the cercarial state by 0.29 mm in 43 days. The greatest capacity for growth on record is for the metacercaria of C.lampsilae which was recorded by Coil (1954) as being able to extend the body length four-fold in 4-6 months.

In this investigation it was found that it was only in extreme cases that the cyst size and that of the contained larva were closely associated and indicative of age. In the majority of cases these measurements showed considerable variation in relation to one another.

The effects of differing hosts upon the growth rate and eventual size attained by the metacercarial body has not received much attention in the literature. Shibue (1954) recovered P.macrobrachicola metacercariae from three genera of Crustacea and published a table of comparative measurements of an unknown number of stained and mounted larvae from each host. If the contractile range given in the account for living specimens obtained from one of the Crustacean genera is taken as a guide, it would appear that little evidence exists in this case to suggest that the differing hosts had any marked effect upon the extent of larval growth. The argument cannot be taken further in view of the lack of available data concerning the period of infection. In this investigation there did not seem to be any association between the species of the hosts and

PULLOUT

the eventual size attained by the larvae. Records of the growth rate of the metacercarial body could not be obtained due to lack of material. Although Gorgoderid cysts have been recovered from the liver and gonads of Crustacea, the viscera and gills of Molluscs and the haemocoel of insect larvae and adults, there is no record that the nutritional value of the location has any effect upon the growth of the larva concerned. Significantly, however, progenesis was reported on two occasions occurring in larvae encysted in the liver of Crustacea. This suggests, firstly, that in the Gorgoderids the cyst wall is at least semi-permeable and secondly, particularly in Phyllodistomum, that the metacercariae are largely incapable of effectively utilising the few nutrients obtained from the body fluids of their molluscan and insect hosts.

Proportionally, the slight size increase which was recorded for the metacercariae recovered during this study was the result of greater relative growth taking place in the posterior region. The range in proportional ratios reflects both the actual variations occurring at different ages and contraction effects, because specimens were measured in a slightly contracted as well as a relaxed condition as a result of their inactivity and their reaction upon fixation.

The mean sucker sizes indicate that these structures are larger in the metacercarial stage than in the cercaria but the range of the dimensions overlaps to some considerable extent. As in the case for the cercaria, the acetabulum exceeds the overall size of the oral sucker at all times and is noticeably wider. A comparison of the metacercarial and cercarial sucker ratios (based upon the measurement of the greatest

diameters of the organs in both living and mounted material) indicates that both suckers are growing at a comparable rate and are maintaining a similar proportional difference between them to that established in the cercaria. Thomas (1958) recorded the sucker ratios (presumably based upon the greatest diameters [?]) of P.simile as 1:1.03-1:1.19 for the cercaria, 1:1.07-1:1.15 in the 14 day old metacercaria and 1:1.09-1:1.14 in the 21 day old larva. The sucker growth rates therefore appear to be equivalent at this stage in this species also. When compared with the material studied in this investigation, the sucker sizes of P.simile are larger, with the size ranges overlapping those of the living specimens but not in the mounted state.

The value of the sucker ratio in larval phases may be confined to giving an indication of growth trends and any basic proportional differences which may be maintained between the two organs. The range of the ratios is noticeably high and can be drastically altered by contraction, and to place any greater significance upon small differences in this measurement at this stage would be unwise.

The width of the excretory bladder of the metacercaria in the species under investigation is considerably less than in the cercarial stage. This change is associated with a decrease in the size of the cells lining the bladder as the result of loss of cystogenous secretion. The bladder does not gradually increase in size as the larva ages, due to the extrusion of waste products into the bladder cavity or into excretory cells. As previously stated, a crystalline form of waste is absent although common in many Gorgoderids, and appears to be particularly

Table 14.

The Metacercaria

| Region | Living material (mm) | Mounted material (mm) | Thomas (1958) (mm) |
|--|--|--|--|
| Body length | 0.552 (0.348-0.840) | 0.347 (0.300-0.428) | (0.41 - 0.75) |
| Contractile range | 0.220 | - | - |
| Anterior region minus V/S | 0.241 x 0.097 (av) (0.165-0.348 x 0.055-0.154) (r) | 0.155 x 0.083 (av) (0.128-0.204 x 0.053-0.126) (r) | (0.205 - 0.38) (r) 1 |
| Posterior region plus V/S | 0.264 x 0.102 (av) (0.198-0.367 x 0.055-0.158) (r) | 0.193 x 0.080 (av) (0.154-0.239 x 0.044-0.110) (r) | * (0.29 - 0.435) (r) 1 |
| Ratio - Anterior region: Posterior region Length | 1:1.095 (av) (1:0.875-1:1.556) (1r) | 1:1.246 (av) (1:0.839-1:1.713)(1r) | * 1:1.106-1:1.415 max/min record - (1r) 14 days 1:0.970-1:1.145 max/min record - (1r) 21 days |
| Width | 1:1.052 (av) (1:0.900-1:2.029)(wr) | 1:0.964 (av) (1:0.750-1:1.367)(wr) | |
| Oral sucker | 0.0855 x 0.0621 (av) (0.0587-0.110 x 0.0403-0.088) (r) | 0.0745 x 0.0549 (av) (0.0630-0.0861 x 0.0418-0.0684) (r) | - (0.0825-0.130 x 0.0800-0.125) (r) |
| Ventral sucker | 0.0930 x 0.0869 (av) (0.0623-0.128 x 0.0623-0.117) (r) | 0.0747 x 0.0686 (av) (0.0592-0.0882 x 0.0567-0.0765) (r) | - (0.0900-0.155 x 0.0900-0.135) (r) |
| Ratio Oral sucker:Ventral sucker - Length rel. to B/A | 1:1.088 (1:0.851 - 1:1.241) (r) | 1:1.003 (1:0.774 - 1:1.134) (r) | - |
| O/S:V/S - width rel. to B/A | 1:1.399 (1:1.118 - 1:2.273) (r) | 1:1.249 (1:1.100 - 1:1.548) (r) | - |
| GD (rel. to organ) | 1:1.088 (1:0.851 - 1:2.273) (r) | 1:1.003 (1:0.774 - 1:1.548) (r) | * (1:1.091-1:1.192) *(r) M/m (l) (1:1.091-1:1.263) (r) M/m (w) |
| Excretory bladder | 0.177 x 0.028 (av) | 0.111 x 0.023 (av) | 0.176 x ? (d) |
| Excretory bladder as % of posterior region width length | 29.2 63.7 | 26.21 55.61 | ? 52.11 (d) |
| Reproductive organs (rel. to organ) | | | |
| Anterior testis | 0.0337 x 0.0265 (av) (0.0204-0.0459 x 0.0204-0.0357) (r) | 0.0290 x 0.0245 (av) (0.0204-0.0408 x 0.0173-0.0357) (r) | 0.0571 x 0.0428 (d) |
| Posterior testis | 0.0354 x 0.0271 (av) (0.0255-0.0459 x 0.0153-0.0357) (r) | 0.0321 x 0.0254 (av) (0.0245-0.0459 x 0.0163-0.0357) (r) | 0.0408 x 0.0214 (d) |
| Ovary | 0.0311 x 0.0245 (av) (0.0204-0.0408 x 0.0204-0.0367) (r) | 0.0265 x 0.0228 (av) (0.0204-0.0357 x 0.0133-0.0357) (r) | 0.0643 x 0.0408 (d) |
| Vitellaria (left and right) | 0.0176 x 0.0126 (av) (0.0102-0.0255 x 0.0102-0.0204) (r) | 0.0195 x 0.0130 (av) (0.0143-0.0357 x 0.0102-0.0306)(r) | 0.0381 x 0.0190 (d) (+ duct ?) |

Abbreviations: O/S = oral sucker
V/S = ventral sucker
av = average (for the numbers involved, see p.)
r = range
w = width
l = length
rel. to organ = measurements are taken relative to the dimensions of the organ concerned regardless of the body axis position
rel. to B/A = measurements are taken relative to the body axes regardless of the main axes of the organ concerned

All measurements are in mm. The living and mounted specimens were not necessarily identical.

The measurements from Thomas (1958) were taken from the main account and Fig. 18 where the sign (d) is given. Thomas gave the following record for the sucker ratio, but did not state which diameters

were involved:

14 days following encystment - 1:1.07-1:1.15
21 " " " " - 1:1.09-1:1.14

* measurements calculated from available data

max/min record = calculated from the maximum and minimum data given for body lengths

M/m = calculated on the maximum and minimum sucker measurements (assumed to be equivalent to the greatest diameters)

associated with those flukes where the definitive host is Amphibian (P. solidum (Goodchild, 1943), Gorgoderina and Gorgodera spp. including the Gorgodera studied in this investigation).

As a result of the reduction in width of the excretory bladder the reproductive organs can be seen more easily within the metacercaria than the preceding phase. On average, the ovary is smaller than either of the testes and the posterior testis is the largest structure. There is, however, an overlap in the dimensions recorded for all the main reproductive organs, even the vitellaria. From individual to individual the posterior testis varies from being larger, smaller or equalling the size of the anterior body. In the mounted material there were fewer specimens measured than in the living state, and this partially accounts for the apparent lack of shrinkage and anomalous size differences in the range records. The vitellaria are transparent bodies devoid of content and, when measuring these organs, it is sometimes difficult to determine the termination of the tapering gland and the beginning of the duct. The larger size recorded for vitellaria from mounted specimens probably reflects the inclusion of a greater part of the duct system which is more easily discernible under these conditions. The measurements on p. 162 show that the average size of the reproductive organs from metacercariae obtained from insect larval hosts was larger than those recovered from Mollusca. The size ranges, however, overlap to a marked extent. More material would be required to ascertain whether the reproductive system of flukes occupying insect larval hosts develops at a more rapid rate than when encystment occurs in molluscs. If this proved to be the

case it might suggest that a nutritional difference existed and/or that the employment of the molluscan host at this stage is of recent origin in the evolution of the life cycle of this species.

The reproductive organs occupy an equivalent position to that found in the adult. They vary from a spherical to an ovoid shape, usually bearing a smooth contour, but are occasionally lobed. The uterus and male ducts are distinct and, as in the cercaria, the genital pore is perforated. The dimensions of the reproductive organs bear no close relationship to either cyst, body or sucker sizes except at the extreme limits of the ranges.

Thomas did not record the size of the reproductive organs of P. simile metacercariae. Measurements obtained from his diagram (Fig. 18, 1958) (Given in Table 14, p. 165) suggest that in comparison with the species examined in this investigation the ovary exceeds the size of the testes and that the anterior testis is larger than the posterior organ. The dimensions attained in a 21 day old larva exceed those recorded for any of the mixed age population studied here as regards the ovary and anterior testis, but the dimensions of the posterior organ are paralleled. The significance of such a difference is doubtful. The size relationships of the reproductive organs vary from individual to individual and, for comparative purposes, average measurements and ranges of larger numbers should be used.

At no time has precocious development of eggs been recorded for British or European metacercarial Phyllodistomes. Progenesis reported

by Wu (1938) for P.lesteri and P.srivastavai by Rai (1963) occurred in larvae encysted in the liver or genital organs of a Crustacean host. The possibility of nutritional differences and a permeable cyst wall may explain these reports. An unencysted feeding progenetic metacercaria was reported by Du in 1930 from the stomach of a Crustacean, and the development of the gametes would appear to be resultant of receiving additional food supplies in excess of the stored food reserves of the metacercaria. Maximum development of the reproductive system was reported to take place within 6 weeks of encystment in C.lampsilae by Coil (1954), but gamete development was never observed under these conditions. Groves (1945) reported that P.solidum metacercariae develop more slowly during the winter months, even under experimental conditions of little temperature variation, and suggested that this delay may represent a form of overwintering in that species.

The penetration glands are clearly visible in metacercariae of all ages. The total length of the glands and their ducts may differ on average from that of the anterior region as little as 0.0042 mm in mounted larvae. Under these circumstances the position of the glands in the body differs little from the cercarial state. The greatest deviation is marked by the anterior position of a few of the glands protruding beyond the level of the gut bifurcation. The glands may also be entirely extra-caecal, a condition which may occur in the cercarial state but which was not noted, probably due to the relatively low numbers examined. In medium to large sized larvae the glands may be entirely anterior to the gut bifurcation and clustered around the oesophagus, or they may lie in similar positions to those found in the cercaria. There seems to be

no relationship to the position of the glands and the overall size of the fluke. Perhaps the original variation in position recorded for the cercaria was too limited and the range found in the metacercaria is merely a reflection of this rather than the result of differing growth rates. After a certain period, whilst growth of the penetration ducts ceases, it continues in the anterior region of the body. This certainly accounts for the apparent anterior movement of the glands in later stages. The glands appeared to contain fluid in some specimens, but it was so transparent in nature that any generalisation inferring that the glands are still active following encystment cannot be made with certainty.

Thomas (1958) stated that penetration glands were visible in the newly formed P.simile metacercaria but not in the adult. Coil (1954) regarded the persistence of the glands for over four months and the ducts for far longer in C.lampsilae metacercariae. In this investigation the glands can be traced easily in young adults containing no eggs. This latter finding is in contrast to the condition reported for P.singulare where, according to Lynch (1936), the glands remain conspicuous in the sexually mature fluke.

(d) Effect upon the secondary intermediate host

The presence of a maximum 7 cysts had no effect upon the insect larva. The cysts are soft and small and do not interfere with the functioning of the thoracic musculature, and the imago develops normally. Perforation of the gut wall by Phyllodistome larvae caused the formation of scar tissue but no signs of discomfort. The host larvae are capable of surviving a large number of gut perforations by both Gorgoderid and

Plagiorchiid metacercariae without apparently suffering any adverse effects. Gorgoderid infected insect nymphs and larvae are capable of supporting large numbers of trematodes (Haematoloechus) and protozoan parasites (Gregarines) and still metamorphose successfully.

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METACERCARIAL STRUCTURE

Diagram A:

Ventral view of a metacercaria obtained from Sphaerium corneum
(mounted specimen).

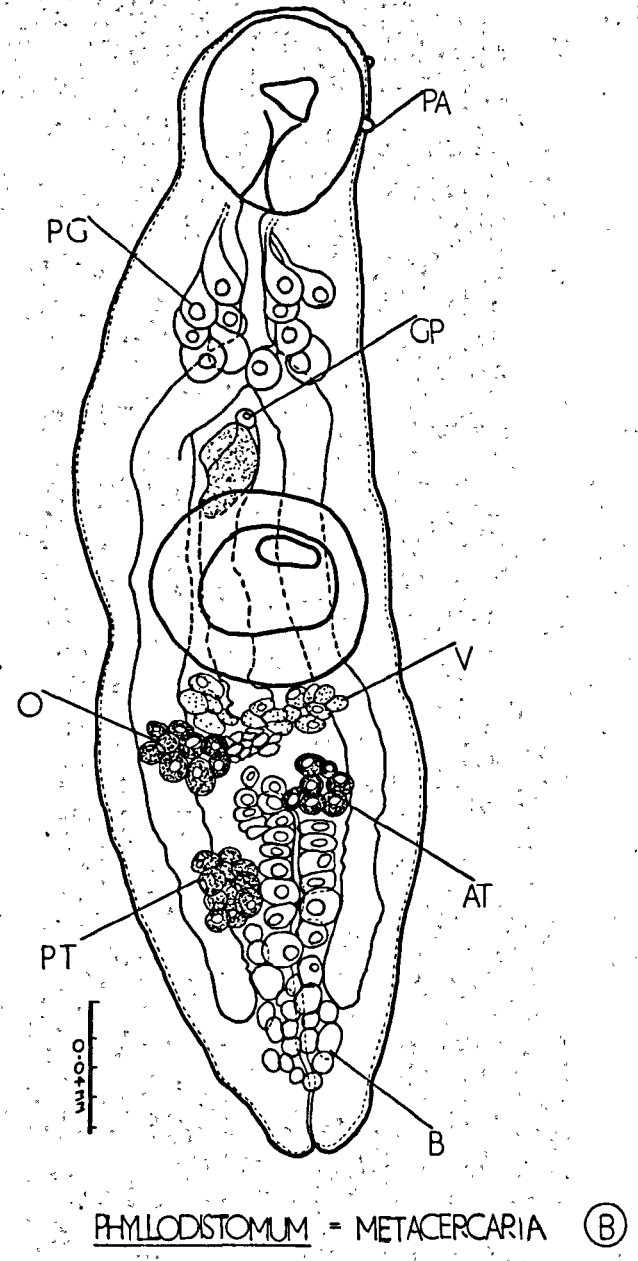
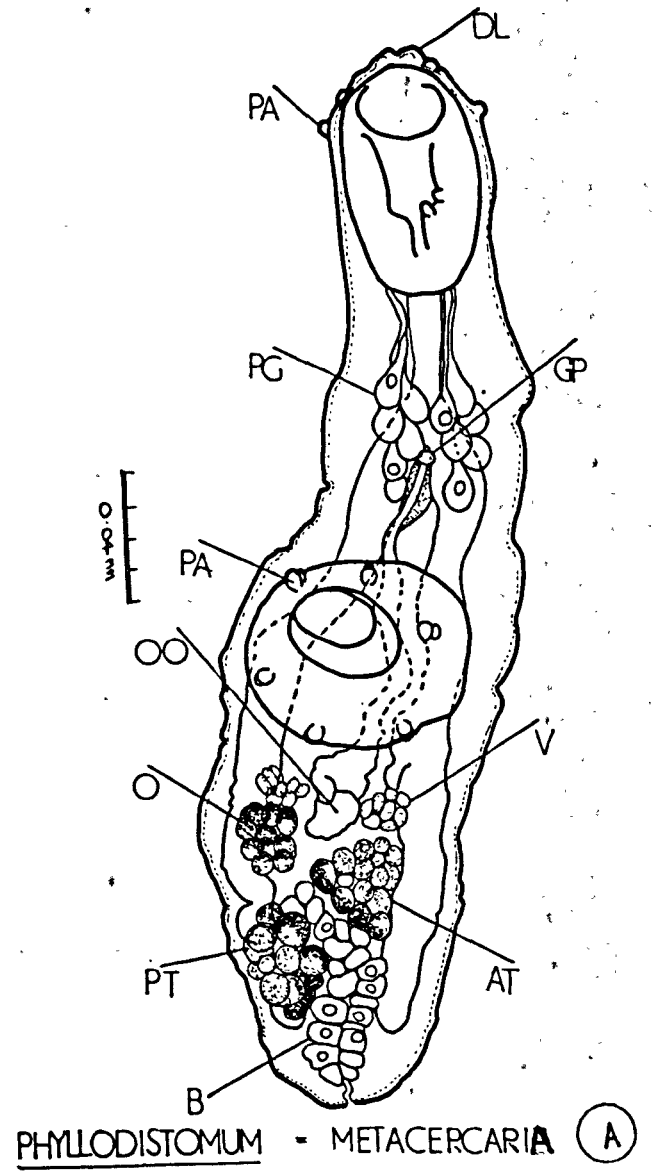
Diagram B:

Ventral view of a metacercaria obtained from the experimental
infection of Phryganea striata (mounted specimen).

Abbreviations used in diagrams A and B:

- DL Dorsal lip
- PA Papilla
- PG Penetration glands
- GP Gonopore
- OO Ootype
- O Ovary
- V Vitelline gland
- AT Anterior testis
- PT Posterior testis
- B Excretory bladder

[both diagrams were drawn with the aid of a projector]



SECTION 7 PHYLLODISTOMUM IN THE DEFINITIVE VERTEBRATE HOSTSub-section 7A Experimental infection of the definitive host

The experiments which were attempted are summarized on p. 175, Table 15. In Series A Phyllodistome cercariae were offered to sticklebacks of various sizes. Small, immature fish were readily attracted to the active cercariae and these were ingested. Fish reaching a size of 3 cms or more responded less readily to such diminutive prey and the introduction of cercariae by pipette served to focus their attention on the larvae and successful ingestion occurred. In A(e) a long term experiment paralleling the events of nature was set up to test the possibility that ^{older} fish may serve as intermediate hosts for the metacercarial stage (where infection would presumably take place by inhalation) and so serve as infective agents for large carnivorous species such as pike. The experiment was terminated prematurely by the death of the fish as the result of an infection of Dermocystidium gasterostei and attendant secondary fungal infections and, due to lack of material, was not repeated.

In Series B two main methods were employed in feeding Phyllodistome metacercariae to potential vertebrate hosts. Metacercariae wrapped in molluscan tissue were successfully fed to a single Rana temporaria and two Gasterosteus aculeatus. Neither host readily accepted inanimate food and the frog therefore had to be force fed. The fish, however, had been established in the laboratory for some time and were used to hand feeding, and even accepted metacercariae wrapped in pellets of

bread. Where insect larvae were utilised these were usually offered entire. All insect larvae were transferred from the infection experiments discussed on pp. 138-144 and carried a potential but unknown Gorgoderid infection derived from either an experimental or natural source. On one occasion a Phyllodistomum metacercaria was dissected out of an experimentally infected Trichopteran host, wrapped in tissue and fed directly to a minnow.

All fish utilised in the experiments were known to be free from Gorgoderid infection. The younger forms were laboratory bred and the older specimens were collected from uninfected areas. Unfortunately, the frogs were required at a period when the supply firm was having difficulty in providing Amphibia, and only six Rana temporaria from an unknown but single source were available. Three of these were examined for Gorgoderids and found to be free from infection. The remaining three then had to be used for infection experiments following examination of their urinary waste over a period of one month which indicated that a mature Gorgoderid infection was absent.

If the infection experiments were successful it was considered that either the Gorgodera or Phyllodistomum specimens would differ considerably in size from any which might already be established in the amphibian urinary system. The infective material was scarce and the insect nymphs and larvae were reaching the adult phase at an earlier period than in nature due to laboratory conditions. It was therefore decided that, despite the unsatisfactory nature of the experiment,

Table 15: Infection experiments involving the definitive host (A) Gasterosteus aculeatus

Series A: Use of Phyllodistome cercariae

| No. of fish | Age on examination | Conditions of the experiment | Results on post-mortem |
|-------------|--|--|--|
| (a) 4 | 1+ yrs. 4-5 cms long. 2 male | Each fish offered (and readily accepted) approximately 100+ cercariae enclosed in sporocysts and embedded in the soft tissues of half a 1.5 cm <u>Sphaerium corneum</u> . Cercariae apparently fully developed | Examination 1 month and 2 months later revealed no <u>Phyllodistome</u> infection |
| (b) 1 | Under 1 yr. immature 2½ cms long. male | Fish placed in small container. Offered 4 fully developed (naturally released) cercariae - readily accepted. Large (1.5 cm) infected <u>S. corneum</u> known to be releasing cercariae placed in container with fish. Mollusc died 12 days later. Daily examination of the water, but no cercarial bodies recovered. | Examination 21 days after the experiment began showed the fish to be uninfected by <u>Phyllodistomum</u> |
| (c) 1 | Under 1 yr. immature 3 cms long. female | Total of 18 fully developed cercariae offered to fish over a period of 2 days - accepted | Examination 28 days later revealed no <u>Phyllodistome</u> infection |
| (d) 1 | Under 1 yr. immature 2¾ cms long. male | 3 dozen fully developed cercariae placed in small tank with fish - 15 bodies recovered | Examination 14 days later revealed no <u>Phyllodistome</u> infection |
| (e) 7 | 1+ years 4.2-5.0 cms long. 4 males 3 females. Beginning to breed | 6 infected <u>S. corneum</u> placed in fish tank prior to cercarial release. <u>Phyllodistome</u> development proceeded and cercarial release occurred 50 days after the beginning of the experimental period. It was intended that the experiment should run for at least 6-8 months, paralleling the natural cycle | Haplosporidial infection etc. killed fish after 2 months. Fish uninfected by <u>Phyllodistomum</u> |

Series B: Use of Phyllodistome metacercariae

| | | | |
|--------|---|---|---|
| (a) 1 | 1+ years 5.5 cms long female nearing breeding condition | Fish readily accepted 39 sporocysts embedded in the soft tissues of half <u>S. corneum</u> . 10+ metacercariae were ingested by this method | Examination 14 days later revealed 1 juvenile <u>Phyllodistomum</u> in the bladder |
| (b) 1 | 1+ years 5.25 cms long female | Fish ingested soft tissues of <u>S. corneum</u> containing 15 metacercariae encysted in 22 sporocysts | Uninfected 14 days later |
| (c) 1 | 1+ years 4.25 cms long male | Metacercariae were extracted from sporocysts - stained with neutral red to aid visibility - and wrapped in small pellets of bread. 4 metacercariae readily accepted. 9 metacercariae offered 3 months later were spat out and lost | Post-mortem 5 months later revealed no <u>Phyllodistomum</u> infection |
| (d) 15 | 1+ years 4-6 cms long 7 male, 8 female | Over a period of many months insect larvae which were (possibly) infected with <u>Phyllodistomum</u> either experimentally or naturally were fed entire to selected individuals. A total of 12 larvae were used in this way (sources - infection expts A, B, pp. 142-3) <u>Cloeon dipterum</u> , <u>Ischnura elegans</u> , <u>Coenagrion puella</u> | Examination of the fish on their death (from 1-5 months later) revealed no <u>Phyllodistomum</u> infections |

Infection experiments involving the definitive host (B) Phoxinus phoxinus

| | | | |
|-------|-------------------------|--|---|
| (a) 1 | -1 year 3.5 cms long | 1 <u>Phyllodistomum</u> metacercaria adhering to the gut wall of an experimentally infected <u>Phryganea grandis</u> (p. 143) was wrapped up in gut tissues and muscle of the invertebrate host and offered to the fish. The metacercaria was ingested | Fish dies 6 months later (bacterial infection), uninfected by <u>Phyllodistomum</u> |
|-------|-------------------------|--|---|

Infection experiments involving the definitive host (C) Rana temporaria

| | | | |
|-------|------|--|---|
| (a) 1 | male | (1) 10+ <u>Phyllodistomum</u> metacercariae encysted in sporocysts and molluscan tissue force-fed; 4 metacercariae lost in process (2) 14 insect larvae (<u>Coenagrion puella</u> and <u>Ischnura elegans</u>) potentially infected either naturally or experimentally by <u>Gorgodera</u> and/or <u>Phyllodistomum</u> metacercariae (see pp. 142-143) ingested by frog over a period of 2½ months | Male (A) juvenile (very small) Gorgoderids recovered: 1 - rectum (live) 9 - ureters (live) 2 - bladder (live) Moribund forms: 3 - in ureter by vesicula seminalis 1 - in vesicular duct |
|-------|------|--|---|

| | | | |
|-------|------|--|--|
| (b) 2 | Male | 33 larvae (<u>Coenagrion</u> and <u>Ischnura</u>) fed to frogs as imago formed from experiments on pp. 142-3. Potential <u>Gorgodera</u> and/or <u>Phyllodistomum</u> infections. Ingested over a period of 5 months | Male (B) Bladder - 1 Gorgoderid (juvenile, medium sized) recovered Male (C) 7 - bladder (large adults) 1 - 'urethra' (large adult) |
|-------|------|--|--|

Series C: Use of Gorgodera metacercariae (A) Gasterosteus aculeatus

| | | | |
|--------|--|---|--|
| (a) 1 | 5½ cms long mature female | <u>Gorgodera</u> metacercaria dissected from <u>Phryganea grandis</u> wrapped in tissue and given to fish. 1 <u>Gorgodera</u> metacercaria from <u>Phryganea striata</u> 14 days later fed as above, + 3 insect larvae from experiments on pp. 142-143. | Not infected - died 1 month later from fungus infection |
| (b) 15 | 1+ years as in B(d) 4-6 cms long 7 male 8 female | 13 nymphs and larvae fed to fish (<u>Ischnura elegans</u> and <u>Coenagrion puella</u>) | Examination of the fish on their death (from 1-5 months later) revealed no <u>Gorgodera</u> infections |

preliminary infections should be attempted. Further infection experiments of a more controlled nature were planned for a later date but infected stock became unobtainable. Further examination of 50 frogs from the same suppliers by students, some months later, indicated that there was no Gorgoderid infection. The collection area was not, however, fully guaranteed as being identical with the previous experimental one.

Results.
Phyllodistome cercariae do not appear to be able to encyst within the sticklebacks following ingestion or inhalation. Phyllodistome metacercariae experience difficulty in establishing themselves in the urinary system of the definitive host and considerable losses occur. A single fluke was recovered 14 days after ingestion (experiment Ba) from the bladder of a female Gasterosteus. Over 10 metacercariae were fed to the fish on this occasion while smaller (4) and larger (15+) numbers proved unsuccessful. No Gorgoderids were obtained from the definitive host following ingestion of potentially infected insect nymphs or larvae. This was possibly due to the low density of metacercarial infection because insect larvae must play an important role in the completion of the Phyllodistome life cycle in nature.

Numerical losses of infective forms were also reported by Thomas (1958) for P. simile. He obtained a maximum return of 22 and a minimum of 2 flukes from over 100 infective organisms introduced into Salmo trutta. Goodchild (1948) noted similar losses for Gorgoderia amplicava where the flukes became entangled with food and were unable to extricate themselves.

The frogs in experiment Series BC were found to be infected by Gorgoderids. One (A) was killed after 2½ months and the other two (BC) after 5 months. The first frog (A) contained both moribund and living juvenile forms, the latter occupying the rectum, ureters and the bladder. This frog had been exposed to two Gorgoderid parasites, Gorgodera and Phyllodistomum, in an attempt to establish a double infection. The Phyllodistomes (10+) were derived from sporocysts embedded in molluscan tissue and force fed. All other infection was by means of naturally ingested insect nymphs from the experiments discussed on pp. 42-3. Of the remaining two frogs, one (C) carried an infection of 8 mature Gorgodera and the other (B) contained one immature Gorgodera in the bladder. The extremely small juvenile Gorgoderids recovered from (A) could not be readily identified whilst alive as belonging to the genus Gorgodera. The moribund juveniles were too decomposed to allow for their identification into either the Phyllodistomum or Gorgodera genus. The description and measurements of these flukes are given in Section 9, pp. 351-402 together with a more detailed account of the overall parasitic load of the frogs which was naturally or experimentally induced as a result of the feeding experiments. Attempts to infect Gasterosteus with Gorgodera failed.

Conclusion

The life cycle of this species of Phyllodistomum can be completed experimentally and possibly also in nature by infection of the definitive host following ingestion of parasitised Sphaerium corneum. Under

natural conditions, however, the role of insect larvae (Phryganea grandis) and nymphs (Ischnura elegans) is probably of great importance, especially where the infection of either young fish or a small, stunted population is involved.

Gorgodera, in contrast to Phyllodistomum, normally infects amphibian hosts and does not possess a metacercaria which encysts precociously in the molluscan host. The life cycle is completed using secondary intermediate hosts (p. 136) which appear from the available evidence to be more numerous than in the case of Phyllodistomum. The two cycles remain distinct at the primary and definitive host levels but partially compete during the secondary intermediate phase.

Sub-section 7B An examination of the definitive host and the related

Phyllodistome infection

(a) Method of investigation

Phyllodistomes were recovered from four collection areas in the Counties of Essex, Middlesex and Surrey over the period 1961-1963 (a detailed description of these regions is given on pp.112-123). The definitive hosts Gasterosteus aculeatus (L) and G. pungitius (L) (formerly Pygosteus pungitius) Family Gasterosteidae were collected by hand net and brought live into the laboratory. A selection was kept in aquaria for experimental purposes whilst others, wherever possible, were killed and examined on a monthly basis in an attempt to trace the parasites' cycle in nature. The fish were killed by breaking their necks and were thoroughly examined for all parasites located on the external surface, gills, in the buccal cavity, eye, body wall, body cavity, swim bladder, gut regions and their associated glands in addition to the cloaca, bladder, kidneys, reproductive ducts and ureters. The results of these investigations for parasites other than Phyllodistomum are given in Section 8, pp.322-351. In an effort to establish the seasonal variation in diet of the definitive host the gut contents were examined (see p.201) and the results, where possible, related to general parasitic load. In addition soft-bodied larvae etc., collected from the same environment, were fed to fish to ascertain their acceptability and to supplement data concerning diet. The total length of the host (including the tail fin), the sex, maturity and phase of reproductive activity were noted. The age of the host was judged according to size (and maturity during breeding

season) in relation to the overall size range of the population of the collection area. The Otoliths were not studied.

(b) Results

The results of the examination of vertebrate fauna are given on pp. 181-201.

Table 16. Summary of the examination of vertebrate fauna

(1) Teleostei

| Genus | Bushy Park | Essex | Newdigate | Wanstead Park | Highams Park | Staines Aquaduct | Windermere | Epping Forest | Total |
|--------------------------------------|------------|--------|-----------|---------------|--------------|------------------|------------|---------------|---------|
| <u>Gasterosteus aculeatus</u> | 85(+P) | 54(+P) | 148(+P) | 17(+P) | 15 | - | - | 5(+P) | 324 |
| <u>Gasterosteus pungitius</u> | 77(+P) | - | - | - | - | - | - | - | 77 |
| <u>Phoxinus phoxinus</u> | 2 | - | 9 | - | - | - | - | 5 | 16 |
| <u>Esox lucius</u> | - | - | - | - | - | - | 8 | - | 8 |
| <u>Perca fluviatilis</u> | - | - | - | - | - | 15 | - | - | 15 |
| <u>Rutilus rutilus</u> | 1 | - | 2 | - | - | 15 | - | 5 | 23 |
| <u>Abramis brama</u> | - | - | - | - | - | 3 | - | - | 3 |
| <u>Carassius carassius</u> | - | 2 | - | - | - | - | - | - | 2 |
| <u>Gobio gobio</u> | 1 | - | - | - | - | - | - | 1 | 2 |
| Total | 166 | 56 | 159 | 17 | 15 | 33 | 8 | 16 | 470 |
| (2) Anura | | | | | | | | | |
| <u>Rana temporaria</u> (tadpoles) | 12 | - | - | - | - | - | - | - | 12 |
| adults | 1 | - | 6(+50)* | 1 | - | - | - | - | 8(+50) |
| (3) Urodela | | | | | | | | | |
| <u>Triturus vulgaris</u> | - | 1 | - | - | - | - | - | - | 1 |
| | 13 | 1 | 6(50) | 1 | - | - | - | - | 21(+50) |

(+P) = Phyllodistomum infection present

* = exact location of British sources may have varied

(N.B. G.pungitius = 8 to 10 spp. of sticklebacks included)

Table 17. Seasonal incidence of the trematode *Phyllodistomum* in *Gasterosteus* spp.

Area (1) Bushy Park

| Month | No. of fish examined | | | No. of fish infected | | | Percentage infected | | | Total no. of flukes recovered |
|-----------|----------------------|------------|----------|----------------------|----------|----------|---------------------|-------------|--------------|-------------------------------|
| | Total | 3 sp. | 8+ sp. | Total | 3 sp. | 8+ sp. | Total | 3 sp. | 8+ sp. | |
| Nov. 1961 | 5 (5A) (0B) | 1A | 4A | 1A | 0A | 1A | 20A | 0A | 25A | 1 |
| Dec. 1961 | 19 (19A) (0B) | 3A | 16A | 9A | 3A | 6A | 47.3A | 100A | 37.5A | 52 (1-27) |
| Jan. 1962 | 10 (10A) (0B) | 1A | 9A | 2A | 0A | 2A | 20A | 0A | 22.3A | 3 (1-2) |
| Feb. 1962 | 0 (0A) (0B) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mar. 1962 | 11 (11A) (0B) | 7A | 4A | 3A | 3A | 0A | 27.3A | 42.8A | 0A | 8 (1-5) |
| Apr. 1962 | 4 (4A) (4B) | 0B | 4B | 0B | 0B | 0B | 0B | 0B | 0B | 0 |
| May 1962 | 14 (4A) (10B) | 0A 1B | 4A 9B | 3A 1B | 0A 0B | 2A 1B | 21.4A 1B | 0A 1B | 50A 11.2B | 9 (1-5) |
| June 1962 | 24 (24A) (0B) | 15A | 9A | 1A | 0A | 1A | 4.16A | 0A | 11.2A | 1 |
| Jul. 1962 | 46 (31A) (15B) | 27A 11B | 4A 4B | 3A 1B | 3A 0B | 0A 0B | 6.52A 1B | 11.2A 0B | 0A 0B | 10 (1-8) |
| Aug. 1962 | 14 (14A) (0B) | 9A | 5A | 0A 1B | 0 | 0 | 0A 1B | 0 | 0 | 0 |
| Sep. 1962 | 11 (0A) (11B) | 8B | 3B | 0A 1B | 0 | 0 | 0A 1B | 0 | 0 | 0 |
| Oct. 1962 | 4 (4A) (0B) | 2A | 2A | 0A 1B | 0 | 0 | 0A 1B | 0 | 0 | 0 |

| | | | | | | | | | | |
|--------------|-----|----|----|----|---|----|------|------|-------|----|
| Total | | | | | | | | | | |
| System B | 40 | 20 | 20 | 1 | 0 | 1 | 2.5 | 0 | 5 | 3 |
| System A | 122 | 65 | 57 | 21 | 9 | 12 | 17.2 | 13.8 | 21.05 | 81 |

Area A + B. 162 No. of flukes recovered for area. 85 77 22 9 13 13.58 10.58 (1-27) 16.88 (1-5) 84 (1-27) (3 sp = 54) (8+ sp. = 30)

Area (2) Collectors' area in Essex

| | | | | | | | | | | |
|--------------|----|----|---|----|----|---|-------|-------|---|------------|
| Nov. 1961 | 22 | 22 | 0 | 20 | 20 | 0 | 90.91 | 90.91 | 0 | 112 (1-18) |
| Feb. 1962 | 32 | 32 | 0 | 29 | 29 | 0 | 90.6 | 90.6 | 0 | 225 (1-72) |
| Total | 54 | 54 | 0 | 49 | 49 | 0 | 90.7 | 90.7 | 0 | 337 (1-72) |

Area (3) Wanstead

| | | | | | | | | | | |
|--------------|----|----|---|---|---|---|-------|-------|---|---------|
| Jul. 1962 | 10 | 10 | 0 | 2 | 2 | 0 | 20 | 20 | 0 | 2 (1-) |
| Oct. 1962 | 7 | 7 | 0 | 2 | 2 | 0 | 28.57 | 28.57 | 0 | 6 (1-5) |
| Total | 17 | 17 | 0 | 4 | 4 | 0 | 23.53 | 23.53 | 0 | 8 (1-5) |

Area (4) Newdigate (stream fauna)

| | | | | | | | | | | |
|--------------|---------------|----|---|----|----|---|-------|-------|---|------------|
| Aug. 1962 | 16 (mixed) | 16 | 0 | 4 | 4 | 0 | 45 | 45 | 0 | 12 (1-7) |
| Sep. 1962 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oct. 1962 | 10 | 10 | 0 | 5 | 5 | 0 | 50 | 50 | 0 | 39 (1-20) |
| Nov. 1962 | 9 (selected) | 9 | 0 | 8 | 8 | 0 | 88.89 | 88.89 | 0 | 55 (1-19) |
| Dec. 1962 | 4 (selected) | 4 | 0 | 3 | 3 | 0 | 75 | 75 | 0 | 58 (10-34) |
| Jan. 1963 | 3 (selected) | 3 | 0 | 3 | 3 | 0 | 100 | 100 | 0 | 151 (9-97) |
| Feb. 1963 | 3 (selected) | 3 | 0 | 3 | 3 | 0 | 100 | 100 | 0 | 67 (10-38) |
| Mar. 1963 | 2 (selected) | 2 | 0 | 2 | 2 | 0 | 100 | 100 | 0 | 23 (8-15) |
| Apr. 1963 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| May 1963 | 12 (selected) | 12 | 0 | 7 | 7 | 0 | 58.34 | 58.34 | 0 | 135 (1-54) |
| June 1963 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Jul. 1963 | 1 (selected) | 1 | 0 | 1 | 1 | 0 | 100 | 100 | 0 | 6 |
| Total | 60 | 60 | 0 | 36 | 36 | 0 | 60 | 60 | 0 | 546 (1-97) |

N.B. Pond fauna, Newdigate - total of 88 3 sp. sticklebacks examined. No *Phyllodistomum* infection.

The fish collected in the Newdigate area from Nov. 1962 onwards were selected specimens of 1+ years of age. Fish netted at all other times were random samples.

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The seasonal incidence of Phyllodistomum in areas 1-4 (an illustration of Table 17)

Abbreviations:

- % popln.inf. = percentage of the total sample of the population to be infected
- MTHS = months of the year from November 1961 to July 1963
- EW = period of extreme winter conditions
- FB = period when the fish were breeding
- D = dam in operation
- C = the times when weed clearance took place
- SA = beginning of the period when only selected fish (i.e. large adults) were sampled

Area 1 - Bushy Park (System A only)

Gasterosteus aculeatus records represented by .

G. pungitius records represented by @

Average readings for both species recorded as (•) and the points are joined by thick lines

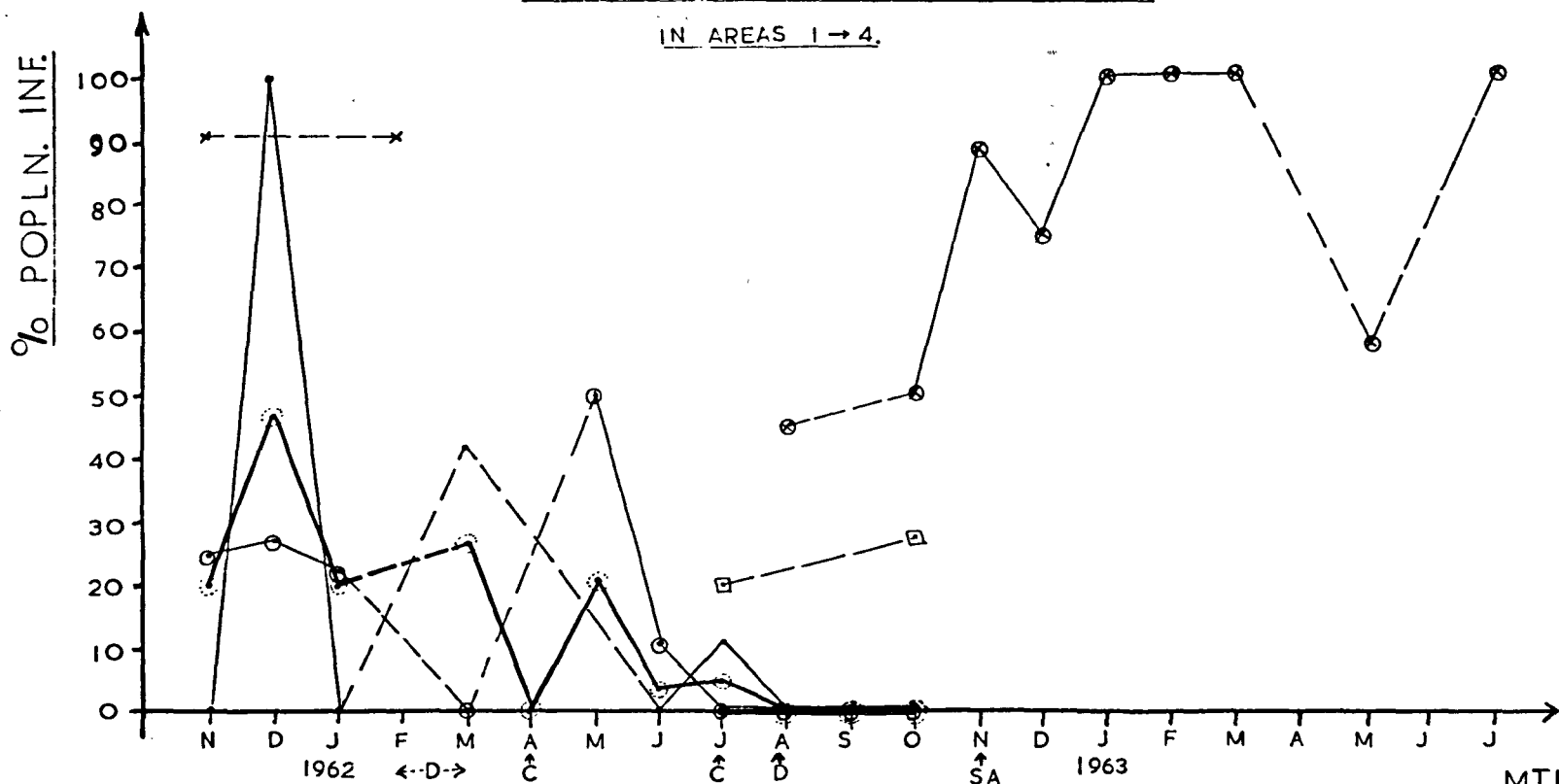
Area 2 - Essex Gasterosteus aculeatus records represented by x

Area 3 - Wanstead Gasterosteus aculeatus records represented by [•]

Area 4 - Newdigate Gasterosteus aculeatus records represented by @

Consecutive readings are linked by unbroken lines. Where records were unavailable in consecutive months the points are joined by broken lines. [Where the same record occurs more than once, this is signified by a double ring around the relevant symbol]

SEASONAL INCIDENCE OF PHYLLODISTOMUM
IN AREAS 1-4.



MTHS.

Table 18. Summary - average numbers of Phyllodistomum per infected individual per month

| Month | Area 1 Bushy Park | Area 2 Essex | Area 3 Wanstead | Area 4 Newdigate |
|----------------|----------------------|-----------------|--------------------|---------------------|
| November 1961 | 1 | 5.6 | - | - |
| December 1961 | 5.8 | - | - | - |
| January 1962 | 1.5 | - | - | - |
| February 1962 | - | 7.4 | - | - |
| March 1962 | 2.7 | - | - | - |
| April 1962 | - | - | - | - |
| May 1962 | 3 | - | - | - |
| June 1962 | 1 | - | - | - |
| July 1962 | 3.3 | - | 1 | - |
| August 1962 | - | - | - | 3 |
| September 1962 | - | - | - | - |
| October 1962 | - | - | 3 | 7.8 |
| November 1962 | - | - | - | 6.9 |
| December 1962 | - | - | - | 19.3 |
| January 1963 | - | - | - | 50.3 |
| February 1963 | - | - | - | 33.3 |
| March 1963 | - | - | - | 11.5 |
| April 1963 | - | - | - | - |
| May 1963 | - | - | - | 19.3 |
| June 1963 | - | - | - | - |
| July 1963 | - | - | - | 6 |

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The seasonal numerical incidence of Phyllodistomum in areas 1-4 (an illustration of Table 18)

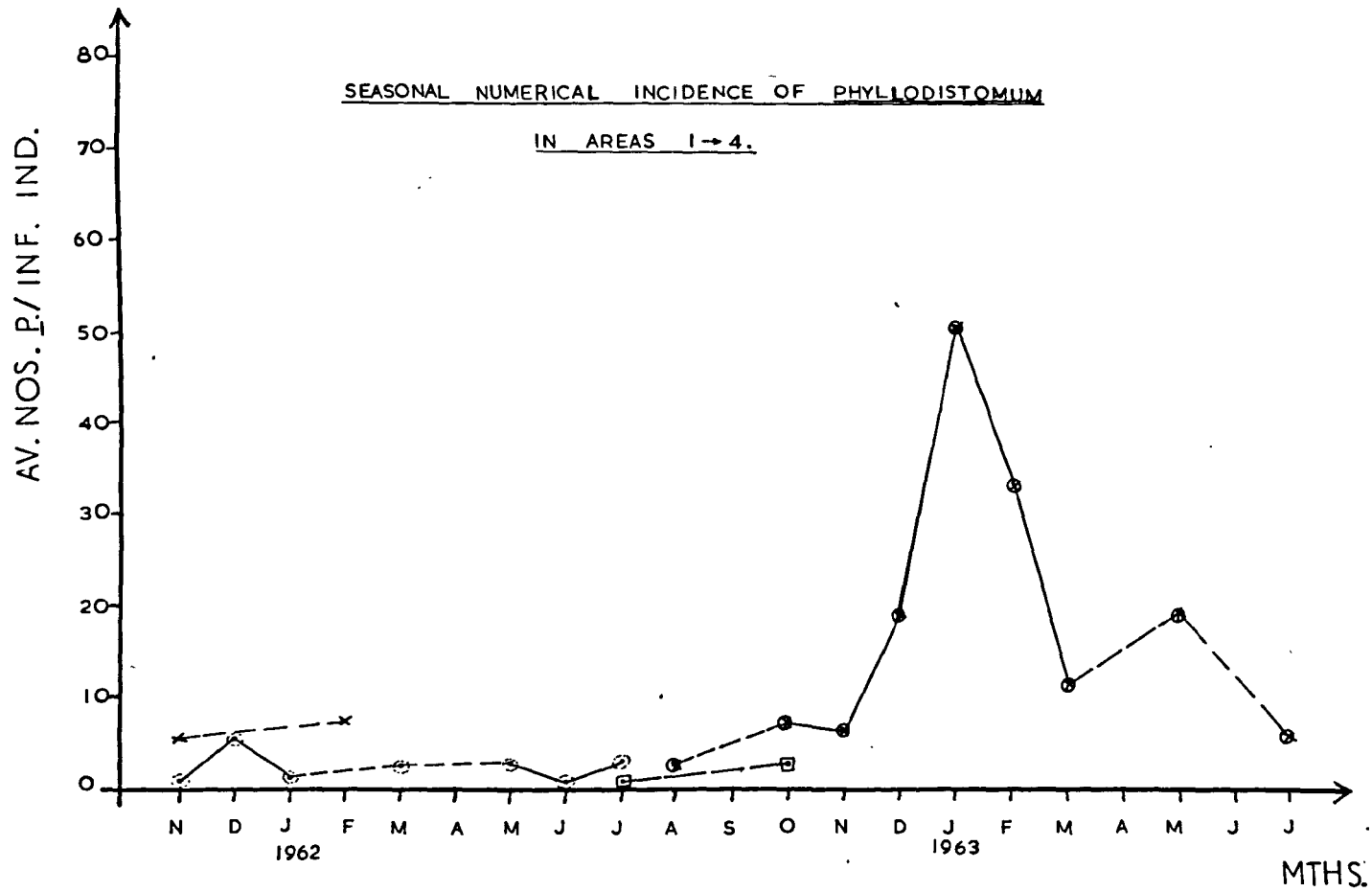
The graph shows the average number of flukes recovered per infected individual (av.no. P/Inf.Ind) per month (Mths) from November 1961 to July 1963.

Area 1 - Bushy Park Total population recorded (i.e. results for both the 3-spined and 8+ spined species are included). The combined records are represented by ⊙

Area 2 - Essex The records for the 3-spined species are represented by x

Area 3 - Wanstead The records for the 3-spined species are represented by □

Area 4 - Newdigate The records for the 3-spined species are represented by @



Seasonal incidence of Phyllodistomum in relation to the sex of the host (the graph is an illustration of Tables 19 and 20)

Abbreviations and symbols:

% Inf. percentage of fish found to be infected each month (Mth) with Phyllodistomum over the period November 1961 to July 1963

⊖ represents records for female fish

⊗ represents records for male fish

Only continuous lines join consecutive records

⊖E record for 3-spined fish (Essex area)(female fish)

⊗E record for 3-spined male fish (Essex area)

⊖B record for 3-spined female fish (Bushy Park) [System A]

⊗B record for 3-spined male fish (Bushy Park)
(Thicker lines join the points on the graph)

⊖8B record for 8+ spined female fish (Bushy Park) [System A]

⊗8B record for 8+ spined male fish (Bushy Park)

⊖W record for 3-spined female fish (Wanstead)

⊗W record for 3-spined male fish (Wanstead)

⊖N record for 3-spined female fish (Newdigate)

⊗N record for 3-spined male fish (Newdigate)

SEASONAL INCIDENCE OF PHYLLODISTOMUM IN RELATION TO THE SEX OF THE HOST.

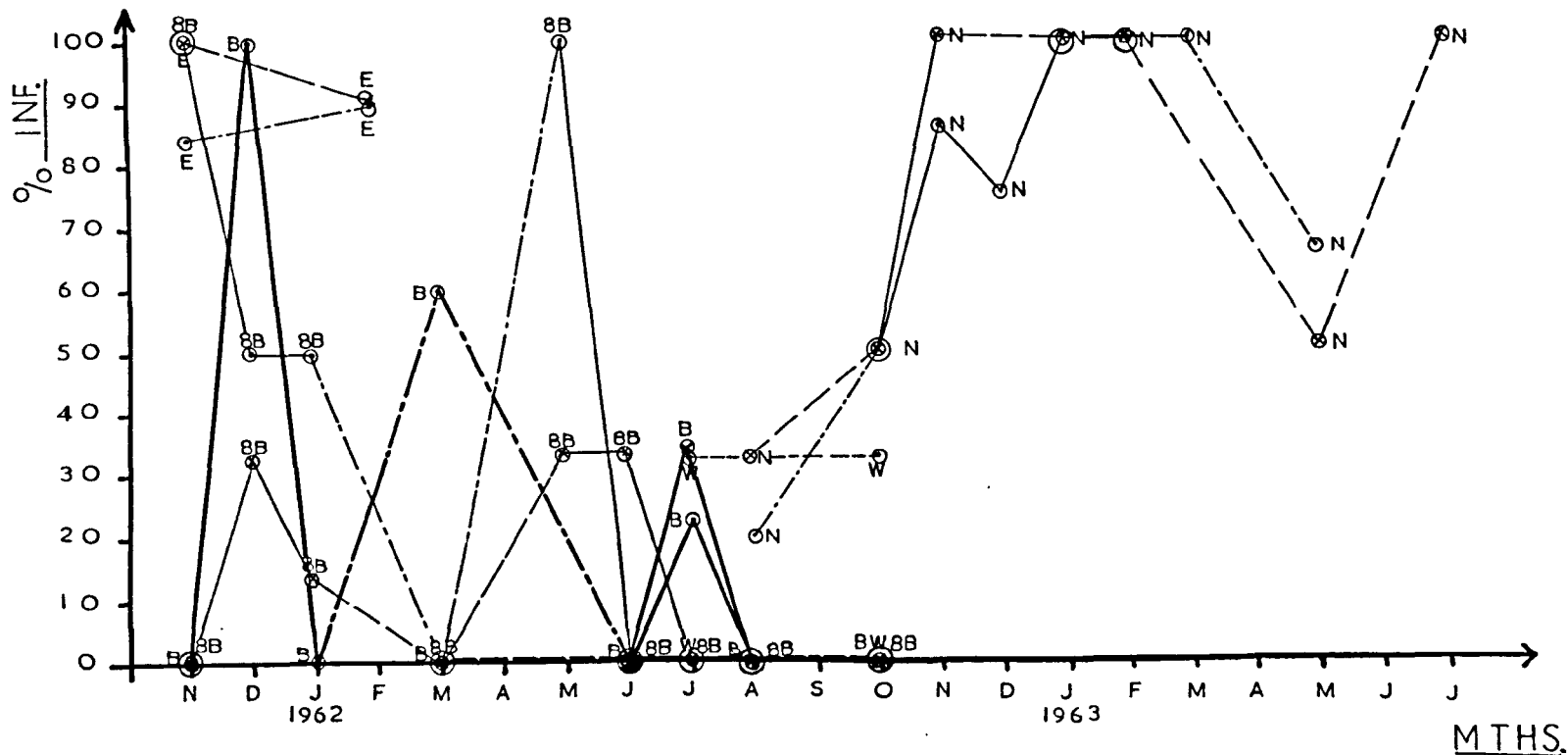


Table 19. Incidence of the trematode Phyllodistomum in relation to the sex of the definitive host

Area (1) Bushy Park 1. Male Gasterosteus spp.

| Month | No. of fish examined | | | No. of fish infected | | | Percentage infection | | | Total no. of flukes recovered (range) |
|--------------|-----------------------|-----------------------|-----------------|----------------------|-----------------|-----------------|-------------------------|--------------------|--------------------|--|
| | Total | 3 sp. | 8+ sp. | Total | 3 sp. | 8+ sp. | Total | 3 sp. | 8+ sp. | |
| Nov. 1961 | 3A | 0 | 3 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| Dec. 1961 | 12A | 0 | 12 | 4 | 0 | 4 | 33.4 | - | 33.4 | 10 (1-4) |
| Jan. 1962 | 7A | 0 | 7 | 1 | 0 | 1 | 14.28 | - | 14.28 | 1 |
| Feb. 1962 | 0A | 0 | 0 | 0 | 0 | 0 | 0 | - | - | 0 |
| Mar. 1962 | 4A | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apr. 1962 | 2B | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| May 1962 | $\frac{5A}{9B}$ | $\frac{0A}{1B}$ | $\frac{3A}{5B}$ | $\frac{1A}{0B}$ | $\frac{0A}{0B}$ | $\frac{1A}{0B}$ | $11.12\frac{A}{B}$ | $\frac{A}{0B}$ | $\frac{33.4A}{0B}$ | $\frac{5A}{0B}$ |
| June 1962 | 3(17*)A | (14)* | 3 | 1 | 0 | 1 | 33.4 (5.8)* | 0 | 33.4 | 1 |
| July 1962 | 10(25*) $\frac{A}{B}$ | $\frac{3A(+15*)}{4B}$ | $\frac{1A}{2B}$ | $\frac{1A}{0B}$ | $\frac{1A}{0B}$ | $\frac{0A}{0B}$ | 10 $\frac{A}{B}$ (3.3)* | $\frac{33.4A}{0B}$ | $\frac{0A}{0B}$ | 1 |
| Aug. 1962 | 2A | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sep. 1962 | 3B | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oct. 1962 | 2A | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | | | | | | | | | | |
| System B | 17 | 7 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| System A | 40 (69*) | 7 (36*) | 33 | 8 | 1 | 7 | (11.5)* | 14.2 (2.7)* | 21.2 | 18 (1-5) |
| Area A + B | 57 (86*) | 14 (43*) | 43 | 8 | 1 | 7 | 14 (9.3)* | 7.14 (2.3)* | 16.28 | 18 (3 sp = 1) (8+ sp = 17) (1-5) |

N.B. * = total when immature fish, too young to be infected, are included in record

Area (1) Bushy Park 2. Female Gasterosteus spp.

| | | | | | | | | | | |
|--------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-------------------|--------------------|--------------------|---|
| Nov. 1961 | 2A | 1 | 1 | 1 | 0 | 1 | 50 | 0 | 100 | 1 |
| Dec. 1961 | 7A | 3 | 4 | 5 | 3 | 2 | 71.4 | 100 | 50 | 42 (1-27) |
| Jan. 1962 | 3A | 1 | 2 | 1 | 0 | 1 | 33.3 | 0 | 50 | 2 |
| Feb. 1962 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | 0 |
| Mar. 1962 | 7A | 5 | 2 | 3 | 3 | 0 | 42.2 | 60 | 0 | 8 (1-5) |
| Apr. 1962 | 2B | 0 | 2 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| May 1962 | $\frac{5A}{9B}$ | $\frac{0A}{0B}$ | $\frac{1A}{4B}$ | $\frac{2A}{2B}$ | $\frac{0A}{0B}$ | $\frac{1A}{1B}$ | $40\frac{A}{B}$ | $-\frac{A}{B}$ | $\frac{100A}{25B}$ | 4 ($\frac{3A}{1B}$) |
| June 1962 | 7A | 1 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| July 1962 | 21 $\frac{A}{B}$ | $\frac{9A}{7B}$ | $\frac{3A}{2B}$ | $\frac{2A}{2B}$ | $\frac{2A}{0B}$ | $\frac{0A}{0B}$ | 9.5 $\frac{A}{B}$ | $\frac{22.3A}{0B}$ | $\frac{0A}{0B}$ | 9 (1-8) |
| Aug. 1962 | 12A | 8 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sep. 1962 | 8B | 6 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oct. 1962 | 2A | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | | | | | | | | | | |
| System B | 23 | 13 | 10 | 1 | 0 | 1 | 4.3 | 0 | 10 | 1 |
| System A | 53 | 29 | 24 | 13 | 8 | 5 | 24.5 | 27.5 | 20.8 | 65 (1-27) |
| Area A + B | 76 | 42 | 34 | 14 | 8 | 6 | 18.42 | 19.04 | 17.6 | 66 (3 sp = 53) (1-27) (9+ sp = 13) (1-5) |

Table 20. Incidence of the trematode Phyllodistomum in relation to the sex of the definitive host

Area 2 - Collector's area in Essex (3 sp. Gasterosteus male and female)

| | Total no. of ♂'s examined | Total no. of ♂'s infected | % infection ♂'s | No. of flukes recovered + range | Total no. of ♀'s examined | Total no. of ♀'s infected | % infection ♀'s | No. of flukes recovered + range |
|---------------|------------------------------|------------------------------|--------------------|--|------------------------------|------------------------------|--------------------|--|
| November 1961 | 9 | 9 | 100 | 49(1-18) | 13 | 11 | 84.61 | 63(2-16) |
| February 1962 | 12 | 11 | 91.67 | 83(1-36) | 20 | 18 | 90 | 142(1-72) |
| Total | 21 | 20 | 95.24 | 132(1-36) | 33 | 29 | 87.88 | 205(1-72) |

Area 3 - Wanstead

| | | | | | | | | |
|--------------|---|---|---|---|----|---|-------|--------|
| July 1962 | 4 | - | - | - | 6 | 2 | 33.34 | 2 |
| October 1962 | 1 | - | - | - | 6 | 2 | 33.34 | 6(1-5) |
| Total | 5 | - | - | - | 12 | 4 | 33.34 | 8(1-5) |

Area 4 - Newdigate: stream fauna

| | | | | | | | | |
|----------------|----|----|-------|----------|----|----|-------|------------|
| August 1962 | 6 | 2 | 33.34 | 3(1-2) | 10 | 2 | 20 | 9(2-7) |
| September 1962 | - | - | - | - | - | - | - | - |
| October 1962 | 8 | 4 | 50 | 36(1-20) | 2 | 1 | 50 | 3 |
| November 1962 | 2 | 2 | 100 | 14(4-10) | 7 | 6 | 85.71 | 41(1-19) |
| December 1962 | - | - | - | - | 4 | 3 | 75 | 58(10-34) |
| January 1963 | 1 | 1 | 100 | 9 | 2 | 2 | 100 | 142(45-97) |
| February 1963 | 1 | 1 | 100 | 19 | 2 | 2 | 100 | 48(10-38) |
| March 1963 | - | - | - | - | 2 | 2 | 100 | 23(8-15) |
| April 1963 | - | - | - | - | - | - | - | - |
| May 1963 | 6 | 3 | 50 | 3 | 6 | 4 | 66.67 | 132(7-54) |
| June 1963 | - | - | - | - | - | - | - | - |
| July 1963 | 1 | 1 | 100 | 6 | - | - | - | - |
| Total | 24 | 14 | 58.34 | 90(1-20) | 36 | 22 | 61.12 | 456(1-97) |

Bushy Park area - System A. The results of examination of the fish population from November 1961 to October 1963 (the graphs are an illustration of Tables 21 and 23)

Graph BP - 3sp A refers to results obtained from the examination of Gasterosteus aculeatus

Graph BP - 8+sp A refers to the results obtained from the examination of G. pungitius (hosts bearing from 8-10 spines are included in this species)

Abbreviations used in both graphs:

- Av.Nos.P./Ind the average numbers of Phyllodistomes recovered per infected host individual
- Av.Fish Lth.-cms average length of the fish given in centimeters (including the tail fin)
- MTHS months of the year

Symbols used in both graphs:

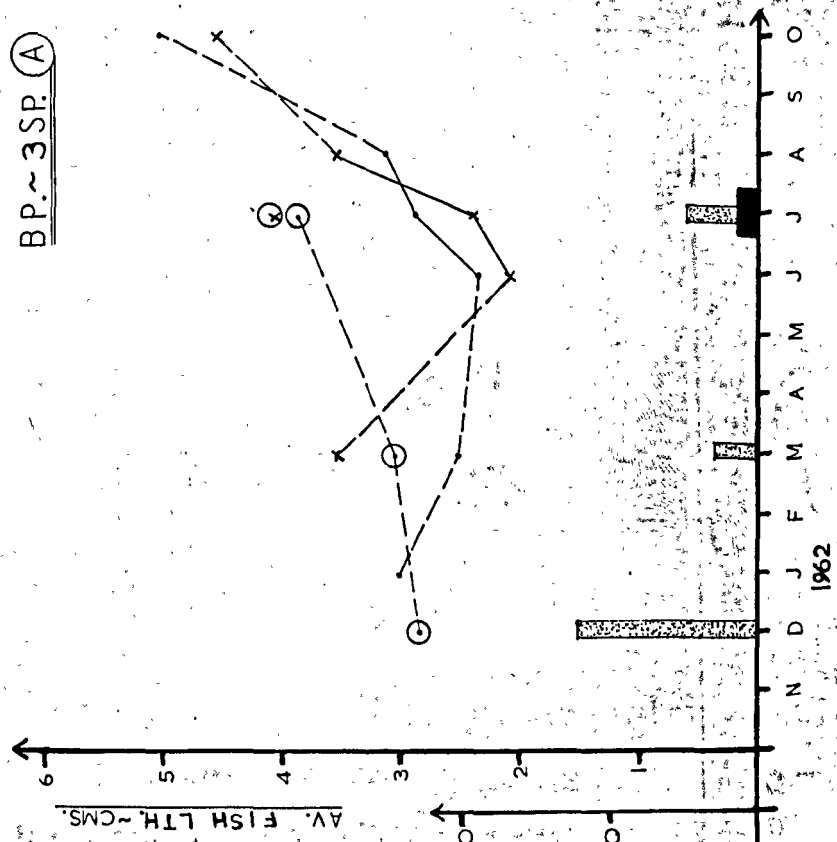
- ⊗ records for infected males
- x records for uninfected males
- ⊙ records for infected females
- records for uninfected females

Average numbers of Phyllodistomes recovered from female fish represented by stippled columns

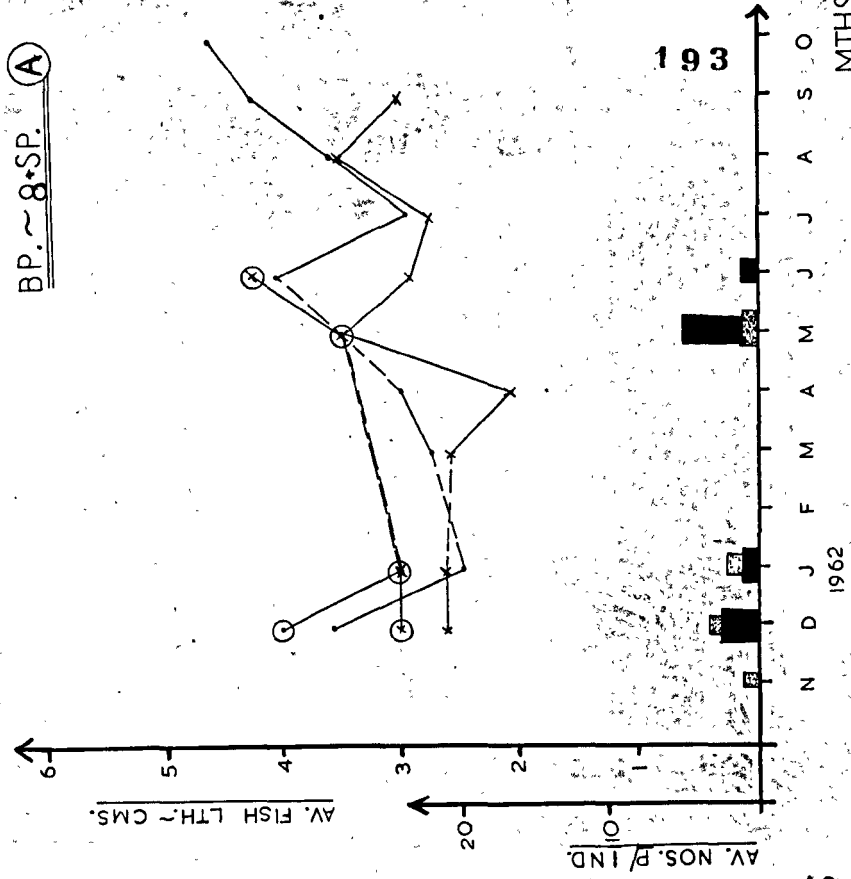
Average numbers of Phyllodistomes recovered from male fish represented by opaque columns

N.B. All consecutive readings are joined by continuous lines. Where consecutive monthly readings were not available the records are connected by broken lines

BP. ~ 3 SP. (A)



BP. ~ 8 SP. (A)



MTHS

1962

1963

MTHS

PL

Graph N-3SP illustrates the results of the examination of the 3-spined stickleback population of Newdigate during the months August 1962 to July 1963 (the graph is an illustration of Tables 21 and 23, pp. 196, 198)

Abbreviations

AV.Nos.P./Ind. = average numbers of Phyllodistomum recovered per infected individual
Av.Fish Lth.-cms = average length of the fish host given in centimeters
MTHS = months of the year

Symbols:

- ⊗ records for infected males
- X records for uninfected males
- ⊙ records for infected females
- records for uninfected females

Numbers of trematodes recovered from females represented by stippled columns

Numbers of trematodes recovered from males represented by opaque columns

N.B. Only consecutive readings are joined by unbroken lines

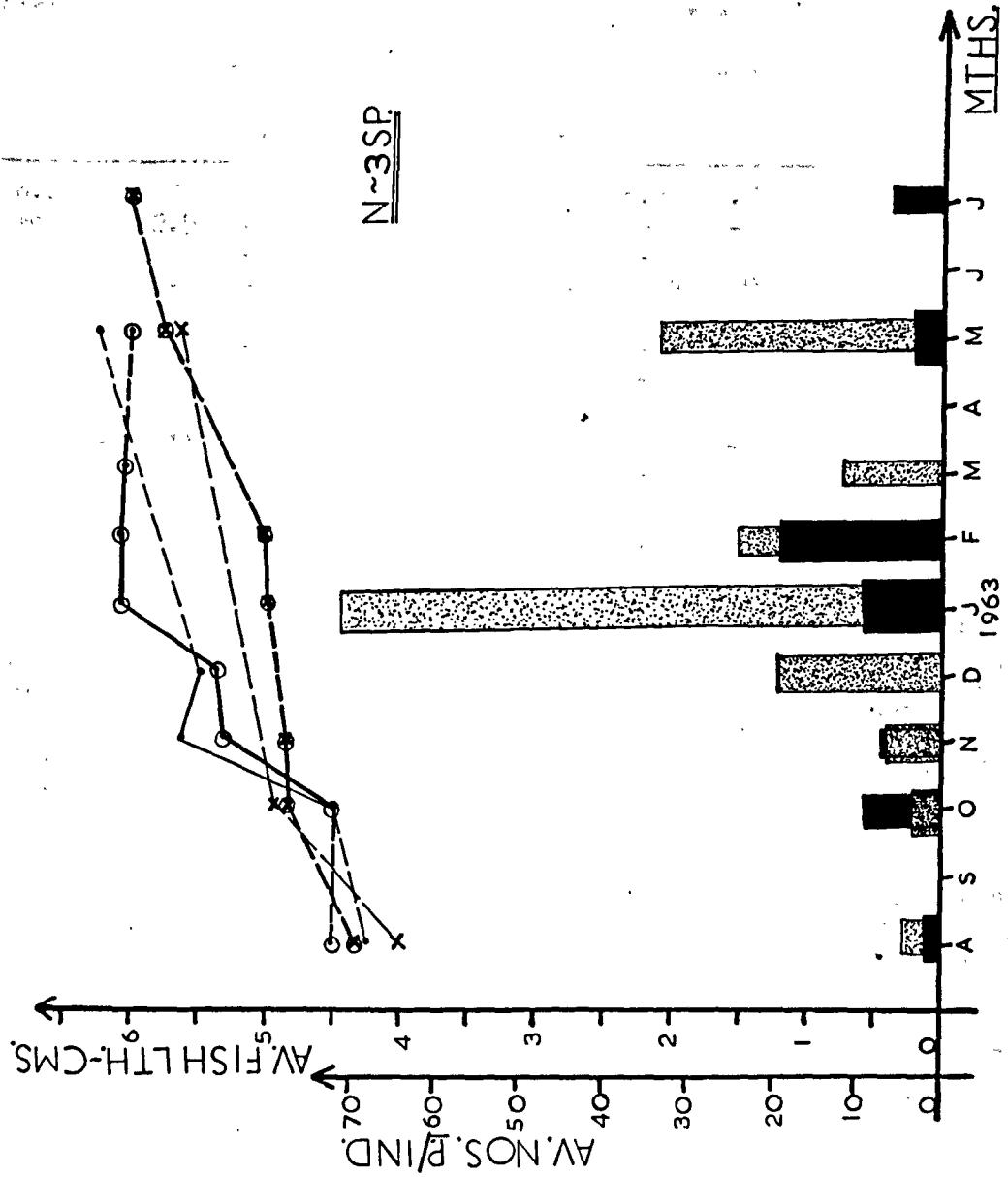


Table 21. Seasonal incidence: average numbers of Phyllodistomum present per infected individual per month

Host - Gasterosteus aculeatus, G. pungitius

| Month | Area 1 Bushy Park | | | | | | Area 2 Essex | | Area 3 Wanstead | | Area 4 Newdigate | |
|-----------|----------------------|-----------------|-----------------|--------|-------------------|-----------------|-----------------|--------|--------------------|--------|---------------------|--------|
| | Male | | | Female | | | Male | Female | Male | Female | Male | Female |
| | T | 3 sp. | 8+ | T | 3 sp. | 8+ | | | | | | |
| Nov. 1961 | 0 | - | 0 | 1 | 0 | 1 | 5.45 | 5.73 | - | - | - | - |
| Dec. 1961 | 2.5 | - | 2.5 | 8.0 | 12 | 3 | - | - | - | - | - | - |
| Jan. 1962 | 1 | - | 1.0 | 2.0 | 0 | 2 | - | - | - | - | - | - |
| Feb. 1962 | - | - | - | - | - | - | 7.54 | 7.89 | - | - | - | - |
| Mar. 1962 | 0 | 0 | 0 | 2.67 | 2.67 | 0 | - | - | - | - | - | - |
| Apr. 1962 | 0 | - | 0 | 0 | - | 0 | - | - | - | - | - | - |
| May 1962 | 5* | 0 | $\frac{B0}{A5}$ | 2 | - | $\frac{B3}{A1}$ | - | - | - | - | - | - |
| June 1962 | 1* | 0 | 1 | 0 | 0 | 0 | - | - | - | - | - | - |
| July 1962 | 1 | $\frac{B0}{A1}$ | 0 | 4.5 | $\frac{B0}{A4.5}$ | 0 | - | - | 0 | 1 | - | - |
| Aug. 1962 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | 1.5 | 4.5 |
| Sep. 1962 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | - | - | - | - |
| Oct. 1962 | 0 | 0 | 0 | 0 | 0 | 0 | - | - | 0 | 3 | 9* | 3 |
| Nov. 1962 | - | - | - | - | - | - | - | - | - | - | 7* | 6.84 |
| Dec. 1962 | - | - | - | - | - | - | - | - | - | - | - | 19.34 |
| Jan. 1963 | - | - | - | - | - | - | - | - | - | - | 9 | 71.00 |
| Feb. 1963 | - | - | - | - | - | - | - | - | - | - | 19 | 24.00 |
| Mar. 1963 | - | - | - | - | - | - | - | - | - | - | - | 11.5 |
| Apr. 1963 | - | - | - | - | - | - | - | - | - | - | - | - |
| May 1963 | - | - | - | - | - | - | - | - | - | - | 3 | 33 |
| June 1963 | - | - | - | - | - | - | - | - | - | - | - | - |
| July 1963 | - | - | - | - | - | - | - | - | - | - | 6 | - |

0 no infection

- no fish examined that month

* occasions when male individuals carried a larger average number of flukes when compared with females

Table 22. Summary of results

3 spined sticklebacks

| <u>Area 1 - Bushy Park</u> | <u>Total</u> | <u>Males</u> | <u>Females</u> |
|-----------------------------------|----------------|--------------|----------------|
| Number examined | 56 (85*) | 14 (43*) | 42 |
| Number infected | 9 | 1 | 8 |
| % infection | 16.07 (10.58*) | 7.14 (2.3*) | 19.04 |
| Number of flukes recovered | 54 | 1 | 53 (1-27) |
| Average number of flukes per fish | 6 | 1 | 6.625 |

Area 2 - Essex

| | | | |
|-----------------------------------|-------|------------|------------|
| Number examined | 54 | 21 | 33 |
| Number infected | 49 | 20 | 29 |
| % infection | 90.74 | 95.24 | 87.88 |
| Number of flukes recovered | 337 | 132 (1-36) | 205 (1-72) |
| Average number of flukes per fish | 6.88 | 6.6 | 7.58 |

Area 3 - Wanstead

| | | | |
|-----------------------------------|-------|---|---------|
| Number examined | 17 | 5 | 12 |
| Number infected | 4 | - | 4 |
| % infection | 23.53 | 0 | 33.34 |
| Number of flukes recovered | 8 | - | 8 (1-5) |
| Average number of flukes per fish | 2 | - | 2 |

Area 4 - Newdigate

| | | | |
|-----------------------------------|--------|-----------|------------|
| Number examined | 60 | 24 | 36 |
| Number infected | 36 | 14 | 22 |
| % infection | 60 | 58.34 | 61.12 |
| Number of flukes recovered | 546 | 90 (1-20) | 456 (1-97) |
| Average number of flukes per fish | 15.167 | 6.428 | 20.73 |

8+ spined sticklebacks

Area 1 - Bushy Park

| | | | |
|-----------------------------------|-------|----------|----------|
| Number examined | 77 | 43 | 34 |
| Number infected | 13 | 7 | 6 |
| % infection | 16.88 | 16.28 | 17.6 |
| Number of flukes recovered | 30 | 17 (1-5) | 13 (1-5) |
| Average number of flukes per fish | 2.307 | 2.428 | 2.167 |

| | | | |
|---|------|------|-------|
| Total av. no. of flukes per fish - area 1, 3 sp and 8+ sp | 3.82 | 2.25 | 4.714 |
|---|------|------|-------|

| | | | |
|-------------------------------|-------|----------------|-------|
| Av. % infection 3 sp area 1-4 | 45.37 | 54.68 (32.71*) | 51.22 |
| 8+ sp area 1 | 16.88 | 39.53 | 17.6 |

| | | | |
|---|-------|-------------|-------|
| Total <u>Gasterosteus</u> spp area 1-4 inc. | 31.44 | 29.90 (28*) | 43.95 |
|---|-------|-------------|-------|

| | | | |
|----------------------------|------|------|-------|
| Av. no. of flukes per fish | | | |
| 3 sp area 1-4 | 9.64 | 6.57 | 11.40 |
| 8+ sp area 1 | 2.31 | 2.43 | 2.17 |

| | | | |
|--|------|------|-------|
| Total <u>Gasterosteus</u> spp. area 1-4 inc. | 8.78 | 5.71 | 10.65 |
|--|------|------|-------|

* signifies immature fish (too young to be infected with Phyllodistomum) also examined and included in the figures

Table 23. Host size relationships and *Phyllodistomum* infections

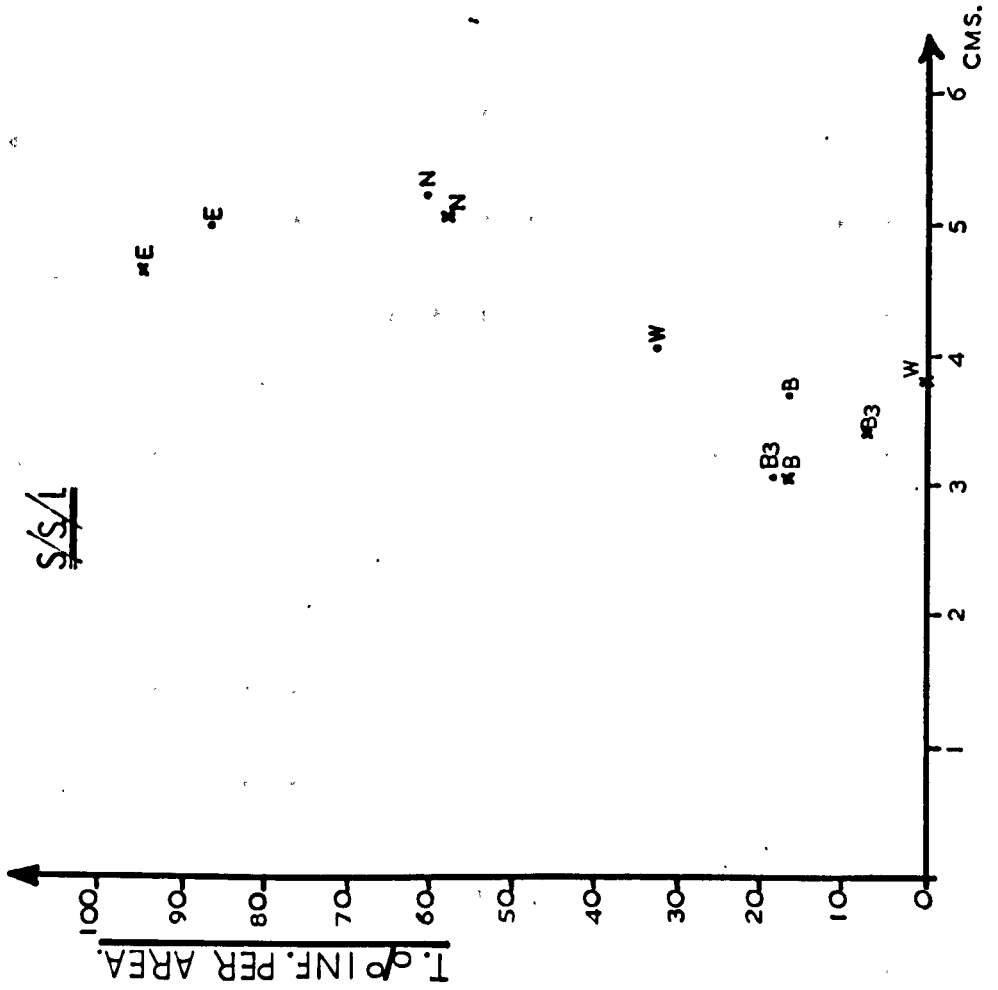
| Area 1 - Bush Park <i>Gasterosteus pungitius</i> | | | | | | |
|--|--|-------------------------------------|--|--|---------------------------------------|--------|
| Month | Total av. length for population | Average length infected male | Average length uninfected male | Average length uninfected female | Average length infected female | |
| Nov. 1961 | ? | ? | ? | ? | ? | ? |
| Dec. 1961 | 2.98A | 3.00 | 2.62 | 3.58 | 4.00 | |
| Jan. 1962 | 2.69A | 3.00 | 2.62 | 2.50 | 3.00 | |
| Feb. 1962 | 0 | 0 | 0 | 0 | 0 | |
| Mar. 1962 | 2.67A | 0 | 2.60 | 2.75 | 0 | |
| Apr. 1962 | 2.74B | 0 | 2.12 | 3.00 | 0 | |
| May 1962 | <u>4.11</u> - <u>3.50A</u> <u>4.39B</u> | <u>3.5</u> <u>3.5A</u> <u>OB</u> | <u>4.00</u> <u>3.5 A</u> <u>4.25B</u> | <u>4.25</u> <u>0 A</u> <u>4.25B</u> | <u>4.5</u> <u>3.5A</u> <u>5.5B</u> | |
| June 1962 | 3.72A | 4.25 | 2.92 | 4.10 | 0 | |
| July 1962 | <u>3.56</u> <u>2.87A</u> <u>4.25B</u> | <u>0</u> <u>0U</u> <u>OB</u> | <u>3.75</u> <u>2.75A</u> <u>4.25B</u> | <u>3.45</u> <u>2.92A</u> <u>4.25B</u> | 0 | |
| Aug. 1962 | 3.55A | 0 | 3.50 | 3.58 | 0 | |
| Sep. 1962 | 3.84B | 0 | 3.00 | 4.25 | 0 | |
| Oct. 1962 | 4.62A | 0 | 0 | 4.62 | 0 | |
| Gasterosteus aculeatus | | | | | | |
| Nov. 1961 | ? | ? | ? | ? | ? | |
| Dec. 1961 | 2.84A | 0 | 0 | 0 | 2.84 | |
| Jan. 1962 | 3.00A | 0 | 0 | 3.00 | 0 | |
| Feb. 1962 | 0 | 0 | 0 | 0 | 0 | |
| Mar. 1962 | 3.04A | 0 | 3.50 | 2.52 | 3.08 | |
| Apr. 1962 | OB | 0 | 0 | 0 | 0 | |
| May 1962 | <u>4.0</u> <u>0A</u> <u>4.0B</u> | 0 | <u>4.0</u> <u>0A</u> <u>4.0B</u> | 0 | 0 | |
| June 1962 | 2.22A | 0 | 2.04 | 2.33 | 0 | |
| July 1962 | <u>3.12</u> <u>2.81A</u> <u>3.89B</u> | <u>4.0</u> <u>4.0A</u> <u>OB</u> | <u>2.79</u> <u>2.35A</u> <u>3.87B</u> | <u>3.23</u> <u>2.89A</u> <u>3.89B</u> | <u>3.87</u> <u>3.87A</u> <u>OB</u> | |
| Aug. 1962 | 3.19A | 0 | 3.5 | 3.11 | 0 | |
| Sep. 1962 | 3.44B | 0 | 3.62 | 3.37 | 0 | |
| Oct. 1962 | 4.75A | 0 | 4.50 | 5.00 | 0 | |
| Area 2 - Essex <i>Gasterosteus aculeatus</i> | | | | | | |
| Nov. 1961 | 4.62 | 4.47 | 0 | 4.75 | 4.64 | |
| Feb. 1962 | 4.94 | 4.81 | 4.25 | 4.75 | 5.08 | |
| Area 3 - Wanstead <i>Gasterosteus aculeatus</i> | | | | | | |
| July 1962 | 4.27 | 0 | 3.94 | 4.00 | 5.5 | |
| Oct. 1962 | 3.54 | 0 | 3.62 | 3.50 | 3.5 | |
| Area 4 - Newdigate <i>Gasterosteus aculeatus</i> | | | | | | |
| Aug. 1962 | 4.27 | 4.37 | 4.00 | 4.31 | 4.50 | |
| Sep. 1962 | 0 | 0 | 0 | 0 | 0 | |
| Oct. 1962 | 4.80 | 4.81 | 4.94 | 4.50 | 4.50 | |
| Nov. 1962 | 5.31 | 4.87 | 0 | 5.62 | 5.35 | |
| Dec. 1962 | 5.42 | 0 | 0 | 5.50 | 5.40 | |
| Jan. 1963 | 5.75 | 5.0 | 0 | 0 | 6.12 | |
| Feb. 1963 | 5.75 | 5.0 | 0 | 0 | 6.12 | |
| Mar. 1963 | 6.12 | 0 | 0 | 0 | 6.12 | |
| Apr. 1963 | 0 | 0 | 0 | 0 | 0 | |
| May 1963 | 5.90 | 5.75 | 5.67 | 6.25 | 6.00 | |
| June 1963 | 0 | 0 | 0 | 0 | 0 | |
| July 1963 | 6.00 | 6.00 | 0 | 0 | 0 | |
| Summary | | | | | | |
| Totals | 3 sp. | Area 1 8+ sp. | 3 + 8+ sp. | Area 2 | Area 3 | Area 4 |
| Av. length | 3.02A B | 3.25A B | 3.12A B | 4.81 | 3.97 | 5.15 |
| Av. male length | 3.40A B | 3.12A B | 3.23A B | 4.64 | 3.83 | 5.04 |
| Av. female length | 3.08A B | 3.73A B | 3.32A B | 4.92 | 4.04 | 5.23 |
| Av. length inf. male | 4.00 | 3.79 | 3.82 | 4.66 | 0 | 5.07 |
| Av. length uninf. male | 4.26 | 3.04 | 3.81 | 4.25 | 3.84 | 4.79 |
| Av. length inf. female | 3.17 | 4.00 | 3.45 | 4.91 | 4.50 | 5.58 |
| Av. length uninf. " | 3.06 | 3.38 | 3.18 | 4.75 | 3.79 | 5.59 |

100

Graph SS/1 illustrates the relationship between the average total size of the fish population of each area (T.POPLN.SZE) in relation to the percentage of that population to be infected (T.%INF.PER AREA) as regards the sex of the host (the graph illustrates Tables 22 and 23, pp. 91-4)

Abbreviations and symbols:

| | |
|----|----------------------------|
| X | male fish record |
| . | female fish record |
| E | Essex |
| N | Newdigate |
| W | Wanstead |
| B3 | 3-spined fish, Bushy Park |
| B | 8+ spined fish, Bushy Park |



T. POPLN. SZE.

Table 24. Seasonal variations in diet of Gasterosteus spp. (post-mortem results)

| | Bushy Park | | | Essex | | Newdigate | | | Wanstead | | Highams | |
|--------------------------------------|------------|--------|--------|--------|--------|-----------|--------|--------|----------|-----------------|---------|------------------|
| | Spring | Summer | Winter | Spring | Winter | Spring | Summer | Winter | Summer | Winter (Oct) | Summer | Winter (Oct.) |
| Annelida - Oligochaeta | + | + | + | - | + | - | + | + | + | - | - | - |
| Crustacea - Amphipoda | - | + | - | - | - | - | - | - | - | - | + | + |
| Branchiura | - | + | - | - | - | - | - | - | - | - | - | - |
| Cladocera | - | + | + | + | + | + | + | + | - | + | + | - |
| Copepoda | - | + | + | + | + | + | + | + | - | + | + | - |
| Isopoda | - | + | - | + | - | - | - | - | - | - | - | - |
| Ostracoda | + | + | + | - | + | + | + | + | + | + | - | - |
| Insecta - Coleoptera | - | + | - | + | + | - | - | + | + | - | - | - |
| Ephemeroptera | - | + | - | - | - | - | - | - | - | - | - | - |
| Hemiptera | - | + | + | - | - | - | - | - | - | - | - | - |
| Diptera | - | + | + | - | - | + | - | + | - | + | + | + |
| Trichoptera | - | - | - | + | + | - | - | - | - | - | - | - |
| Plecoptera | - | - | - | - | - | - | - | + | - | - | - | - |
| Unidentifiable arthropod remains | - | + | + | - | + | - | - | + | - | - | - | - |
| Arachnida - Acarina | - | + | + | - | - | - | + | - | - | - | - | - |
| Mollusca - Lamellibranchiata | - | + | - | - | - | - | + | - | - | - | - | - |
| Gasteropoda | - | + | - | - | - | - | - | + | - | - | + | + |
| Vertebrata - <u>Gasterosteus</u> spp | - | - | - | - | - | - | - | - | - | - | + | - |
| Vegetation/Desmids/Diatoms | - | + | + | + | + | + | + | + | + | - | - | - |

c) Discussion

In Bushy Park repeated interference with the water system by draining and weed clearance (previously discussed on p. 112) resulted in marked faunistic changes. The Sphaerium corneum population was not large and began to decline following damming operations carried out during February and March. Prior to this period, in January, a single specimen had been recovered which had been carrying a Phyllodistome infection. As the numbers of Sphaerium began to fall a further infected specimen was recovered in April, but as in the previous case no fully developed cercariae or metacercariae were formed. Sphaerium were very difficult to recover in the late summer months and a second dam in August, reducing both the level and the rate of flow of the water, eradicated the population. As was the case in the laboratory, the infected Lamellibranchs appear to be particularly sensitive to environmental change and infected Sphaerium were probably absent from the collection areas from approximately May or June 1962. The only metacercarial-infected insect nymph to be recovered was consequently found early in the year in March. The percentage of fish infected with this parasite (Table 17 and graph , p. 184) began to fall steadily after May, which marked the beginning of the breeding season, when such an initial decrease is to be expected as a result of a higher percentage of young fish entering the population. Their early feeding habits do not expose them to Phyllodistome infection as they are sustained by a diet of planktonic forms, but later they ingest Dipteran larvae and small Oligochaetes. As they increase in size, omnivorous

tendencies become more pronounced, the main restriction being the size of the prey. Fish no more than approximately 2-3 months old were found to be infected by this Gorgoderid. The fluke probably gained entry by means of either small, very young Zygopteran or Ephemeropteran nymphs or Dipteran larvae. Although Phyllodistomum was never recovered from the latter source it still remains as a theoretically possible route. Vickers (1941) recovered C. macrocerca from such an intermediary host and Gorgodera was found in a Dipteran larva during this investigation (p. 136). In Bushy Park fish produced during the summer of 1962 had no means of becoming infected by Gorgoderids so that young fish, although reaching a fair size, when collected during August, September and October carried no Phyllodistome infections. Older breeding fish of that year had died off as the result of the epizootics which had occurred following a rise in temperature associated with a long dry spell and the artificially lowered water level.

An interesting point of difference between the two water systems investigated in this area was the absence of Gorgoderid infection from three-spined sticklebacks in System B while the nine-spined specimens from this river were parasitised. A higher percentage of the nine-spined population in System A carried Phyllodistomes, but in contrast to the three-spined fish there were fewer trematodes per individual. The reason for such differences is not known. There appears to be little to no variation in diet preferences and the urinary systems of the two species are essentially similar. The nine-spined system is somewhat smaller, the ureters are comparatively shorter and the urinogenital pore is quite

diminutive. The rectal and urinogenital openings occupy an identical position relative to one another and the tissue folding between the two apertures is sufficiently similar to offer no additional migratory hazard to the fluke in either case.

The Newdigate samples varied markedly in size from month to month. The first sample in which Phyllodistomum was detected was taken in August and consisted of fauna from a number of ponds (subsequently found to be free from infection) and the stream (where Gorgoderids occurred). As a result the total percentage of infection for August was correspondingly low (Table 17 and graph , p.154). The October sample was representative of the stream fauna but succeeding samples consisted of selected adults so the percentage of infection is higher than would be the case in nature. The parasite in an adult fish population is present throughout the year. The severe winter of 1962-1963 had no effect upon the degree of infection of Newdigate fish. Larval stages were recovered from the lamellibranch fauna in most months until this latter source was exhausted. Fully developed cercariae or metacercariae were recovered in August, October, November, December and February, and metacercariae were dissected from insect nymphs and larvae during March. Incompletely developed cercariae were recovered from molluscs sampled in January 1962 and in 1963. As the numbers of hosts involved were so low it was impossible to say if the parasite overwintered by means of extended development. The fact that fully developed cercariae and metacercariae were recovered from Sphaerium supplied from the Essex area in February 1962 would indicate that this was not the case and that cercarial release and meta-

cercarial encystment continue throughout the year in all seasons. There may be a greater tendency to encyst within the mollusc during the winter months however. This has been found to be the case for Gorgoderina vitelliloba according to Lees (personal communication).

Table 18 (and graph , p.187) illustrate the average number of Phyllodistomes recovered from individuals in an infected population. Shown in this way, Bushy Park fauna yields a constantly low result and even Essex with a high percentage of the population infected possessed on average a low density per individual. The selected fauna from Newdigate reflected a widely fluctuating Gorgoderid density bearing little relationship to the percentage of the population which was infected. The highest number of flukes recovered from a single Gasterosteus aculeatus from areas 1, 2, 3 and 4 were 27, 72, 5 and 97 respectively. In practically all cases the population of flukes was of mixed ages, the January count from Newdigate being the only one to be composed of a high proportion of juveniles.

Tables 19 and 20 illustrate the incidence of the fluke Phyllodistomum in relation to the sex of the host and the season. These results are summarised in Table 21 where it can easily be seen that in the case of the three-spined stickleback the female harbours on average more trematodes than the male. In the nine-spined species, however, the male just supplants the female. Generally a higher number of female fish were present in the area sample and a larger percentage were infected than was the case for the males. The only exception occurred in area 2

where females still harboured on average more flukes per individual despite a lower percentage infection in the female population. Tracing the percentage infection of females and males in detail from month to month shows that (graphs, p.189) while the female is more highly infected for the majority of the time the male exceeded this state in November (Essex, Newdigate), July (Bushy) and August (Newdigate). In February in Essex and Newdigate both sexes carried practically equal percentage infections.

Looking more closely at the number of trematodes carried per individual per month (Table 21) in relation to the sex of the host revealed that only on four occasions (in May and June 1962 for ten-spined sticklebacks and in October and November for three-spined sticklebacks) did male fish exceed the parasitic load of the females. These variations bear no relationship to the increasing and decreasing production of host reproductive hormones through the season.

The summary on p.194 at the end of Table 23 illustrates that in all but one of the communities studied the female fish reached a greater length than the male. With the exception of three-spined sticklebacks in Bushy Park infected females were larger than infected males, and in most cases the infected females also exceeded the uninfected stock of both sexes. The atypical result from Bushy Park may be the result of the low numbers of infected three-spined fish recovered from this district. A comparison of Tables 22 and 23 illustrates that in districts harbouring larger fish there is a likelihood of more Phyllodistomes being recovered per individual. However, this relationship is not a clear one as can be seen in graph 55/1. Plotting the total average size of the population

against the percentage infected, a general trend can be discerned where the larger the fish constituting the population the higher the percentage infection will be. This generalisation does not hold true for selected adults from Newdigate. This therefore suggests that the previous data is merely indicating the percentage of adults present in the total population and that another relationship exists between wholly adult fauna. Where the male percentage infected population exceeds the female, as noted previously (p.206) the numbers of flukes recovered from the two sexes parallel the general trend in most cases, with the female carrying a greater parasitic load. However, one exception occurs in the November 1962 record at Newdigate. Here, although the females reach a greater size, more parasites were recovered on average from the males.

Conclusion

The following generalisations can be made concerning *Phyllodistome* infections in sticklebacks:

1. There is no immunity developed to this infection and fish continue to acquire parasites throughout their lives.
2. It follows therefore that the larger and older the fish, the greater the parasitic load.
3. The presence of the parasite has no stunting effect upon the growth of the host at any stage.
4. Females are more numerous and appear to attain greater overall length than the males and so acquire more parasites per individual. In addition a greater percentage of the total female population is parasitized than in the case of the males.

5. There is no ^{detectable} seasonal variation in the cycle of Phyllodistomum whether it is during the larval stages or within the definitive host. The hormonal state of the fish of either sex has no effect upon the parasite numbers.

(NB. Van Cleave and Mueller (1932) and Slusarski (1958) reported a similar lack of seasonal variation in the adult P. superbum and P. simile populations).

Sub-section 7C The trematode Phyllodistomum(a) Method of investigation

In Phyllodistomum-infected hosts the parasite was observed in situ through the translucent unopened ureter and bladder walls. In some cases the entire urinary system was fixed in F.A.A. in situ, immediately upon display, then removed and embedded in wax. Longitudinal and transverse sections were cut at 6-8 μ and stained in Mayers' haematoxylin and eosin, Mallory's aniline blue, Best's carmine, Tworts stain and mucicarmine.

Other Phyllodistomes were removed after their exact location had been noted and were examined live. The flukes were fixed by plunging active specimens into F.A.A. solution. This fixation technique was satisfactory as regards all stages of fluke recovered from the definitive host and avoided pressure effects. In toto preparations were stained with aceto-alum carmine and mounted in Canada balsam.

All measurements of living and mounted material were carried out using an ocular micrometer. Relaxant techniques were not utilised when acquiring a collection of mounted specimens. (The effects of these agents are discussed on p.403). The majority of species of Phyllodistomes have been described from material where relaxants were not used prior to fixation and even the use of a standard fixation technique gives highly variable results due to the contractability of the fluke. It was considered that the comparison in this investigation of a variety of accounts where the fixatives were rarely identical would be made easier if, in a large sample of collected specimens, the greatest variation

possible could be achieved whilst retaining a standard technique. It was thus hoped that within one species the total range of normal variation could be recorded and form a basis for establishing a truly representative range for these extensile trematodes. By taking a large enough sample it was also hoped to include the other variables such as crowding effects in different hosts.

Specimens used for measurement were taken from five differing water systems associated with the Thames. They were derived from Gasterosteus aculeatus and in the case of Bushy Park from G.pungitius also. It was possible that more than one species of Phyllodistomum had been encountered during this investigation and it was therefore necessary, in the first instance, to examine the specimens recovered from each area separately. A summary of these results is given on p.213, and the relationships between the various dimensions are shown in more detail in graphs on pp.215-231.

(b) Preliminary results obtained from the examination of area samples

In all the Bushy Park data (pp.215-231) it will be noted that in no case does there appear to be any separation into two divisions, although flukes from both host species are represented. As a result of close comparison of the two sets of figures for flukes recovered from 3- and 8-spined sticklebacks, it was decided to include these as a single species on page 213 because the dimensions of all measurable characters overlapped, and in addition no anatomical differences could be traced.

These results demonstrate the lack of apparent host effect upon the overall measurements attained by the trematodes and lack of host specificity. However, the different fish species could affect the growth or development rate of the flukes to varying extents and this would not be shown by the records.

The results portrayed in the measurements on p. 213 suggest that all the flukes recovered during this investigation belonged to a single species. The largest sample to be measured was obtained from Essex, and this single population exhibited a wide species variation. Practically all the measurements shown on p. 213 for flukes from other areas fall within the Essex range. In a very few specimens obtained from Newdigate, Wanstead and Epping, the posterior region exceeded the value given for Essex specimens and ^{this} slight size difference was reflected (as would be expected) in a greater extreme value for some of the reproductive organs. The overlap of all other measurements between the area samples indicates that this apparent difference in the results is at least partially a contractile effect, and illustrates some of the variations which can be obtained despite the use of a standard fixation technique.

The record for the sucker ratios given either as a mean value or as ranges appears to indicate that there is a considerable variation between the trematode populations of the collection areas. The results, however, merely reflect that the proportions of the three main age groups which constitute each population differ in the samples collected. These age groups (juveniles, trematodes with an intercaecal uterus and

those with an extracaecal uterus) are numerically defined in Section 10 (p. 445), where it can be seen that at each stage the sucker sizes (as measured by their diameters) vary from approximating equality to where the acetabulum exceeds the oral sucker by a maximum $1\frac{1}{2}x$ (in juveniles and young flukes) to more than $2x$ the size (in adults). This range in each age group explains the dispersal shown in graphs BL/S 1-3.

Information forming the basis for the diagnostic description given on pp. 232-245 in this section and the detailed discussion on taxonomy (Section 10, p. 409) was obtained from material examined on an area basis. A generalised account co-relating this data was written after it had been ascertained that, in addition to the measurements, all morphological variations due to age, contraction etc. were identical in all area samples. Larval stages were not available from all collection sources but comparison of such specimens that were recovered did not reveal differences in either development or dimension. It is certain that variations exhibited by a single species have been observed.

SIZE RELATIONSHIPS

The relationship between the total length of the body and the maximum width of the posterior region of Phyllodistomum

Graph B/W (1)

Newdigate records are represented by x

Bushy Park records are represented by ⊙

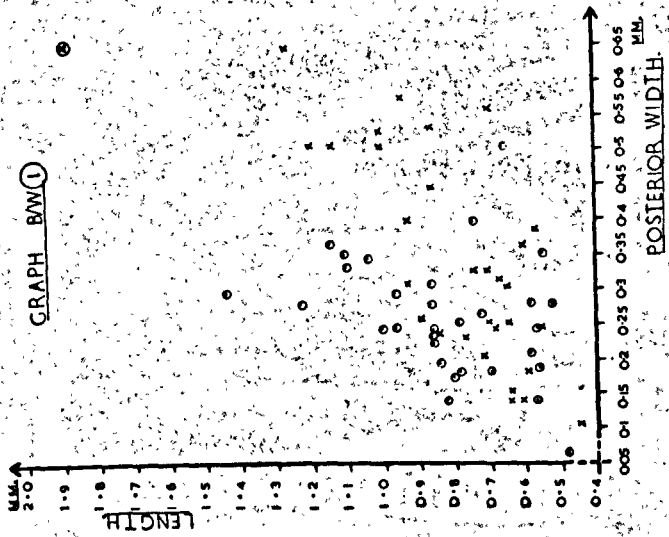
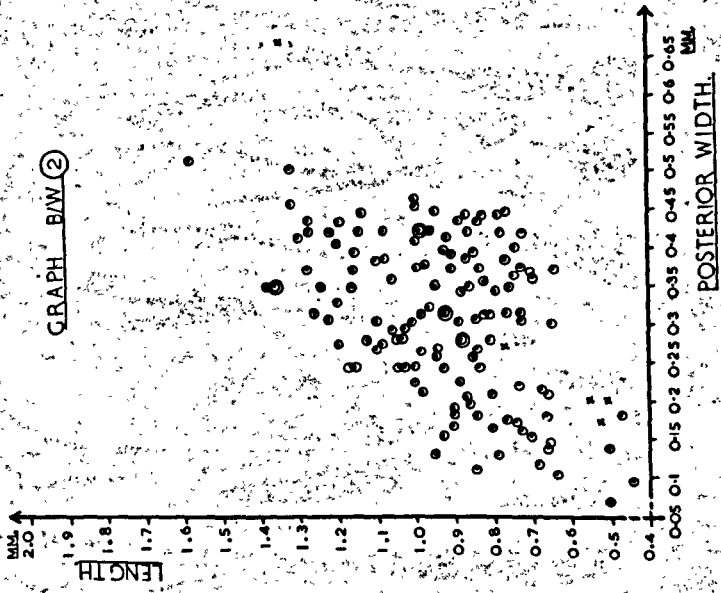
Epping Forest records are represented by ⊗

Graph B/W (2)

Essex records are represented by ⊙

Wanstead records are represented by x

(where the same record occurs more than once, this is signified by a double ring around the relevant symbol)



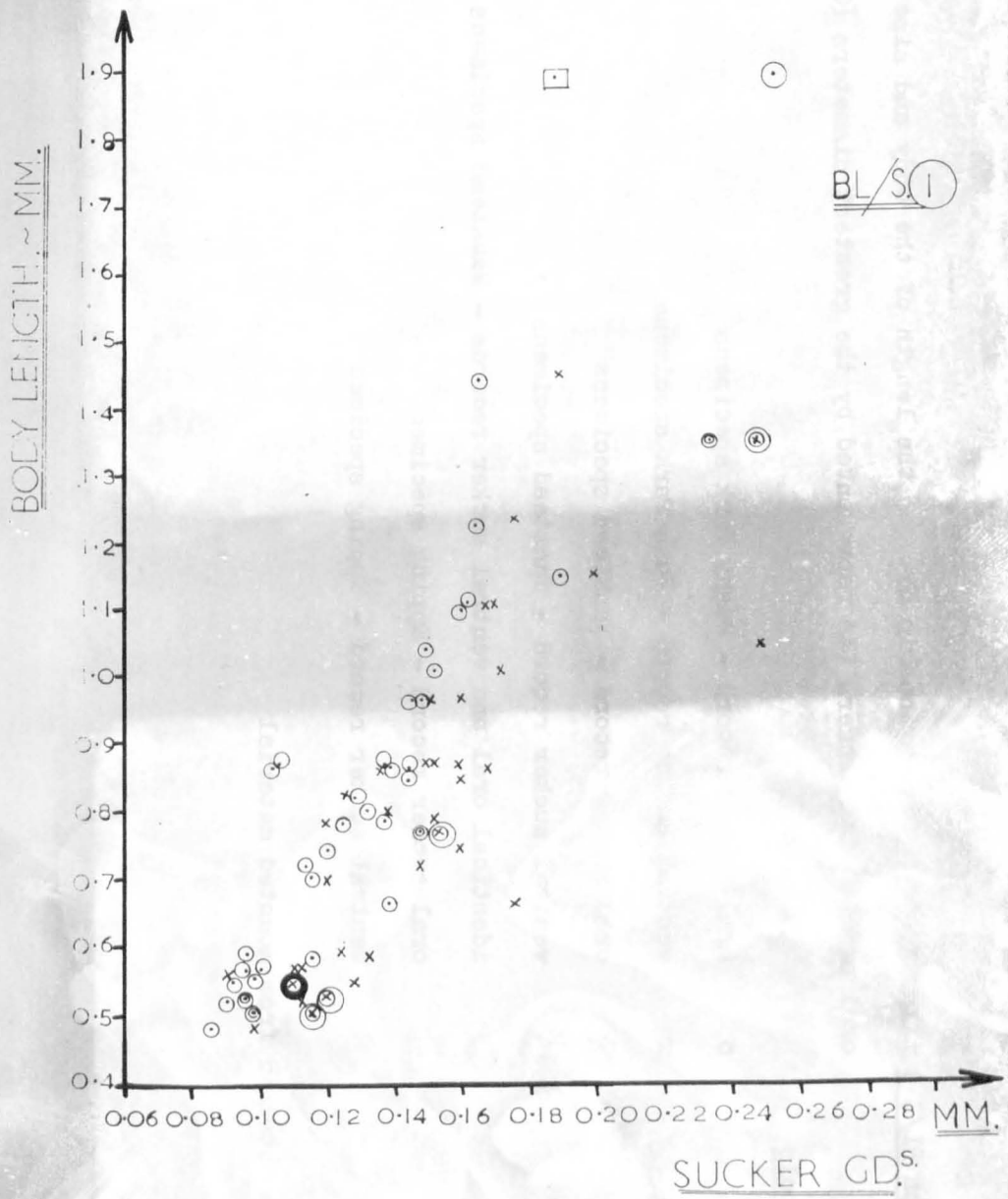
216
15

Graph BL/S 1 illustrates the relationship between the length of the body and size of the oral and ventral suckers (as represented by the greatest diameters [GD'S])

Symbols

- oral sucker record - Bushy Park specimens
- x ventral sucker record - Bushy Park specimens
- ⊙ oral sucker record - Wanstead specimens
- ⊗ ventral sucker record - Wanstead specimens
- ⊗ identical oral and ventral sucker records - Wanstead specimens
- ◻ oral sucker record - Epping specimen
- ventral sucker record - Epping specimen

Data obtained from mounted material



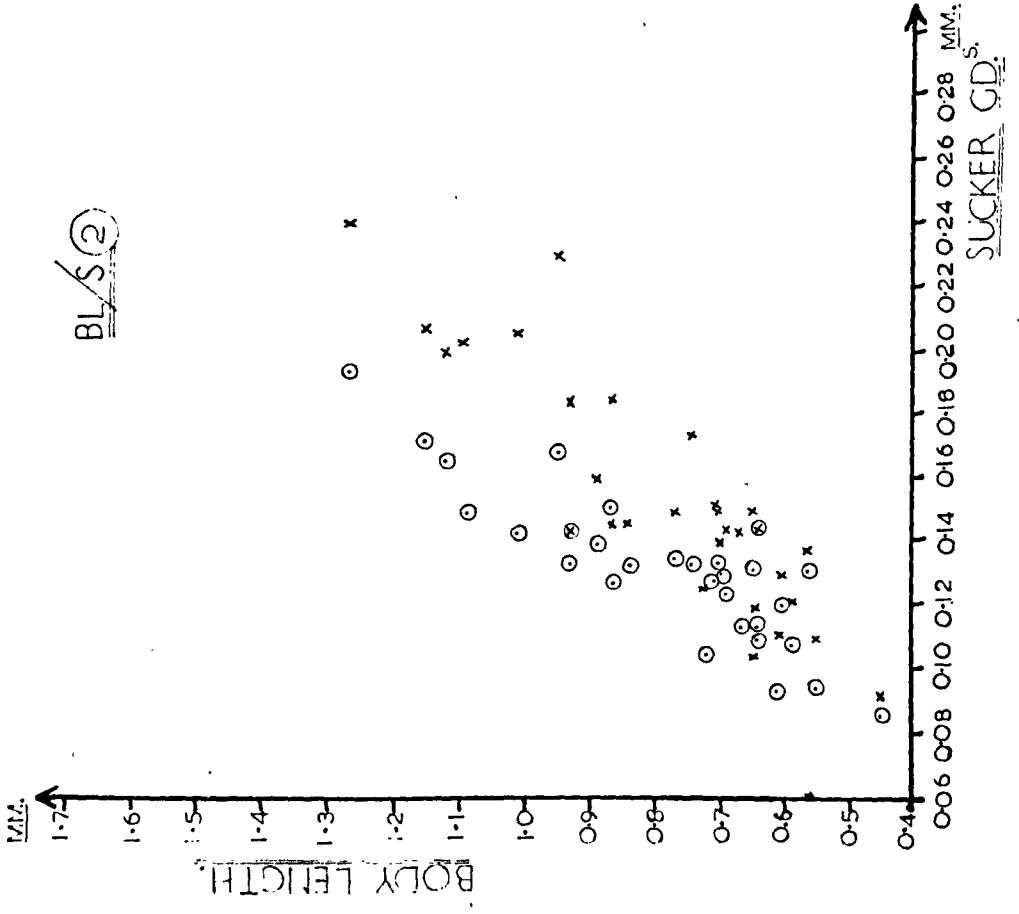
Graph BL/S 2 illustrates the relationship between the length of the body and the sizes of the oral and ventral suckers (as represented by the greatest diameters [GD's])

Symbols

- Newdigate area - oral sucker record
- x Newdigate area - ventral sucker record
- ⊗ identical oral and ventral sucker ratios

Data obtained from mounted material

BL/S ②



720
130

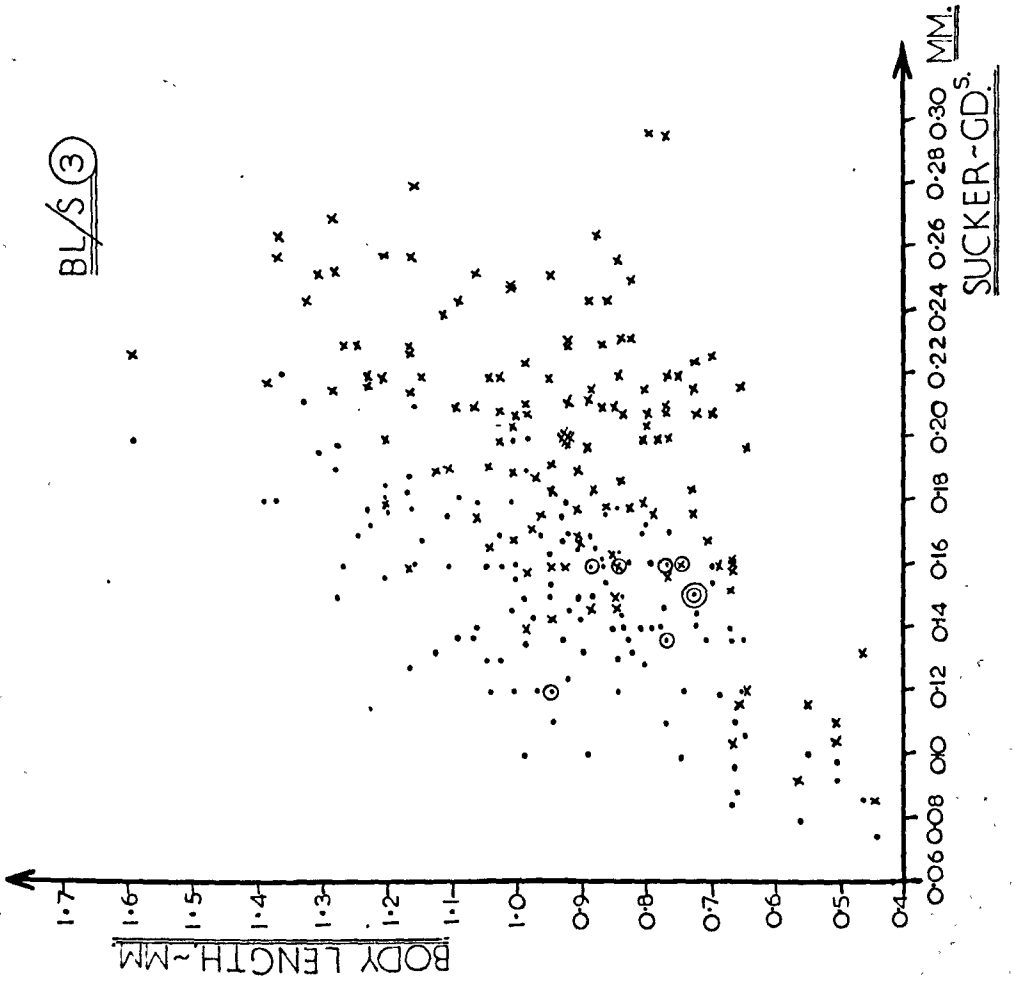
Graph BL/S 3 illustrates the relationship between the body length and the sizes of the oral and ventral suckers (as represented by the greatest diameters [GD's])

Symbols

- oral sucker record - Essex specimen
- x ventral sucker record - Essex specimen
- o oral sucker record appears twice
- ⊙ oral sucker record appears three times
- ⊗ oral and ventral sucker records coincide

Data obtained from mounted material

BL/S ③

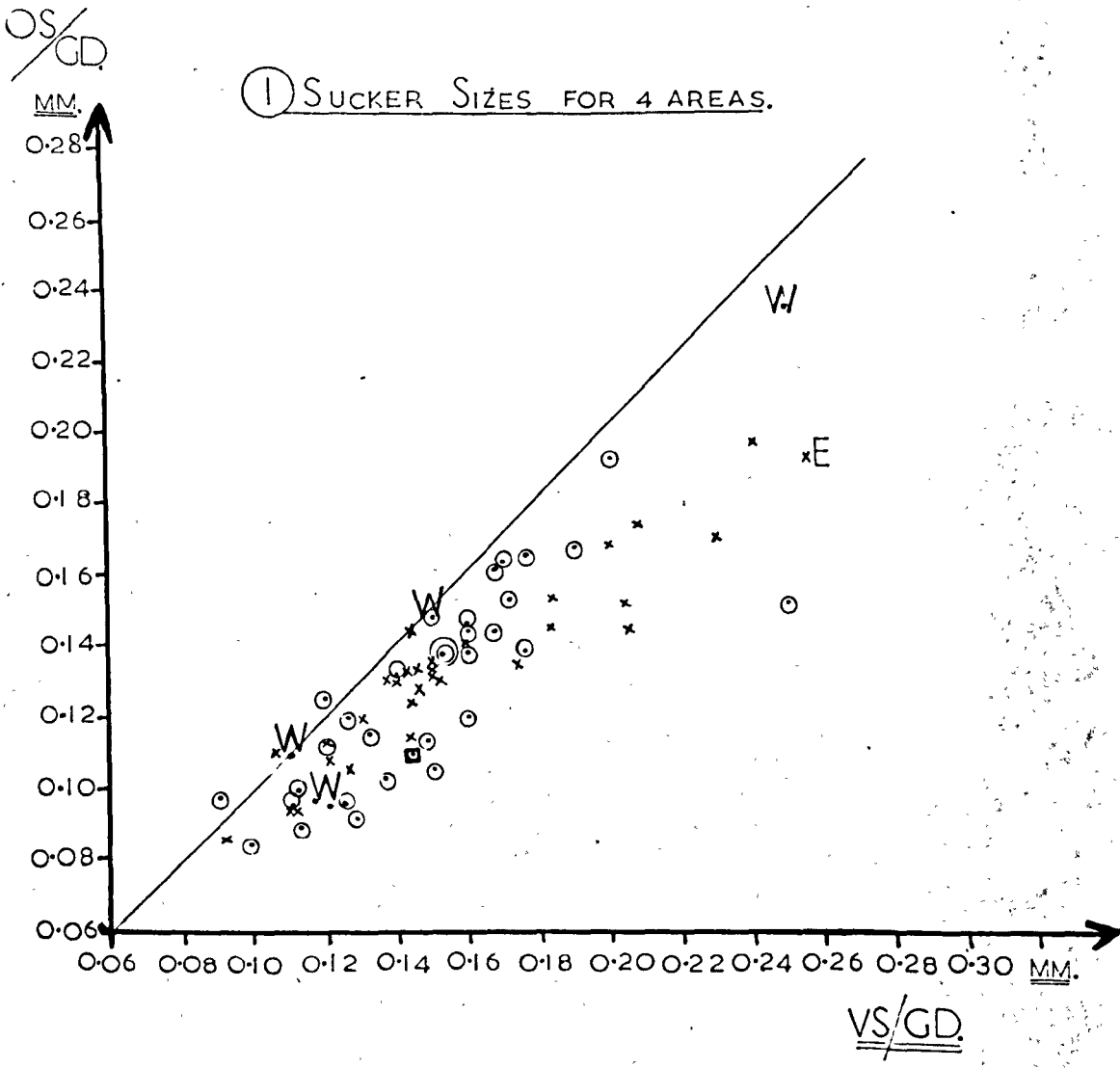


1. Sucker sizes for four areas

The graph illustrates the relationship between the oral and ventral suckers of mounted specimens as represented by their greatest diameters. The diagonal line indicates the point at which the diameters of the two organs are equal.

Abbreviations and symbols

| | |
|--------|--|
| O/S.GD | the oral sucker record-greatest diameter |
| V/S.GD | the ventral sucker record-greatest diameter |
| MM | millimeters |
| ⊙ | Bushy Park record |
| x | Newdigate record |
| ⊗ | Newdigate and Bushy Park records coincide |
| ⊙ | Bushy Park recorded twice |
| W | Wanstead record |
| • | |
| ∩ | Wanstead and Bushy Park records coincide |
| xE | Epping record |
| □ | record for the experimentally established Phyllodistome - 14 days in the definitive host |



2. Sucker sizes - Essex

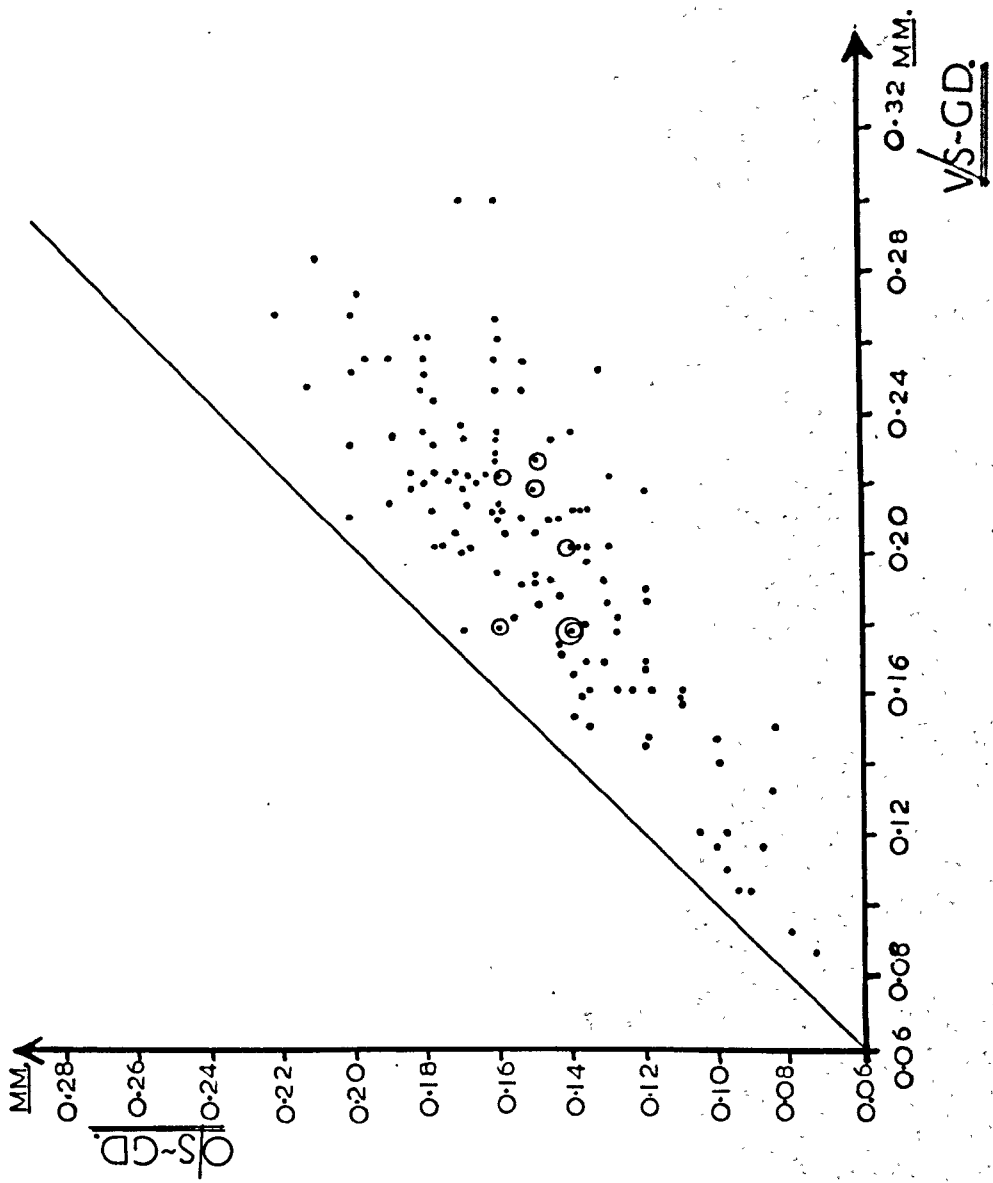
The graph shows the relationship between the oral and ventral suckers of mounted specimens from the Essex area.

Abbreviations and symbols

| | |
|--------|--------------------------------------|
| O/S.GD | the oral sucker-greatest diameter |
| V/S.GD | the ventral sucker-greatest diameter |
| . | a single record |
| o | two identical records |
| ⊙ | three identical records |

The line represents the point of equality for the two organs

② SUCKER SIZES ~ ESSEX.

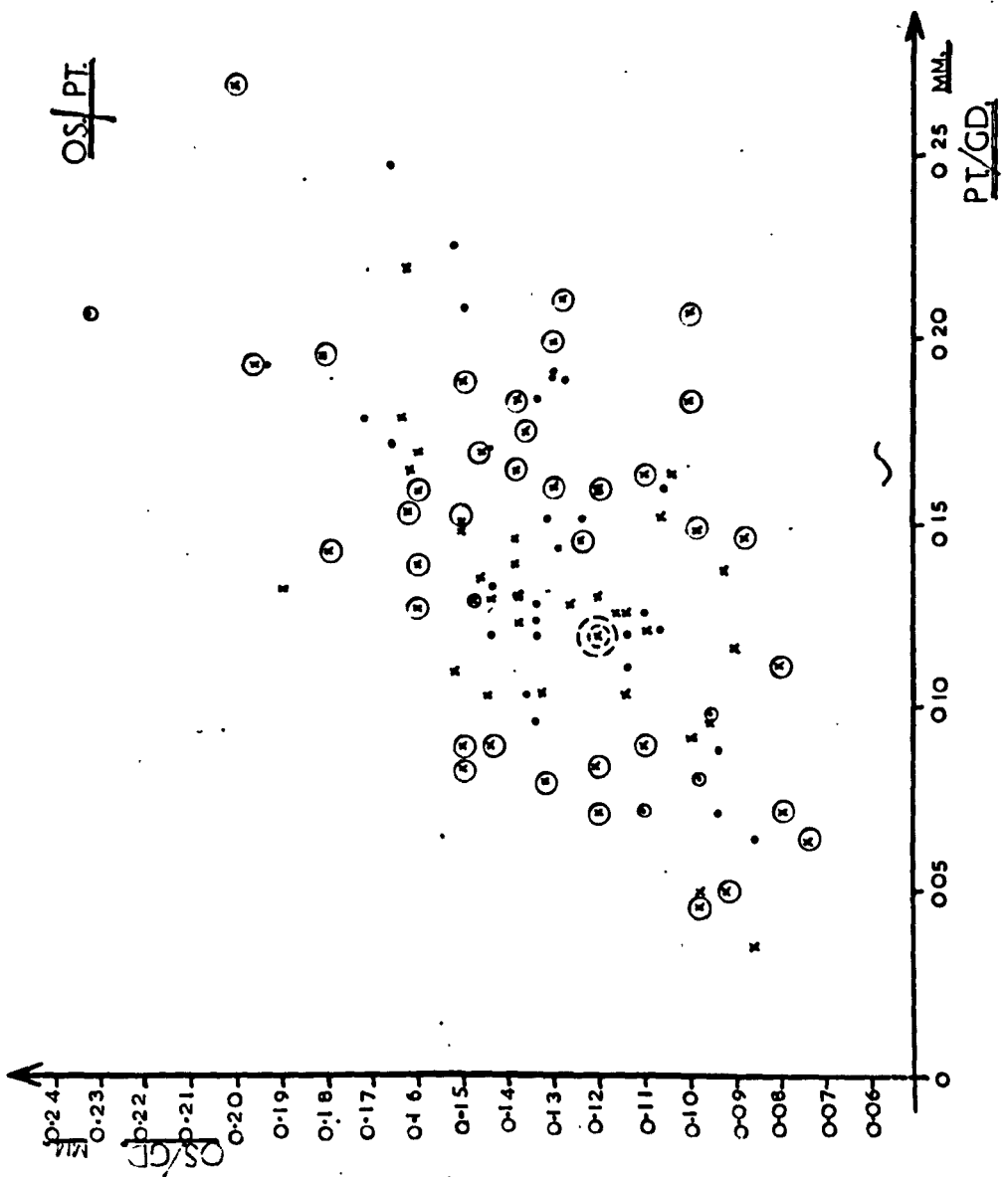


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Graph OS/PT illustrates the relationship between the size of the oral sucker and the posterior testis. The measurements of both organs are based on their respective greatest diameters. The oral sucker was used instead of the body size in order to minimise contractile effects.

Abbreviations and symbols

| | |
|-------|--|
| PT/GD | posterior testis-greatest diameter |
| OS/GD | oral sucker-greatest diameter |
| x | Bushy Park record |
| . | Newdigate record |
| (x) | Bushy Park and a double Essex record |
| (x) | Essex record (40 specimens only) |
| (x) | Newdigate and Essex records coincide |
| o | Wanstead record |
| o | Wanstead and Bushy Park records coincide |



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SIZE RELATIONSHIPS - THE REPRODUCTIVE ORGANS

Graphs 1-3 illustrate the size relationships shown by the main reproductive organs
(ref. p. 213 - mounted material)

Abbreviations and symbols

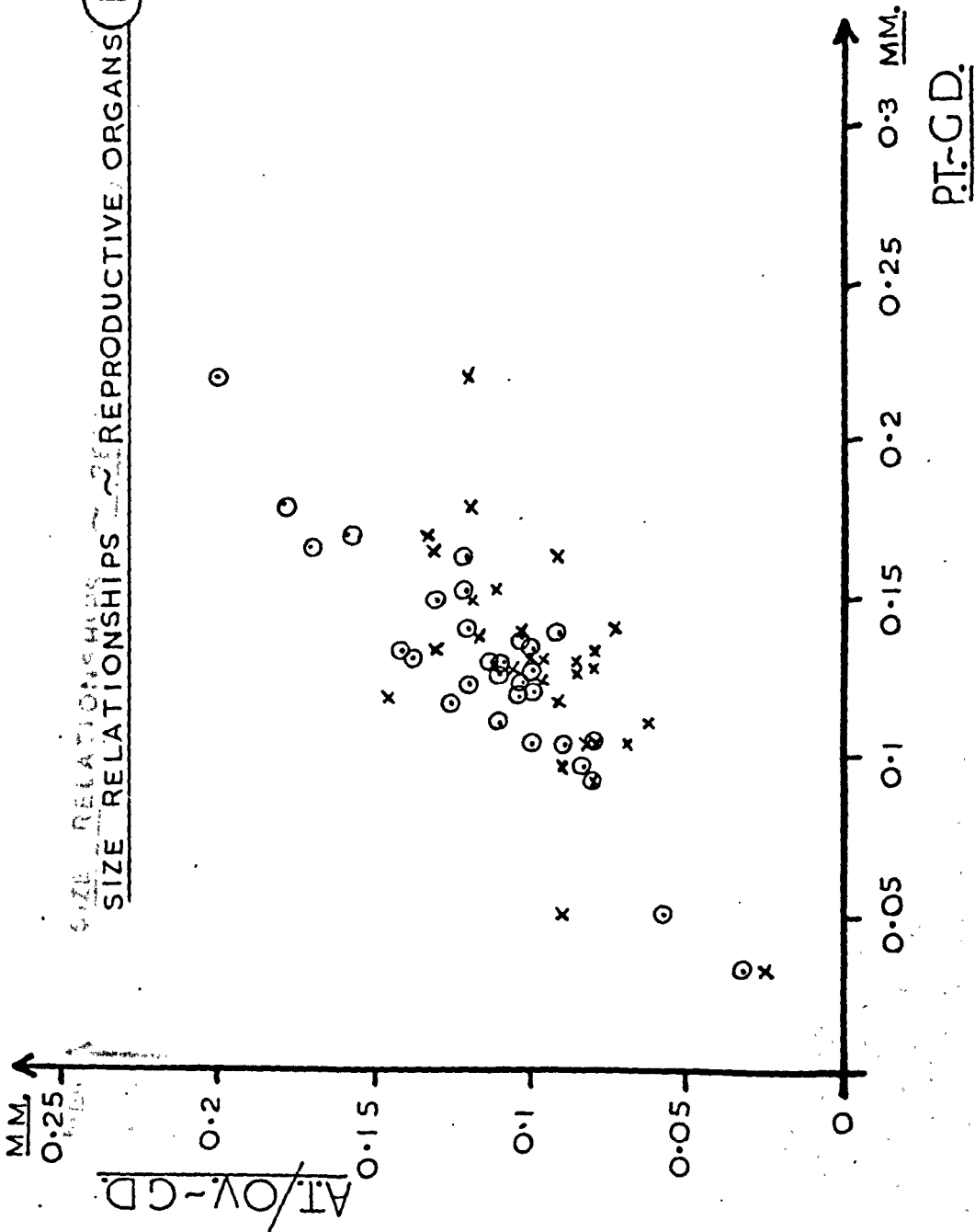
AT anterior testis
OV ovary
PT posterior testis
GD greatest diameter of the organ
MM millimetre

| | | | |
|--------------------------------|--------|------------|--|
| Graph 1 | Area 1 | Bushy Park | anterior testis record represented by ⊙ ovarian record represented by x |
| Graph 3 (50 specimens only) | Area 2 | Essex | anterior testis record represented by ⊙ ovarian record represented by x |
| Graph 2 | Area 3 | Wanstead | anterior testis record represented by Δ ovarian record represented by ∇ |
| | Area 4 | Newdigate | anterior testis record represented by ⊙ ovarian record represented by x |
| | Area 5 | Epping | anterior testis record represented by ⊙ ovarian record represented by ⊗ |

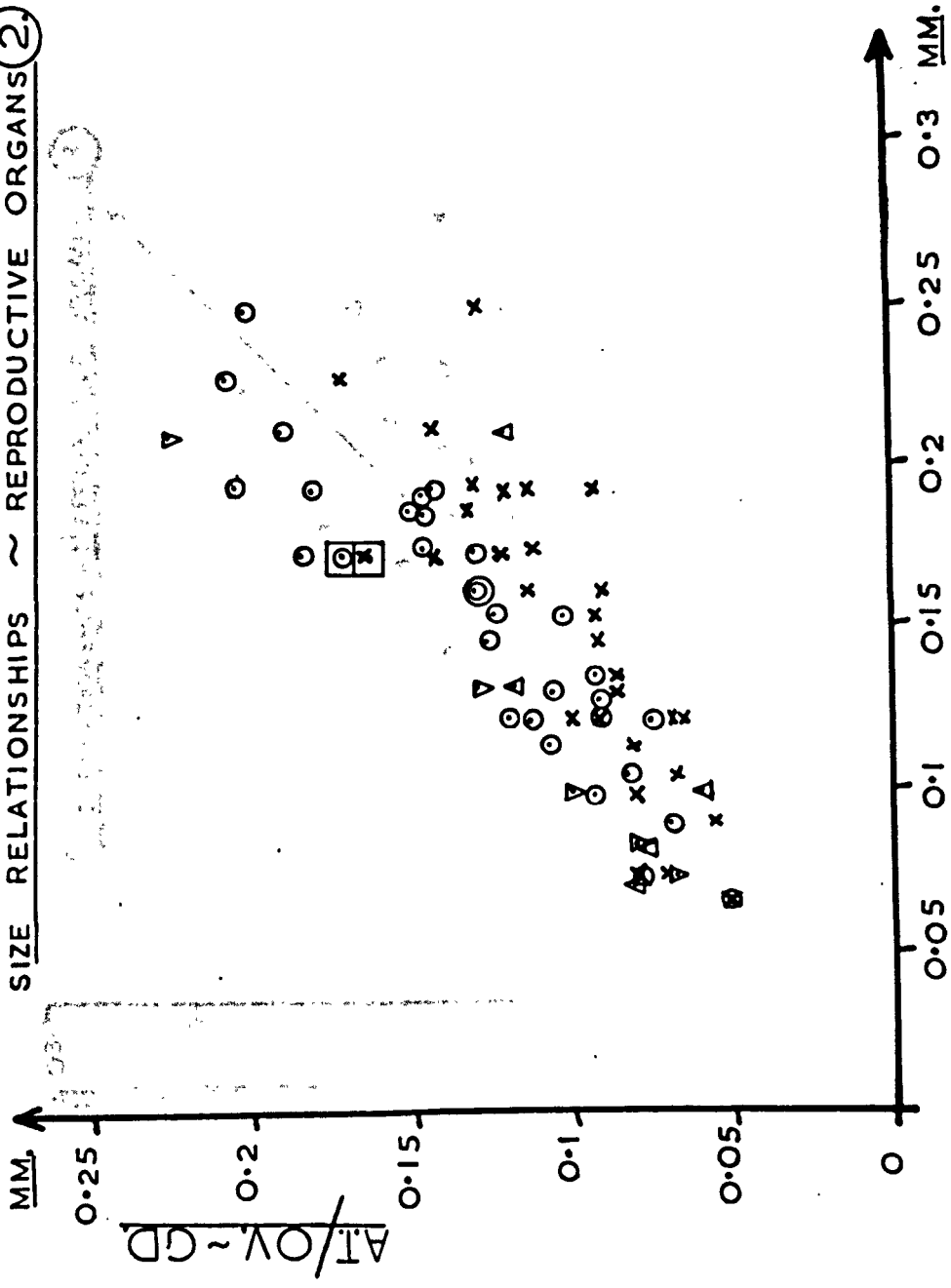
⊙ indicates that the records for the anterior testis were duplicated

⊗ indicates that the records for the ovary and anterior testis coincided

SIZE RELATIONSHIPS BETWEEN REPRODUCTIVE ORGANS (1)

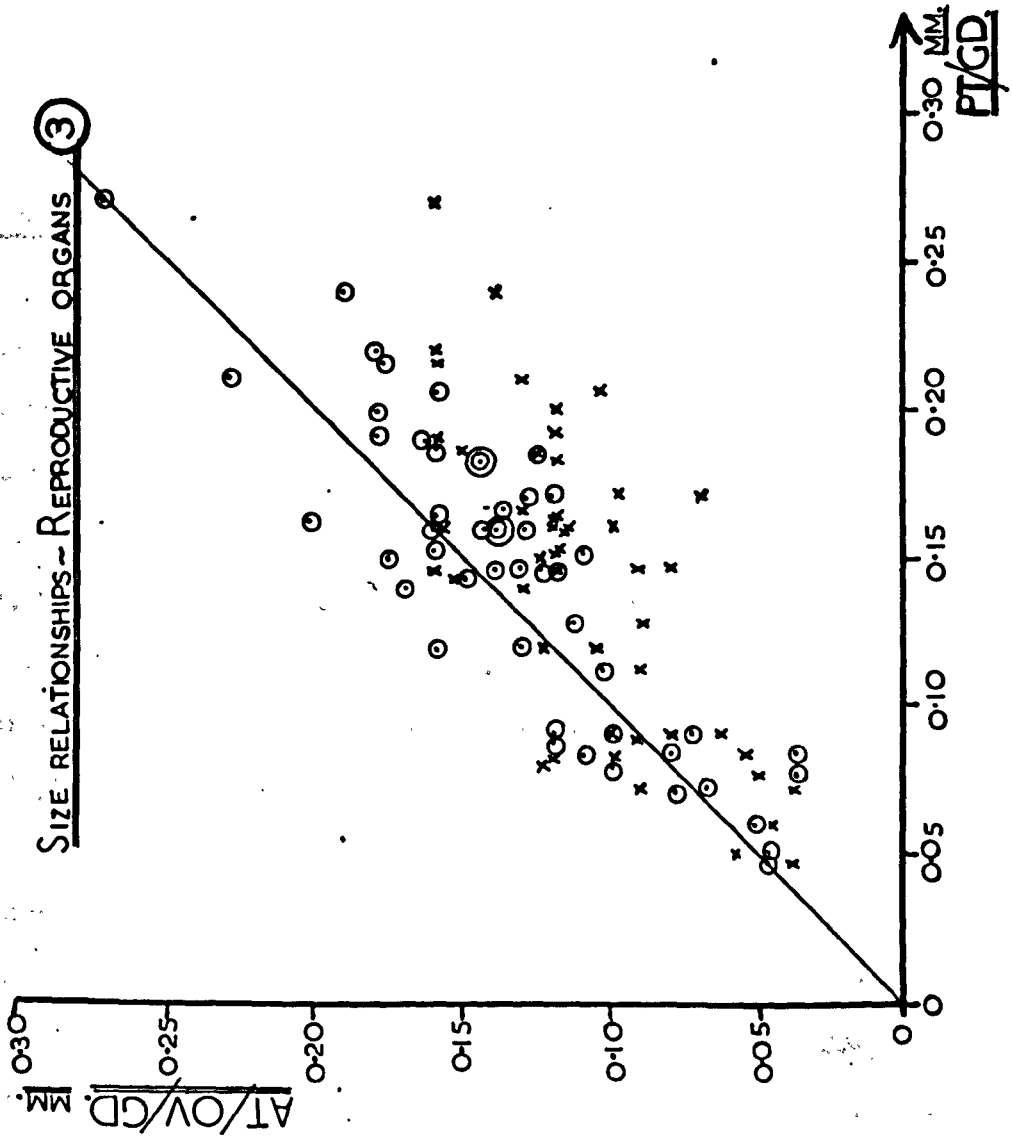


SIZE RELATIONSHIPS ~ REPRODUCTIVE ORGANS (2)



P.T. ~ GD.

SIZE RELATIONSHIPS ~ REPRODUCTIVE ORGANS ③



C) Detailed Description of the Trematode in the Definitive Host(Ref: Numerical Analysis)

- | | |
|---|--------------|
| (1) Area collections - | page 213 |
| (2) All stages of the Life Cycle on a comparative basis with <u>Gorgodera</u> - | page 399-401 |
| (3) Mounted collection compared with <u>P. simile</u> - | page 443 |
| (4) Age groups - the diagnostic features - | page 445 |

Body form: A sub-cylindrical narrow forebody extends anterior to the acetabulum while behind the ventral sucker the body expands to form a dorso-ventrally flattened foliate region. In juvenile specimens the width of the latter approximates that of the forebody but in older specimens it is transversely expanded to accommodate the reproductive organs and eggs. The outline varies according to the contractile phase, both regions ranging from a narrow elongated form to an almost ovoid disc particularly posteriorly. The posterior notch to which former authors have attached some importance can be present or absent in all phases of movement. Its appearance is dependent solely upon the contraction or relaxation of the sphinctoral muscles surrounding the excretory pore together with the body musculature of the area. The movement of the two regions is largely independent and is discussed in detail under the section on behaviour (page 266).

Flukes recovered from the definitive host ranged from a size overlapping that of the metacercaria to a maximum of 3.15 mm. in the live state. The largest mounted fluke from the collection of 200 measured 1.890 mm. All subsequent measurements will refer to this

collection. The trematodes appear to mature when reaching a size between 0.500 and 0.800 mm in length. The area of maximum width lies in the posterior region but its exact position varies according to the contractile phase between the ovarian level and that of the two testes. The dimensions of the two regions vary with age and the degree of contraction. The ratio of the anterior to the posterior width ranging from 1:0.80 - 1:3.70 over 200 mounted specimens illustrates that the posterior region increases progressively in width (see graphs B/W (1)(2), page 215) paralleling the development of the reproductive organs and more especially that of the uterus. The regional length ratio similarly increases with age but is subject to great variation due to contraction.

Suckers. The oral sucker is a muscular terminal organ surrounding the ventral mouth. Basically an elongated structure it can vary from a globular to an ovoid shape during contractions which are usually associated with body movement.

The acetabulum also exhibits a series of shape changes. When attached to the substratum it flattens and its diameters increase. Upon release the sucker thickens dorso-ventrally and the diameters are consequently shortened. In volume or area the oral sucker is always smaller than the acetabulum in individuals recovered from the definitive host. The accuracy of results obtained from the classical method of comparing sucker sizes by measuring only one diameter, either parallel to the body axis or the organ, is debatable. Instances in graphs 1 and 2 (pages 223 and 225) where the two suckers

appear equal or where the anterior organ exceeds acetabular size indicate that a single diameter measurement can give inaccurate results during certain phases of contraction. The marked difference between ratios obtained for a comparison of the suckers' greatest diameters and their width reflects the usual pronounced elongation of the oral sucker in mounted specimens. The average sucker ratios obtained for the larval stages and the total trematode population taken from the definitive host (pages 399-401) indicate that the acetabulum increases in size at a faster rate than the oral sucker and that the initial difference found in the cercarial stage is not only maintained but accentuated.

Cuticle. The ridged cuticle is completely devoid of armature and differs in no way optically from the thin, transparent layer formed at the cercarial stage. The sucker cavities lack spines but in a few cases what appeared to represent such protuberances were noted in living specimens. Subsequent examination of sectioned material revealed nothing and it is possible that the structures observed were merely areas of compacted cuticular ridging associated with localised contraction.

Papillary structure. The presence of cuticular papillae are a noticeable feature of this trematode species. Their structure is illustrated on page 236. All the papillae examined lacked a protruding bristle of the type described by Sturges (1897) for P.(D) patellare.

They are rounded permanent structures covered by a fine cuticular extension which can be lifted off the papilla under adverse osmotic

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A DIAGRAMMATIC REPRESENTATION OF PAPILLARY STRUCTURE

Each diagram is a drawing of the papillae as seen in mounted sections and examined under an oil immersion lens.

Diagram A:

An extended marginal papilla of a young fluke.

Diagram B:

A contracted marginal papilla of an older fluke.

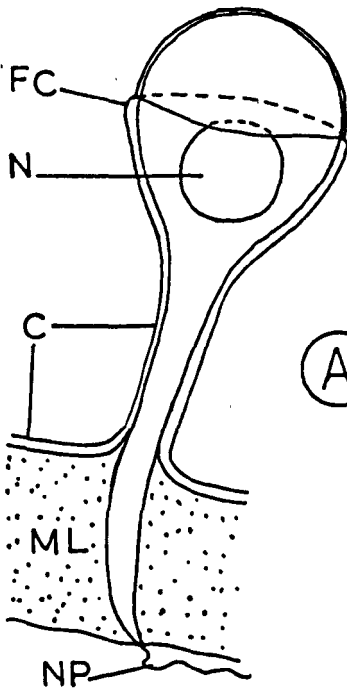
Diagram C:

A section of the acetabular rim showing a double papilla.

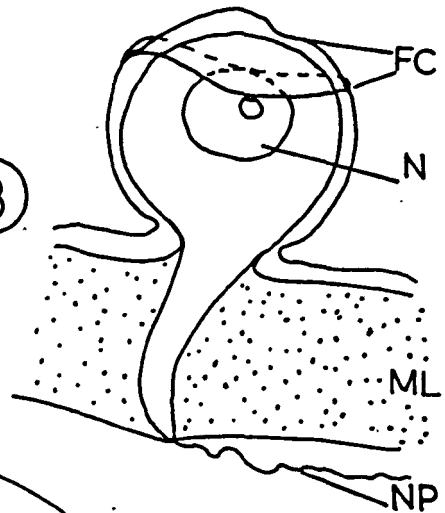
Abbreviations utilised in diagrams A - C:

- C = Cuticle
- FC = Folded cuticle
- ML = Muscle layers
- N = Nucleus
- NP = Nerve process
- RC = Ridged cuticle - this is a continuation of the ridging found over the general body surface

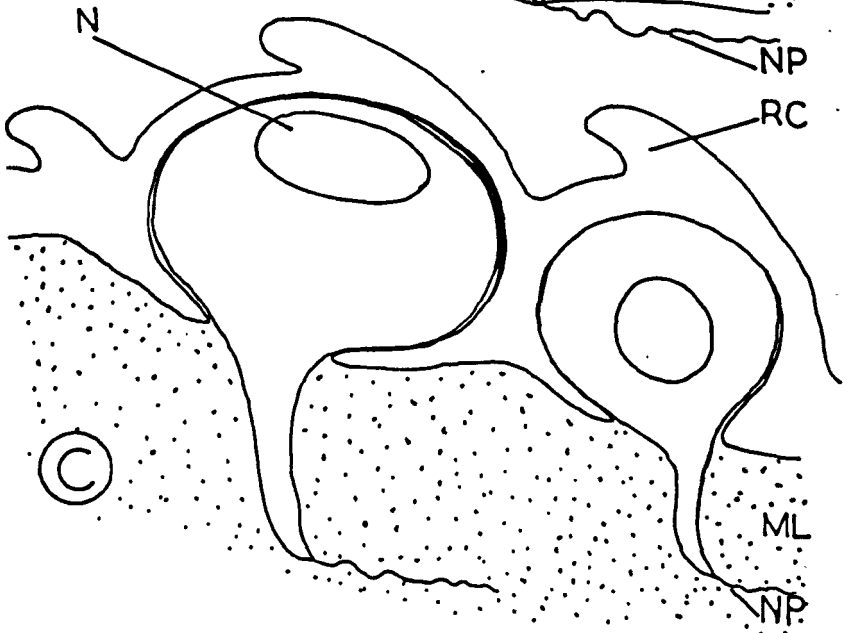
PHYLLODISTOMUM-
PAPILLAE.



(A)



(B)



(C)

conditions. The bulge of cuticle so obtained retains the imprint of the papillary pattern.

Papillary pattern. The adult fluke is covered with numerous papillae which are arranged in a characteristic pattern retained without alteration throughout the development of the trematode from the cercarial state. The arrangement of these papillae is shown in the diagrams on pages 239 and 241. For descriptive purposes they can be divided into three principal series, (1) the marginals, (2) the dorsal and (3) the ventral systems.

Marginal system - The most noticeable papillae are those situated upon the extreme body margins. These are arranged in a single row from the anterior to the posterior end. 4 papillae on either side of the oral sucker are followed by 4 lying opposite one another on either side of the 'neck' region, the last positioned anterior to the ventral sucker. Opposite the ventral sucker are 2 papillae on each side of the body followed by 8 pairs in the posterior region, 8 on each side extending from the ventral sucker to the excretory pore position. (Formula A - P = 4+4+2+8 per side.)

Dorsal system - Over the dorsal surface of the oral sucker are 4 papillae. The first pair lie opposite the second pair of marginal papillae whilst the second pair lie between the 3rd and 4th marginals. In the 'neck' region the most anterior dorsal pair of papillae also alternate with the marginals but the remaining 3 pairs are more or less opposite the latter. Situated on either side of the mid line in the anterior region are 3 pairs of double papillae which alternate with the marginals.

HYLLODISTOMUM - ARRANGEMENT OF THE PAPILLAE
ON THE DORSAL AND VENTRAL SURFACES

The two drawings represent the adult phase, but identical patterns are found on both the cercarial and metacercarial stages.

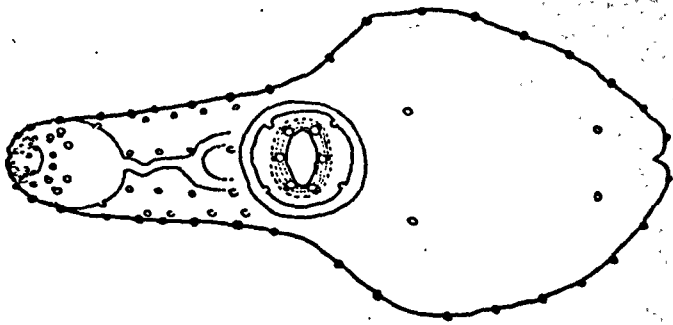
In both diagrams:

Marginal papillae - are drawn as solid black structures.

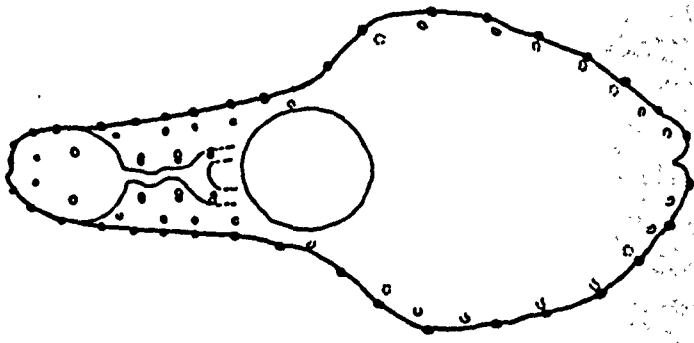
Dorsal }
Ventral } papillae - are drawn in outline only.

Additional papillae - (sporadic occurrence) are drawn with a broken outline.

(Information for these diagrams was obtained from the observation of numerous living specimens. The diagrams are not drawn to scale.)



PHYLLODISTOMIUM ~ VENTRAL SURFACE
PAPILLARY ARRANGEMENT.



PHYLLODISTOMIUM ~ DORSAL
PAPILLARY ARRANGEMENT.

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DIAGRAMMATIC REPRESENTATION OF THE VENTRAL SUCKER
PAPILLARY PATTERNS

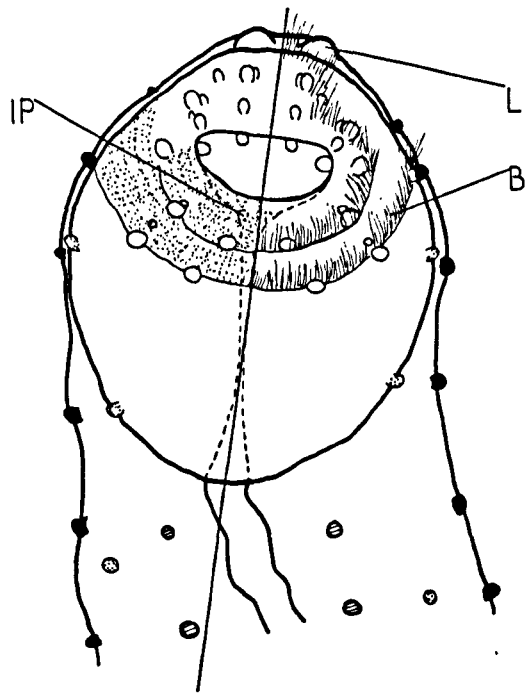
In both figures the side to the left of the dividing line represents the condition found in Phyllodistomum. The right-hand side of each diagram represents the condition found in Gorgodera. It will be noted that the two are identical except for the presence of minute bristles in Gorgodera.

In the oral sucker diagram:

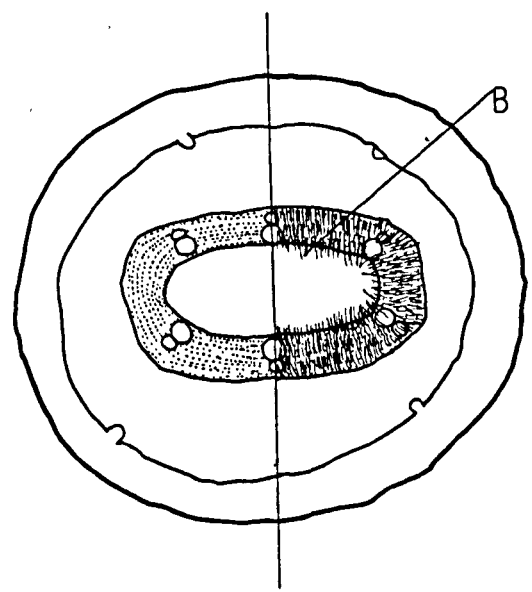
- Marginal papillae - are drawn as solid black structures.
- Outer ventral row - are stippled.
- Inner ventral row - are cross-hatched.

Abbreviations:

- L = dorsal lip
- B = bristle or spine
- IP = inner papillae (can be protruded forwards)



ORAL SUCKER~VENTRAL SURFACE



VENTRAL SUCKER~VENTRAL SURFACE.

PAPILLARY ARRANGEMENT.

Opposite the ventral sucker on each side lies one dorsal papilla alternating with the marginals. Posteriorly there are always 6 (sometimes 8) papillae on each side distributed towards the dorsal margin and occupying various positions in relation to the extreme marginals. This dorsal series of papillae found throughout the anterior and posterior regions of the body are difficult to distinguish from the true marginals during movement. (Formula $A - P = 2 + \frac{4}{3D} + 1 + (6-8)$ per side (D signifies a double papilla)).

Ventral system - As in the dorsal system there are two rows of papillae anteriorly, one lying close to the body margin and one row occupying a more median position. Overlying the oral sucker on each side are two papillae of the more marginal ventral series (stippled in the diagram, page 241). Behind these are 4 papillae roughly alternating with the true marginals on either side. Medially 4 pairs of single papillae alternate with the marginal ventral series.

In the posterior region there are only 4 papillae; one pair immediately behind the ventral sucker and the second situated more posteriorly in the region of the third marginal papillae from the posterior end. These are completely distinct from the anterior ventrals, are slightly more median and cannot be confused with the true marginals. (Formula $A - P = 2 + \frac{4}{4} + 2$ per side.)

The ventral oral sucker pattern is highly complex. Surrounding the mouth are 4 papillae plus 2 (labelled 'IP' in the diagram on page 241), which are deeper set and lie within the buccal cavity, but which are protruded during feeding and other movements to the level of those

surrounding the mouth rim. Posterior to the mouth are 2 rows of papillae arranged in two crescents, the ends of which terminate in the first and second anterior marginals. The position of these two rows is marked by a ridge in the surface of the oral sucker. Both rows consist of a double papillae on each side and two single papillae medially. Anteriorly also there are two rows of papillae involving double and single forms, but there is no ridging. 2 single papillae of the inner row on each side are exactly opposite the double papillae of the outer row, the latter consisting of 4 papillae on each side. This outer row consists of 2 single papillae alternating with 2 double structures on each side. Protruding anteriorly, beyond all the papillate structures, are two muscular lips. Formula = total papillae present = 26.

$$\text{Total A - P} = 8+4+6+4+4. \quad (\text{One side} = 4+2+3+2+2 = \frac{2}{2} + 2+3 + \frac{1}{1D} + \frac{1}{1D})$$

(diagram, page 241)

Ventral sucker papillary arrangement is much simpler and consists merely of two rows. An inner row of double papillae arranged around the rim of the sucker cavity. These 6 structures are so arranged in a ridged muscular region that 2 would be bisected by a median anterior-posterior dividing line. On either side of such a line are 2 single papillae situated on a muscular ridge within the sucker cavity.

Total formula = 6D + 4. (Diagram page 241)

The excretory system. The arrangement of the excretory tubules and the numbers of flame cells remain constant from the cercarial stage to the adult. The system is illustrated on page 245.

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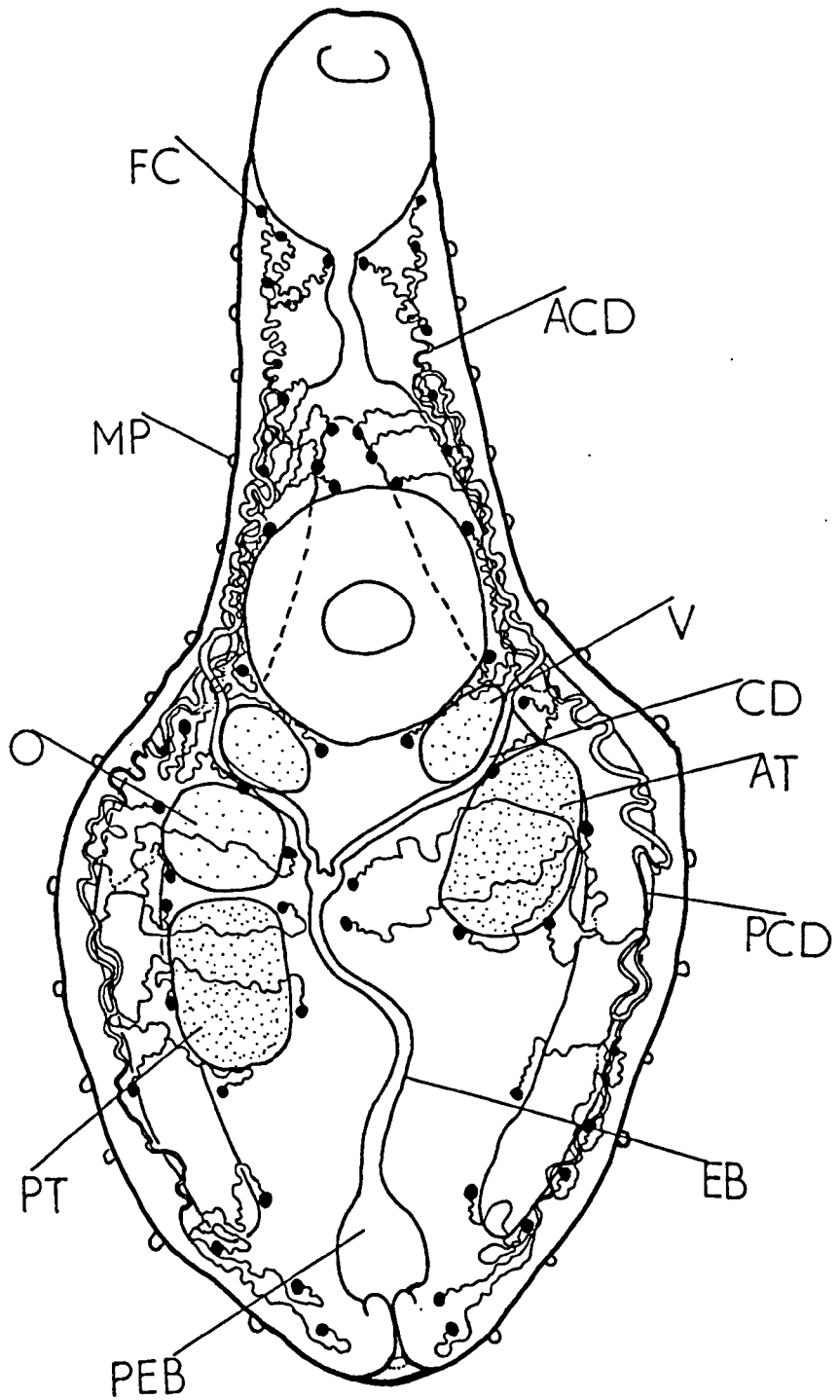
DIAGRAMMATIC REPRESENTATION OF THE EXCRETORY SYSTEM
OF HYLLODISTOMUM

The diagram represents the adult phase, but the system is identical to that found in the cercaria and metacercaria.

Abbreviations:

- FC = flame cell
- ACD = first accessory collecting duct
- MP = marginal papilla
- V = vitelline gland
- O = ovary
- CD = common collecting duct
- AT = anterior testis
- PCD = posterior region of main collecting duct
- PT = posterior testis
- EB = excretory bladder
- PEB = region of the posterior expansion
of the excretory bladder

(Information for this diagram was obtained from the examination of numerous living specimens placed into dilute horse serum and stained with neutral red.)



PHYLLODISTOMUM ~

EXCRETORY SYSTEM.

From the anterior end of the gently curved excretory bladder arise two main collecting ducts. These continue anteriorly, passing generally behind both vitellaria but occasionally one duct may cross a vitellarium or the ovary or anterior testis of one side. The two ducts continue forwards following a tortuous path on either side of the ventral sucker until they reach the gut bifurcation. Here they each give rise to one accessory branch passing forwards and the larger duct coils back in close proximity to the ascending common branch. When the posterior edge of the ventral sucker is reached, the posterior main collecting ducts continue along the outer edges of the gut caecae to terminate in the region of the blind caecal endings. The flame cell pattern on each side of the body is $(4+5)+(5+5+4+4) = 27$. The total number of flame cells present equals 54.

The degree of coiling of the main tubules varies according to the contractile phase of movement. The excessive coiling anteriorly reflects the extensile nature of this region. The position of the flame cells varies but slightly from individual to individual.

The excretory bladder extends anteriorly to a point level with the ovary and anterior testis, giving rise to a small protrusion at its extreme anterior end beyond the point at which the two collecting ducts arise. (The point of union between the bladder and common collecting ducts is identical in form to that described for P. lohrenzi, Catoptroides lacustri and Gorgoderina amplicava by Byrd et al (1940) but distinct from the shape assumed by Gorgoderina tanneri.) The ducts tend to curve slightly before passing forwards. This characteristic

arrangement (depicted on page 245) can be changed during extensile movements when the two ducts are drawn sharply forwards. Posteriorly the excretory bladder is rhythmically expanded by an increase in fluid content which subsides upon relaxation of the rosette-like sphincter muscle guarding the short duct leading to the subterminal dorsal excretory pore. The bladder, in specimens from the definitive host, occupies approximately the same percentage in length of the posterior region as in the metacercaria but is proportionally narrower (see page 400 and page 401). This is mainly associated with the increase in width of the flukes' body paralleling the development of the reproductive system and particularly the uterus. However, the actual bladder measurement is considerably narrower than the equivalent structure in the larval phase reflecting the complete loss of cystogenous material from the bladder cells of juveniles and adults.

The Gut and Digestion. The mouth is situated ventrally and opens into a short oesophagus. This region is highly muscular and is bounded by a noticeable inner sheath of circular muscles and a longitudinal set forming a thin outer layer which is progressively depleted as the bifurcation is approached. This is the only markedly muscular area in the digestive system as the simple dilatable caecae are poorly served. The caecal walls are lined by a single layer of flattened or almost columnar cells whose shape depends not only upon the phase of body and caecal contraction but in addition to the stage of digestion. The cells ingest particles in an amoeboid fashion by protruding one or two lobose extensions per cell out into the gut cavity. These supple extensions are washed back and forth in the contained caecal fluid

during rhythmic gut contractions. A similar phenomenon was noted by Looss (1894) for D. cygnoides.

In sections of flukes killed in situ the processes of digestion were further studied and the gut cells could be seen engulfing multiplicative stages of Myxosporidia and transitional epithelial material of the host system. One to two epithelial nuclei and cytoplasmic fragments can be ingested at one time. Surrounding the cell protrusions and lining the gut is what appears to be a mucoid layer containing numerous small particles. Many of these were also found floating freely within the gut cavity. The sticky secretion along the gut lining probably serves to entangle a quantity of cellular debris and, if enzymes are present in this layer, may represent the site for extra-cellular digestion. Undigested material is voided via the mouth with the additional assistance of the body musculature.

The oesophagus is short and in relaxed specimens is slightly sinuous in nature. It is, however, readily converted into a straight channel by body extensions. In cases of extreme contraction, the oesophageal longitudinal musculature can reduce the length of the tube without bending it aided by the compensatory expansion of the region of the caecae which form the bifurcation where the walls at such times become crenate in outline. Under these circumstances the body wall accommodates by becoming serially folded over the ventral sucker. The oesophagus and the anterior region is so extensile that the gut bifurcation may appear to lie from mid-way between the two suckers to a point definitely nearer the ventral sucker. Similarly, the distance

from which the caecae terminate from the posterior extremity varies according to the phase of contraction and more particularly with the presence or absence of the posterior notch. The caecae generally terminate anterior to the excretory sphincter however. Following a slightly sinuous course laterally down the body, they are normally equal in length. There is little variation within the species concerning this character, although the two caecae may differ very slightly in length from one another. In one out of two hundred individuals this difference was so pronounced that one branch was of normal length whilst the second terminated anterior to both testes. The position of the testes in the posterior region is not constant and their relationship to the point at which the caecae terminate is consequently highly variable. The only generalisation which can be made is that the gut either terminates close to the hind margin of the posterior testis or may extend for varying distances beyond this reproductive zone.

Penetration glands. These glands are still visible in both the juveniles and young laying adults. In some juveniles (see page 251) including the 14 day-old fluke established experimentally in the definitive host the glands were situated in an inter-caecal position whilst in other juveniles they were extra-caecal and situated around the gut bifurcation. The total length of the glands and their ducts differs from that of the anterior region in juveniles by a greater amount than in the case of the cercaria or metacercaria (0.042 - 0.048 mm). The penetration complex itself, however, is longer than

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PHYLLODISTOMUM JUVENILE TAKEN FROM THE
BLADDER OF GASTEROSTEUS ACULEATUS

Detailed diagram of the ventral view of a
mounted specimen drawn with the aid of a projector

Abbreviations:

PA = Papilla

CS = Oral sucker

CG = 'Cephalic cells'

PD = Ducts of the penetration glands

OE = Oesophagus

GP = Gonopore

PG = Penetration glands

VES = Vesicular seminalis

VD = Vas deferens

VE = Vasa efferentia

VS = Ventral sucker

V = Vitelline gland

OO = Ootype

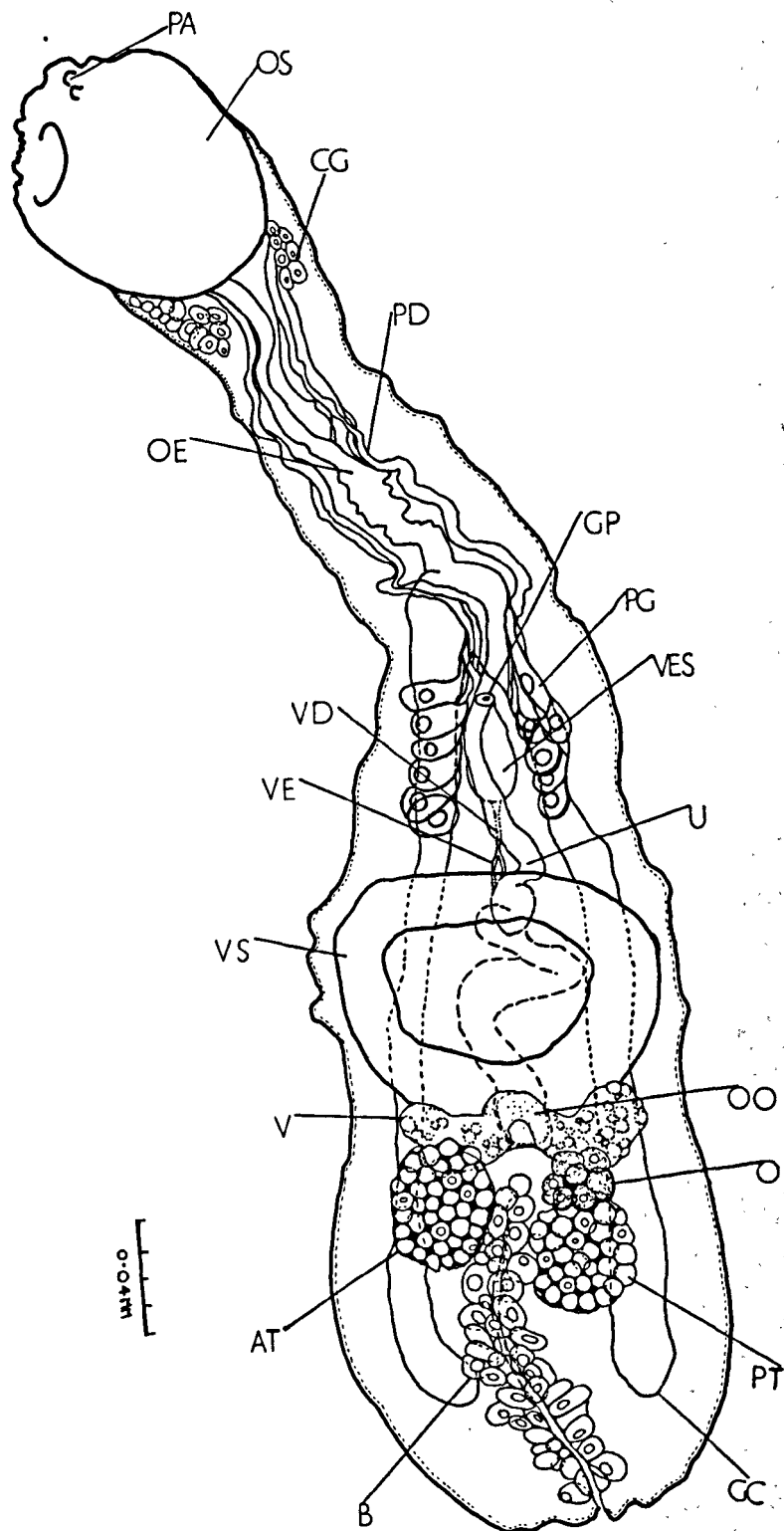
AT = Anterior testis

O = Ovary

PT = Posterior testis

B = Excretory bladder

GC = Termination of the gut caecum



PHYLLOSTOMUM - JUVENILE.

those found in larval stages, and as the duct system retains its characteristic coiled nature and is not straightened in any way, it follows that the glands, although non-functional, continue to grow for some time. In adults with few eggs and a little developed uterus, the gland position varies from the region of the gut bifurcation to a point anterior to the nerve ganglion. The difference between the length of the anterior region and that of the glands plus their ducts, when occupying the latter anterior position, ranged between 0.11 mm - 0.15 mm. Growth in the shortened complex had obviously ceased and the eosinophillic staining of the nuclei indicated that degenerative changes were taking place. The glands disappear completely in older, well developed adults but the ducts remain visible for a much longer period, retaining their coiled state throughout. Similar traces of this complex were noted by Steelman (1938) for the adult P. caudatum but Lynch (1936) described apparently functional glands in the mature P. singlare.

Nervous system. This system is identical to that found in the cercarial and metacercarial stages. The anterior cerebral ganglia, linked by a transverse commissure, represents a concentration of small cells which appear rounded in cross section. Their nuclei are small so that under low power the ganglia appear as predominantly eosinophillic structures. Processes emitting from the ganglia pass to multi-branched cells which lead in turn to the muscle sheaths of the body wall, etc. Large anterior and posterior nerve trunks arise from each ganglion and serve the two suckers. A less conspicuous lateral

branch arises on either side, mainly serving the body wall. The anterior ganglia lie ventrally on each side of the oesophagus at a short distance from the oral sucker and anterior to the gut bifurcation. Anterior to the nervous concentration on either side of the posterior margin of the oral sucker lie clusters of small, darkly staining cells which are without connection with the nervous system (illustrated on page 251). They probably represent a localisation of interstitial bodies. Other cells found in larval stages, such as the eosinophilic cells prominent in the cercaria, are lacking in the juvenile and adult.

Reproductive system. The hermaphrodite reproductive system occupies most of the posterior region. Various points eluded to in the text are illustrated on pages 264, 263, 265.

In practically all cases, except those of atrophy, all the reproductive organs are in contact with the posterior gut caecae either along their entire lateral margins or a small part of the latter. Occasionally the caecum may completely overlies some of these organs. In only one case out of 200 was this rule found to be inapplicable to all stages of contraction.

The space system between the organs in the posterior region can vary according to the contractile phase. The shape of the organs can also change during the extremes of body movement (see page 265).

Female system. The vitellaria are paired structures situated immediately postero-lateral to the acetabulum. According to the contractile phase of the specimen, these organs can lie at some distance from the sucker or come to overlies it dorsally. They may be

completely distinct from other reproductive organs in the vicinity or they may touch or overlap one or both of these structures. The outline of the vitellaria varies from a spherical to an ovoid shape possessing slight to deep marginal indentations. The two organs do not always acquire the same shape or degree of indentation. The longitudinal axes, although commonly obliquely inclined to that of the body, may be completely transverse or parallel to the latter and the direction of the axes of a pair of vitellaria sometimes differ.

In juveniles the vitellaria appear as colourless spaces devoid of cellular content. They soon become filled with cells containing refractile material which spreads down the vitelline ducts towards the ventrally placed reservoir. This obscures the delineation of the tapering gland from its duct and makes accurate measurement difficult. These organs undoubtedly undergo shape changes during growth. The production of vitelline material in juveniles and its passage into the uterus slightly precedes oocyte release mechanisms.

The ovary may possess a spherical or an ovoid outline (a shape which is commonly but not exclusively found in juveniles) or it may assume a sparsely or multi-marginally indented form. The degree of lobing does not necessarily bear any relationship to the indentation found in the other reproductive organs of the individual.

The ovary is smaller on average than either of the two testes. It is amphitypic, remaining on the right side in 52% of the specimens examined. In only one case out of 200 mounted specimens was the ovary found to be in a state of atrophy. A fluke reaching 1.015 mm in length

possessed an ovary measuring 0.064 x 0.040 mm which is little larger than that found in the metacercaria. The testes reacted to stains normally and measured 0.11 x 0.08 mm. The uterus, although extending to an inter- and extra-caecal position was not highly coiled and contained very few eggs which did appear to be developing however, suggesting that the ovary was still functional. In one case the ovary was completely absent, apparently having never developed. The uterus was normal and extended inter- and extra-caecally. It contained non-embryonate eggs, the shells surrounding vitelline material only.

The oviduct arises from a central or eccentric position in the ovary and pursues an oblique course towards the mid-line. The arrangement of the female ducts has already been discussed and are illustrated on page 19 . The uterus in metacercariae and juveniles extends from the oötype dorsally over the ventral sucker to the genital pore. As the uterus increases in length, the initial descending uterine branch develops ventrally as a single loop between the reproductive organs of the posterior region. In cases where the reproductive organs remain closely applied to one another, the uterus begins to coil in the region behind the posterior testis. Where the ovary and testes are separated, the dorsal ascending limb coils between these structures before passing forwards. The ascending limb of the uterus commonly passes between the ovary and testis of one side and vitellarium and testis of the other, but rarely between the ovary and vitellarium. Following varying degrees of basal coiling the uterus finally passes extra-caecally. The uterus always passes medially down

the fluke and then, from the region behind the posterior testis, begins to grow extra-caecally. It does not enter this region primarily from between the reproductive organs. The coils pass extra-caecally from one side to the other crossing the extreme posterior region and rarely remain on the same side to repeat an extra-caecal loop. These coils pass anteriorly for varying distances, some reaching as far forwards as the outer vitelline margins. Secondary features occur as the ventral descending limb begins to coil between the reproductive organs and the ascending limb coils in the space between the vitellaria and the ventral sucker, even extending extra-caecally at this point in a few specimens.

The extra-caecal development of the uterus provides only an approximate guide to the reproductive age of the individual. Eggs are first produced by trematodes in which the uterus is entirely inter-caecal and usually slightly coiled basally (diagram D page 263). The stage at which extra-caecal loops first appear varies and may be delayed in specimens where the reproductive organs are widely separated (cf. diagram B, C, page 264). For example some flukes reaching up to 1.162 mm in length possessed uterine coils which simply overlaid the caecae and never extended into the true extra-caecal zone while others measuring only 0.525 mm in length already possessed extra-caecal coiling. The uterus merely increases to occupy available space coiling from side to side within the body until the posterior region is practically filled. In old flukes, eggs often obliterate the margins of the reproductive glands making their measurement impossible,

but in well over 200 specimens examined the coils were never observed directly crossing the latter.

The uterus extends anteriorly and empties into an extremely small genital atrium. There is no increase in the muscle layers surrounding the terminal portion of the uterus and it cannot accurately be described as a metraterm.

The genital pore is median and ventral. Its exact position varies with the degree of contraction of the anterior region, but it usually remains closer to the ventral sucker than the oral. It is situated immediately posterior to the gut bifurcation.

The male system. The two testes vary in shape from spherical, ovoid or triangular bodies to elongate structures commonly lying at tandem in the posterior region. Their outlines may be smooth, crenate or deeply lobed. The two bodies do not always possess a similar shape or outline and their main axes need not coincide. In extreme cases the longitudinal axis of one testis may be parallel to the main body axis whilst the other lies at right angles to it.

The testis position relative to one another in this species is moderately variable but changes with the contractile state of the individual and, to a lesser extent, with the specimen's age. The anterior testis may lie diagonally opposite and completely in front of the posterior organ. Within a population every variation occurs, from the above position to one where the two structures are almost parallel in the transverse plane with the anterior margin of that testis lying on the opposite side to the ovary just protruding beyond the margin of its

partner. In 6 specimens (mostly juveniles) from the collection of 200 the two testes were arranged one behind the other, either completely free or with overlapping median borders and with the lateral margins of the posterior testis touching the gut caecae on both sides of the body. It would appear that there is a tendency for the two testes to move transversely further apart as the fluke develops but this feature is easily masked by contractile effects. The anterior testis occupies a position lateral to the ovary and is, on average, smaller than the posterior testis, but larger than the female organ. It can overlap the vitellarium of that side and/or the ovary, or it may remain distinct from both structures.

Abnormalities were more common in the male system (see illustrations on page 263). One small fluke, measuring 0.50 mm long, developed without a posterior testis. Sixteen cases of testal atrophy were recovered from 200 specimens; 4 of these involved only the anterior organ; 4 only the posterior one and in 8 both testes were affected. The atrophied organs were smaller, in some cases separated from the caecal wall, and reacted feebly to stains. All cases of atrophy were recovered from fish which were over one year old and the flukes included were 1 mm or more in length (when mounted). It is possible that the longevity of the trematode approximates that of its host in nature, that is $1\frac{1}{2}$ to 2 years in the collection areas utilised.

The vas efferens pass dorsally and anteriorly to unite close to the anterior margin of the acetabulum. The vas deferens is short and dilates to form a large bipartite vesicular seminalis which occupies a

considerable amount of the space between the anterior margin of the acetabulum and the gut bifurcation. The longitudinal axis of the body and the vesicle generally coincide. The smaller chamber of the vesicle lies anteriorly and bends ventrally towards the genital pore. (The uterus at this point lies ventral to the vesicle.) Surrounding the smaller chamber and close to the uterus lie a cluster of what may serve as prostatic cells. Leading from the anterior vesicular chamber is a short, ventrally directed muscular ejaculatory duct which opens into the small genital atrium.

The sperm are active organisms consisting of a large head region and fibrillate tail. They are found alive throughout the male and female systems and are particularly concentrated in the male vesicle chambers. Sperm are voided live from the genital pore in small strings bound together in a transparent slightly glutinous liquid.

The genital atrium is muscular and protrusible in the form of a rosette. An eversible cirrus or penis is absent.

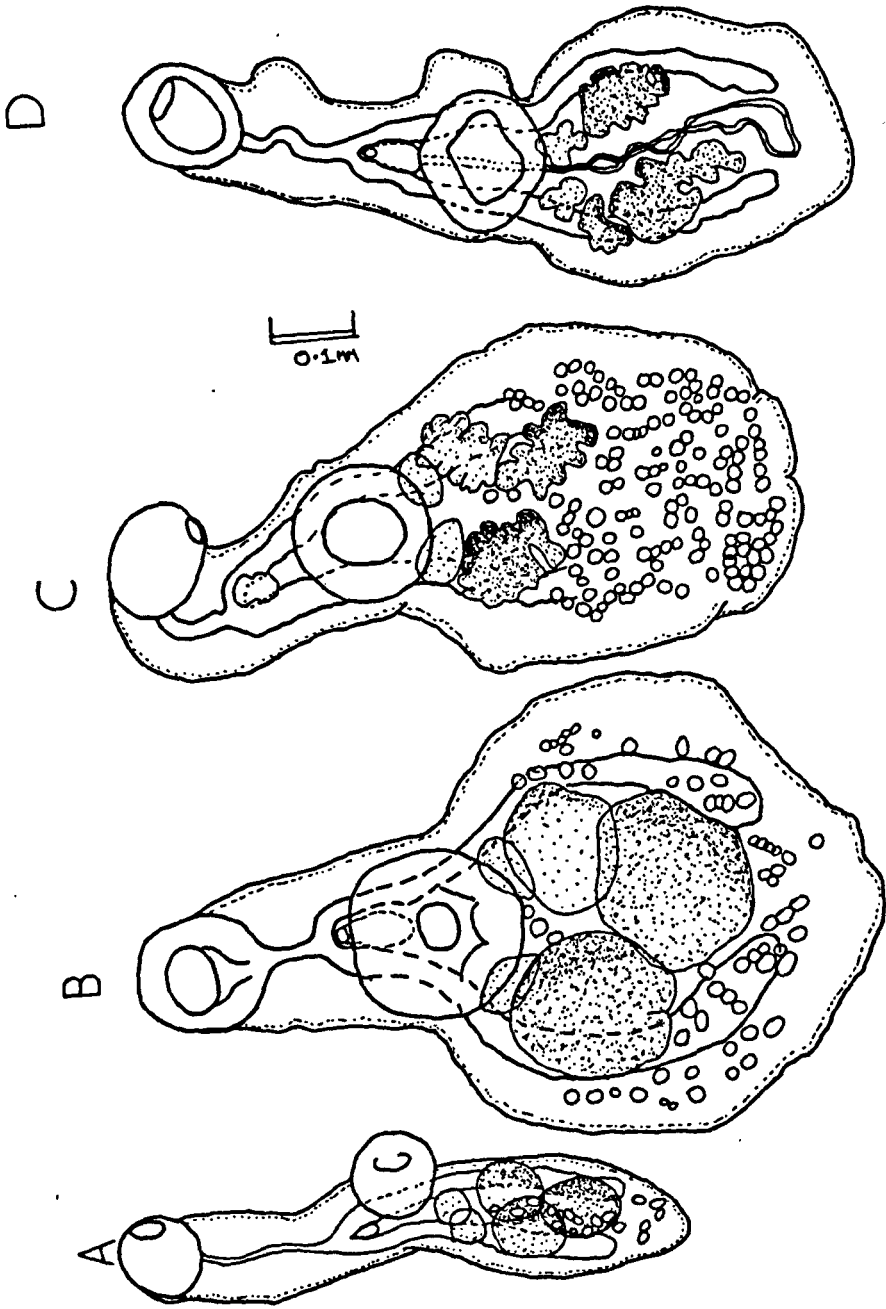
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THE DIAGRAM ILLUSTRATES SOME OF THE DIFFERENT GONADIAL POSITIONS ATTAINED AND MORE PARTICULARLY THE DIFFERENT DEGREES OF LOBATION IN SELECTED MOUNTED PHYLLODISTOMUM ADULTS

The diagrams are viewed from the ventral aspect and were all drawn to the same scale with the aid of a projector.

- Specimen A: Shape - All the reproductive organs are spherical in outline.
 Position - All the reproductive organs are in contact marginally with the gut caecum of their respective side; the posterior testis, however, is in contact with both the caecae. The ovary and two testes overlap one another marginally but the vitellaria remain distinct from the anterior male and female organs, although overlapping each other in the mid-line.
- Specimen B: Shape - The vitellaria are egg-shaped and the remaining organs are only roughly spheroidal, the anterior testis bearing a single indentation posteriorly.
 Position - The vitellaria, whilst remaining separate from one another, overlap the anterior male and female organs whilst the latter both marginally overlie the posterior testis. All the organs are in contact marginally with the gut caecum of their respective side.
- Specimen C: Shape - The vitellaria are egg-shaped and smooth in outline as in specimens A and B. The ovary and two testes are deeply indented.
 Position - The reproductive organs are situated in an identical position relative to the acetabulum as in A, B and D, but there is a proportionally larger amount of tissue posterior to the organs than in the other cases. The vitellaria, remaining separate, overlap or touch the anterior organs; the ovary and posterior testis are in contact marginally. All the reproductive organs are in contact with the gut caecum of their respective sides.
- Specimen D: Shape - All the reproductive organs possess indented margins.
 Position - The reproductive organs remain separate. All the organs remain in contact with the gut caecum of their respective side except for the left vitellarium which is distinct. (Eggs were present in this specimen but were not drawn, the outline of the uterus being shown only.)

- N.B. 1) The variable position of the gonopore in relation to the acetabulum.
 2) The different percentage of the posterior region occupied by the reproductive organs in specimens A, B and D when compared with C. This difference has resulted in the early extra-caecal development of the uterus in B and in extensive basal coiling in C.



GONADIAL LOBATION.

262

DIAGRAMS OF SELECTED MOUNTED SPECIMENS OF PHYLLODISTOMUM
ILLUSTRATING CASES OF ATROPHY AND THE NON-DEVELOPMENT OF
REPRODUCTIVE ORGANS

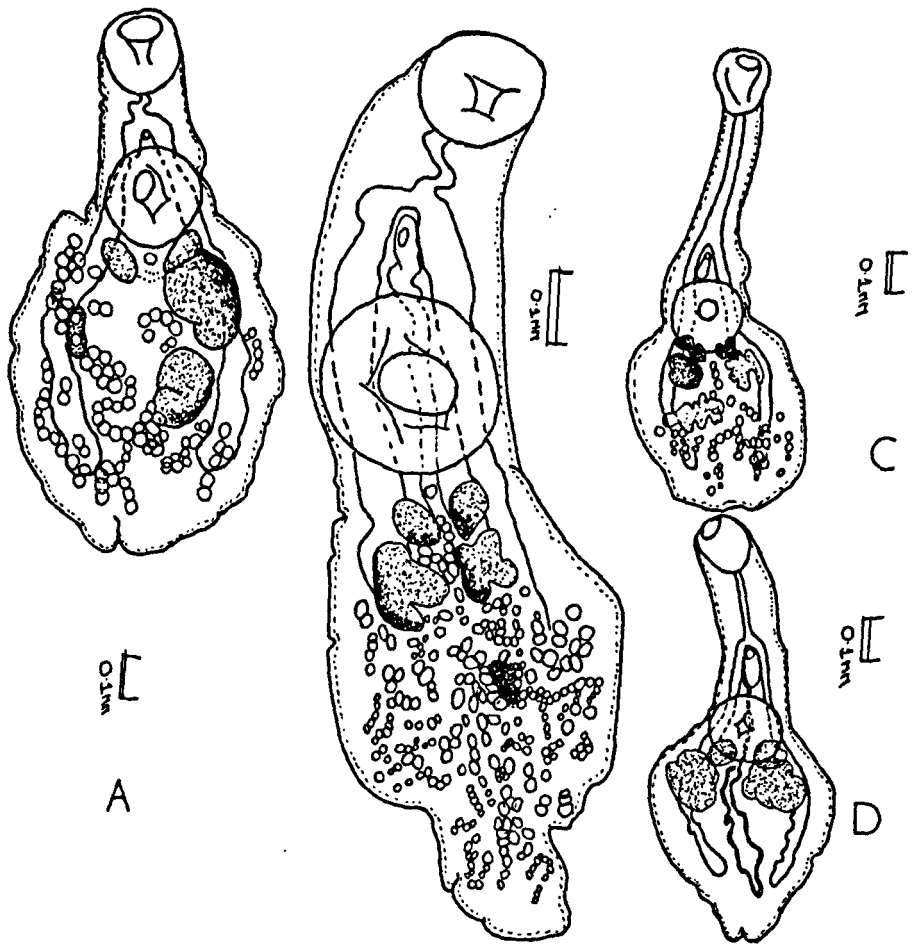
Specimen A: Atrophy of the anterior testis. Eggs in the uterus were fertilised and developing normally.

Specimen B: Atrophy of the posterior testis to a pale staining remnant with no definite outline. Eggs within the uterus were fertilised and developing normally.

Specimen C: Both testes atrophied, but not reduced appreciably in size, giving a feeble reaction to stains, causing them to remain so pale as to be barely measurable. Uterus was filled with a high percentage of unfertilised eggs and a few apparently normal ones.

Specimen D: Posterior testis completely absent - probably never developed. Eggs in the uterus were fertilised.

(The specimens are drawn to scale from the ventral aspect with the aid of a projector.)



REPRODUCTIVE ATROPHY & NON-DEVELOPMENT.

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TEN MOUNTED SPECIMENS OF HYMLODISTOMUM FIXED AND SELECTED TO
SHOW IN SEQUENCE SOME OF THE POSITIONS ASSUMED DURING MOVEMENT
WHEN IN THE LIVE STATE

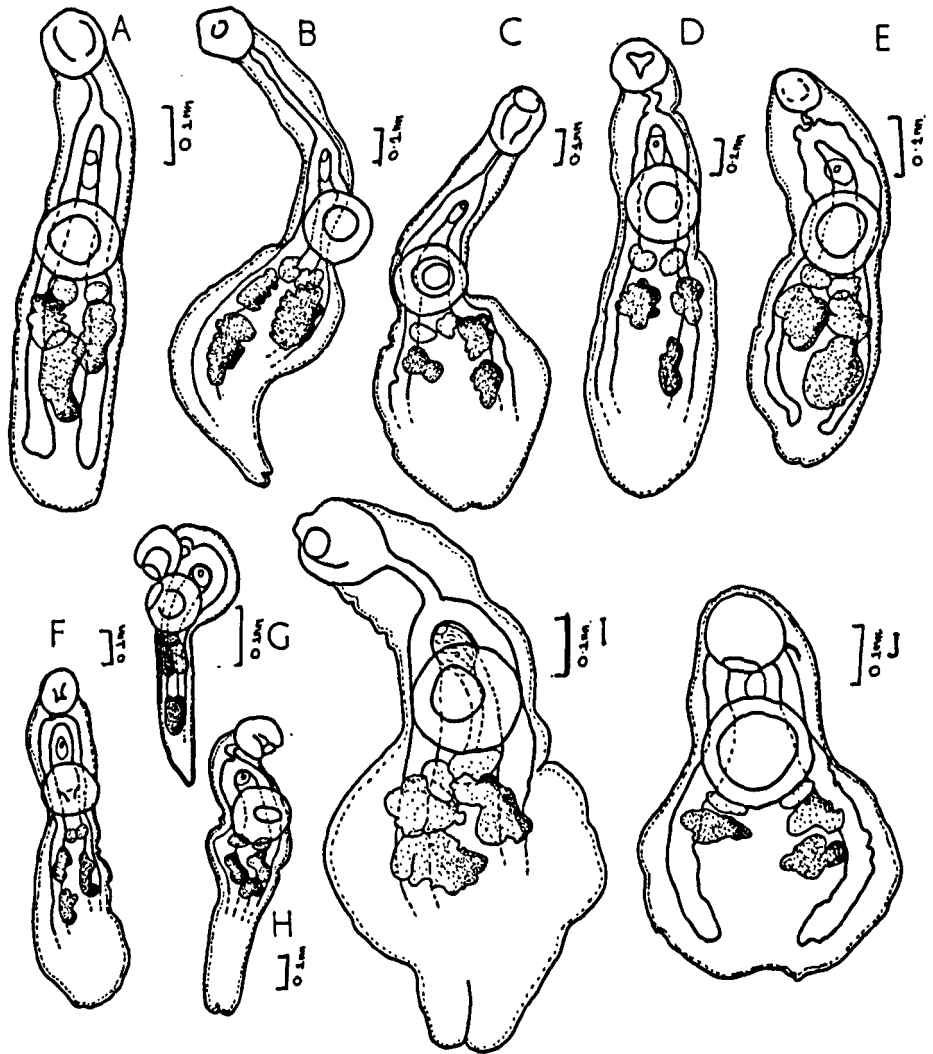
(All specimens were viewed from the ventral aspect (except G)
and were drawn to scale by the aid of a projector.)

- Position A: Relaxed specimen. (Note the close approximation of all the reproductive organs.)
- Position B: The anterior region is extended and bent to the animal's right while the acetabulum remains attached. The posterior region has just begun to swing back towards the left. The anterior section of the posterior region is expanded whilst the posterior extremity is somewhat extended.
- Position C: Anterior region is extended and bent to the left. The posterior region has remained in a near stationary median position in the expanded state. The ventral sucker is attached to the substratum.
- Position D: The anterior sucker is attached and the anterior region, as a result, is beginning to contract and shorten. The posterior region is relaxed. (Note the wide separation of the gonads and compare with A and E).
- Position E: Similar to D - note the lateral expansion of the anterior region as it shortens and the fact that it remains straight in this case.
- Position F: Detachment of the ventral sucker. As the acetabulum is drawn forwards the posterior region is beginning to elongate - this process begins immediately posterior to the sucker.
- Position G: Semi-lateral view illustrating how the two suckers are brought together with the anterior region bent into the form of a U. Posterior region fully extended.
- Position H: Attachment of the ventral sucker and release of its oral counterpart. The anterior region is beginning to straighten and elongate. The posterior region is beginning to expand again starting in the region immediately behind the acetabulum.
- Position I: Anterior region extended, bent to the right, and actively probing. Ventral sucker attached. Posterior region continuing to expand with only the posterior extremity still extended and possessing a well-defined posterior notch.
- Position J: Oral sucker recurved ventrally during probing activity. The posterior expansion now completed.

Gonadial Position:

The position of the gonads relative to one another varies from specimen to specimen, but the following changes were observed to occur in single living specimens as a result of movement:

- 1) From condition E to J on posterior expansion.
(N.B. Expansion of this type does not inevitably result in organ separation as a comparison of I and J illustrates.)
- 2) From H or I to F on extension of the posterior region. There is also a tendency for the main gonadial axis to be re-orientated during extremes of muscular activity, e.g. in the most elongated specimens the main (longitudinal) reproductive axes tend to parallel that of the body, but in expanded specimens (e.g. J) they parallel the transverse body axis.



CHANGES IN BODY SHAPE & GONADIAL POSITION ASSOCIATED
WITH LOCOMOTION, ETC.

d) The Living Fluke - Movement.

d.1) Method of investigation:

Observations were made upon living material at all stages of the life history. The trematode body carries out a series of movements which are identical from the cercarial to the adult phase. The stages recovered from the definitive host were observed by means of a binocular microscope, both within a water-filled watchglass following their release from the host and through the translucent walls of the unopened, undisturbed host system within the body cavity of a freshly killed host. Behaviour was identical in both situations.

These trematodes are capable of a wide range of contractions and undergo characteristic shape changes both whilst actively progressing and when remaining in one location.

d.2) Non-progressive movements:

Throughout these contractions the ventral sucker remains attached to the substratum. The extensile anterior region acts as a sensitive probe which can be pushed into the smaller urinary tributaries down which the main body of the fluke is incapable of progressing. The oral sucker never reaches the kidney cells however, even in the smallest specimens which have just invaded the host's system. The dorsal lip is depressed ventrally and protruded so that it skims lightly over the epithelial surface. The papillae probably serve a tractile function in detecting patches of loosened cells. When a suitable area is located the lip is pushed by a series of short movements amongst the epithelial cells lining the ureters dislodging the

loose tissue. The oral sucker aids this process by a plucking movement. The anterior region can be extended anteriorly or be swung from side to side curving round to pass beneath or above the posterior region. A whole series of random probing movements are carried out, followed at intermittent intervals by complete contraction. The anterior probe may contract parallel to the longitudinal body axis, the region remaining entirely in the dorso-ventral plane. In these circumstances the body wall is folded back over the ventral sucker area in a series of ridges and the anterior region is considerably widened. Commonly, however, the anterior probe is arched dorsally upon contraction, and there is little posterior accommodation. Consequently the anterior region is transversely distended to a lesser extent in these circumstances. During probing movements the fluke is able to swivel around upon its ventral attachment point so that the anterior region can probe in a complete circle. The ventral sucker can also alter its position very slightly by sliding over the host tissue thus increasing the immediate range of the probe while minimising the chance of sudden removal.

The posterior region undergoes a series of movements showing a certain degree of independence from those of the probe. It can be contracted fully, emphasising the posterior notch or completely obliterating it, whilst remaining parallel to the longitudinal body axis. Periods of contraction may be followed by partial or complete extension. An extensile wave passes from the acetabular region posteriorly and following the elongation of the body a wave of relaxation in the same direction results either in a relaxed, widened

area or triggers a further contraction. In the relaxed state this region can be swung from side to side in the same or opposite direction as the anterior end. The region can also exhibit, whilst remaining practically immobile and relaxed, a series of small contraction and relaxation movements involving the formation and loss of the posterior notch. Occasionally the smooth outlines of the posterior region may be lost and a lobed or crenate margin obtained. This condition can occasionally also be obtained by directing a stream of water from a pipette over this region. The body wall crinkles up during the next contractions, but resumes a smooth outline when the treatment ceases.

Both sections of the body can be lifted dorsally away from the substratum. In the anterior region this can occur in the extended or contracted state whereas, posteriorly, it occurs more commonly during and immediately following extension. The two regions can each be lifted upwards to a maximum of 45 degrees, either separately or in unison, leaving the ventral sucker firmly attached and protruding ventrally. During such non-progressive movement rarely do both regions extend simultaneously.

d.3) Progression.

The first stages in locomotion begin as the ventral sucker^{is} attached to the epithelium and the anterior region is extended. Following exploratory movements the oral sucker is closely applied to a particular site and by sucking up a small portion of the epithelium into the sucker cavity becomes firmly attached. The ventral sucker is then detached, and the anterior region curved dorsally and shortened thus bringing the acetabulum in close apposition to the oral sucker. The ventral sucker

can be applied directly or slid posteriorly for a short distance before becoming firmly attached by sucking a small area of epithelium into the cavity. As the acetabulum is brought forwards, the posterior region is elongated, usually raised and then relaxed. Finally the region is contracted and lowered following acetabular attachment. The oral sucker is detached and the anterior region is extended prior to the next forward movement. The series of movements performed during progression are illustrated on page 265.

e) The Living Fluke - Egg laying behavioure.1) In Situ

The copious flow of urine from the host usually precludes the recovery of eggs from the excretory system. On rare occasions, however, a few eggs were collected from the uretal tributaries of freshly killed hosts and more commonly from the bladder. Although the trematodes release eggs into whatever region of the excretory system they happen to be occupying, they seem to have a tendency to lay within the bladder whenever possible. In all cases where fish had been kept in the refrigerator overnight pending examination, eggs were only recovered from the bladder although the urinary system of the host sheltered flukes in both the ureters and terminal expansion of the system.

Egg laying movements of the fluke occur in a definite sequence. With the extension of the anterior region, eggs travel up the uterus from the more posterior loops, occasionally assisted by slight contractions of the posterior portion of the body. As the eggs approach the genital pore the anterior region ceases its lateral probing movements and contracts strongly causing eggs to pass out via the gonopore. Alternatively, once the eggs have reached the level of the genital opening uterine contractions and the accompanying flow of uterine fluid and eggs may be sufficient to cause laying without the necessity of further bodily contractions. If more than one egg is laid the embryos are released in small groups of between 2 - 6, with the eggs briefly adhering to one another, before parting.

The following experiments were devised in order to obtain information concerning the numbers of eggs released by these trematodes and the effects of temperature on egg laying. Infected fish were placed

in large conical funnels filled with water. The bottom of the funnel was blocked by a cork to prevent the fish entering the narrow tubular portion of the funnel and this was perforated by a glass tube of smaller bore. A short length of rubber tubing was attached to the base of the funnel and closed by a clip. Tubifex in a small feeding bowl were placed in the funnel and the water was gently aerated so that it was not violently disturbed. A total of six fish were used in the experiment. They had been kept for periods ranging from 1 - 4+ months and were fully accustomed to laboratory conditions. They were all at least one year old and measured 6 cms. or more in length. Transferred from stock tanks to the funnels which contained water at the same temperature, they were given a continuous supply of identical food to that they had originally received in the tanks. The fishes' activities were normal and their behaviour patterns were not varied in any way as a result of their transfer to the new environment.

One fish was placed into each funnel and left for exactly 24 hours. After this period the fish were transferred to temporary containers in water at the same temperature. The food bowls and corks were removed, disturbing the water as little as possible, and the aerators were taken away. Water from the food bowls was emptied into the funnels and the bowls were refilled and examined for any miracidia. The outer surfaces of the bowls and the cork and tube were also washed and the washings searched. The funnels were left for between 5 - 10 minutes in a vertical position and then 5 cc samples were taken from the base, the water being passed into graduated small transparent plastic containers. Since miracidia are positively geotropic (see page 62) it was found that the

majority of the miracidia were recovered within the first three samples taken. It was possible, by using an angled funnel, that some miracidia as a result of avoiding behaviour were remaining in the lower regions of the main body of water and were not entering the tube. It was assumed that downward suction upon opening the clip would draw down any miracidia in this basal area of the funnel so that they would be included in the samples. Although trial procedure showed that following six consecutive equal-volume samples all the miracidia could be accounted for, specimen examinations were made throughout the total water volume at all subsequent four-sample intervals. Following examination the funnels were washed through, the washings checked and then carefully cleaned. The fish plus the water in which it had been placed were then put into the funnel, the container washed and the washings placed in the funnel so that continuity of the experiment was maintained. The above procedure, although involving frequent handling of the fish, only caused alarm to the host on one occasion when, upon urination following transference, the fish dislodged a trematode, thus reducing that particular experimental period to a week. Otherwise the experiment was continued for a fortnight, counting taking place every 24 hours. Two fish of different sex were used in the experiment under conditions of high summer temperatures, whilst three fish (including one male) were examined under colder winter conditions.

Results.

The results are tabulated in Tables 25 and 26 and illustrated in the graph on page 277. (All eggs referred to in the account were viable and enclosed active miracidia). It can be seen that there is no direct

relationship between the temperature and the numbers of eggs laid per day. The only effect that temperatures of this order may have is an indirect one in lengthening or shortening the time taken for eggs to develop. It has been previously established experimentally, using trematodes isolated from the host, that at 1°C flukes become motionless and are incapable of laying. As 2°C (35.6°F) mobility returns and the fluke is once more capable of releasing eggs and the process continues at temperatures as high as 21°C (69.8°F). In the experiments summarised on Table 25 flukes were laying in water temperatures of 70°F under natural conditions. It is therefore unlikely that temperature of either extreme ever prevents laying in nature.

Table 25

| Date | Female Fish - 1 | | Male Fish - 1 | | Temperature (fahrenheit) |
|------------|-------------------------------------|--|-------------------------------------|--|-----------------------------|
| | Numbers of Mira- cidia recovered | | Numbers of Mira- cidia recovered | | |
| 5.6.62. | 13 | | 0 | | 62 |
| 6.6.62. | 3 | | 0 | | 62 |
| 7.6.62. | 19 | | 1 | | 64 |
| 8.6.62. | 38 | | 3 | | 63 |
| 9.6.62. | 32 | | 5 | | 69 |
| 10.6.62. | 61 | | 22 | | 63 |
| 11.6.62. | 24 | | 7 | | 64 |
| 12.6.62. | 30 | | 8 | | 63 |
| 13.6.62. | 49 | | 0 | | 69 |
| 14.6.62. | 15 | | 0 | | 69 |
| 15.6.62. | 3 | | 3 | | 69 |
| 16.6.62. | 8 | | 6 | | 69 |
| 17.6.62. | 47 | | 1 | | 70 |
| 18.6.62. | 4 | | 4 | | 70 |
| Totals 346 | | | 60 | | Av. 67.2°F. |

P.M. -

Phyllodistomum - 15 recovered.P.M. - Phyllodistomum - 1 recovered.

| Date | Female Fish - 23 | | Male Fish - 2. | | Date | Numbers of Miracidia recovered | Temperature (fahrenheit) |
|-----------|--------------------------------------|-----------------------------|--------------------------------------|-----------------------------|-------------|--------------------------------------|-----------------------------|
| | Numbers of Miracidia recovered | Temperature (fahrenheit) | Numbers of Miracidia recovered | Temperature (fahrenheit) | | | |
| 24.1.63. | 2 | 56 | 8.1.63. | 7 | 56 | | |
| 25.1.63. | 0 | 55 | 9.1.63. | 1 | 58 | | |
| 26.1.63. | 1 | 54 | 10.1.63. | 2 | 58 | | |
| 27.1.63. | 6 | 54 | 11.1.63. | 1 | 54 | | |
| 28.1.63. | 5 | 59 | 12.1.63. | 11 | 52 | | |
| 29.1.63. | 3 | 60 | 13.1.63. | 1 | 50 | | |
| 30.1.63. | 2 | 62 | 14.1.63. | 0 | 55 | | |
| 31.1.63. | 2 | 60 | 15.1.63. | 16 | 57 | | |
| 1.2.63. | 1 | 59 | 16.1.63. | 10 | 59 | | |
| 2.2.63. | 2 | 58 | 17.1.63. | 0 | 60 | | |
| 3.2.63. | 1 | 53 | 18.1.63. | 0 | 59 | | |
| 4.2.63. | 0 | 57 | 19.1.63. | 1 | 59 | | |
| 5.2.63. | 0 | 58 | 20.1.63. | 1 | 59 | | |
| 6.2.63. | 1 | 61 | 21.1.63. | 3 | 60 | | |
| Totals 26 | | Av. 57.6°F. | 54 | | Av. 56.8°F. | | |

P.M. - Phyllodistomum - 10 recovered. P.M. - Phyllodistomum - 6
recovered.

(22.1.63. 3 59)

Table 26.FEMALE FISH - 2A

| Date | Number of Miracidia Recovered | Temperature (degrees fahrenheit) |
|-----------------|-------------------------------|--|
| 17.1.63. | 0 | 60 |
| 18.1.63. | 1 | 59 |
| 19.1.63. | 2 | 59 |
| 20.1.63. | 1 | 59 |
| 21.1.63. | 5 | 60 |
| 22.1.63. | 7 | 59 |
| 23.1.63. | 14 | 59 |
| <u>Total 30</u> | | <u>Av. 59.3°F.</u> |

P.M. Phyllodistomum = 11 recovered.

FEMALE FISH - 3

| Date | Number of Miracidia Recovered | Temperature (degrees fahrenheit) |
|----------------|-------------------------------|--|
| 5.1.63. | 0 | 54 |
| 6.1.63. | 0 | 52 |
| 7.1.63. | 0 | 56 |
| 8.1.63. | 0 | 56 |
| 9.1.63. | 0 | 58 |
| 10.1.63. | 1 | 58 |
| 11.1.63. | 0 | 54 |
| 12.1.63. | 1 | 52 |
| 13.1.63. | 1 | 50 |
| 14.1.63. | 0 | 55 |
| 15.1.63. | 0 | 57 |
| 16.1.63. | 0 | 59 |
| 17.1.63. | 0 | 60 |
| 18.1.63. | 0 | 59 |
| <u>Total 3</u> | | <u>Av. 55.7°F.</u> |

P.M. Phyllodistomum = 97 recovered

276
5
3

EGG LAYING EXPERIMENTS

GRAPHS FF1 to FF3 illustrate Tables 25 and 26.

Abbreviations and Symbols:

Nos. M. = Numbers of miracidia recovered.

°F - Temperature in degrees Fahrenheit.

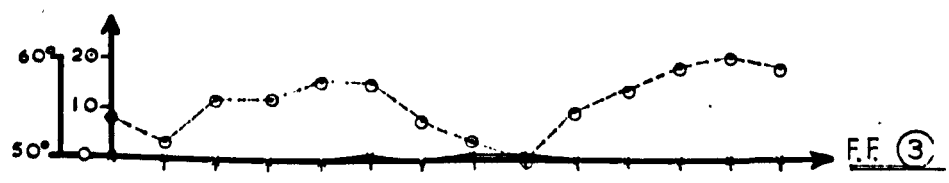
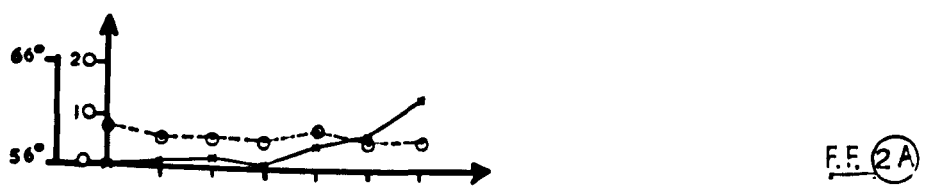
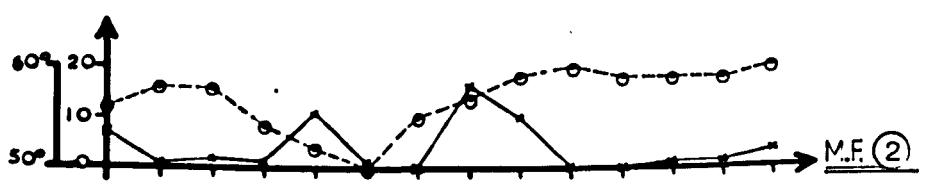
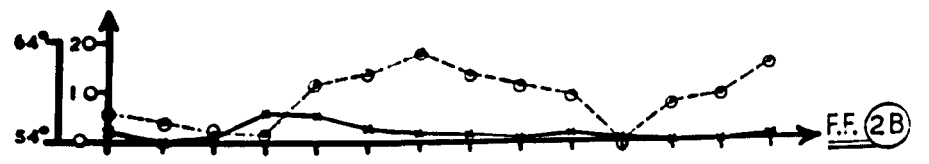
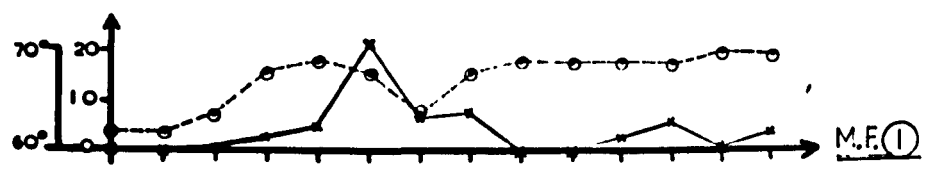
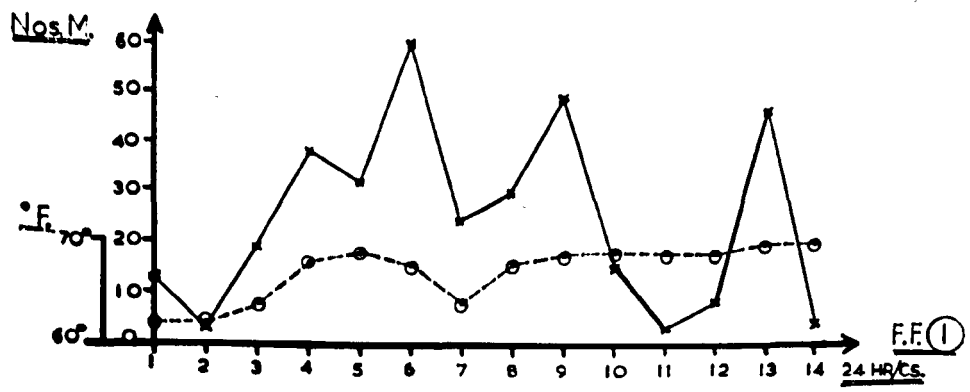
24 Hr/cs = Record taken every 24 hours.

FF 1; FF 2B; FF 2A; FF 3; - Female fish 1; 2B; 2A and 3.

MF 1; MF 2; - Male fish 1 and 2

o - Temperature record - jointed by broken lines.

x - Miracidial record joined by unbroken lines.



EGG LAYING - EXPERIMENT

In female fish number 3 it will be seen that a total of 97 flukes recovered on post-mortem (P.M.) at the end of the experiment released only three miracidia over a period of a fortnight at an average temperature of 55.7°F. The reason for this lies not in the temperature effect but that the flukes were mostly juveniles and that only six possessed formed eggs in the uterus. From the stage of development of the eggs it would appear that only one fluke was actually laying and that it was probably in the very early stages of the lifetime of egg production. The total length of this single trematode when mounted was 0.595 mm and the posterior region measured 0.385 x 0.182 mm, (an area of 0.07087 sq.mm.). Egg release at such an early stage bears no immediate relationship to daily temperature variations. It is not a continuous process and, in this case, there were as many as five days intermission. It would seem from this that ovarian activity must be a rhythmic process where periods of production alternate with periods of rest.

Examination of the number of miracidia obtained from male fish 1 illustrates that this alternating rhythmic activity is maintained in the mature large fluke. A single trematode laid 60 eggs at an average temperature of 67.2°F over a period of 14 days. Two days when no eggs were laid were followed by six days of production, two days rest and then further production. This trematode, which will be subsequently referred to as A, had a total length, (when mounted) of 1.211 mm, the posterior region measuring 0.777 x 0.560 mm (an area of 0.435 sq.mm.). The posterior region was therefore 6.28x larger than the same region in the young fluke from female 3. For the sake of simple initial comparison it can be assumed that the numbers of eggs produced is proportional

Table 27.

Egg Laying Behaviour

| Host | Nos. of flukes present | Nos. of Miracidia per 7 days | Area of P.R. sq. mm. | Area of P.R. cf. w. A. (sq.mm.) | The.laying rate when = A | Calc.laying rate (av.) | Recorded laying rate |
|----------------|------------------------|------------------------------|----------------------|---------------------------------|--------------------------|------------------------|----------------------|
| Male 1 (HT) | 1 (= A) | 22;38; | 60 | 0.435 | - | - | 60/14 days |
| Female 1 (HT) | 15 | 190;156; | 346 | 0.196(av) | 2.215x(s) | 27.09/14 days | 23.06/14 days |
| Female 2B (LT) | 10 | 19;7 | 26 | 0.2696 (av) | 1.614x(s) | 37.19/14 days | 2.6/14 days |
| Female 3 (LT) | 1 | 1;2 | 3 | 0.0703 | 6.28x(s) | 9.764/14 days | - |
| Male 2 (LT) | 6 | 23;31; | 54 | (0.3886) | (1.12x(s)) | (53.6/14 days) | 9/14 days |

Theoretically:

| | | | | | | | | |
|----------------|---------|--------|----|--------|-----------|---------------------------------|---|---|
| Female 2A (LT) | 1 (- x) | 11;23; | 34 | 0.7825 | 1.799x(L) | 107.9/14 days (53.95/7 days) | - | - |
|----------------|---------|--------|----|--------|-----------|---------------------------------|---|---|

Abbreviations:

- (HT) - High Temperature experiment.
- (LT) - low Temperature experient.
- (av) - Average
- (s) - smaller than A.
- (L) - larger than A.

only to the size of the posterior region of a fluke and that there are no other variables involved (the results of such calculations are summarised in Table 27, page 279). Theoretically, on this basis, when A was the same size as the fluke from the female host 3 it would have been producing from 9 to 10 eggs per fortnight. It would seem from these results, that an average difference of 11.5°F has a marked effect upon the whole process of egg production, development and laying. Temperature is not the only factor which has to be considered, however, since a steady increase in ovarian output with age and addition to uterine length is to be expected.

The female host 1 carried 15 trematodes from which 346 miracidia were recovered in 14 days at an average temperature of 67.2°F. The total average length of the flukes was 1.1434 mm (range 0.896 - 1.330 mm) with a mean posterior region measuring 0.735 x 0.267 mm (an area of 0.1964 sq.mm). This is approximately 2 times (2,215 x) less than the equivalent area of A. Theoretically, when A possessed a posterior region of 0.1964 sq.mm. it could have released an average 27.09 eggs over a 14 day period. Assuming that equal contributions were made by all the 15 trematodes present, a production rate of 23.06 eggs per fortnight can be calculated. The discrepancy between the two theoretical rates can easily be explained on the grounds that the mean value obscured the fact that there were three size groups within the collection of 15, indicating three separate periods of infection. Three of the flukes exceeded the posterior regional record for A whilst five were considerably

below this figure. The greater number of smaller specimens in the sample probably reduced the total output masking the higher production rates of the three larger trematodes.

Female fish 1 and male host 1 were both collected from the Essex area and were kept in the same tank for $4\frac{1}{2}$ months prior to the egg laying experiment. The only differences which might have had some effect upon the trematodes during 5 months in the laboratory were the differing sexes of the two hosts and some degree of crowding in the female. These factors obviously did not affect production. Despite the fact that the trematodes had been kept under similar conditions in the two fish for over one-quarter of their total expected span of life, the periods of maximum-, minimum- and non-production did not coincide, each fluke apparently retaining a degree of individuality (C.F. graph F.F.I. and M.F.I. and Table 25) even within the female. Lack of co-ordination has the advantage that the chances of maintaining a continual discharge of eggs from the host are increased and the disadvantage of the resting period in the ovarian cycle is reduced to a considerable extent within a relatively small fluke population.

Female fish 2B was infected by 10 trematodes which released 26 eggs in 14 days at the low average temperature of 57.6°F . The flukes attained a mean length of 0.786 mm (range 0.644 - 0.994 mm) and a mean posterior regional size of 0.581×0.464 mm (= an area of 0.2696 sq.mm). Thus, on average, these flukes possessed a posterior region 1.614 times smaller than trematode A. When A had a posterior region measuring only 0.2696 sq.mm. in area it would have been theoretically capable of

producing 37.19 eggs every 14 days according to previous results. In the case of the flukes collected from the female host 2B however, the average rate per individual was much lower - being only 2.6 eggs over the same period. Since maximum and minimum periods of production will rarely coincide for all the flukes present, it follows that this low result cannot be explained in these terms. Neither can the varying sizes of the flukes satisfactorily explain the situation. It can be concluded that in this case temperature has had a marked retardation effect on both ovarian production rates and miracidial development. This conclusion is further supported by results obtained from the female host 2A and male host 2.

The data placed under the heading of "female host 2A and 2B" refer to the same fish but to differing parasite loads. The fish forcibly urinated upon handling following a week-long experiment (2A) and lost a single trematode, which will be referred to as "X". The experiment was continued but the difference in subsequent releases was marked. X measured 1.632 mm long with a posterior region of 0.977×0.800 mm (area 0.7825 sq.mm). In 2A eggs left the host at a rate of 30 a week. In 2B with the loss of X only 26 miracidia were recovered in a fortnight from 10 flukes. The average laying rates were respectively 2.73 eggs per 7 days and 2.6 eggs per 14 days. Theoretically, a fortnight's production by the 11 flukes in 2A would have produced 60 eggs in 14 days. If ten of these were laying at a rate of 2.6 eggs each over this period X would be producing 34 eggs in 14 days. In comparison, X possessed a posterior region 1.799 times

larger than the similar area in A and theoretically should have been capable of laying 107.9 eggs over the fortnightly period. The main explanation for such a discrepancy between these results is the effect of temperature.

The 6 flukes from the male host number 2 released 54 eggs over a period of 14 days at an average temperature of 56.3⁰F. The theoretical average laying rate, assuming equal production from all trematodes present, equalled 9 eggs each per fortnight. The infected host was utilised in other experiments and was not examined until 4½ months later when the flukes had reached an average length of 0.948 mm (range 0.807 - 1.122 mm) with average posterior region measurements of 0.591 x 0.658 mm (an area of 0.3886 sq.mm). Obviously, at the time of laying, they had a posterior region of smaller dimensions than either A or X. The rate of laying was minimal and typical of the results obtained at lower temperatures.

Within the uterus of A there were 391 eggs at the termination of the experiment. Laying at the rate recorded and assuming all the eggs to be fertile, it would take over three months for the last of these eggs to be released which appears to be an excessive time for miracidial development, particularly at such high temperature. The probable reasons for this result are discussed in the general conclusions concerning egg laying sequences on page 288. Whatever the temperature within any one fortnight, there are at least two periods when egg release is minimal or completely lacking. In young flukes this rest period has been recorded as extending, under natural conditions, for

as much as 5 days/^{OR}when the fluke is kept outside the host. 8 days, whilst in older stages it is reduced to 2.

e.2) Egg laying behaviour under experimental conditions, the trematode being removed from the host.

Phyllodistomes can be kept alive in the laboratory for varying periods, their longevity when free from the host largely depending upon the temperature, their age and activity. Extremely young trematodes never survive for long, probably as a result of their lower food reserves. The addition of host urinary cellular waste or diluted horse serum has little to no effect upon them since they appear unable to use such material to any advantage.

During this investigation a few of the trematodes which were kept at room temperatures in frequently changed tapwater laid viable eggs and a still smaller minority remained alive for as long as a week maintaining egg laying activity throughout. One fluke A(s), measuring 1.662 mm in length, laid a total of 48 viable eggs in 7 days at a laboratory temperature averaging 68°F. The eggs were counted at 24 hour intervals and were produced in a fluctuating series which included a single interval of non-production and which closely paralleled the results obtained from the previous egg production experiments, (Miracidial numbers were: 10, 14, 6, 9, 0, 2, 17). A slightly larger fluke B(s) measuring 1.785 mm and recorded at the same temperature produced (at 24 hourly intervals) 40, 15, 7, 8, 7, 0 viable eggs - a total of 77 in six days. Successful results of this type occurred infrequently. If a trematode proceeded to lay on removal from its host it generally did so on one occasion only and a laying sequence was not revealed.

It was observed, on the few occasions that fish had been kept overnight in the refrigerator prior to examination, that the contained flukes had been stimulated to lay. It was possible that the utilisation of a sudden depression and subsequent rise in temperature might provide a method of obtaining eggs and miracidia in quantity when required and prevent undue loss of adult material by dissection. In order to test this theory the following experiment was attempted:-

Trematodes, observed to contain miracidia, were placed into solid watch glasses containing tapwater and a few drops of horse serum to lower the freezing point. These vessels were put into a container, packed around with ice cubes and the whole apparatus covered and left at room temperature for 24 hours. The longevity of each fluke depended upon the relative length of time spent at the high room temperatures (which were quickly attained during summer months); its age and activity following ^{the} stimulation of temperature change. Young flukes in general survived only one temperature depression but the larger specimens usually survived 2-3 repetitions of the above experimental procedure. The total range of temperatures obtainable by these rather crude methods paralleled the extremes likely to be experienced by the fluke in nature reaching -1°C (30.2°F) to $+21^{\circ}\text{C}$ (69.8°F).

Results.

The results were extremely variable. The majority surviving one temperature depression were stimulated to lay under these conditions indicating that this was a moderately successful means of obtaining eggs. They released from 2 to 23 viable eggs, containing active

miracidia, in 24 hours, giving a result which was completely compatible with the data obtained from the previous egg production experiments. A very few larger specimens, however, produced a totally unexpected quantity of eggs. One fluke (1A) laid 170 eggs, 92 of which proved to be infertile or vitelline in origin. A group of six flukes (6B) from one host, which were kept together during the experiment, released 850 eggs in 24 hours. The presence of, and continuing contact with other trematodes in the water, stimulated this group to greater muscular activity than solitary specimens. Perhaps as a consequence of this they laid more eggs (140 of which were infertile or vitelline in origin) and became moribund after only 24 hours. The results obtained from trematodes surviving 3 and 4 temperature depressions are given in Table 28.

Table 23.

| Nos. of flukes utilised. + fresh ice - T°C de- pression No.1. | Record of the number of Miracidia recovered 24 hours later. Then +fresh ice - T°C depression No.2. | Record of the number of Miracidia recovered 24 hours later. Then +fresh ice T°C depression No.3. | Miracidial Numbers recovered 8 hrs. later | Miracidial Numbers recovered 24 hrs. later |
|--|--|--|---|--|
| 1) | 30 | 0 | - | None - fluke moribund |
| 1) | 25 | 0 | - | " |
| 1) | 18 | 4 | - | " |
| 1) | 9 | 16 | - | " |
| 1) from one host (9C) | 6 | 10 | - | " |
| 1) | 0 | 15 | - | " |
| 1) | 0 | 4 | - | " |
| 1) | 19 | 5 | - | " |
| 1) | 0 | 21 | - | " |
| 1) from one host (1D) | 48 | 81 | 40 fluke moribund | - |
| 1 from one host (1E) | 24 | 41 | - | 87 |
| 1 from one host (1F) | 87 | 0 | - | None - fluke moribund |

Total: (9C) 9 flukes from one host released 178 viable eggs in 48 hrs.
 (1D) 1 fluke " " " " 169 " " in 56 hrs.
 (1E) 1 fluke " " " " 152 " " in 72 hrs.
 (1F) 1 fluke " " " " 87 " " in 48 hrs.

In the case of the first 9 trematodes all from one host (9C), the response to 3 variations in temperature was to lay eggs in quantities similar to those recovered during the previous egg production experiments. The next three flukes shown in the Table 23 produced unexpectedly high numbers of viable eggs over a period ranging from 48 to 72 hours.

As noted previously, under these unnatural conditions, the trematodes were, in addition, stimulated to part with eggs which appeared to be either in their early stages of development, infertile or composed entirely of vitelline material and the numbers (which in this case were not recorded) tended to increase towards the termination of the fluke's life. This suggests that there is a progressive breakdown in co-ordination between vitelline and ovarian production rates and that the smaller products are being washed through the uterus at a more rapid rate than the normal developing eggs. Under natural conditions all subsequent development of immature eggs is curtailed by bacterial action.

General Conclusions on Egg Laying Sequences. (Sections e.1 and e.2).

From results obtained from 6B it would seem that a large Phyllo-distome ovary has the ability to produce at least 5 fertile eggs every hour at certain times in the ovarian cycle (i.e. 1 egg passing through the ootype every 12 minutes). This potential is not continually realised however. With maximum uterine capacities in the range of 500 - 600 eggs a fluke may, under stress, be able to release approximately up to 1/5 or 1/6 of this quantity in 24 hours. Under natural conditions it would appear as if this maximum potential is seldom realised in this way.

The ovarian rhythm cannot be over-sensitive to small temperature differences because of the relatively long period taken for miracidial development (seemingly 3 months) and restricted uterine capacity. The

ovarian rhythm must basically consist of maximum and minimum periods of production each possessing rest sequences. The ovary retains the plasticity to react to a sustained temperature change of the magnitude encountered in basic seasonal changes. This sudden burst of maximum activity could occur particularly in the early Spring months (e.g. February/March) resulting in the release of increased numbers of larvae in the early Summer (May). Parasitic development in the mollusc population would occur whilst the fish hosts were breeding and the young fish, then too small to be infected, would be increasing rapidly in size. When the latter reach 2 - 3 months old and become large enough to acquire Phyllodistome infection (August onwards) the flukes would be infecting insect larvae and be readily available for entry into the definitive host. It is possible that such an ovarian response to rising temperatures occurs each year. The fact that this is not reflected in the numbers of flukes recovered seasonally from the definitive hosts is probably due to numerical seasonal changes in the hosts involved in the life cycle. The increase in the population density of the 2 intermediate hosts during the summer increases the food material available for the definitive host and the subsequent rise in the number of fish present may obscure the pronounced effects of the egg response and the multiplication phases of the trematode. In nature it also appears that the fluke has the ability to retain eggs in the uterus over a period of a few days close to the time when they are about to be released. This may result in the maximum egg release being spread over several days so that the numbers of eggs released per 24 hours may

not reflect the actual rate at which the oöcytes left the ovary (e.g. 1E, 1D Table 28).

All the flukes tested in the stress experiments, which gave maximum results, were obtained from hosts which had lived through a substantial temperature change approximately 3 months previously. The fluke (1A), which laid 170 eggs at the end of April, was taken from a host which had been brought into the higher temperatures of the laboratory at the beginning of February from cold lake waters. The six flukes (6B) and 1D, 1E, 1F were collected at the end of November and kept in the laboratory until the beginning of March. 9C were taken from a host which had spent two months in the laboratory and which did not show any responsive increase in miracidial numbers when examined. All other flukes were obtained from stock collected and immediately examined in the months of August and November. The two large flukes referred to on page 284 (A(S) and B(S)) were from hosts collected and examined in July. The latter released the maximum number of eggs (40) to be recorded under natural conditions for a 24 hours period.

This retention of general sensitivity to large temperature changes is an adaptation of some importance to the maintenance of a steady population density of this trematode and illustrates the ideal relationship to life in a poikilothermic host.

The stress experiments did cause flukes to lay with a little more certainty than other methods. The experiments also caused maximum release to occur during a minimum period of time in a few cases, but otherwise natural phenomena were recorded. The reason that previous egg production experiments had not revealed the true potential of these

trematodes is related to the timing of the experiments. The fish examined at lower temperatures (Male 2, Female 2A and 2B) had been brought into the laboratory only one month previously and insufficient time had elapsed for the maintained temperature change to affect the numbers of eggs laid. The two fish examined at higher temperatures had been previously kept in the laboratory for $4\frac{1}{2}$ months and the parasites had probably passed through the period of maximum output and so the level of output recorded was a basic response to summer temperatures. Summer laying rates exceed those attained during the winter and are associated with faster miracidial development.

f). The Living Fluke - General behaviour in the definitive host.

f.1) Migrating juveniles

The region of the vertebrate gut in which ectopia takes place is unknown in this species. Although the process of natural excystment was not observed, it seems likely, in view of the fact that the cyst wall is permeable, that transference to an acid or alkaline environment would stimulate the trematode to attempt to escape and tear the wall apart rather than to passively await its enzymic digestion and dissolution. Goodchild (1948) found this to apply to Gorgodera amplicava when, after placing cysts into dilute hydrochloric acid solution (PH:1.5) the metacercariae were stimulated to excyst actively.

During this investigation young Phyllodistomes were not recovered from any environment other than the urinary system. Various authors, however, have recovered migrating flukes both during feeding experiments and general investigations and it would appear from the reports that the trematodes are released in the intestine, rarely in the stomach, and progress by means of their own activity towards the anus. Van Cleave and Mueller (1932) reported ectopic Phyllodistomum superbum from the gut of Esox lucius, Percina caprodes zebra and Percopsis omnisco-maycus. Bhalerao (1937) recovered immature specimens (P. lewisi according to Figulevsky (1953)) from the intestine and stomach of Indian fish of 3 genera. Ssinitzin (1901) recovered excysted specimens of a species he referred to as P. folium from the intestine of Carassius vulgaris and Abramis brama only two hours after feeding metacercarial cysts to these fish.

In this investigation, during the experimental infection of Amphibia

with Gorgoderia young specimens were recovered from the rectum and cloacal region. These young flukes were firmly fixed to the gut wall by means of their suckers and were progressing in the direction of the urinary openings. This observation is identical to that made by Goodchild in 1948 concerning Gorgoderia amplicava following metacercarial excystment in the stomach or intestine of several Amphibian hosts. Nematodes recovered from the rectal contents of the frogs in contrast to the Gorgoderia were entangled in the mucus and detritus. According to Goodchild (1948) failure by young Gorgoderia amplicava specimens to extricate themselves from digesting material in the gut may have accounted for some of the considerable losses involved when infecting the definitive host. The age and activity of the juvenile is of obvious importance during this phase and it is likely that those individuals with markedly reduced reserves are more inclined to fail at this time and to be swept through the gut.

The time taken for Gorgoderida to pass through the alimentary canal of the definitive host and reach the urinary system will vary principally according to the temperature. The shortest time recorded for infection was 8 hours which was achieved by the juveniles of Gorgoderia amplicava and reported by Goodchild (1948). Ssnitzin (1901) recovered P. folium from the urinary ducts of Carassius vulgaris and Abramis brama some 24 hours after metacercarial ingestion and Thomas (1958) recorded an identical migration time for P. simile in Salmo trutta. P. solidum migrated through Desmognathus fuscus fuscus in 24 hours (Goodchild 1943) and Crawford (1940) reported a similar time for an

unknown Phyllodistome species in experimental Bufo boreas boreas and Ambystoma tigrinum. It would appear that the differing lengths of the alimentary systems in Fish and Amphibia have little effect upon Gorgoderid migration times and that most species of Gorgodera, Gorgoderina and Phyllodistomum are capable of reaching the urinary system within 24 hours of metacercarial ingestion.

Phyllodistomes, utilising Fish hosts, have been reported from a variety of locations other than the urinary system. Few such reports however represent the true feeding location of the parasite. For example, P. carangis was recovered from the washings obtained from the body cavity following the dissection of the marine teleost Caranx ruber by Manter (1947); it was also recovered from the posterior dorsal region of the body cavity of Citula dorsalis by Bravo-Hollis and Manter (1957); P. (Distoma) conostomum was reported from the gills and oesophagus of Coregonus oxyrhynchus by Olssen (1876) and Jaiswal (1957) recovered an unknown species of Phyllodistomum from the body cavity of Labeo fimbriata and P. parorchium from an identical location in Glossogobius giuris. In the case of the latter two authors, the material which was used was not examined in fresh condition. Olssen, according to Nybelin (1926) frequently used fish which died some considerable time before examination and Jaiswal obtained his material from the markets and fishery departments of Hyderabad, India. The kidney region rapidly degenerates following the death of the host and could easily give rise to conditions where flukes were found wandering in the body cavity following their release by degeneration from the urinary system. Meyer, on the other hand, reported in 1958 that he found an immature species of Phyllodistomum

in the hepatic ducts of Catostomus c. commersoni. He considered it to be a migrating form of the species P. lysteri previously described from the same host by Miller in 1940. Whether the flukes could have survived until maturity in such an abnormal situation is open to conjecture but the voluminous nature of the caecae noted by Miller as being atypical of any familiar group suggests that the longevity of the fluke was limited in the anomalous location.

Several records of Gorgoderids recovered from unusual locations in Fish hosts whilst fully adult have been reported. Gupta (1951) obtained P. singhiai from the intestine of Mastacembelus armatus in the district of Lucknow and in 1953 the same author recovered P. vittatusi from the intestine of Macrones vittatus in Assam. Kaw (1950) obtained living mature specimens of P. loossi from the body cavities of two species of Schizothorax in Kashmir apparently from freshly collected material.

Similar reports of both migrating phases as well as mature flukes taken from unusual locations exist for Amphibian hosts. Dhalerao in 1937 reported mature P. shandrai specimens occurring in the rectum of Rana tigrina although Kaw (1950) recovered specimens of this fluke from the bladder of the same host. The parasite may have become dislodged during urination or had wandered following the death of the host. In an unusual report Joyeux and Baer (1934) stated that three mature specimens of Gorgoderina capensis were taken from the muscles of the ventral body wall of Rana esculenta where the worms appeared to be migrating through the tissue. They concluded that these flukes, previously recorded by these authors from the urinary bladder of the same host species, matured precociously before reaching their definitive habitat, indicating a

presumed migration through the host's coelom following penetration of the gut. It was noted that there was apparently no host reaction induced. This is at variance with Goodchild's experimental data obtained in 1954 when he transplanted specimens of Gorgoderia amplicava and Gorgoderina attenuata into the coelomic cavity of several amphibian hosts. The flukes were eventually encapsul^{at}ed by host tissue and histolysed by diapedetic phagocytes. The fact that prior to encapsulation they appeared unable to penetrate the urinary system from this adverse location, even though many were found to be crawling over it, further supports the theory that at no time do Gorgoderids belonging to the Phyllodistomum, Gorgoderia or Gorgoderina genera penetrate the alimentary wall to attain their correct infection site in the urinary system. (Goodchild's work has recently been supported by Mitchell (1966) in a series of transplantation experiments using Gorgoderina vitelliloba). When occupying a favourable position in the ureters species with large and powerful suckers can penetrate into the mesonephic tissue of the host but do not pass beyond this point. Some species, mostly belonging to the genus Phyllodistomum, are incapable of penetrating so deeply. Dependence upon enzymes either disgorged from the gut or the properties of cercarial penetration glands retained to the adult phase (as reported by Lynch (1936) for P. singulare) appear to be limited to feeding activities such as loosening epithelial cells from the urinary lining as suggested by Goodchild (1943). The penetrative ability of these flukes seems to be solely a function of sucker size and strength. As a result, all Gorgoderids in the completion of their life cycle are forced to migrate down the entire length of the vertebrate gut and are incapable

of penetrating the muscle coats of the gut wall. The Joyeux and Baer report of 1934 was explained by Odlaug in 1937 as the result of the inanition of the hosts giving rise to adverse physiological conditions.

Anatomical differences between the arrangement of the anal and genital apertures within the Fish and Amphibia affect both the success and site of initial infection.

In the Fish-infesting Gorgoderids the migration must take place between the anal region into the external environment and then to the urino-genital opening. This hazardous period is probably one of the main causes for the frequently reported large differential between the numbers of metacercariae ingested and the number of flukes recovered from the urinary system. In Gasterosteus spp. the anal area is a slightly protruding circular region consisting of a radiating series of ridges and folds. Immediately posteriorly there is a second protrusion around the opening of the urino-genital system. The opening is situated in the anterior section of this latter region lying on the periphery of the anal ridged zone. A migrating fluke, upon reaching the anal opening, would be able to pass directly to the urinary system and be protected on either side by anal ridging provided it took a mid-ventral path. If it followed any of the other ridges it would be directed away from the excretory openings. It might be thought that, in common with the Turbellaria, a marked rheotactic sensitivity would be found in trematodes adapted to living in regions where continual fluid flow was a characteristic of the environment. It could be assumed that the flukes would turn towards the source of flow and minimize

the risk of being dislodged from or moving out of the urinary system. During the external migratory phase, however, such behaviour would be distinctly disadvantageous. The anus lies anterior to the urino-genital opening and flukes, exhibiting positive rheotaxis of the type described above, would turn away from the correct infection site. Isolated flukes did not exhibit noticeable rheotactic responses under experimental conditions and it can be assumed that successful migration from the gut to the urinary system is accomplished purely by chance for there are few signs to aid the fluke during this period. The epithelium does not change in character until the urino-genital channel has been entered and any discharge from the urinary system to which the trematode might be sensitive would be carried posteriorly and away from the anal region.

In the Amphibia the cloaca is well developed, so reducing some of the hazards involved in the later stages of the migration. The bladder cloacal aperture is, however, distinct from the urino-genital openings in the male and in the female the oviducts also open separately into this region. The migrating juvenile thus has a greater element of choice than is the case for a Fish host. According to Goodchild (1943) P. solidum crawls from the cloaca of Desmognathus fuscus fuscus towards the bladder orientating itself by means of some sensitive appreciation of the direction of beat of the cloacal cilia. Goodchild (1943) suggested that the strength of this ciliary action may be such that in some cases it prevents flukes from entering, (adhering and progressing through?) the cloaca. Juvenile Gorgodera amplicava, following their failure to reach the intestinal wall of the experimental host Triturus v. viridescens,

(due to a mucosal barrier) were thought by Goodchild to fail in their attempt to "enter" the cloaca for this reason.

During this investigation juvenile flukes were recovered from the bladder, lower and upper extremities of the ureters of Gasterosteus spp. At no time were they obtained from the reproductive ducts of either the male or female whatever their respective breeding condition. The urinary system of Gasterosteus spp. consists of two elongate mesonephri drained by numerous small, branched tubules leading into two main ureters or mesonephric ducts which lie along the outer edges of the kidneys for most of their length except for an extreme anterior expansion (head kidney) which is connected to the rest of the organ by a narrow isthmus on either side. Phyllodistomes never penetrate this anterior expansion. The two ureters pass posteriorly, maintaining a dorsal position and empty into the anterior expanded region of the "bladder". The latter is not homologous to the similarly named structure found in higher vertebrates such as the Amphibia and represents a terminal expansion of the mesonephric ducts and, as a result, is histologically identical to these structures. The bladder lies dorsal to both the rectum and the reproductive ducts. In both the male and female the two reproductive tubes unite a short distance before opening into the extreme terminal portion of the urinary tract. The single duct so formed, even in the mature breeding fish, has an extremely narrow internal diameter. Young flukes would experience great difficulty in entering such a system and in adhering to the glandular lining. As a result of this arrangement, the trematodes rarely come in contact with either semen or oviducal secretions. Situated to either side of the

posterior region of the kidneys in the region of the bladder in both sexes are clusters of spheroidal accessory tissue into which the trematodes never penetrate.

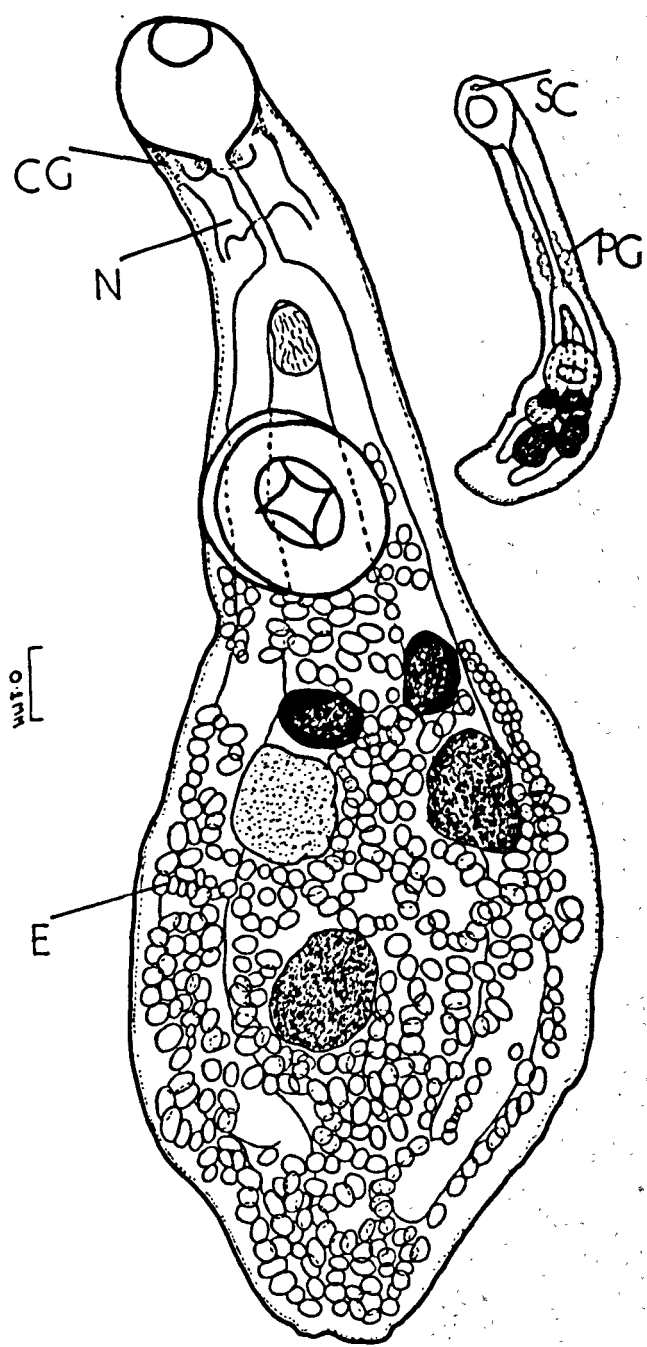
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DIAGRAM ILLUSTRATING THE SIZE RANGE OF PHYLLODISTOMUM
SPECIMENS RECOVERED FROM THE URINARY BLADDER OF THE
DEFINITIVE HOST - GASTEROSTEUS ACULEATUS.

Both specimens are drawn to the same scale from the ventral aspect. In both the juvenile and the laying adult the vitellaria are drawn with a solid outline; the ovary is lightly stippled and the testes heavily stippled. (Diagram drawn with the aid of a projector using mounted specimens).

Abbreviations:

- SC = Stylet chamber
- CT = "Cephalic gland cells"
- N = "Cerebral" ganglion
- PG = Penetration glands
- E = Eggs in utero



IMMATURE & MATURE SPECIMENS FOUND
IN THE BLADDER.

In the Amphibia Gorgoderids have been frequently recovered from the reproductive tract of the host. This reflects some lack of sensitivity on the part of the fluke and suggests that correct orientation at this juncture is again largely a matter of chance. Goodchild reported in 1948 and 1950 that Gorgodera amplicava and Gorgoderina attenuata were found in the oviducts and were numerous in the seminal vesicles. During this investigation Gorgoderid specimens were similarly recovered from the seminal vesicles of Rana temporaria. The specimens, however, were moribund and none were sexually mature. The success of the Gorgoderids upon entry into the reproductive tracts of their hosts, particularly the female system, does not appear to have been fully investigated, but Goodchild's report in 1950 that in the preferential sequence of invasion sites for Gorgodera amplicava and Gorgoderina attenuata, the oviducts are placed last may signify that flukes do not do well in these systems. This may relate as much to the histology of the oviduct wall as to the composition of its fluid content.

Upon entry into the urinary bladder of the Gasterosteus host, the juvenile Phyllodistomes do not undergo any definite migratory route towards the kidneys as has been reported for several Amphibian species. The smallest flukes to be recovered were extracted from the bladder and, although young forms were frequently recovered from branches of the urinary tributaries, the only significance which can be attached to this is a reflection of the greater ease with which these flukes can penetrate into these areas. It is also possible that this position in

the lateral branches of the ureters confers an advantage upon the young flukes, since they are less likely to be swept away by spasmodic urine flow when in this situation. Flukes in the bladder are particularly vulnerable and can be dislodged following forcible urination in both Fish and Amphibian hosts. During this investigation a fully mature *Phyllodistome* was lost in this way during an experiment (see page 282) and Goodchild (1948) reported the loss of young *Gorgodera amplicava* specimens from the bladder of *Rana catesbeiana* under similar conditions.

In the Amphibia most *Gorgoderids* seem to exhibit a sequential preference for certain sites in the urino-genital system. Goodchild (1948, 1950) reported that juvenile *Gorgodera amplicava* and, supporting Rankin's observations of 1938, that *Gorgoderina attenuata* migrate initially into the Wolffian ducts and then into the bladder where they mature. In 1948 he stated that *G. amplicava* remained in the ureters for at least two weeks where, on occasions, they became packed together in such numbers as to cause the upper ends of the ducts to become distended. Crawford (1940) observed flukes which he assumed to belong to the genus *Phyllodistomum* migrating to the kidney (presumably the ducts?) of *Bufo boreas boreas* remaining in this location for two weeks and then moving to the bladder where they attained sexual maturity in five weeks.

f.2) General pathology of the urinary system - The effects and inter-relationships of Phyllodistomum and other organisms infecting the mesonephros (with notes on other Gorgoderids).

Once Phyllodistomes, in Gasterosteus aculeatus and G. pungitius have entered the urinary system, they are entirely confined to areas lined by transitional epithelium which includes the mesonephric ducts, their tributaries and the bladder. The transitional epithelium to which the trematodes cling has an extensile nature and is reduced from a multi-layered, folded state to a flattened strip, a single cell deep, on dilation of the bladder or ducts. The cells glide over one another and are capable of considerable deformation without loss of function. The superficial cells are large, occasionally binuclear, and may be covered with microvilli as are the epithelial cells of the bladder of Dufo marinus (Bentley, 1966). Supporting the epithelium is a thin strip of connective tissue surrounded by muscle layers which basically vary in thickness and composition according to region. The latter layers gradually increase in width from the origin of the ducts in the kidney down towards the bladder, reaching their maximum around the bladder and "urethra". Distension or contraction of the bladder or lower parts of the ureters does, of course, alter the thickness of the respective layers.

Damage to the urinary system of a vertebrate usually produces changes which can be traced in some cases by direct observation or, more generally, by examination of tissue sections under the microscope. There are several pathological conditions which might be expected to occur as a result of Gorgoderid infection, particularly where large numbers of trematodes are involved. Sections of uninfected, lightly

infected (1 - 12 flukes present) and heavily infected material (40 flukes present) were studied in addition to the direct examinations made during dissection in an attempt to trace the effects which these trematodes were having upon their hosts. The results of their feeding habits; the degree of crowding; the extent of their migration; the length of the association and the toxicity and irritative quality of the parasites' waste products were some of the factors considered.

The greatest number of flukes recovered from a single Gasterosteus during this investigation was 97, but these were largely juveniles. They were taken from a mature female fish (Newdigate) measuring 6.25 cms in length. 40 trematodes occupied the bladder and the remaining 28 and 29 were recovered from the ureters and their tributaries. A dense infection which had produced more extensive pathological effects at the time of examination was recovered from a mature female fish (6.0 cms long) collected from the Essex area. 60 trematodes, ranging from a medium to a large size, were taken from the bladder and 12 large flukes were recovered from the ureters. In both cases permanent dilation of the urinary tracts was visible to the naked eye. In the first case, where the host possessed a large population of juveniles, the uretal tributaries could be seen to be swollen from the point where they joined the ureter along their passage amongst the kidney tissue as far as their second bifurcation. All other ducts retained a normal diameter. Where populations are composed of various numbers of larger flukes different degrees of swelling occur. A few, very large trematodes or a dense population of mixed sizes are capable of permanently dilating the ureters

for more than three-quarters of their length, while the tributaries and terminal portions of the main ducts remain normal. In such cases the bladder could also be permanently distended. The most common region to be affected, however, is the base of the ureters at the point where they enter the bladder and in infections where large trematodes are involved these swollen regions are frequently the only visible signs of damage. Occasionally fish were examined where the ureters were swollen locally at irregular intervals along their length but ^{this} was an unusual feature. Uretal dilation was also noted by Choquette (1943) in Salvelinus fontinalis infected by P. lachancei and in Amphibian hosts of Gorgodera amplicava by Goodchild (1948).

Theoretically, if the flukes were causing a permanent obstruction within the urinary ducts, the accumulation of urine would cause dilation. If this were the case, the muscle coats of the duct wall would show signs of hypertrophy. Since this muscular increase was never found it might be assumed that the trematodes, due to their mobility, were only causing an intermittent blockage, particularly in dense infections where flukes were continually passing over and past one another or turning round in the narrow confines of the ureters. If this was occurring the repeated variation in urinary pressure would eventually stretch the kidney tissue and this would atrophy. In addition, the transitional epithelial layer could be damaged by the release of injurious substances into the urine leading to irritation and epithelial sloughing. Removal of the epithelial layer, whether as the result of such irritation or the feeding habits of the flukes, would result in the underlying tissues being affected and marked host

reaction would be evident. Any interruption in the correct functioning of the kidneys, especially in long term association, might be demonstrated by a condition paralleling dropsy with an increase in the amount of fluid retained in the body tissues. This latter state was never noted in any age of fish carrying either a dense or a light infection for any period of time. Phyllodistomum-infected fish, from their external appearance, appeared to experience no inconvenience resulting from the presence of these parasites.

Interpretation of the pathological picture presented in the sections of infected fish kidney was made difficult by the presence of several unidentified bacterial infections as well as two myxosporidial genera Henneguya and Myxobolus. Some bacterial infections in Newdigate and Dushy Park stock initiated the formation of acid-soluble castes which were easily discernible in squash preparations of fresh kidney tissue. Crystals were also seen occasionally but were regarded as being insignificant because they can be formed under certain conditions when urine is allowed to stand. The presence of either castes or crystals was in no way related to the presence or absence of Phyllodistomum. Occasionally yellow patches of necrotic tissue were visible in the kidney on dissection and various patches of inflammatory host reaction were seen in sections which could not be related either to the trematode infection or the presence of Myxosporidia and were assumed to be associated with some form of bacterial infection. Signs of atrophy in the kidney tissue such as a large percentage of the Malpighian corpuscles in a contracted state and rendered non-functional

were never found. Swollen and greatly enlarged corpuscles were found in all the sections examined and this condition was not associated with trematode infection. The distended structures enclosing an eosinophilic vacuolate colloidal mass indicated the presence of a host antibody reaction.

Tell (1935) in his study of the parasitic fauna of fishes of Lake Vyrtsyarv confirmed the presence of antagonistic relations between the parasites of the urinary bladder of Esox lucius (Myxidium lieberkühni and Phyllodistomum folium). In this investigation Henneguya and Myxobolus occurred in all trematode-infected areas in both single and double infections. There did not appear to be such a relationship in nature between the trematodes and the protozoa in this case, although Phyllodistomum readily ingested Myxosporidial spores. Only a certain percentage of the fish from each area were tested for the presence of Myxosporidia. Of these in fish free from trematodes 76.92% carried Myxosporidia whilst the remaining 23.08% remained totally free from any infection. In fish infected by trematodes and examined for protozoan parasites, 62.5% carried Myxosporidia and 37.5% were uninfected. However, the 62.5% supported 274 Phyllodistomes whereas the 37.5% which were free from additional protozoan load only supported 120. At no time did the numerical density of the trematode infection bear any apparent regular relationship in nature to that of the protozoan population which, however, could only be measured by the comparative density of the spores recovered from kidney smears. In the laboratory, however, provided fish with a dense trematode population were kept for several

months an antagonistic relationship was revealed where without continual re-infection the Myxosporidial population could be steadily decreased or completely eradicated.

Thyllostomes are incapable of penetrating into the mesonephric tissue and migrating through it or of utilising it for nutritive purposes. The lack of atrophic mesonephros associated with these infections indicated, in addition, that the flukes were not causing any form of obstruction in the urinary passages even when large numbers of trematodes were involved. Dilation of the ducts, observed on dissection, was not the result of an increase in urinary pressure but the permanent effects of mechanical stretching of the tissue by the flukes. Primarily, the distance which a fluke may migrate is determined by its size, and this is the reason why the kidney tissue is not exploited. The flukes browse upon sloughed epithelial cells (a process discussed on page 206) and do not migrate into ducts where they experience difficulty of passage unless the infection is sufficiently dense to reduce the available food supply. They are also more active following the stimulation of contact between flukes and this may be a contributory factor controlling the rate at which their reserves are utilised. Mounted sections do not reveal clearly the reason why flukes do not cause some degree of obstruction in the urinary tubules; but observation of the living flukes in situ clarified the matter. In the heaviest infection recorded, 97 juveniles occupied the ureters and tributaries of a single Gasterosteus. In surface view some of the flukes could be seen apparently blocking the tributaries lying with the

anterior probe in one branch and the posterior region in another with the acetabulum at the junction. In the terminal main ducts the flukes were actively passing one over another arranged in series of three or four with individuals moving off into side branches or coming from them. Some were observed to reverse until they found sufficient room to allow them to turn round and then progress in the opposite direction. If the ducts were viewed end-on it could be seen that the flattened body of the flukes, although less pronounced at this stage than in the adult, prevented the ducts from being blocked. During their passage past one another (when they generally avoided clinging to another fluke), the separate extension of each region retained a system of spaces between the two opposed bodies which remained throughout the period of proximity. The swelling of the extremely fine branches was a mechanical effect resulting from repeated stretching during the passage of the wider posterior region after the finer probe. When flukes first come in contact with narrow passages which they cannot penetrate with ease, they usually only push the probe into them. Following the utilisation of most of the sloughed cells in the vicinity the fluke pushes further into these channels pulling the posterior region after them. This section of the body does not become cylindrical during this operation but curves over at the edges so that the through fluid system of the duct remains intact. (This position was also drawn by Ssinitzin (1945) for specimens of P. folium - whilst they occupied the urinary ducts.) After repeated journeys of this kind, the fine muscular layers of the ducts apparently lose some of their elasticity and retain a wider diameter.

Where fish were carrying older infections, dilated urinary ducts were not necessarily present, even though the flukes were considerably larger than the juveniles in the previous case. Older flukes are generally delimited to the bladder as a consequence of their size and a fish carrying one very large trematode or from 19 - 24 medium and small sized flukes at the time of examination would not necessarily have suffered from dilated ducts. The larger trematodes would remain in the bladder and the rest could easily migrate along the ureters and would find sufficient food available to obviate the necessity of probing into the constricted side channels. In infections composed of as many as 7 large trematodes, however, or mixed populations involving from 20 - 72 flukes, where a high percentage of the flukes are of large dimension, dilation of the ducts almost certainly occurs. Such expansion allows the larger fluke to considerably extend its browsing territory. Under these conditions several flukes of mixed sizes, when occupying widened uretal channels, can pass over one another at the one time. The larger flukes bend the plate-like posterior region over into the form of a slit ring whilst the smaller ones can pass onwards without touching the uretal walls. A system of spaces is retained between these moving bodies and no fluke was seen to force its way through and increase the chances of blockage. Each fluke tends to wait until a space presents itself before attempting to progress. In section, such enlarged regions would be expected to give a clear indication of flattening in the epithelial layer and thinned muscle coats with broken elastin fibrils in the thin sheet of areolar connective ^{tissue} below the epithelium. Interpretation of such sections,

however, is difficult. The flukes are stretching tissues which, according to their exact location, normally exhibit a range in extensibility. The permanency of such stretching is attained only after some considerable time and the slight increase in fibrous connective tissue seen in the ureter and bladder walls may indicate that there has been some inelastic repair tissue laid down as support. The muscle coats do not exhibit a readily detectable thinning as a result of mechanical stretching. It can only be concluded that much of the diameter increase is well within the capacity of the extensile tissue and that with the loss of some elasticity it can be accommodated. The parasite can apparently be contained so as to prevent irreparable damage which would give rise to degeneration. Broken elastin fibres were not detected (using Mallory's Aniline Blue where the fibres stain pink or yellow) but localised regions where the muscle fibrils appeared undulatory in outline despite a stretched epithelial layer were noted. Apparently these muscle layers were still functional since their thickness in section remained normal.

Flattening of the epithelial layer was again difficult to determine due to the extensile quality of the tissue. In swollen zones the epithelium appeared to be permanently flattened and a progressive increase towards the normal state could be traced as the narrower distal regions of the ureter were reached. Similar epithelial flattening was reported in Salvelinus fontinalis infected by P. lachancei by Choquette (1948). The epithelium, in this investigation, appeared to be utilised both by the flukes and by an unidentified intra-cellular

organism which occupied the portion of the epithelial cell close to the lumen and away from the cell nucleus. What appeared to be multiplicative stages occurred in well-defined capsules inside the cells and when the single spheroidal particles were passed out via a terminal pore the empty capsule was left as a clear area in the living host cell. The particles floated freely in the urine and were found in lumen of the kidney tubules beyond the transitional epithelium range. They were also found in the gut of Phyllodistomum where they could be seen together with sloughed epithelial cells in various stages of digestion.

On the outer surface of normal transitional epithelium there is a layer of dying cells which is regularly lost and replaced from below. It is amongst these sloughed cells that the Phyllodistomes browse and feed. When tracing these superficial, readily-detachable cells in an infected system by means of sections it is found that, in contrast to an uninfected system, their distribution is patchy and that the layer is absent in the immediate vicinity of the trematodes and around commonly frequented areas such as the ureteral bases. During progression the flukes normally hold the body away from the surface of the host's urinary system with the suckers as the only surface continuously in contact with substratum. In crowded conditions or whilst turning or progressing in a confined space, the fluke may brush off a few of the cells which remain attached to the body and can be seen on collection. The two suckers also tend to detach cells during locomotion. Cellular loss by these latter processes, however, is negligible, and the primary

means by which they are removed is associated with the browsing action of the oral sucker. Observation of living flukes in an unopened urinary system demonstrated how the flukes progressively cleared strip-like areas of superficial epithelial cells from around the anterior probe whilst remaining in one position and then moved on, eventually leaving a trail of raised rings where the suckers grasped the then inextensible tissue of the host. In section the quantity of material lifted into the sucker cavity during locomotion is seen to consist mainly of the epithelial layer and it is not damaged or broken by such extension. This layer always returns to its original position in the living host leaving no trace of the fluke's passage. It is possible that release of enzymic fluid from the gut onto the transitional surface layers may serve to further loosen the superficial cells so supporting the action of the muscular lip and oral sucker. The occasional release of material into the urine, however, is of too spasmodic an occurrence to form a regular part of the ingestion pattern. The products voided from the gut at these times probably represent metabolic waste which, in addition to the excretory products may, if toxic, induce the transitional epithelium by irritation to shed superficial material at a more rapid rate than is normally encountered. The quantities of irritative fluids would be relatively small, and even in dense infections the copious flow of the host urine would prevent the toxicity from reaching an adverse level. The transitional layer remains intact at all times so that any toxic materials cannot penetrate to the underlying tissues and a well-defined host reaction does not ensue.

The transitional epithelial layer in the Amphibian bladder has been shown to be metabolically active, being involved in glycolysis, DPN and citric acid cycles and to possess high enzymic content. (Bentley, 1966). The bladder lining in fish is probably somewhat similar histochemically. Sloughed cells from this layer contain the remains of nuclei and probably represent a reasonable source of breakdown products for the flukes particularly if the sloughing rate has been artificially increased. The necessity for such a source of simple products is evident from a study of the substances likely to be present in the urine. Teleosts produce on average 100 cc of hypotonic urine per kilogramme per day. Within this liquid there is a varying quantity of ions and simple substances such as amino acids, creatine, creatinine, etc., with the majority of the urea and ammonia produced (the fish are ammonotelic) being lost through the gills and less so via the urine. Its composition and PH will not fluctuate so violently as in mammals such as Man because the fish tend to feed almost continuously but the composition will still vary with the type of food. The osmotic concentration of the urine is never high and its fluctuations would cause no undue stress to the trematodes. Indeed Goodchild (1954) found in experiments carried out in vivo that twice the normal salt concentration normally encountered by Gorgoderia amplicava and Gorgoderina attenuata had no effect upon the flukes over a period of 3 - 7 days. Trematodes situated in amphibian bladders are probably more resistant to fluctuations in osmotic pressure than flukes adapted to Teleosts where the host is unable to absorb water from the bladder contents.

Trematodes living in Amphibian hosts are also provided with a higher concentration of urea since most Amphibia are ureotelic.

The only hormones with a molecular structure small enough to be able to enter the host urine in a possibly active state are steroids which might affect the population size and presumably, therefore, the longevity of the trematodes, in accordance with the report made by Lees and Bass (1960) and Lees (1962). Here it was suggested that the presence of the female hormone oestradiol in the fluids of the host led to a depression of the level of parasitisation which was particularly marked in the case of Gorgoderina vitelliloba.

Associated with this data was evidence of a seasonal fluctuation of numbers related to the sex of the host, its breeding condition and feeding activities. In this Phyllodistomum species such variations were not found to occur and any hormones present in the host's urine have no effect upon these trematodes.

Goodchild concluded (1954) that the metabolism of Gorgodera amplicava and Gorgoderina attenuata was of the euryanoxybiotic aerofermentous type but Odlaug (1955) suggested that it was near to oxybiosis due to the parasites' small size and flattened shape. In this species of Phyllodistomum the glycogen reserves did not appear to be remarkably high and this may reflect the fact that the bladder does not represent an entirely anaerobic situation for trematodes which are flattened to an even greater extent than either of the Gorgodera or Gorgoderina genera. Odlaug recorded that the glycogen reserves in species of the two latter genera were low when compared

with intestinal parasites but that they exceeded the value obtained for living parasites.

In conclusion as regards pathogenicity, it would appear that Phyllodistomum, even when infecting the host in large numbers, has few effects which cannot be compensated for by the tissue systems involved. This species approaches the commensal - rather than the parasitic- state and this is indicative of a long association.

As a result of the presence of comparatively weak suckers, it has been shown that these trematodes are unable to invade the mesonephric tissue. This does not apply to the two genera Gorgoderina and Gorgoderina which have been discovered embedded in the kidney tissue of their Amphibian hosts on several occasions. Odlaug reported in 1937 that Gorgoderina amplicava was capable of entering the mesonephric tissue as distinct from the ducts. All such forms he reported as being sexually immature. Goodchild in 1950 corroborated this discovery and found, in addition, that some specimens failing to extricate themselves and pass to the bladder, became encapsulated and remained in this position throughout their adult life. He observed that although both Gorgoderina amplicava and Gorgoderina attenuata were capable of entering the kidney tissue only the former seemed able to develop to maturity in this location. The capsule was eventually constructed internally of a tissue identical in form to that found within the urinary tracts following irritative hyperplasia and, although the encapsulated forms were altered in appearance from those inhabiting the bladder, by feeding upon the mucoid capsule contents seen in the gut caecae, and

The photomicrographs represent longitudinal sections of Phyllodistomum-infected kidneys of Gasterosteus aculeatus.

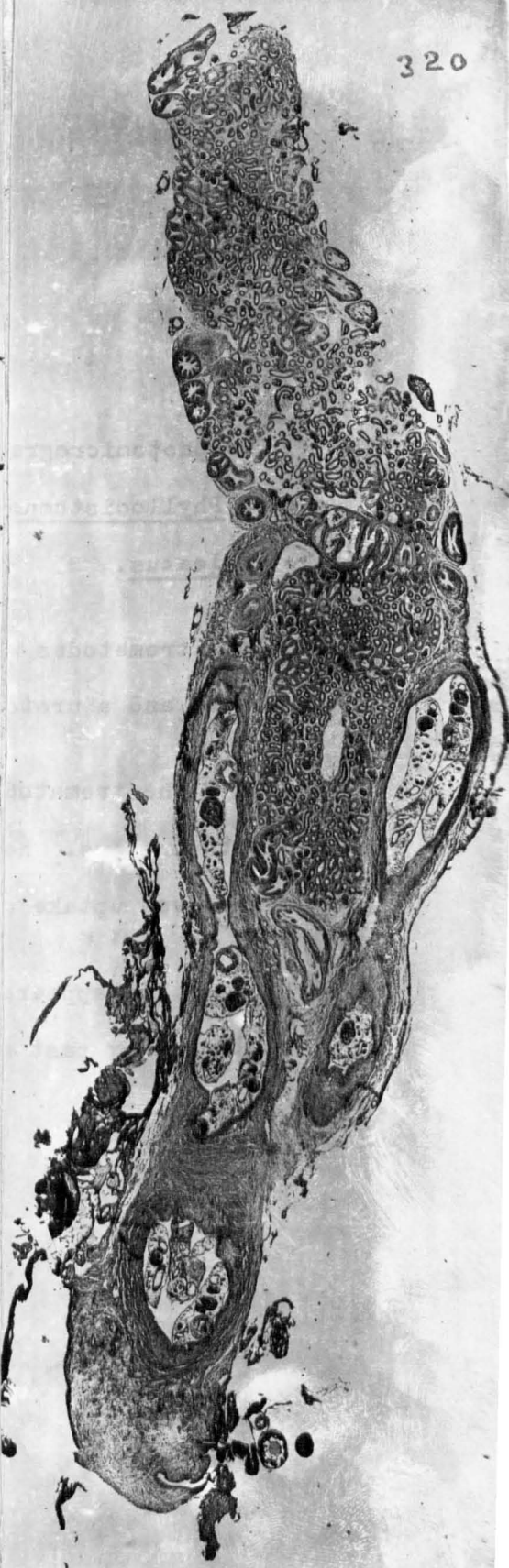
In A trematodes are situated in the bladder, ureteral bases and a ureter.

In B the trematodes are occupying the bladder and both ureters. Note the ventral sucker attachment involves uptake of epithelium only.

N.B. The appearance of the kidney tissue is somewhat altered by past and present Bacterial/Myxosporidial infections.



A



B

I suggest, the hyperplastic transitional epithelial cells lining the latter, the Gorgoderids survived to maturity.

Invasion of the mesonephric tissue by these Gorgoderid juveniles produced marked host reaction and in infections involving several hundred young trematodes, the hosts were adversely affected and a further increase in the numbers of flukes caused their death. Heavy influxes of these parasites apparently causes kidney failure. In this present investigation the suckers of the adult Gorgodera specimens were powerful enough to cause bruising to the living bladder wall of the frog. Whilst they were moving over its surface they caused dilation of the fine capillaries of the wall and occasionally burst these vessel leaving a trail of minute haemorrhages clearly visible under the binocular microscope. Although the frog bladder is a more delicate tissue than the urinary expansion found in Fish, the damage caused was undoubtedly largely the result of the presence of more powerful suckers.

SECTION 8 BRIEF NOTES CONCERNING PARASITES,
OTHER THAN PHYLLODISTOMUM, RECOVERED FROM
VERTEBRATES IN THE COURSE OF THE PRESENT

INVESTIGATION

(a) Method of investigation

The fish were examined as indicated on p. 179. The Microsporidia and Haplosporidia were recorded only when cysts were formed and tissue smears were not made in order to trace these Protozoa. The Myxosporidia were discovered as a result of examination of kidney tissue smears taken from a percentage of most of the monthly collections. No attempt was made to trace any Myxosporidia which may have been situated in other organs of the body. Bacterial infections were noted but not stained or identified. Apart from the infective organisms mentioned above a complete record was made of the parasites encountered during this investigation. Positive identification at the generic level was possible in most cases whilst specific classification is sometimes tentative, as indicated in the species list on pp. 324-6. An attempt was made to determine the characteristic parasitic fauna existing in the differing collection areas and to correlate the seasonal parasitic load with the life cycle of the host and the parasites, the degree of competition and the general effects of different parasitic populations upon the overall sizes attained by the hosts. Finally, the presence or absence of Phyllodistomum was taken into account.

(b) Results of the investigation

The species encountered are listed on pp. 324-326; the incidence of these parasites in the various areas is shown in Table 29, p. 327; this data is expanded into a consideration of the seasonal presence and absence of parasites on the basis of an area comparison in Table 30, p. 328; detailed percentage infections are given in Tables 31 and 32, pp. 329-330 and Table 33, p. 331 shows an additional record for fish hosts other than *Gasterosteus*. Tables 31 and 32 are re-arranged in Table 34 (p. 332) and illustrated in part in the graphs on pp. 334-340. The latter indicate the occurrence of selected infections throughout the year, area data being combined to supplement lack of information. The percentages given in some of the data can only be used as an indication of population trends, since the numbers of fish examined in some months, particularly where the area data is divided, was occasionally quite small (the actual numbers are given in Section 7B). A brief summary of the parasitic infections recovered from *Gasterosteus* spp. is given in tabular form on pp. 341 - 348.

Classification of parasitic forms (other than Gorgoderids) recovered
from fish during this investigation

- C. Mastigophora (Flagellata) [based on Kudo, 1954]
 SC. Phytomastigina
 O. Dinoflagellata Bütschli
 SO. Peridiniinea Poche
 T. Gymnodinioidae Poche
 F. Blastodiniidae Kofoid & Swezy
 G. Oodinium Chatton
- SC. Zoomastigina
 O. Polymastigina Blochmann
 SO. Monomonadina
 F. Tetramitidae Bütschli
 G. Costia Leclerque
- SP. Ciliophora Doflein, 1901 [based on Corliss, 1961]
 C. Ciliata Perty, 1852
 SC. Holotricha Stein, 1859
 O. Hymenostomatida Delage & Hérouard, 1896
 SO. Tetrahymenina Fauré-Fremiet in Corliss, 1956
 F. Ophryoglaenidae Kent, 1880
 G. Ichthyophthirius Fouquet
- SC. Holotricha Stein, 1859
 O. Gymnostomatida Bütschli, 1889
 SO. Cyrtophorina Fauré-Fremiet in Corliss, 1956
 F. Chlamyodontidae Stein, 1859
 G. Chilodonella Strand
- O. Peritrichida Stein, 1859
 SO. Mobilina Kahl, 1933
 F. Urceolariidae Dujardin, 1841
 G. Trichodina Ehr.
- C. Sporozoa [based on Kudo, 1954]
 SC. Cnidosporidia Doflein
 O. Myxosporidia Bütschli
 SO. Platysporea Kudo
 F. Myxobolidae Thélohan
 G. Myxobolus Bütschli (spp)
 G. Henneguya Thélohan
- O. Microsporidia Balbiani
 SO. Monocnidea Léger & Hesse
 F. Nosematidae Labbé
 G. Glugea Thélohan
Glugea anomala (Moniez)

C. Sporozoa

SC. Acnidosporidia Cépède

O. Haplosporidia Caullery & Mesnil

G. Dermocystidium PérezG. Dermocystidium gasterostei Elkan, 1962

P. PLATYHELMINTHES

[based on Dawes, 1946]

C. Trematoda

O. Monogenea

SO. Monopisthocotylea Odhner, 1912

SF. Gyrodactyloidea Johnston & Tiegs, 1922

F. Gyrodactylidae Cobbold, 1864

SF. Gyrodactylinae Monticelli, 1892 (amend. Johnston
& Tiegs, 1922)G. Gyrodactylus Nordmann, 1832 (spp.)

SO. Polyopisthocotylea Odhner, 1912

SF. Diclidophoroidea Price, 1936

F. Discocotylidae Price, 1936

SF. Discocotylinae Price, 1936

G. Diplozoon paradoxum Nordmann, 1832

O. Digenea

SO. Prosostomata Odhner, 1905

F. Diplostomatidae Poirier, 1886 amend.

SF. Diplostomatinae Monticelli, 1888

G. Diplostomulum metacercariaeG. Posthodiplostomulum (Neascus) metacercariae

F. Cyathocotylidae Poche, 1926

SF. Cyathocotylinae Mühling, 1898

G. Cyathocotyle Mühling, 1896 metacercariaeF. Echinostomatidae Looss, 1902 amend. Poche, 1926
or Stiles & Hassall, 1926SF. Echinostomatinae Looss, 1899 amend. Stiles &
Hassall, 1926

G. (?) rediae/cercariae/metacercariae

C. Cestoda

[based on Wardle & McLeod, 1952]

SC. Eucestoda

O. Pseudophyllidea Carus, 1863

F. Dibothriocephalidae Lühe, 1902

G. Ligula Bloch, 1782 - Ligula intestinalis Goeze, 1782
1782 (plerocercoids)G. Schistocephalus solidus Creplin, 1829 (plerocercoids)G. Diphyllobothrium Cobbold, 1858 (?) (plerocercoid)

SC. Eucestoda

- O. Pseudophyllidea Carus 1863
 - F. Triaenophoridae Loennberg, 1889 amend.
 - G. Triaenophorus Rudolphi, 1793
 - Triaenophorus nodulosus Pallas, 1760
- O. Proteocephala Wardle & McLeod, 1952
 - F. Proteocephalidae La Rue, amend. Woodland, 1933
 - SF. Proteocephalinae Mola, 1929
 - G. Proteocephalus filicollis Rudolphi, 1810
 - G. Proteocephalus sp.

P. ACANTHOCEPHALA

- O. Palaeacanthocephala
 - F. Echinorhynchidae
 - G. Echinorhynchus sp.

P. NEMATHELMINTHES

- C. Nematoda
 - SC. Phasmodia
 - O. Ascaroidea
 - F. Anisakidae encysted in abdominal cavity - larval stage
 - O. Strongyloidea - intestinal - males and females present

P. ARTHROPODA

- C. Crustacea
 - SC. Branchiura
 - F. Argulidae
 - G. Argulus foliaceus L.

P. MOLLUSCA

[according to A. E. Ellis, 1947]

- C. Lamellibranchiata
 - O. Eulamellibranchiata
 - SO. Schizodonta
 - SF. Unionacea
 - F. Unionidae
 - SF. Anodontinae
 - G. Anodonta Lamarck, 1799. (glochidia)

FUNGAE

- C. Oomycetes
 - F. Saprolegniaceae
 - G. Saprolegnia sp.

Table 29. The incidence of parasites (other than Gorgoderids) recovered from Gasterosteus species in the various collection areas.

| <u>Genus</u> | <u>Area</u> | | | | | |
|------------------------------|-------------|-------|------|----------|-------|---------|
| | BP(T) | E | H | N(T) | W | Ep. |
| <u>Oodinium</u> | - | - | - | p | - | - |
| <u>Costia</u> | p | - | - | p | p | - |
| <u>Ichthyophthirius</u> | - | - | - | p | - | p |
| <u>Chilodonella</u> | - | - | - | p | - | - |
| <u>Trichodina</u> | p | - | p | p | p | - |
| <u>Myxobolus</u> | p | p | - | p | p | ? |
| <u>Henneguya</u> | p | p | p | p | p | ? |
| <u>Glugea</u> | p | - | - | - | - | - |
| <u>Dermocystidium</u> | - | - | - | p | - | - |
| <u>Gyrodactylus</u> | p | p | p | p | p | - |
| <u>Diplozoön</u> | - | - | - | p | - | - |
| <u>Diplostomulum</u> (m) | p | - | - | - | p | - |
| <u>Echinostome</u> (m) | p | - | - | - | - | - |
| <u>Ligula</u> (p) | p | - | - | - | p | p |
| <u>Schistocephalus</u> | p | - | - | - | p | - |
| <u>Proteocephalus</u> | p | - | - | p | - | - |
| <u>Nematoda</u> | p | p | - | p | - | - |
| <u>Argulus</u> | p | p | - | - | - | - |
| <u>Glochidia</u> | p | p | - | p | - | - |
| <u>Saprolegnia</u> | p | - | - | p | p | - |
| TG = 20 | 15(+p) | 6(+p) | 3 | 14(+p) | 9(+p) | 2+?(+p) |
| (T) av. fish length (cms) | 3.12(T) | 4.81 | 4.93 | 5.15(T)* | 3.97 | (?) |

Abbreviations

- BP(T) Bushy Park (Total area - Systems A & B included in the record)
 E Essex area
 H Highams
 N(T) Newdigate (Total area - stream and pond fauna included in record)
 W Wanstead
 Ep Epping Forest
 (m) metacercariae recovered only
 (p) plerocercoids recovered only
 p parasite present
 - parasite absent
 ? kidneys not examined for Myxosporidia in this case
 (?) average length not known
 * signifies that only larger adults in the population were collected after a certain period - hence the greater average size
 T total
 TG total no. of genera recorded
 (+p) Phyllodistomum present in this area

Table 30. The seasonal presence and absence of certain parasites of Gasterosteus spp. (other than Gorgoderids). An area comparison.

| Area | J | F | M | A | M | J | J | A | S | O | N | D (months) | |
|-------|---|----|---|---|---|---|---|---|---|---|---|------------|----------------------------|
| BP(T) | O | ? | O | O | O | O | O | p | O | O | ? | O | <u>Costia</u> |
| N(T) | O | O | O | O | O | O | O | O | ? | p | O | O | |
| W | ? | ? | ? | ? | ? | ? | p | ? | ? | O | ? | ? | |
| N(T) | - | - | - | - | - | - | - | - | ? | p | - | - | <u>Ichthyophthirius</u> |
| Ep | ? | ? | ? | ? | ? | ? | ? | ? | p | ? | ? | ? | |
| BP(T) | O | ? | O | p | p | p | p | p | p | p | ? | p | <u>Trichodina</u> |
| N(T) | O | O | p | O | p | O | O | O | ? | p | O | O | |
| W | ? | ? | ? | ? | ? | ? | p | ? | ? | p | ? | ? | |
| H | ? | ? | ? | ? | ? | ? | O | ? | ? | p | ? | ? | |
| BP(T) | ? | ? | p | ? | p | p | p | ? | O | ? | ? | ? | <u>Myxobolus</u> |
| N(T) | O | O | p | O | O | O | O | p | ? | O | p | p | |
| W | ? | ? | ? | ? | ? | ? | p | ? | ? | p | ? | ? | |
| E | ? | p | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | |
| BP(T) | ? | ? | p | ? | p | p | p | ? | p | ? | ? | ? | <u>Henneguya</u> |
| N(T) | O | O | p | O | p | O | p | p | ? | O | p | p | |
| W | ? | ? | ? | ? | ? | ? | p | ? | ? | p | ? | ? | |
| H | ? | ? | ? | ? | ? | ? | p | ? | ? | O | ? | ? | |
| E | ? | p | ? | ? | ? | ? | ? | ? | ? | ? | O | ? | |
| BP(T) | O | ? | O | O | p | p | p | O | p | p | O | O | <u>Gyrodactylus</u> |
| N(T) | O | O | O | O | p | O | O | O | ? | O | O | O | |
| W | ? | ? | ? | ? | ? | ? | p | ? | ? | p | ? | ? | |
| H | ? | ? | ? | ? | ? | ? | O | ? | ? | p | ? | ? | |
| E | ? | p | ? | ? | ? | ? | ? | ? | ? | ? | O | ? | |
| BP(T) | O | ? | O | O | O | O | p | p | ? | O | O | p | <u>Diplostomulum (m)</u> |
| W | ? | ? | ? | ? | ? | ? | p | ? | ? | p | ? | ? | |
| BP(T) | O | ? | O | O | O | O | p | O | p | O | O | O | <u>Ligula (p)</u> |
| W | ? | ? | ? | ? | ? | ? | O | ? | ? | p | ? | ? | |
| BP(T) | O | ? | O | O | O | p | p | p | O | p | O | O | <u>Schistocephalus (p)</u> |
| W | ? | ? | ? | ? | ? | ? | p | ? | ? | p | ? | ? | |
| BP(T) | p | ? | p | O | p | O | p | p | p | p | O | p | <u>Proteocephalus</u> |
| N(T) | O | O | p | O | O | O | O | O | O | O | p | O | |
| BP(T) | p | ? | p | O | O | O | O | O | O | O | O | p | Nematodes |
| N(T) | O | O | O | O | O | O | O | p | ? | O | p | O | |
| E | ? | pe | ? | ? | ? | ? | ? | ? | ? | ? | p | ? | |
| BP(T) | p | ? | O | O | p | p | p | p | p | p | p | p | <u>Argulus</u> |
| E | ? | p | ? | ? | ? | ? | ? | ? | ? | ? | p | ? | |
| BP(T) | p | ? | p | p | p | p | O | O | O | O | O | O | <u>Glochidia</u> |
| N(T) | O | O | O | O | O | p | O | O | ? | O | O | O | |
| E | ? | p | ? | ? | ? | ? | ? | ? | ? | ? | p | ? | |
| BP(T) | O | ? | O | O | O | p | O | O | O | O | O | O | <u>Saprolegnia</u> |
| N(T) | p | p | p | O | O | O | O | O | ? | O | p | p | |
| W | ? | ? | ? | ? | ? | ? | O | ? | ? | p | ? | ? | |

Key

- BP(T) Bushy Park records for the total area including systems A and B
- N(T) Newdigate records for the total area including pond and stream fauna
- W Wanstead records
- H Highams records
- E Essex records
- p parasite present
- pe Nematode encysted in body cavity
- O parasite absent
- ?

Table 31. Seasonal incidence of parasites infecting Gasterosteus aculeatus and G.pungitius (other than Gorgoderids) in the Bushy Park area (Systems A & B inc.)

| Month | Ectoparasites | % of fish infected | Endoparasites | % of fish infected | Total no. parasites recovered |
|-----------------------|---------------------|--------------------|---------------------------|--------------------|-------------------------------|
| Nov.1961 ⁺ | <u>Argulus</u> | 20 | <u>Glugea</u> | 80 | |
| Dec.1961 ⁺ | <u>Trichodina</u> | 15.26 | <u>Glugea</u> | 84.21 | |
| | <u>Argulus</u> | 10.53 | <u>Diplostomulum(m)</u> | 21.05 | |
| Jan.1962 ⁺ | | | <u>Proteocephalus</u> | 20 | 2 |
| | <u>Glochidia</u> | 40 | <u>Nematodes</u> | 5.26 | 9 |
| | <u>Argulus</u> | 10 | <u>Glugea</u> | 80 | |
| | | | <u>Proteocephalus</u> | 5.26 | 4 |
| Feb.1962 | ? | ? | <u>Nematodes</u> | 5.26 | 1 |
| Mar.1962 | <u>Glochidia</u> | 63.64 | ? | ? | |
| | | | <u>Glugea</u> | 90.91 | |
| | | | <u>Henneguya</u> | 50* | |
| | | | <u>Myxobolus</u> | 50* | |
| | | | <u>Echinostomes(m)</u> | 18.18 | |
| | | | <u>Proteocephalus</u> | 9.09 | 3 |
| | | | <u>Nematodes</u> | 18.18 | 2 |
| Apr.1962 ⁺ | <u>Trichodina</u> | 100 | <u>Glugea</u> | 33.34 | |
| | <u>Glochidia</u> | 33.34 | | | |
| May 1962 | <u>Trichodina</u> | 92.85 | <u>Glugea</u> | 64.28 | |
| | <u>Gyrodactylus</u> | 14.28 | <u>Henneguya</u> | 88.89* | |
| | <u>Argulus</u> | 85.71 | <u>Myxobolus (spp)</u> | 88.89* | |
| | | | <u>Proteocephalus</u> | 7.14 | 1 |
| Jun.1962 | <u>Trichodina</u> | 95.84 | <u>Glugea</u> | 12.50 | |
| | <u>Gyrodactylus</u> | 8.34 | <u>Henneguya</u> | 50* | |
| | <u>Glochidia</u> | 4.16 | <u>Myxobolus</u> | 50* | |
| | <u>Argulus</u> | 37.5 | <u>Schistocephalus(p)</u> | 12.5 | 2 |
| | <u>Saprolegnia</u> | 4.17 | | | |
| | | | | | |
| Jul.1962 | <u>Trichodina</u> | 94.78 | <u>Glugea</u> | 35.41 | |
| | <u>Gyrodactylus</u> | 17.02 | <u>Henneguya</u> | 100* | |
| | <u>Argulus</u> | 50 | <u>Myxobolus</u> | 100* | |
| | | | <u>Diplostomulum(m)</u> | 36.36** | |
| | | | <u>Schistocephalus(p)</u> | 69.23** | 14 |
| | | | <u>Ligula (p)</u> | 12.5** | 1 |
| | | | <u>Proteocephalus</u> | 29.16 | 40 |
| Aug.1962 ⁺ | <u>Trichodina</u> | 100 | <u>Glugea</u> | 21.42 | |
| | <u>Costia</u> | 7.14 | <u>Diplostomulum(m)</u> | 14.28** | |
| | <u>Argulus</u> | 14.28 | <u>Schistocephalus(p)</u> | 7.14 | 1 |
| | | | <u>Proteocephalus</u> | 28.57 | 6 |
| Sep.1962 | <u>Trichodina</u> | 100 | <u>Glugea</u> | 100 | |
| | <u>Gyrodactylus</u> | 36.36 | <u>Henneguya</u> | 100* | |
| | <u>Argulus</u> | 72.73 | <u>Ligula (p)</u> | 9.09** | 1 |
| | | | <u>Proteocephalus</u> | 36.36 | 12 |
| Oct.1962 ⁺ | <u>Trichodina</u> | 75 | <u>Glugea</u> | 50 | |
| | <u>Gyrodactylus</u> | 100 | <u>Schistocephalus(p)</u> | 25** | 2 |
| | <u>Argulus</u> | 75 | <u>Proteocephalus</u> | 50 | 7 |

Abbreviations and symbols

+ kidneys not examined for Myxosporidia in these months

* % of fish examined for this condition (not necessarily a % of the total sample for the month)

** % of fish old enough to carry the infection, e.g. Schistocephalus, Ligula and Diplostomulum were not recovered from fish measuring up to a total length of 2.5 cms. Fish of this size were therefore not included in the data for these genera. Small proteocephalidean tapes (P.filicollis) were recovered from fish measuring as little as 2 cms in length and the % readings for this genus are based upon the total sample for each month.

(p) plerocercoids recovered

(m) metacercariae recovered

(spp.) more than one species recovered

Nematodes male and female strongyloids recovered from the stomach and intestine

Table 32. Seasonal incidence of parasites infecting Gasterosteus aculeatus (other than Gorgoderids) in the Newdigate area

| Month | Ectoparasites | | | Endoparasites | | | | |
|------------------------|--------------------------------|--|--------------------------|--------------------|--|--|--|--------------------|
| | Pond fauna (various locations) | % of fish infected (no. of parasites recovered in parenthesis) | Stream fauna | % of fish infected | Pond fauna (various locations) | % of fish infected (no. of parasites recovered in parenthesis) | Stream fauna | % of fish infected |
| Oct. 1961 ⁺ | <u>Ichthyophthirius</u> | 87.5 | ? | ? | - | - | ? | ? |
| | <u>Diplozoon</u> | 12.5 (1 pr) | ? | ? | - | - | ? | ? |
| Nov. 1961 ⁺ | - | - | ? | ? | - | - | ? | ? |
| Dec. 1961 ⁺ | - | - | ? | ? | - | - | ? | ? |
| Jan. 1962 ⁺ | <u>Chilodonella</u> | 31.82 | ? | ? | <u>Dermocystidium</u> | 81.82 | ? | ? |
| Feb. 1962 ⁺ | <u>Chilodonella</u> | 50 | ? | ? | <u>Dermocystidium</u> | 100 | ? | ? |
| Mar. 1962 | <u>Trichodina</u> | 42.10 | ? | ? | <u>Dermocystidium</u> | 80 | ? | ? |
| | <u>Chilodonella</u> | 35 | ? | ? | <u>Henneguya</u> | 58.34* | ? | ? |
| | | | | | <u>Myxobolus</u> | 41.67* | ? | ? |
| | | | | | unknown Myxosporidial infection (large round cysts in body wall) | 8.34 | ? | ? |
| | | | | | <u>Proteocephalus</u> (1) | 4.76 | ? | ? |
| Apr. 1962 ⁺ | - | - | ? | ? | <u>Dermocystidium</u> | 100 | ? | ? |
| May 1962 | - | - | ? | ? | <u>Henneguya</u> | 100* | ? | ? |
| Jun. 1962 | - | - | ? | ? | Bacterial inf. (large cysts in body wall - containing rods) | 100 | ? | ? |
| Jul. 1962 | - | - | ? | ? | <u>Henneguya</u> | 33.34* | ? | ? |
| | | | | | <u>Dermocystidium</u> | 100 | ? | ? |
| Aug. 1962 | ? | ? | Bacterial inf. (fin rot) | 5.88 | ? | ? | <u>Henneguya</u> (spp.)* | 16.67 |
| | | | | | | | <u>Myxobolus</u> (spp.)* | 83.34 |
| | | | | | | | Nematodes (5) | 11.76 |
| Sep. 1962 | ? | ? | ? | ? | ? | ? | ? | ? |
| Oct. 1962 | <u>Chilodonella</u> | 66.67 | <u>Trichodina</u> | 30 | <u>Dermocystidium</u> | 100 | ? | ? |
| | <u>Costia</u> | 33.34 | | | | | | |
| | <u>Diplozoon</u> | 12.50 | | | | | | |
| Nov. 1962 | <u>Chilodonella</u> | 40 | <u>Chilodonella</u> | 88.89 | <u>Dermocystidium</u> | 60 | <u>Oodinium</u> | 50 |
| | <u>Saprolegnia</u> | 100 | <u>Saprolegnia</u> | 11.12 | <u>Henneguya</u> | 50* | <u>Henneguya</u> | 100* |
| | | | | | <u>Proteocephalus</u> (1) | 20 | <u>Myxobolus</u> | 100* |
| | | | | | Nematoda (2) | 20 | | |
| Dec. 1962 | <u>Chilodonella</u> | 100 | <u>Chilodonella</u> | 100 | <u>Dermocystidium</u> | 100 | <u>Henneguya</u> | 66.67* |
| | <u>Saprolegnia</u> | 100 | <u>Saprolegnia</u> | 25 | | | <u>Myxobolus</u> | 33.34 |
| Jan. 1963 | <u>Saprolegnia</u> | 100 | <u>Chilodonella</u> | 66.67 | <u>Dermocystidium</u> | 100 | unknown microsporidia found in kidneys | 100* |
| | | | <u>Saprolegnia</u> | 33.34 | | | <u>Oodinium</u> | 50 |
| Feb. 1963 | ? | ? | <u>Saprolegnia</u> | 66.67 | ? | ? | <u>Henneguya</u> | 100* |
| Mar. 1963 | ? | ? | <u>Chilodonella</u> | 33.34 | ? | ? | | |
| | | | <u>Saprolegnia</u> | 50 | | | | |
| Apr. 1963 | ? | ? | ? | ? | ? | ? | ? | ? |
| May 1963 | - | - | <u>Trichodina</u> | 58.34 | - | - | <u>Henneguya</u> | 58.34 |
| | | | <u>Gyrodactylus</u> | 58.34 | | | | |
| | | | <u>Glochidia</u> | 66.67 | | | | |

Seasonal incidence of parasites infecting Gasterosteus aculeatus (other than Gorgoderids) in the Essex area

| Month | Ectoparasites | % of fish infected | Endoparasites | % of fish infected | Total no. of parasites recovered |
|------------------------|---------------------|--------------------|---|--------------------|----------------------------------|
| Nov. 1961 ⁺ | <u>Glochidia</u> | 9.09 | Nematodes | 4.54 | 2 |
| | <u>Argulus</u> | 9.09 | | | |
| Feb. 1962 | <u>Gyrodactylus</u> | 18.75 | <u>Henneguya</u> | 27.27* | - |
| | <u>Glochidia</u> | 3.13 | <u>Myxobolus</u> | 9.09* | - |
| | <u>Argulus</u> | 37.50 | unknown microsporidial infection - cysts in body wall | 5.56 | - |
| | | | Nematode (Ascaroid - encysted larva) | 3.12 | 1 |

Seasonal incidence of parasites infecting Gasterosteus aculeatus (other than Gorgoderids) in the Wanstead area

| Month | Ectoparasites | % of fish infected | Endoparasites | % of fish infected | Total no. of parasites recovered |
|-----------|---------------------|--------------------|----------------------------|--------------------|----------------------------------|
| Jul. 1962 | <u>Trichodina</u> | 80 | <u>Henneguya</u> | 100* | |
| | <u>Costia</u> | 20 | <u>Myxobolus</u> | 100* | |
| | <u>Gyrodactylus</u> | 50 | <u>Diplostomulum</u> (m) | 100 | |
| Oct. 1962 | <u>Trichodina</u> | 71.42 | <u>Schistocephalus</u> (p) | 90 | 12 |
| | <u>Gyrodactylus</u> | 14.28 | <u>Henneguya</u> | 66.67* | |
| | <u>Saprolegnia</u> | 14.28 | <u>Myxobolus</u> | 66.67* | |
| | | | <u>Diplostomulum</u> (m) | 100 | |
| | | | <u>Schistocephalus</u> (p) | 28.57 | 4 |
| | | | <u>Ligula</u> (p) | 85.71 | 36 |

Seasonal incidence of parasites infecting Gasterosteus aculeatus (other than Gorgoderids) in the Highams area

| Month | Ectoparasites | % of fish infected | Endoparasites | % of fish infected | Total no. of parasites recovered |
|-----------|---------------------|--------------------|---|--------------------|----------------------------------|
| Jul. 1962 | - | - | unknown species of microsporidial infection (round cysts in body wall) | 20 | |
| | | | <u>Henneguya</u> | 100* | |
| Oct. 1962 | <u>Trichodina</u> | 100 | unknown species of microsporidial infection (identical to inf. found in July) | 25 | |
| | <u>Gyrodactylus</u> | 50 | <u>Henneguya</u> | 100* | |
| | Bacterial fin rot | 20 | | | |

Abbreviations and symbols

- + kidneys not examined for Myxosporidia in these months
- * % of fish examined for this condition (not necessarily a percentage of the total sample for the month)
- (p) plerocercoid recovered
- (m) metacercariae recovered
- ? no fauna examined in that month from that region
- ecto- or endo-parasites absent

Table 33. Seasonal incidence of parasites of fish other than Gasterosteus spp.

| Area | Month | Host | Ecto- parasites recovered | % fish infected | No. of parasites recovered | Endo- parasites recovered | % fish infected | No. of parasites recovered |
|----------------------------|------------------------|---|---------------------------------|--------------------|----------------------------------|--|--------------------|----------------------------------|
| <u>A. Staines Aquaduct</u> | | | | | | | | |
| | June 1962 ⁺ | Perch - <u>Perca fluviatilis</u> | - | - | - | <u>Cyathoco- tyle (m)</u> | 93.34 | - |
| | | | | | | <u>Echino- rhynchus</u> | 66.67 | 33 |
| | | | | | | <u>Proteo- cephalus</u> | 53.34 | 34 |
| | | Bream - <u>Abramis brama</u> | - | - | - | <u>Cyathoco- tyle (m)</u> | 33.34 | - |
| | | Roach - <u>Rutilus rutilus</u> | - | - | - | <u>Diphyllo- bothrium(p)</u> | 7.14 | 1 |
| <u>B. Newdigate</u> | | | | | | | | |
| | Nov. 1961 ⁺ | Minnow - <u>Phoxinus phoxinus</u> | <u>Diplozoon</u> | 57.14 | 17 pr. | Microsporidial cysts [<u>Glugea(?)</u>] | 28.57 | - |
| | | | ' <u>Neascus</u> '(m) | 42.85 | 53 | | | |
| | Dec. 1961 ⁺ | | <u>Diplozoon</u> | 100 | 30 pr. | Nematode (Ascaroid larva encysted) | 16.67 | 1 |
| | | | ' <u>Neascus</u> '(m) | 66.67 | 77 | | | |
| | May 1963 ⁺ | | Bacterial fin rot | 100 | - | - | - | - |
| <u>C. Bushy Park</u> | | | | | | | | |
| | Apr. 1962 ⁺ | Dace - <u>Leucis- cus leuciscus</u> | <u>Trichodina</u> | 100 | - | - | - | - |
| | | | <u>Gyrodactylus</u> | 100 | - | - | - | - |
| | | Minnow - <u>Phox- inus phoxinus</u> | <u>Trichodina</u> | 100 | - | - | - | - |
| | | | <u>Gyrodactylus</u> | 50 | - | - | - | - |
| | | | <u>Glochidium</u> | 50 | 1 | - | - | - |
| | July 1962 ⁺ | Roach - <u>Rutilus rutilus</u> | ' <u>Neascus</u> ' | 100 | 20 | - | - | - |
| <u>D. Epping Forest</u> | | | | | | | | |
| | Oct. 1961 ⁺ | Roach - <u>Rutilus rutilus</u> | - | - | - | <u>Ligula(p)</u> | 20 | 6 |
| | | | - | - | - | Nematodes | 20 | - |
| | | Gudgeon - <u>Gobio gobio</u> | - | - | - | - | - | - |
| <u>E. Essex</u> | | | | | | | | |
| | Feb. 1962 ⁺ | Carp - <u>Carassius carassius</u> | - | - | - | Microsporidial inf. unidentified | 50 | - |
| <u>F. Windermere</u> | | | | | | | | |
| | Nov. 1961 ⁺ | Pike - <u>Esox lucius</u> | <u>Gyrodactylus</u> | 12.5 | - | <u>Trienophorus</u> | 75 | - |

Key (p) plerocercoid phase
(m) metacercariae
+ kidneys not examined for Myxosporidia

Table 34. The seasonal variation in the percentage of Gasterosteus species infected by selected parasites other than Gorgoderids

TRICHODINA

| Area | J | F | M | A | M | J | J | A | S | O | N | D | Total |
|-------|---|---|-------|-----|-------|-------|-------|-----|-----|-------|---|-------|-------|
| BP(T) | - | ? | - | 100 | 92.85 | 95.84 | 94.78 | 100 | 100 | 75 | ? | 15.26 | 71.16 |
| N(T) | - | - | 42.10 | - | 53.84 | - | - | - | ? | 23.08 | - | - | 11.89 |
| H | ? | ? | ? | ? | ? | ? | - | ? | ? | 100 | ? | ? | 26.67 |
| W | ? | ? | ? | ? | ? | ? | 80 | ? | ? | 71 | ? | ? | 76.47 |

MYXOBOLUS

| | | | | | | | | | | | | | |
|-------|---|------|-------|---|-------|----|-----|-------|---|-------|-------|-------|-------|
| BP(T) | ? | ? | 50 | ? | 88.89 | 50 | 100 | ? | - | ? | ? | ? | 40* |
| N(T) | - | - | 41.67 | - | - | - | - | 83.34 | ? | - | 33.34 | 22.23 | 22.23 |
| W | ? | ? | ? | ? | ? | ? | 100 | ? | ? | 66.67 | ? | ? | 75* |
| E | ? | 9.09 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 9.09* |

HENNEGUYA

| | | | | | | | | | | | | | |
|-------|---|-------|-------|---|-------|----|-------|-------|-----|-------|-------|-------|--------|
| BP(T) | ? | ? | 50 | ? | 88.89 | 50 | 100 | ? | 100 | ? | ? | ? | 45* |
| N(T) | - | - | 66.67 | - | 61.54 | - | 33.34 | 16.67 | ? | - | 66.67 | 66.67 | 39.65* |
| W | ? | ? | ? | ? | ? | ? | 100 | ? | ? | 66.67 | ? | ? | 75* |
| H | ? | ? | ? | ? | ? | ? | 100 | ? | ? | - | ? | ? | 33.34* |
| E | ? | 27.27 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | 27.27* |

COSTIA

| | | | | | | | | | | | | | |
|-------|---|---|---|---|---|---|----|------|---|------|---|---|-------|
| BP(T) | - | ? | - | - | - | - | - | 7.14 | - | - | - | - | 0.61 |
| N(T) | - | - | - | - | - | - | - | - | ? | 8.34 | - | - | 2.03 |
| W | ? | ? | ? | ? | ? | ? | 20 | ? | ? | - | ? | ? | 11.76 |

CHILODONELLA

| | | | | | | | | | | | | | |
|------|----|----|-------|---|---|---|---|---|---|-------|-------|----|-------|
| N(T) | 36 | 50 | 34.34 | - | - | - | - | - | ? | 15.38 | 71.43 | 80 | 34.31 |
|------|----|----|-------|---|---|---|---|---|---|-------|-------|----|-------|

GLUGEA (records based upon the presence of visible cysts)

| | | | | | | | | | | | | | |
|-------|----|---|-------|-------|-------|------|-------|-------|-----|----|----|-------|-------|
| BP(T) | 80 | ? | 90.91 | 33.34 | 64.28 | 12.5 | 35.41 | 21.42 | 100 | 50 | 80 | 84.21 | 48.15 |
|-------|----|---|-------|-------|-------|------|-------|-------|-----|----|----|-------|-------|

DERMOCYSTIDIUM (records based upon the presence of visible cysts)

| | | | | | | | | | | | | | |
|------|-------|-----|----|-----|---|---|-----|---|---|-----|----|-----|-------|
| N(P) | 86.95 | 100 | 80 | 100 | - | - | 100 | ? | ? | 100 | 60 | 100 | 45.45 |
|------|-------|-----|----|-----|---|---|-----|---|---|-----|----|-----|-------|

GYRODACTYLUS

| | | | | | | | | | | | | | |
|-------|---|-------|---|---|-------|------|-------|---|-------|-------|---|---|-------|
| BP(T) | ? | - | - | - | 14.28 | 8.34 | 17.02 | - | 36.36 | 100 | - | - | 12.34 |
| N(T) | - | - | - | - | 53.84 | - | - | - | ? | - | - | - | 4.73 |
| H | ? | ? | ? | ? | ? | ? | ? | ? | ? | 50 | ? | ? | 13.34 |
| W | ? | ? | ? | ? | ? | ? | 50 | ? | ? | 14.28 | ? | ? | 35.29 |
| E | ? | 18.75 | ? | ? | ? | ? | ? | ? | ? | ? | - | ? | 11.12 |

PROTEOCEPHALUS

| | | | | | | | | | | | | | |
|-------|------|---|------|---|------|---|-------|-------|-------|----|----|----|-------|
| BP(T) | 5.26 | ? | 9.09 | - | 7.14 | - | 29.16 | 28.57 | 36.36 | 50 | - | 20 | 16.17 |
| N(T) | - | - | 4.76 | - | - | - | - | - | - | - | 20 | - | 2.25 |

ARGULUS

| | | | | | | | | | | | | | |
|-------|----|------|---|---|-------|------|----|-------|-------|----|------|-------|-------|
| BP(T) | 10 | ? | - | - | 85.71 | 37.5 | 50 | 14.28 | 72.73 | 75 | 20 | 10.53 | 37.65 |
| E | ? | 37.5 | ? | ? | ? | ? | ? | ? | ? | ? | 9.09 | ? | 25.92 |
| Sizes | L | L | ? | ? | LS | MLS | ML | MLS | MLS | ML | L | L | |

GLOCHIDIA

| | | | | | | | | | | | | | |
|-------|----|------|-------|-------|-------|------|---|---|---|---|------|---|------|
| BP(T) | 40 | ? | 63.64 | 33.34 | 7.14 | 4.16 | - | - | - | - | - | - | 9.10 |
| N(T) | - | - | - | - | 61.54 | - | - | - | - | - | - | - | 5.40 |
| E | ? | 3.13 | ? | ? | ? | ? | ? | ? | ? | ? | 9.09 | ? | 5.56 |

N.B. On occasions the monthly sample of fish was small, so that the resulting percentage can be taken only as an indication of the probable result

KEY
 BP(T) Bushy Park area - systems A and B included
 N(T) Newdigate area - stream and pond fauna included
 N(P) Newdigate area - pond fauna only
 H Highams area record
 W Wanstead area record
 E Essex area record
 ? monthly record unavailable
 - parasite absent

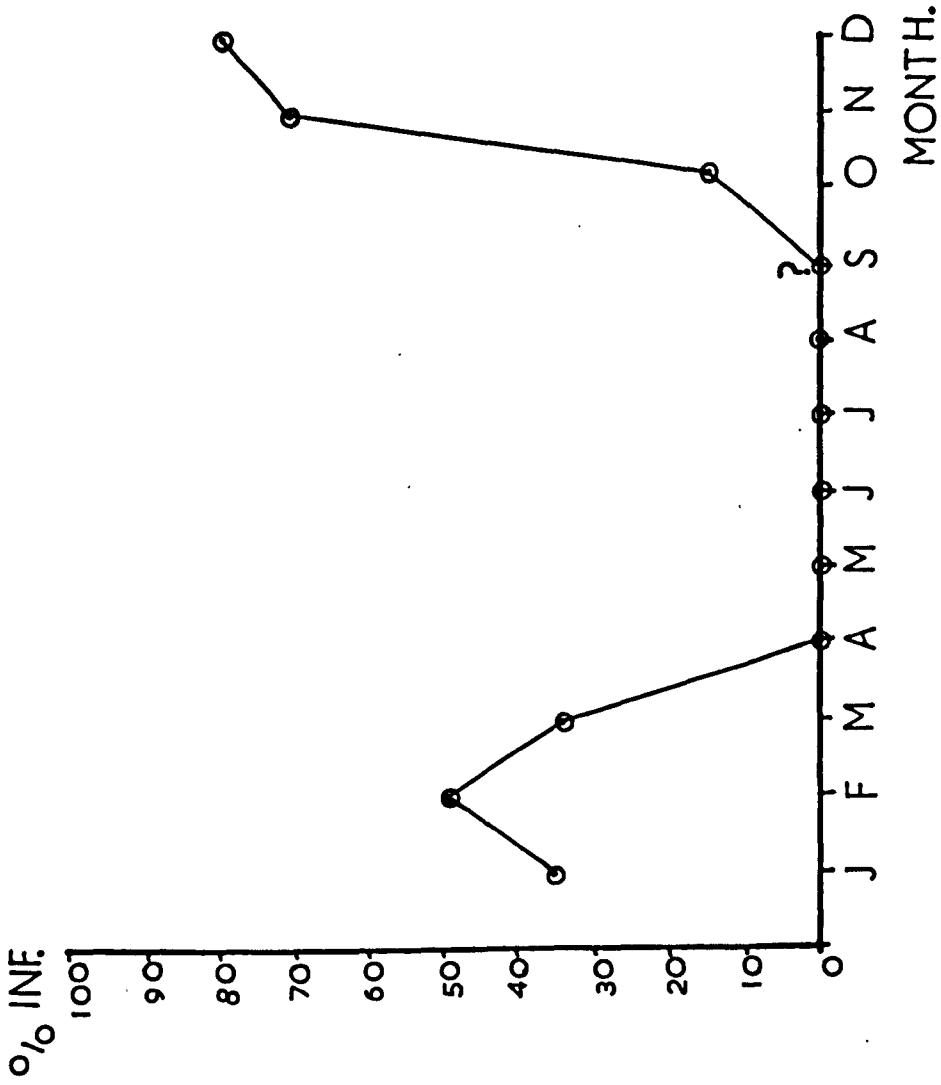
* percentage of fish tested for this condition. Figures not necessarily based on the total monthly sample
Argulus L, large adults; S, small juveniles; M, medium sized specimens

523

The seasonal variation in the percentage of Gasterosteus spp. infected by Chilodonella sp. in the Newdigate area

The graph illustrates Table 34, p. 332, and portrays the typical cycle assumed by this parasite with the numbers increasing in the winter months. The period covered by the graph includes average figures collected during October 1961 - May 1963, arranged according to the seasons.

(? = month when no sample was taken)

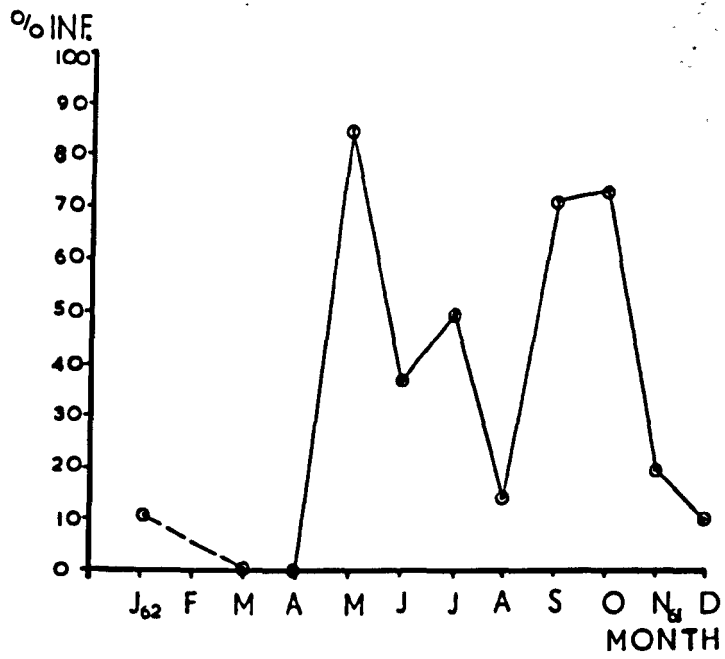


CHILODONELLA SP. ~ SURREY.

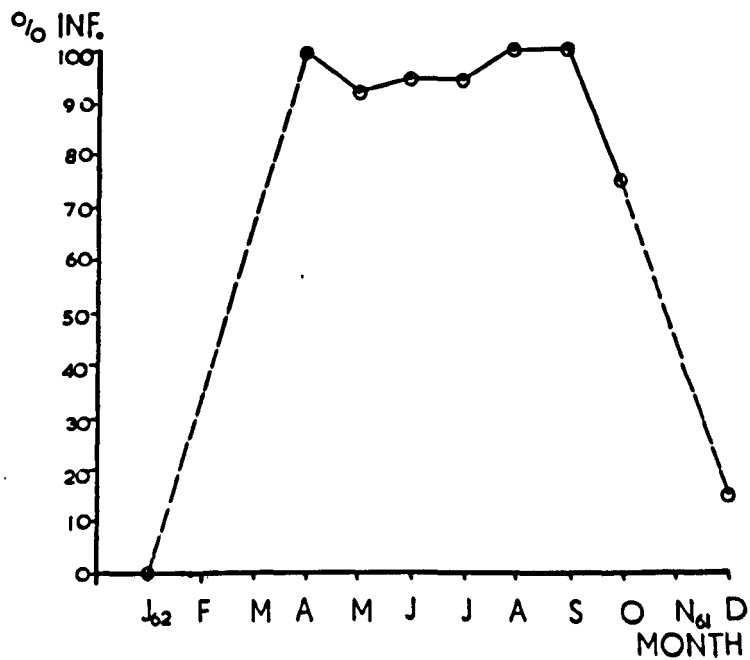
323

The seasonal variation in the percentage of Gasterosteus spp. infected by Argulus foliaceus and Trichodina sp. in the Bushy Park area.

An illustration of Table 34, p.332. In the case of Trichodina the typical cycle was obscured by local conditions so that a high percentage of the fish were infected during the summer when there was a dense fish population and the rate of water flow was lowered. The parasitic density per host, however, tended to increase in the cooler months. Both graphs cover the period November, 1961 - October, 1962, arranged according to the seasons.



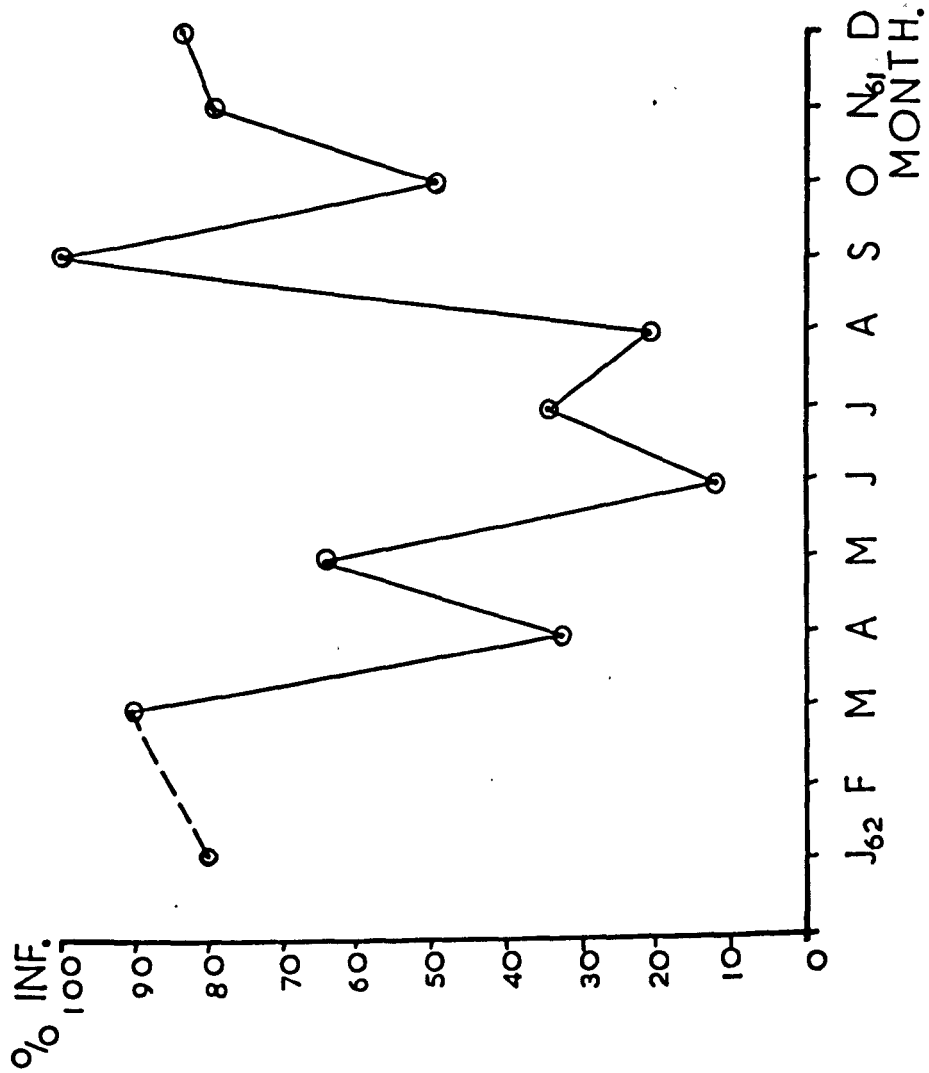
ARGULUS SP. - BUSHY PARK.



TRICHODINA SP. - BUSHY PARK.

The seasonal variation in the percentage of Gasterosteus spp. infected by Glugea anomala in the Bushy Park area

An illustration of Table 34, p.332. The records are based upon the presence of visible cysts and cover the period November 1961 - October 1962 arranged according to the seasons.



GLUGEA SP. ~ BUSHY PARK.

339

The seasonal variation in the percentage of Gasterosteus spp. infected by Proteocephalus filicollis in the Bushy Park area.

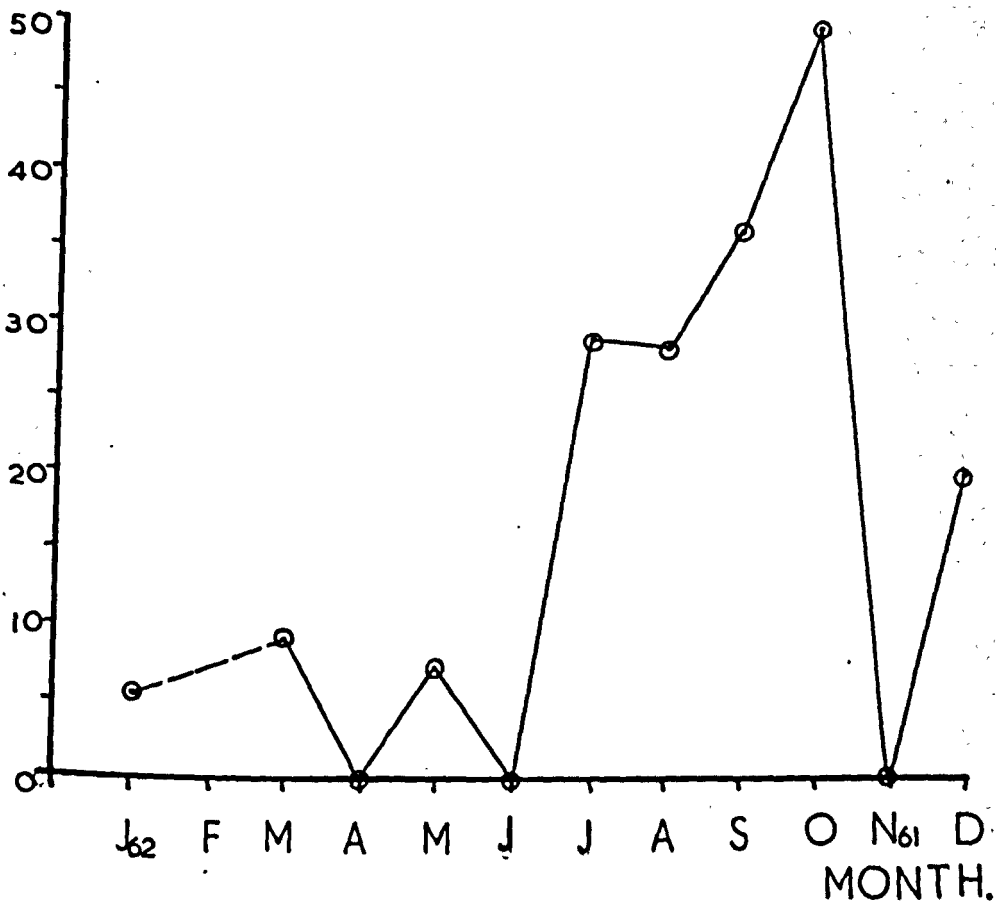
The graph is an illustration of Table 34, page 332.

Abbreviations:

% inf. = the percentage of fish to be infected by the parasite.

The results are arranged according to the season and run from November, 1961 - October, 1962.

% INF.



PROTEOCEPHALUS ~ BUSHY PARK.

Parasites recovered from Gasterosteus spp.

| Genus | Site of infection | Mode of nutrition | Observed effects upon the host: symptoms and reactions | Life cycle note | Remarks and observations |
|-----------------|--|--|---|--|--|
| <u>Oodinium</u> | Attached to skin of body and tail - also fins. An ectoparasite with endoparasitic tendencies | Penetrates skin by means of pseudopodia; digests and absorbs hosts' tissues and fluids | Loss of glossy skin imparts a soft yellow appearance. In one case abnormal behaviour was noted and the host was put to death following pronounced twisting whilst swimming and in obvious distress. Other infected stock died as a result of this infection | Division takes place after the parasite has left the host | Recovered on two occasions and only during the winter months: Newdigate stream in November and February |
| <u>Costia</u> | Attached to gills, skin and fins. True ectoparasitic form | Feeds upon epithelial cells and mucus | Host stimulated to increase mucus production and this may lead to asphyxia. Epithelial losses occur | Rapid re-production by favoured by higher temperatures. (Cyst formation in adverse conditions) | Although obtained from 3 areas, a comparatively rare parasite. Recovered in the months of July, August & October either as a single infection or associated with <u>Chilodonella</u> or <u>Trichodina</u> . Attained maximum density when associated with a heavy infection of <u>Trichodina</u> (possibly the increased destruction of tissue favoured this parasite [?]) |

Parasites recovered from Gasterosteus spp.

| Genus | Site of infection | Mode of nutrition | Observed effects upon the host: symptoms and reactions | Life cycle note | Remarks and observations |
|--------------------------------------|---|---|--|---|--|
| <u>Ichthyophthirius</u> | Beneath epithelium of gills, fins and skin. Skin parasite but endoparasitic | Burrows through tissue of the skin, absorbing nutrients. Does not penetrate sub-cutis | Causes White Spot disease, so called because the adult parasite (encysted in granular and mucoid areas of skin) shows up as a white spot. Gills necrotic with increased mucus content in opercular cavity. Fins ragged | Rapid division in cysts attached to submerged objects in the water. Higher temperatures are optimum for development | Recovered spasmodically from 2 areas (Newdigate ponds and Epping Forest). Probably from sluggish waters - not associated with any other skin parasites. Recovered in September and October |
| <u>Chilodonella</u> (graph p.334) | Attached to gills, fins, skin. True ectoparasitic form | Feeds on mucus and sloughed epithelial cells | Excessive mucus production. In the gill region: this caused asphyxia and resulted in the host's death. Fish appeared blue-white in hue - epithelium deteriorated and secondary infections of <u>Saprolegnia</u> were encountered | Reproduces at low winter temperatures. Rarely recovered in summer when many ciliates die | Characteristic of ponds and streams of Newdigate where the typical cycle is shown with the parasite apparently disappearing in the summer months. This parasite was responsible for the death of fish when associated with <u>Trichodina</u> and <u>Dermocystidium</u> and in addition when it was represented as a single infection |

Parasites recovered from Gasterosteus spp.

| Genus | Site of infection | Mode of nutrition | Observed effects upon the host: symptoms and reactions | Life cycle note | Remarks and observations |
|-------------------------------------|---|--|--|---|--|
| <u>Trichodina</u> (graph p. 336) | Skin, gills, fins. True ectoparasitic form. (Also found on 75% tadpoles in Bushy Park area) | Omnivorous, feeding on bacteria, microorganisms and epithelial cells of skin | Continual rotation of ciliate causes irritation and excessive mucus production. Can cause asphyxia. Fish acquired a blue-white coating, but this was not as noticeable as in other skin complaints | Reproduction favoured by high fish density and slow rate of water flow. Other-wise during the summer the intensity of the infection tends to be reduced | Local conditions obscure typical cycle except in Highams area where the rate of water flow remained relatively high throughout the year. In Wanstead sluggish water and high fish density gave a maximum % of hosts infected during July. In October there was less crowding and a milder <u>Trichodina</u> infection per individual. In Bushy Park a greater proportion of fish were infected by a high density of <u>Trichodina</u> in the cooler months of the year. Found associated with <u>Gyrodactylus</u> - competition between the two organisms limited because <u>high temperatures</u> (as well as dense fish populations and sluggish waters) favour <u>Gyrodactylus</u> . In a high % of cases there appears to be a reciprocal antagonistic reaction between these parasites as regards their success in regions of body (e.g. gills and rest of body) |
| <u>Myxobolus</u> | Spores located amongst kidney tissue. Amoebulae extracellular and intercellular in kidney tubules | - | Some inflammatory reaction may be associated with this condition, but the exact nature of the host reaction was difficult to determine and distinguish from past (microsporidial and/or bacterial) or present (bacterial) infections. Pure infections were not obtained. Does not appear to be fatal | Cysts were never recovered | Generally found in association with <u>Henneguya</u> with which it must compete, although the source of nutrients is such that no antagonistic relationship develops. Probably ubiquitous and present throughout the year |
| <u>Henneguya</u> | Spores located amongst kidney tissue. Amoebulae extracellular and intercellular in kidney tubules | - | As in the case of <u>Myxobolus</u> | Cysts were never recovered | Slightly more common in occurrence than <u>Myxobolus</u> . Other points as given for <u>Myxobolus</u> . Probably ubiquitous and present throughout the year. |

Parasites recovered from Gasterosteus spp.

| Genus | Site of infection | Mode of nutrition | Observed effects upon the host: symptoms and reactions | Life cycle note | Remarks and observations |
|--------------------------------|--|--------------------------|---|--|--|
| <u>Glugea</u> (graph p.338) | Intracellular development. Recovered from all regions, organs & mesenteries of the body except swim-bladder, urinary bladder and heart | Intracellular absorption | Effect depends upon the site of the cyst. Those embedded in the liver, spleen, kidneys, will upset metabolic function. Those embedded in the reproductive organs may affect fertility. Large cysts were recovered close to brain, eye and in buccal cavity. The most important effect would appear to be general loss of condition and retardation of growth, rather than fatality | Spheroidal creamy-white cysts formed, averaging 2 mm across. Maximum 15/ fish. Youngest fish to possess cysts 1.75 cms in length | Found only in Bushy Park area where visible cysts were recovered all year round. Reduction in number of fish infected during the summer is associated with an increase in number of young fish in which cysts had not had time to develop. Externally placed cysts were not observed to burst, leaving bleeding sores, as was the case for <u>Dermocystidium</u> . Possibly release is more gradual. The occurrence of this parasite undoubtedly caused the Bushy Park faunal average length to be reduced below the value of the similar measurement for the Wanstead area. |
| <u>Dermocystidium</u> | Entire body covered with what appears to be superficially situated cysts - actually parasites occupy the dermis & overlying epidermal layers become transparent so that cysts appear to be superficial | - | Fish apparently unaffected during development of cysts. When these began to burst, leaving bleeding sores, the fish died. Mass mortality occurred as a direct result of this infection, the fish dying before all the cysts on the body had ruptured. The fatal nature of this disease was demonstrated on fish kept in the laboratory for several months, and contrasts with Elkan's observations (1962) | Elongate white cysts, 2-3 mm long | This parasite was not responsible for mass mortality in Bushy Park. Occurred in the Newdigate area and only in pond fauna. Probably present throughout the year. May & June records being the result of a small fish sample |
| <u>Gyrodactylus</u> | Roams freely over skin, gills, fins. A true ectoparasitic form | Feed on epithelial cells | Excessive mucus production can result in asphyxia. Appears to be more fatal when dealing with fish in aquaria than in nature | Viviparous. Mass reproduction favoured by warm temperatures & high fish density | Obtained during the summer months from 3 areas. It was recovered from only one batch of fish from Newdigate stream & this infection was heavy. Possibly this represented a recent migration from the lakes to the stream following contamination of the lake by this parasite, during restocking procedure. Parasite frequently associated with <u>Trichodina</u> . Some reciprocal antagonism concerning the success on gills or body |

| Genus | Site of infection | Mode of nutrition | Observed effects upon the host: symptoms and reactions | Life cycle note | Remarks and observations |
|--------------------------|---|-----------------------------|--|--|---|
| <u>Diplozoon</u> | Attached to gills. A true ectoparasite | Feeds upon epithelial cells | Irritation results in excessive mucus production. Up to 10 pairs (recovered from one individual during this investigation) appeared to be well tolerated | Adults in permanent copula | Recovered from the Newdigate pond area & only once infecting <u>Gasterosteus</u> (1 pr). Appears to have a preference for <u>Phoxinus</u> . Found in October, November and December only. |
| <u>Diplostomulum</u> (m) | Recovered from the lens of the eyes. Endoparasitic | - | Causes parasitic cataract resulting in the destruction of lens tissue. Density of infection in the lenses can differ so that the fish may retain partial sight in one eye whilst being completely blind in the other. The fish's ability to locate food is affected, resulting in general loss of condition, and other parasites (especially of the skin) are favoured | Developing in <u>Limnaea stagnalis</u> ; cercariae are released the skin of the fish host and migrate to the eye. Metacercariae exhibit preference for the lens and remain unencysted. Final hosts piscivorous birds | Recovered from Wanstead & Bushy Park areas in July and October, July, August and December respectively. The eyes of young fish (under 2.5 cms long) were not infected. This parasite appears to be favoured under conditions where water rate of flow is slow & where the rate of progression of fish is reduced by <u>Schistocephalus</u> infections. Young fish of under 2.5cms were not affected by this latter parasite. Both young & adult fish occupied a position near to the surface of the water, feeding on planktonic forms. Either young fish could avoid these cercariae or they were equally affected and the migrating stages were not recorded |
| <u>Echinostomes</u> (m) | Encysted in the skin of the buccal cavity. Endoparasitic. (Also found embedded in internal gills of 50% of tadpoles in Bushy Park area) | - | No marked reactions had taken place. Perhaps the cysts had not been present long enough to evoke encapsulation processes | Develop in rediae in <u>Limnaea pereger</u> ; the small cercariae are inhaled or eaten by a wide variety of vertebrate and invertebrate organisms (see Section 5) | Recovered on two occasions from small fish which had inhaled (or attempted to ingest) the cercariae |

Parasites recovered from Gasterosteus spp.

| Genus | Site of infection | Mode of nutrition | Observed effects upon the host: symptoms and reactions | Life cycle note | Remarks and observations |
|----------------------|--|-------------------|--|---|--|
| <u>Ligula</u> (p) | Plerocercoids recovered from the body cavity. Endoparasitic | Absorption | In <u>Gasterosteus</u> the plerocercoids did not attain sufficient size to demonstrate features normally characteristic of Ligulosis | Copepodid & Piscine intermediate hosts + definitive hosts = Piscivorous birds | Recovered from Bushy Park, Wanstead and Epping areas. Totally unimportant in Bushy Park where only 2 plerocercoids were obtained and these were too small to affect the fish. 36 were recovered from Wanstead but all were extremely small & associated in some cases with <u>Schistocephalus</u> (maximum taken from 1 individual = 15). Similarly only 4 were recovered from Epping Forest sticklebacks. <u>Ligula</u> attained a large size only in roach where the fish was considerably distorted. <u>Ligula</u> does not appear to do well in <u>Gasterosteus</u> species. Plerocercoids recovered in July, September and October only |

Parasites recovered from Gasterosteus spp.

| Genus | Site of infection | Mode of nutrition | Observed effects upon the host: symptoms and reactions | Life cycle note | Remarks and observations |
|------------------------------|--|-------------------|--|---|---|
| <u>Schisto- cephalus</u> | Plerocercoids recovered from the body cavity. Endoparasitic | Absorption | Pronounced swelling & distortion of the body whether there are 1-3 larvae present. A single larva can reach a length in excess of that of its host. Pressure effects are considerable. The gut may be constricted, the gonads regressed and the liver abnormal. Fish swim close to the surface of the water and have difficulty in progressing | As for <u>Ligula</u> . The presence or absence of this parasite depends to some extent on the local mig- ratory habits of the ducks & herons. The former are resident in Bushy Park area, whilst the latter appear to be more mobile, only occasionally using the Park as a nesting area | Recovered from Wan- stead & Bushy Park in July & October, June, July, August & October respectively. The peak number of plerocercoids occurred in July in both areas, subse- quently decreasing in colder weather. This parasite was not recovered from young fish under 2.5 cms in length. Plerocercoids were recovered from the gut of 3 cm long specimens prior to their penetration into the body. Why this should be the case when both host size groups are feeding freely on plankton is unknown |

Parasites recovered from Gasterosteus spp.

| Genus | Site of infection | Mode of nutrition | Observed effects upon the host: symptoms and reactions | Life cycle note | Remarks and observations |
|---|---|---|--|---|---|
| <u>Proteocephalus</u> (graph p. 340) | Rectum & intestine Endoparasites | Absorption | Maximum numbers recovered from adult fish = 9; a maximum 3 young tapes were recovered from fry of 2.5 cms in length and only 1 taken from smaller fish. No apparent damage to host occurs at these levels of infection | Classical life cycle involves a copepodid intermediary host (for the procercoid stage) & a vertebrate (for the plerocercoid phase). Plerocercoids either have the ability to migrate from the body cavity to the gut or reach this situation as a result of cannibalism | Recovered from Bushy Park & Newdigate pond fauna. Located in 1 or 2 ponds only in the latter area. In Bushy Park a maximum number of fish were infected during the summer months, particularly latterly. Young fish from 2 cms upwards were infected |
| Nematoda (a) <u>Strongyloidea</u> | Attached to stomach wall or free in contents (bursate ♂'s & ♀'s present) Also in intestine (♂'s & ♀'s present) | Probably ingest gut contents | Maximum numbers per host = 9. No apparent damage caused with these densities of infection | ? | Recovered from pond and stream fauna of Newdigate, Bushy Park and Essex. Sporadic occurrence throughout the year |
| (b) <u>Ascaroidea</u> <u>F. Anisakidae</u> | Encapsulated in abdominal cavity lying against rectum and urinary passages: large larval stage | - | Single larva found per fish. Although apparently healthy the fish died. Whether this was due to this infection or not could not be ascertained | Life cycle probably as follows: eggs in faeces shed into water & developed to 2nd stage juvenile before infecting fish. Larva encysts in liver and mesenteries of intermediate fish host, becoming adult in piscivorous forms such as pike | Recovered from an Essex fish. (Also from <u>Phoxinus phoxinus</u> in Newdigate) |
| <u>Argulus</u> (graph p. 336) | On skin and fins. A true ectoparasitic form | Pierces skin; injects toxin and sucks body fluids | Puncture marks were easily visible & left fish susceptible to secondary infection involving <u>Saprolegnia</u> . Fish, irritated by biting action, attempted to rid themselves of parasite by swimming up and down the aerator currents in aquaria. Success resulted in the fish either attempting to eat the parasite or actively avoiding the free-swimming form. Death resulted in one case from ingestion of parasite which attached to roof of buccal cavity and caused asphyxia (and toxic poisoning?) | Eggs laid on submerged objects. Breeds rapidly in higher temperatures of summer producing a new generation approximately every two months. Adults overwinter | Recorded from Bushy Park & Essex only. Probably present throughout the year. Typical cycle revealed young form being recovered from May-September, and mating adults occasionally found on the fish. Max. numbers recovered from one individual = 17 larvae or 11 large adults. The reason for the reduction in the number of fish infected during August is not clear |
| <u>Glochidia</u> (<u>Anodonta</u>) | Embedded in fins, operculum, gills and body. Endoparasitic form | Absorbs nutrients? | Host reaction limited to enclosing glochidium in fibroid cyst. (A form of immunity following heavy infestations has been noted in other fish and may apply in this case). Fish supported up to 20 larvae without any adverse effects being exhibited | - | The single record from Newdigate (stream) reflects the fact that the low population of <u>Anodonta</u> is collected from the stream during cleaning and a permanent adult population is not allowed to build up. The gradual fall in the percentage of affected fish in the Bushy Park area was also the result of clearance, drainage etc. In Essex only a small population of <u>Anodonta</u> was present throughout the year |

1) Summary

Parasites of Gasterosteus spp.

No parasite proved to be ubiquitous but Gyrodactylus and Henneguya were recovered from 5 collecting areas and Myxobolus and Trichodina from 4. (It is probable that the Myxosporidia did occur in all the sites investigated but were not detected). Six parasites were unique to a single area and in some cases were restricted to one water system within the region. These were Oodinium (Newdigate stream), Chilodonella (Newdigate ponds and stream), Glugea (Bushy Park A and B), Dermocystidium (Newdigate ponds), Diplozoon (Newdigate ponds) and Echinostome metacercariae (Bushy Park System A).

1.2) Conclusion

The stunted Bushy Park fauna indicated that fish infected by the largest number of parasitic genera attained the lowest average length. The Newdigate fish, however, did not reflect this situation since they attained the maximum average length whilst carrying only one parasitic genus short of the Bushy Park total. The reason for this lies in the pathogenicity of the parasites concerned under the different area environmental conditions, and more particularly the composition of the parasitic fauna and not the total number of genera present. It would appear from a comparison of the species list for Bushy Park and Wanstead with that for Newdigate that the parasites having the most stunting effect upon growth are Schistocephalus, Diplostomulum and, in the case of Bushy Park, Glugea. Trichodina infected a higher percentage of the stunted

fauna than was the case for Newdigate. Argulus may also have adversely affected the Bushy Park fish under such conditions.

The parasitic load of the host and its resulting condition had no direct effect upon the tissue feeding Phyllodistomum population. In the way it affected the size of the host and therefore its food intake, however, the total parasitic load did indirectly affect this trematode. The largest fish on average came from Newdigate and the population supported the greatest total numbers of Phyllodistomes recovered (see p.197). Essex fish carried the next maximum total and the results from Wanstead and Bushy Park fauna followed in sequence. Obviously there is no immunity acquired as regards this parasite. When Henneguya and Myxobolus are associated with Phyllodistomum infections, they appear to provide a greater source of nutrients rather than to compete with the trematodes. Perhaps they achieve this indirectly by increasing epithelial loss. The spores, however, constitute part of the trematodes' diet.

Section 9 - Gorgoderids other than Phyllodistomum recovered
during this investigation

a) Method of investigation

Gorgoderid cercariae and metacercariae were discovered during the routine investigation of Newdigate fauna (pages 125-136). The cercariae were recovered from a molluscan host distinct from that harbouring Phyllodistomum (page 130). These cercariae were fed to a number of potential secondary hosts (pages 142-143) and the results of these experiments and the routine examination of the fauna revealed that the cercariae and metacercariae belonged to the same species and probably to the genus Gorgodera. In order to determine whether the reproductive rudiments remained characteristic of Gorgodera or changed to the Gorgoderina pattern in the adult phase (as recorded for Gorgoderina attenuata Stafford by Rankin (1938)) an attempt was made to infect both fish and frogs with the parasite (pages 142-3 and 175). It proved impossible to obtain local Newdigate frogs at the time when the limited Gorgodera material was available. The suppliers were experiencing difficulty in collecting frogs over this period so that a sample of only six could be used. As this sample was too small to determine whether a portion of this frog population was carrying a small Gorgoderid infection of the same or a different species the juveniles and adults obtained at post mortem following feeding experiments are considered separately in the following discussion and their measurements given on pages 382, 389-394. All adults and juveniles recovered however appear to belong to the same species and to be derived only from experimental material. Running parallel with the Gorgoderid

infection experiments were those involving Haematoloechus carried as free metacercariae wandering in the Haemocoel of Odonata (pages 142-143). The adult trematodes were recovered in a graded series and their size was proportional to the length of the period of infection. The infection success and growth rate of this secondary association showed interesting differences from the Gorgoderids. Attempts to infect fish with Gorgodera (pages 142, 143, 175) failed.

The parasite is probably not indigenous to the Newdigate area but may have been introduced when ailing imported stock from other parts of Britain and the Continent were released into the estate grounds.

All stages were examined, where possible, in both a living and mounted state utilising identical methods to those already described for Phyllodistomum.

Gorgodera sp. - a brief description of the stages of the life cycle recovered during this investigation in comparison with Phyllodistomum.

b) Sporocyst generation

These are large, recurved, elongate white sacs embedded in the gill lamellae of Sphaerium lacustre. The larger structures containing fully developed cercariae measure from 1.837-2.345 mm in length and from 0.35 to 0.455 mm in width.

c) The fully developed cercaria

The measurements of living and mounted material are given on page 353. The cercariae may be classified as macrocercous. They belong to the so-called 'Gorgodera' group erected by Sewell in 1922, the chamber occupying not more than one-sixth of the total length of the tail.

Detailed measurements of Gorgodera cercariae:

A) The Cercarial Body

1) Total length

Average length of 5 specimens (relaxed - slightly extended state)
Maximum extensile range recorded for 1 individual from the fully contracted to the extended position
Minimum length recorded for a contracted specimen
Maximum length recorded for an extended specimen

a) Living material

= 0.440 mm range 0.356-0.532 mm.
= 0.420 mm.
= 0.256 mm.
= 0.700 mm.

b) Mounted material

= 0.364 mm range 0.282-0.513 mm.

2) Regional Proportions

Measurement of the anterior region (excluding V/S) of 5 specimens (contracted - slightly extended state)
Measurement of posterior region (V/S included) of 5 specimens (contracted - slightly extended state)
Ratio: average anterior: posterior region

a) Living material

= 0.187 x 0.140 mm (0.136-0.238 x 0.117-0.158mm)
= 0.253 x 0.140 mm (0.220-0.312 x 0.121-0.165mm)
= 1:1.353
= 1:1.000

Maximum extensile range recorded (each region measured separately) in one individual - anterior region
- posterior region

= 0.292 mm
= 0.191 mm

b) Mounted material

Measurement of the anterior region (excluding V/S) of 10 specimens (contracted - slightly extended state)
Measurement of the posterior region (including V/S) of 10 specimens (contracted, slightly extended state)
Ratio: Average anterior region: posterior region - length
- width

= 0.139 x 0.130 mm (0.103-0.202 x 0.114-0.147mm)
= 0.225 x 0.172 mm (0.172-0.312 x 0.0934-0.132mm)
= 1:1.613
= 1:0.900

3) Sucker sizes

Measurements of the O/S of 5 specimens (relative to organ and main body axes)
Measurement of the V/S of 5 specimens (relative to the organ and main body axes)

a) Living material

= 0.086 x 0.078 mm (0.082-0.097 x 0.066-0.081mm)
= 0.105 x 0.102 mm (0.087-0.128 x 0.077-0.110mm)

Ratio: O/S: V/S (relative to main body axes) length
(relative to main body axes) width
(relative to the organ) greatest diameter

= 1:1.223 (1:1.063-1:1.371)
= 1:1.298 (1:1.049-1:1.576)
= 1:1.223 (1:1.063-1:1.371)

b) Mounted material

Measurements of O/S from 10 specimens (relative to the body axes)

= 0.0823 x 0.0693 (0.0735-0.0980 x 0.0622-0.0775mm)

Measurements of V/S from 10 specimens (relative to the body axes)

Ratio: O/S: V/S (relative to main body axes) length
(relative to main body axes) width
(relative to the organ) greatest diameter

= 0.0903 x 0.0805 (0.0765-0.102 x 0.0714-0.0969mm)
= 1:1.092 (1:1.013-1:1.202)
= 1:1.162 (1:1.029-1:1.301)
= 1:1.092 (1:1.013-1:1.202)

4) Excretory bladder sizes

Average measurement of bladder from 5 specimens
Average measurement of the posterior region of the 5 specimens above
Average percentage of the posterior width occupied by the bladder
Average percentage of the posterior length occupied by the bladder

a) Living material

= 0.145 x 0.0678 (0.128-0.158 x 0.0513-0.0734mm)
= 0.261 x 0.142 (0.220-0.312 x 0.121-0.165mm)
= 47.72%
= 55.45%

b) Mounted material

Average measurement of the bladder from 5 specimens
Average measurement of the posterior region of the 5 specimens above
Average percentage of the posterior width occupied by the bladder
Average percentage of the posterior length occupied by the bladder

= 0.132 x 0.0624 (0.110-0.147 x 0.055-0.0734mm)
= 0.239 x 0.115 (0.187-0.312 x 0.106-0.132mm)
= 54.09%
= 56.5%

5) Cercarial stylet

Total size range from 5 selected specimens: length
Depth at the base (lateral view)
Depth of keel at the level of the wings - lateral view

a) Living material

= 0.0306-0.0326 mm (Av. = 0.0310mm)
= 0.0061-0.0071 mm (Av. = 0.00612mm)
= 0.0061-0.0082 mm (Av. = 0.00813mm)

B) Cercarial tail

1) The cercarial ring

Average size (l x b parallel to body axes) 5 specimens measured

a) Living material

= 0.0892 x 0.125 mm (0.0734-0.110 x 0.0917-0.143mm)

2) The cercarial chamber

Average size (l x b parallel to body axes) 5 specimens measured

a) Living material (cercarial body free)

= 0.183 x 0.202 mm (0.154-0.257 x 0.176-0.216mm)

3) Tail stem

Average size of 5 specimens

a) Living material

= 3.075 x 0.213 mm (2.887-3.307 x 0.183-0.257mm)

Abbreviations: O/S = oral sucker
V/S = ventral sucker
l x b = length and breadth

Living and mounted specimens are not necessarily identical.

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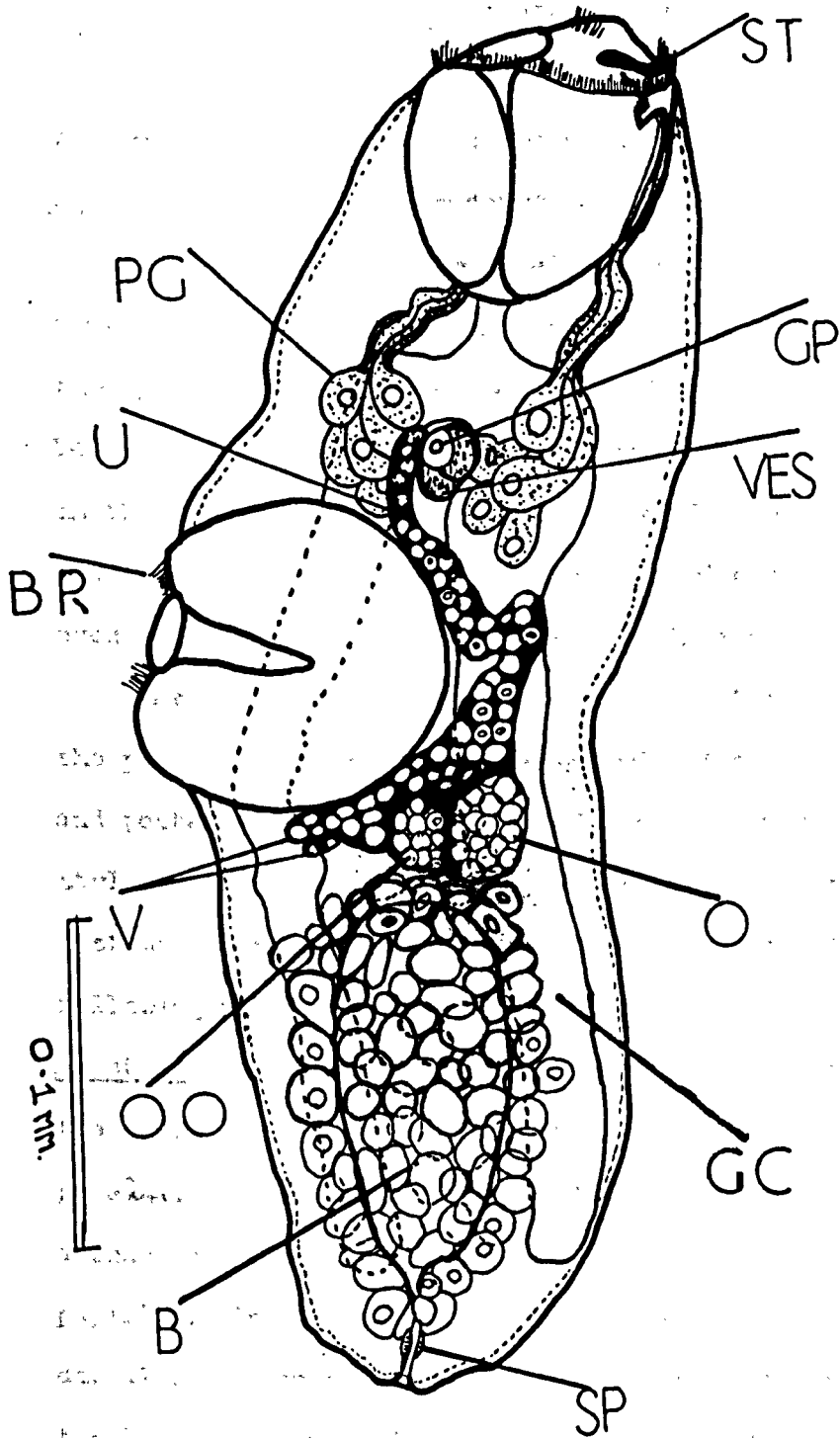
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GORGODERA - CERCARIA

Ventro-lateral view of a mounted specimen
drawn with the aid of projector

Abbreviations:

ST = Stylet
PG = Penetration gland cell
GP = Gonopore
U = Uterine anlage
VES = Anlage of the vesicula seminalis
BR = Bristles
V = Vitellaria
O = Ovary
OO = Ootype complex- rudiment
GC = Gut caecum
B = Bladder
SP = Sphincter guarding excretory opening



CORCODERA = CERCARIA

c 1) The Cercarial Body (illustrated on page 355)

The cercarial body is muscular and highly contractile and its regional proportions are consequently variable. Basically similar to Phyllodistomum in its construction it is, however, less dorsoventrally flattened and bounded by a finely ridged cuticle which is patterned in a manner similar to a human palm print and has no parallel ridges of the type previously described. One of the noticeable differences between the two genera is the presence of dense refractile cytoplasm in the Gorgodera specimens which rendered observation of the excretory and reproductive rudiments in living specimens virtually impossible even with the aid of horse serum as a clearing agent.

The papillary pattern on the body (as far as it could be determined) the position of the main excretory collecting ducts and their anterior and posterior branches; the papillary pattern over the suckers; the stylet; the form of the gut and nervous system are identical with the systems described for the Gorgodera metacercaria on page 372. The following features are characteristic of the cercaria alone.

Bristles Concentric rings of bristles or spines are situated on both the oral and ventral suckers. These are arranged as illustrated on page 241. On the ventral sucker the bristles form a circular band around the acetabular opening and are bounded by a ring of six double papillae. On the oral sucker there are two circlets of spines, a distal ring surrounding the mouth passing on to the ventral surface of the lip and a proximal incomplete circlet extending only over the ventral surface. Both lines of bristles correspond to the main circles

and half circles of the papillae. The bristles project vertically beyond the latter and appear to impede at this stage any tactile sensory function which the papillae may serve. They are, however, easily detached and are lost during the examination of the cercaria and are apparently also readily detached from the fluke under natural conditions. Following the loss of the bristles the position of their points of insertion is marked by fine stippled areas on the suckers. The periphery of each zone is delineated by a ridge the position of which corresponds to the ridges found on Phyllodistomum suckers and is in line with identical papillary patterning. Stippled areas were also noted on Phyllodistomum suckers and are probably equivalent to the sensory nodular area described for the acetabulum of C. donecerca by Goodchild (1939) and also noted by Ssinitzin (1905) and Krull (1935). Although the true function of the bristles is not apparent in this case, the small spines recorded on the tail termination and in the sucker cavities of P. simile cercariae by Thomas (1958) and on the tail tip of C. macrocerca (Vickers, 1941) serve to increase the cercaria's power of fixation. It is possible that in the past many species of Phyllodistomum possessed spines which were developed to an equivalent degree to the state attained today by Gorgodera and that during their evolutionary history these structures have been progressively reduced and lost. If the spines' functions are primarily fixational the reduction in Phyllodistomes may be associated with the trend towards shortening the life cycle and encysting in the molluscan host. The early attainment of more powerful suckers by Gorgodera may

obviate the need for spines and account for the ready manner in which they are lost. It is doubtful, in the case of Gorgodera and Phyllodistomum, that sucker bristles originally possessed a tactile function which was progressively superceded by the use of papillae resulting in the reduction of the spines seen today. If this had been the case, it might be expected that the numbers of acetabular papillae would differ between the two genera examined and that a greater remnant of the spinose condition would occur in Phyllodistomes than in the probably more advanced Gorgodera (if the larval stages can be considered to evolve at an equivalent rate to the adult which is doubtful). The presence of spines in C. conica (C.P. solidum) Goodchild, 1939, parallels the condition found in Gorgodera. The two life cycles do not include an encysted stage in the mollusc and both terminate in Amphibian hosts having lost the sucker bristles at the metacercarial phase. The impermanent nature of the bristles in the Gorgodera specimens examined does not indicate a sensory function in this case. On one occasion the bristles were retained following encystment within the intermediate host but normally they are lost well in advance of this stage. Similar spines or hairs to those described for Cercaria conica by Goodchild (1939) were indicated for C. steelmani by Baker (1943). Both authors considered the structures to be sensory.

Penetration glands These are clusters of six large gland cells located on either side of the terminal genital rudiments below the gut bifurcation. They lie either completely inter-caecally or overlap each caecum. There were insufficient numbers of cercariae recovered to

determine whether any considerable variation in gland position occurred. Occupying an apparently identical position to the *Phyllodistome* glands the sinuous path and local dilations of their ducts also closely reflect the latter's condition. The ducts open separately into the stylet chamber on either side of the stylet wings. Unlike *Phyllodistomum*, however, the gland cells contain conspicuous coarse eosinophyllic granules.

The excretory bladder

This is a noticeable feature of the cercaria. It is a large median structure with its opening guarded by a sphincter leading to a short canal and a dorsal excretory pore. Unlike *Phyllodistomum* the bladder expands anteriorly and the average measurement of its maximum width is greater than that attained by the latter. The bladder cells are elongate and situated with their longitudinal axes at right-angles to the main bladder axis. They open into the bladder on the side opposite the nucleus and are packed with eosinophyllic granules which, again unlike *Phyllodistomum*, appear to be clustered into small groups.

Other Gland Cells

Scattered throughout the body on the dorsal and ventral surfaces are a few very large cells containing fine eosinophyllic granules. The cells are slightly concentrated immediately posterior to the ventral sucker. Their multi-branched protoplasmic extensions eventually form fine ducts opening to the exterior. As in *Phyllodistomum* these cells are probably cytogenous in function.

Reproductive System The darkly-staining genital primordia consist of

what can be interpreted as two vitellaria, an ootype complex, ovary and genital strand, the latter extending dorsally behind the ventral sucker to the anterior expansion opposite the developing genital pore area. The genital cord anterior to the ootype is always coiled. The peripheral position and dense packing of the bladder nuclei hampered attempts to trace the male anlage and no sign could be found either in the living specimens, whole mounts or sections, of the testal rudiment. This condition is in direct contrast to Phyllodistomum cercariae where the entire reproductive system is visible when the larva is fully formed.

c 2) The Tail (illustrated on page 362)

The cercarial body is attached by a relatively broad stalk-like area to a tail which is divisible into a proximal cuticular ring, a hollow cercarial chamber and a long, distal extension.

The cuticular ring is a transparent structure surrounding the point of attachment of the cercarial body to the tail. It possesses a similar range of shapes to that found in Phyllodistomum and differs little in size from the latter. Its function is to allow for the entry of the cercarial body into the chamber and, as previously described, the ring is lost when this occurs.

Extending from the posterior end of the cercarial body, immediately ventral to the excretory opening, are strands of longitudinal muscles which pass centrally to a cylindrical sheath through a squat, conical, cuticular chamber, little differentiated in width from the main tail stem. The main bulk of the longitudinal muscles, however, take their origin from a slightly expanded point in the sheath where the latter

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GORGODERA - CERCARIAL TAIL

A diagrammatic representation of the regions of the cercarial tail as it appears in the live state.

Abbreviations:

CH/Prox.STEM

- = Chamber and proximal region of the stem
- CR = Cuticular ring
- CB = Cercarial body - posterior end
- S = Stalk attaching the cercarial body to the tail: NB Longitudinal muscle strands passing into the ventral surface of the cercaria.
- MA = Thickened zone at proximal end of globe. The site for the attachment of the longitudinal muscles of the stem.
- CH = Chamber (cuticle thickened)
- G = Globe of compact small cells
- NC = Nerve cell.
- C = Cuticle of the stem (finer than in chamber)

MID STEM

- = Diagrammatic representation of the tapering distal section of the median zone of the stem.
- LM = Longitudinal muscle blocks
- CM = Circular muscles

DIST.STEM

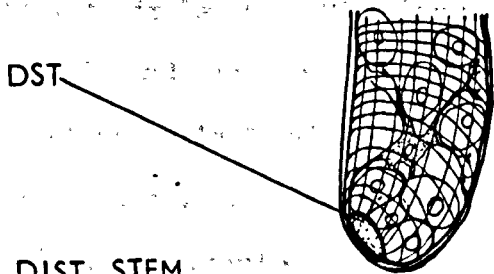
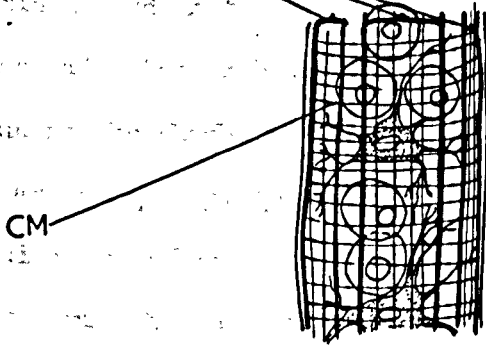
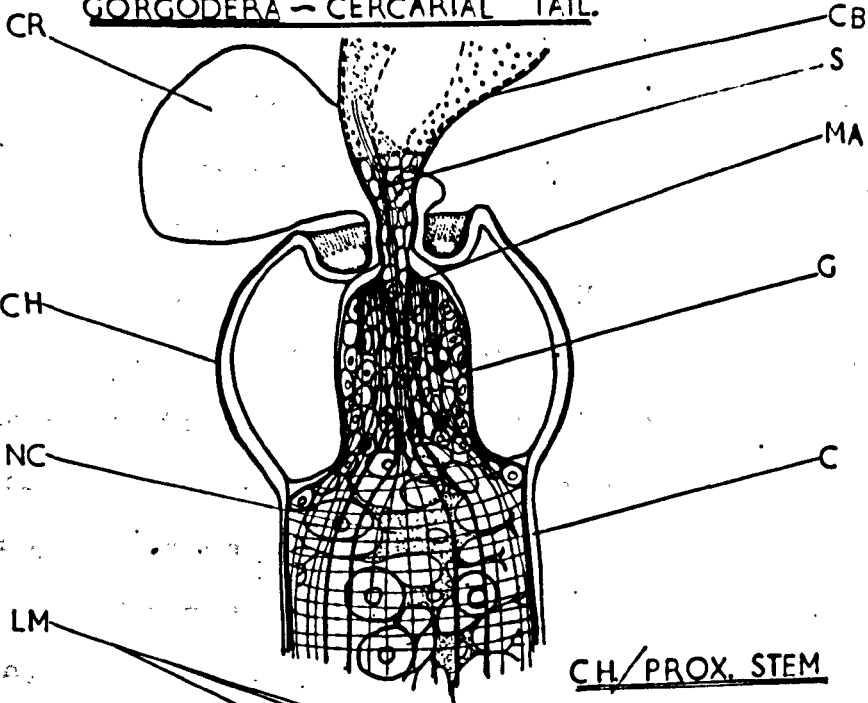
- = Distal portion of the stem
- DSC = Disc found at the stem tip

SECT. DIST. STEM

- = Line diagram of a section of the stem tip in a contracted specimen
- RC = Wrinkled cuticle
- DST = Disc attached to cuticle
- LM = Longitudinal muscle bands passing onto the disc

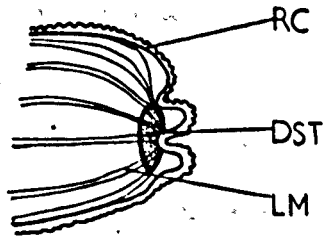
(N.B. The central canal is omitted as it is only visible in mounted specimens.)

GORGODEBA - CERCARIAL TAIL.



DIST. STEM

MID. STEM



SECT. DIST. STEM

comes in close contact proximally with the thickened cuticle of the chamber. A ring-like thickened area on the outer surface of the sheath thus serves for muscle attachment whilst only a few strands of muscle continue past this point into the cercarial body. The point of muscle attachment coincides with a proximal depression in the chamber cuticle similar to that found in Phyllodistomum and similarly caused by the contractions of the tail muscles. Unlike Phyllodistomum, the chamber has no localised proximal cuticular thickening but is of uniform thickness throughout. Small, darkly staining cells are compacted proximally into the central chamber sheath or strand. The strand does not occupy as large a percentage of the chamber as in Phyllodistomum, being more cylindrical or ovoid in shape. The cells within the strand crossing the chamber are so densely packed that nerve cells could not be distinguished in this region.

The tail stem is bounded by a noticeably finer cuticle than that surrounding the chamber. The smooth cuticle is not folded below this region as in Phyllodistomum but is close fitting throughout its length. Longitudinal muscles, arranged in distinct bands, extend from the chamber strand to the tail tip and retain their banded arrangement throughout their length. Circular muscles lie internally to the longitudinal bands and are found throughout the tail stem. The large parenchymatous cells found in the stem diminish in number as the tail tip is approached. Large, granular, multi-branched inter-connecting nerve cells, concentrated slightly anteriorly but distributed throughout the tail, serve the muscle fibres. Such large and superficial nerve

cells could not be found in the tail stem of the less active Phyllodistomum cercariae. In transverse and longitudinal sections the centre of the tail appears to be filled with eosinophillic material perforated by a canal with fine brightly staining walls. This canal can not easily be seen in the living specimen although it runs the entire length of the tail. A similar canal was not found in Phyllodistomum.

The smooth, rounded, distal end of the tail is converted in contracted specimens into a concave, sucker-like structure. This is the result of contraction of the longitudinal muscles which retracts the terminal ovoid, disc-like area of cuticle to which they are attached. Whether this action functions as a method of temporary attachment (as mentioned by Vickers (1941) for C. macrocerca) could not be elucidated since the few specimens of fully-developed cercariae were not seen to attach themselves. The tail was not found to be adhesive in any of the specimens examined.

c 3) Cercarial Behaviour

Fully mature cercariae are released from the mollusc via the exhalant siphon. Whether they escape whilst the cercarial body is free or already within the chamber is not known. The violent contractions of the longitudinal muscles of the tail provide the propulsive force and also cause the body to be withdrawn into the chamber. It therefore seems likely that withdrawal follows release avoiding excessive damage to the host. Cercariae enclosed within the chamber are capable of living for more than 24 hours after their escape. They are active

swimmers. During effective forward progression the long tail is thrown into a sinuous, anteriorly progressing curve which causes the anterior body to be lashed from side to side in the water. In this way the cercaria rises and progresses a short distance and then slowly sinks, the tail twisting erratically and performing an attractive rather than a locomotive function. They continue to swim sporadically in this fashion for some time.

The cercariae were normally recovered, with a few exceptions, during the routine examination of molluscan hosts. Under these conditions they were found intertwined in active wriggling masses. If such intertwining takes place under normal conditions, it would render these large cercariae highly conspicuous to fish and insect larvae alike and provide a method for mass infection. Insufficient numbers of naturally released cercariae were recovered in order to test this but the few that were obtained remained unattached to any object for over 16 hours. It is more probable that these cercariae, in contrast to Phyllodistomum represent more of a dispersal phase and that their large size and activity renders them sufficiently attractive to several carnivorous hosts without the necessity for crowding.

The cercarial body whilst within the chamber is bent over upon itself and remains fairly static in contrast to the active form in Phyllodistomum. When released from the tail the cercarial body is capable of moving about by means of its suckers for some time. Upon maximum contraction, the entire body is converted into an ovoid shape which is rounded in cross-section, and unlike any form assumed by

Phyllodistomum. The anterior and posterior regions are both highly contractile and move independently in a manner similar to the description on pages 20-9. Upon extreme extension both regions are capable of producing a flattened margin and the posterior region can widen to a leaf-like equivalent of the Phyllodistome state. A posterior notch can be absent or prominent according to the contractile phase. The anterior region is utilised as an extensile probe in exactly the same manner as described for Phyllodistomum. The body however, always tends to be thicker in transverse section and this becomes particularly noticeable upon extreme contraction.

d) Gorgodera - The Metacercaria

d 1) The Cyst

In contrast to Phyllodistomum these metacercariae possessed no tendency to encyst precociously within the molluscan host. Cysts were recovered from the thoracic haemocoel (segments 1-3) of Odonata (Zygoptera, Anisoptera), Trichoptera and Diptera (page 36). The position of the cyst and the degree of penetration varied to the same extent as in Phyllodistomum and the cysts were recovered from the oesophageal wall, the haemocoelic surface of the oesophagus and from the internal surface of the terga. The sizes of the cysts obtained from various hosts are recorded on page 368. Although the Gorgodera mean value exceeds that given for Phyllodistomum on page 154 the ranges overlap. Metacercarial cysts grow considerably whilst within the insect host but increase in size is a slow process. Cysts recovered from Trichoptera which had been kept in the laboratory for two months at cool temperatures, were little

different from those obtained following an experimental infection of twenty-four hours duration. The data recorded from Odonatan hosts indicates that the metacercariae are able, in time, to double their original cyst dimensions.

In contrast to Phyllodistomum, the cyst wall consists of a series of well-defined laminated layers of varying thickness. Its total width is gradually increased as the cyst ages ranging from 0.0061 mm in cysts measuring 0.213 x 0.213 mm to 0.0102 mm in a cyst of 0.427 x 0.400 mm. The initial external layers are thinner than those nearer to the fluke and this may be an effect brought about by stretching during growth. The outer layers can be peeled off from the surface of the intact cyst in a series of strands. The cysts are rounded in top view but the majority appear to be more markedly ovoid laterally than is the case for Phyllodistomum. The wall, when seen from this lateral aspect, is thickened at one point. This region assumes the appearance of a complete ring around the cyst reaching a maximum 0.031 mm across. These thickenings were still present in the torn remains of the cyst wall and were the result of neither pressure nor optical effects. The cyst wall, as in Phyllodistomum, is permeable despite its greater thickness and allows the passage of vital dyes. It fits closely around the larva leaving a much smaller space system than is characteristic of Phyllodistome metacercariae. This space restriction does not affect the degree of activity of the encapsulated fluke.

Gorgodera - Metacercariae cyst sizes: (selected specimens giving an overall total of 25)

| <u>Host</u> | <u>Cyst size</u> | | | |
|-------------------------------------|------------------|------------------------|------------------------------|--|
| <u>ODONATA</u> | | | | |
| Zygoptera - <u>Ischnura elegans</u> | 0.257 x 0.227 mm | Natural infections | E/A | |
| | 0.213 x 0.201 mm | " " | " | |
| | 0.367 x 0.337 mm | " " | " | |
| | 0.330 x 0.301 mm | " " | " | |
| | 0.385 x 0.359 mm | " " | " | |
| | 0.337 x 0.337 mm | " " | " | |
| <u>Coenagrion puella</u> | 0.409 x 0.373 mm | " " | " | |
| | 0.400 x 0.400 mm | " " | " | |
| | 0.330 x 0.315 mm | " " | " | |
| | 0.264 x 0.246 mm | " " | " | |
| | 0.436 x 0.356 mm | " " | Host *2 months in laboratory | |
| | 0.427 x 0.400 mm | " " | Host *3 months in laboratory | |
| | 0.367 x 0.345 mm | " " | E/A | |
| <u>Enallagma cyathigerum</u> | 0.352 x 0.345 mm | " " | E/A | |
| Anisoptera - <u>Aeschna cyanea</u> | 0.220 x 0.205 mm | " " | E/A | |
| | 0.202 x 0.184 mm | " " | " | |
| | 0.202 x 0.147 mm | " " | " | |
| <u>TRICHOPTERA</u> | | | | |
| <u>Phryganea grandis</u> | 0.205 x 0.198 mm | " " | Host *3 months in laboratory | |
| | 0.224 x 0.187 mm | " " | Host *2 months in laboratory | |
| | 0.220 x 0.191 mm | Experimental infection | } of 24 hours duration | |
| | 0.220 x 0.220 mm | " " | | |
| | 0.209 x 0.209 mm | Natural infections | E/A | |
| <u>Phryganea striata</u> | 0.213 x 0.213 mm | " " | E/A | |
| | 0.209 x 0.201 mm | " " | " | |
| <u>DIPTERA</u> | | | | |
| <u>Chaoborus</u> sp. | 0.330 x 0.297 mm | " " | E/A | |

Range:

| | | |
|--------------|----------------------------------|------------------------------------|
| Zygoptera: | 0.213 - 0.436 x 0.201 - 0.400 mm | } 0.202 - 0.436 x 0.147 - 0.400 mm |
| Anisoptera: | 0.202 - 0.220 x 0.147 - 0.205 mm | |
| Trichoptera: | 0.205 - 0.224 x 0.187 - 0.220 mm | |
| Diptera: | 0.330 x 0.297 mm | |

Average:

| | | |
|----------------------|----------------------------------|---------------------|
| Odonata - Zygoptera: | <u>Ischnura elegans</u> | - 0.315 x 0.294 mm |
| | <u>Coenagrion puella</u> | - 0.370 x 0.341 mm |
| | (<u>Enallagma cyathigerum</u>) | - 0.352 x 0.345 mm) |
| Anisoptera: | <u>Aeschna cyanea</u> | - 0.208 x 0.179 mm |
| | <u>Phryganea grandis</u> | - 0.216 x 0.201 mm |
| | <u>Phryganea striata</u> | - 0.211 x 0.207 mm |
| Diptera: | (<u>Chaoborus</u> sp.) | - 0.330 x 0.297 mm) |

Mean (25 cysts) =
0.306 x 0.284 mm

Abbreviations: E/A = examined on arrival.

* = hosts were kept from 2 - 3 months in the laboratory during the winter months of November, December, January and February.

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GORGODERA - METACERCARIA

The ventral view of a mounted specimen drawn with the aid of a projector.

Abbreviations:

AN = Anterior nerve branch

PG = Penetration gland cell

PN = Posterior nerve branch

GP = Gonopore

U = Uterine coiling

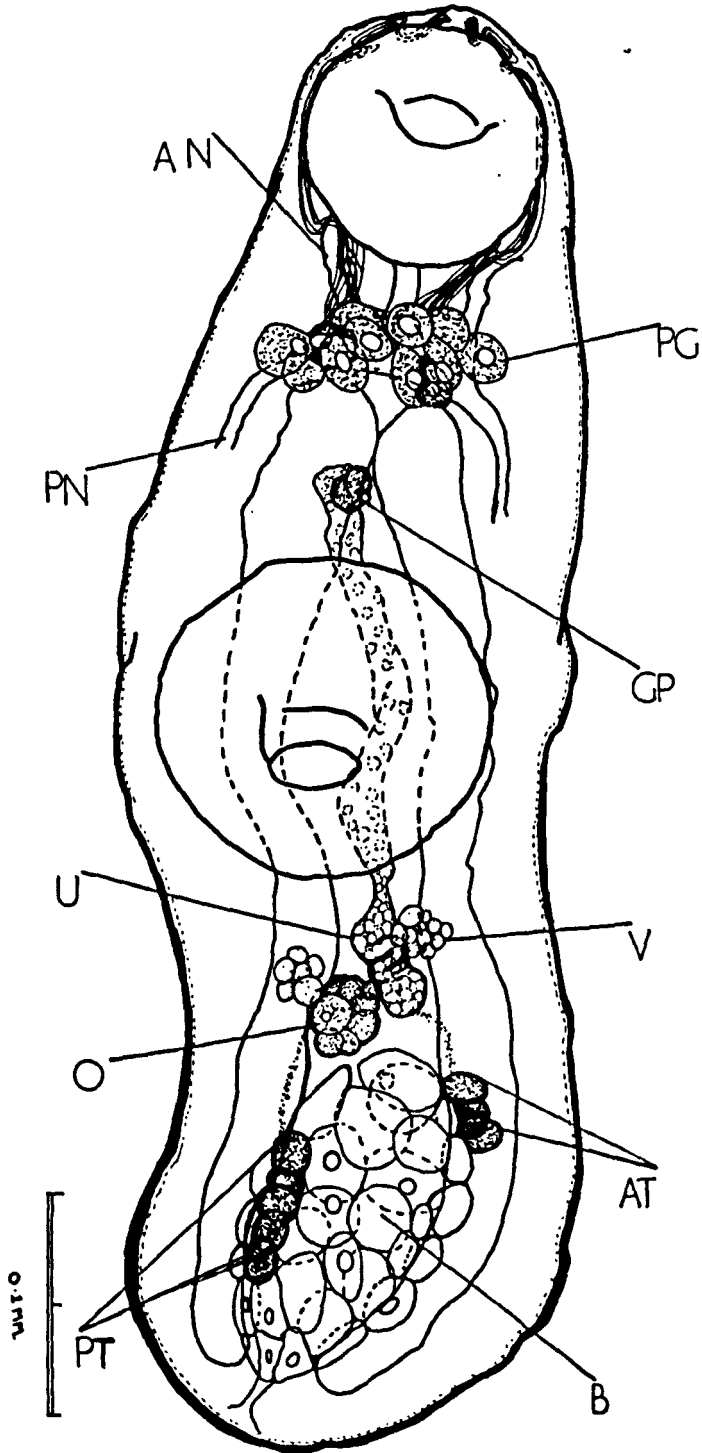
V = Vitellaria

O = Ovary

AT = Anterior testis - 4 lobes

PT = Posterior Testis - 5 lobes

B = Bladder (the concretions are lost during fixation and staining processes)



GORGODERA - METACERCARIAE

Detailed measurements of Gorgodera metacercariae:

1) Total length

Average length of 10 specimens from Odonatan hosts
(specimens - contracted - fully extended state)
Average length of 5 specimens from Trichopteran hosts
(specimens - contracted - relaxed state)
Maximum extensile range recorded for one individual
from the fully contracted to the extended position
Minimum length recorded for a contracted specimen
Maximum length recorded for an extended specimen

a) Living material
= 0.867 (0.462-1.290 mm)
= 0.647 (0.462-0.752 mm)
= 0.712 mm (Odonatan host)
= 0.459 mm (Trichopteran host)
= 0.445 mm (Odonatan host)
= 0.293 mm (Trichopteran host)
= 1.290 mm (Odonatan host)
= 0.752 mm (Trichopteran host)

Average length of 10 specimens from Odonatan and
Trichopteran hosts (contracted - slightly extended state)

b) Mounted material
= 0.596 mm (0.323-0.774 mm)

2) Regional proportions

Measurement of the anterior region (excluding V/S) of
10 specimens (contracted - extended state)
Measurement of the posterior region (including V/S)
of 10 specimens (contracted - extended state)
Ratio: Average anterior: posterior region - length
width
Maximum extensile range recorded for one individual
(each region measured independently) - anterior region
posterior region

a) Living material (Odonatan/Trichopteran hosts)
= 0.297 x 0.161 mm (0.147-0.667 x 0.073-0.222)
= 0.400 x 0.197 mm (0.230-0.622 x 0.092-0.293)
= 1:1.348
= 1:1.224
= 0.444 mm
= 0.267 mm

Measurement of the anterior region (excluding V/S) of
10 specimens (contracted - slightly extended state)
Measurement of the posterior region (including V/S) of
10 specimens (contracted - slightly extended state)
Ratio: Average anterior: posterior region - length
width

b) Mounted material (Odonatan/Trichopteran hosts)
= 0.236 x 0.148 mm (0.084-0.320 x 0.092-0.213)
= 0.360 x 0.156 mm (0.238-0.453 x 0.103-0.231)
= 1:1.524
= 1:1.056

3) Sucker sizes

Measurement of the O/S of 20 specimens (relative to organ)
Measurement of the V/S of 20 specimens (relative to organ)
Ratio: O/S: V/S relative to B/A length
relative to B/A width
relative to organ - greatest diameter
Measurements of O/S of 10 specimens (relative to organ)
Measurements of V/S of 10 specimens (relative to organ)
Ratio: O/S: V/S relative to B/A length
relative to B/A width
relative to organ - greatest diameter

a) Living material (Odonatan/Trichopteran hosts)
= 0.118 x 0.0990 (0.0816-0.173 x 0.0510-0.147)
= 0.147 x 0.139 (0.0826-0.201 x 0.0612-0.172)
= 1:1.419 (1:0.750-1:1.556)
= 1:1.432 (1:1.200-1:1.569)
= 1:1.250 (1:1.000-1:1.428)
b) Mounted material (Odonatan/Trichopteran hosts)
= 0.108 x 0.0945 (0.0867-0.128 x 0.0660-0.117)
= 0.136 x 0.127 (0.0953-0.165 x 0.0770-0.158)
= 1:1.256 (1:1.084-1:1.482)
= 1:1.341 (1:1.167-1:1.50)
= 1:1.257 (1:1.084-1:1.482)

4) Excretory bladder size

Average measurements of bladder from 5 specimens
Average measurements of the posterior region of the
5 specimens above
Average percentage of the posterior width occupied
by the bladder
Average percentage of the length of the posterior
region occupied by the bladder
Average measurements of bladder from 5 specimens
Average measurements of the posterior region of
the 5 specimens above
Average percentage of the posterior width occupied
by the bladder
Average percentage of the posterior length occupied
by the bladder

a) Living material (Odonatan/Trichopteran hosts)
= 0.191 x 0.0816 mm (0.102-0.267 x 0.055-0.918)
= 0.447 x 0.170 mm (0.348-0.622 x 0.0917-0.238)
= 49.10%
= 42.74%
b) Mounted material (Odonatan/Trichopteran hosts)
= 0.187 x 0.0980 mm (0.150-0.227 x 0.0633-0.194)
= 0.411 x 0.178 mm (0.324-0.453 x 0.133-0.231)
= 55.09%
= 45.46%

5) Metacercarial stylet

Mean length from 3 specimens obtained from Ischnura elegans
Mean length from 6 specimens obtained from Coenagrion puella
Mean length from 3 specimens obtained from Aeschna cyanea
Mean length from 3 specimens obtained from Phryganea grandis
(natural and experimental infections)

a) Living material
= 0.0356 mm (0.0306-0.0337 mm)
= 0.0328 mm (0.0316-0.0357 mm)
= 0.0258 mm (0.0255-0.0265 mm)
= 0.0275 mm (0.255-0.0306 mm)

Overall mean length of 15 specimens

Mean measurement for stylet base - 10 specimens side view
(all specimens from various hosts overlap in values)
Mean measurement of stylet keel - depth at wings side
view - 10 specimens (all specimens from various hosts
overlap in values)
Mean measurement of stylet across the wings - dorsal view
- 5 specimens (all specimens from various hosts overlap
in values)

= 0.0309 mm (0.255-0.0357 mm)
= 0.00633 mm (0.00510-0.00816 mm)
= 0.00714 mm (0.00612-0.00816 mm)
= 0.00612 mm (0.00510-0.00714 mm)

6) Reproductive organs

Measurements taken from 5 specimens (Odonatan/Trichopteran hosts)
Testis A - divided into 4.
Mean measurement of each testis lobe = 0.0226 x 0.0195 mm (range 0.0168 - 0.0367 x 0.0147 - 0.0255 mm)
Testis B - divided into 5.
Mean measurement of each testis lobe = 0.0249 x 0.0202 mm (" 0.0147 - 0.0408 x 0.0126 - 0.0255 mm)
Ovary - mean measurement = 0.0367 x 0.0347 mm (" 0.0296 - 0.0418 x 0.0306 - 0.0378 mm)
Right vitellaria = 0.0332 x 0.0277 mm (" 0.0275 - 0.0408 x 0.0255 - 0.0306 mm)
Left vitellaria = 0.0379 x 0.0291 mm (" 0.0286 - 0.0459 x 0.0210 - 0.0306 mm)
Vitellaria - total mean = 0.0356 x 0.0284 mm (" 0.0275 - 0.0459 x 0.0210 - 0.0306 mm)
Ovary - amphitypic - situated on the right in 50% of the cases (10 specimens)
Anterior testis composed of 5 fragments in 40% of the cases (5 specimens)
Posterior testis composed of 5 fragments in 60% of the cases (5 specimens)
Vesicula seminalis (10 specimens) = 0.0397 x 0.0256 mm (range 0.0210 - 0.0525 x 0.0126 - 0.0378 mm)

Abbreviations:

O/S = oral sucker
V/S = ventral sucker
Rel. to B/A = measurements are taken relative to the main body axes
disregarding those of the organ concerned

All measurements are taken relative to the organ or regional axis, except where stated otherwise and are in mm
Living and mounted specimens are not necessarily identical

d. 2) The Metacercarial Body (illustrated on page 370)

The metacercaria possesses a muscular, highly contractile body bounded by a cuticle patterned in an identical fashion to that described for the cercaria.. The cytoplasm is more transparent than that of the earlier larva enabling more structural details to be seen but it retains a greater density than any Phyllodistome stage. The Gorgodera metacercaria is slightly larger on average than the corresponding cercarial stage but the regional proportions are approximately the same. Although on average the suckers are larger the two larval size ranges overlap. Proportionally the ventral sucker is growing considerably faster than the oral sucker and this is shown in both the average ratios and their ranges.

Papillary pattern

The rounded body margin and the depressed nature of the papillae made the marginal series difficult to discern and differentiate from the structures belonging to the dorsal and ventral sequence.

Marginals

On each side of the body posterior to the oral sucker there are 4 papillae and 2 on either side of the acetabulum. Posterior to the ventral suckers are 6 - 8 papillae on each side.

Dorsal papillae

Observations concerning these papillae in this series are incomplete.

Ventral papillae

There is a double row running on either side of the anterior probe but only the beginning of each row could be accurately positioned. The

outer and inner series began in an identical position relative to the marginals as previously described for Phyllodistomum. A single pair of papillae were found opposite the posterior pair of marginals on either side of the acetabulum.

Sucker papillae

Primary attention was paid to the ventral sucker patterns. These were more easily seen against the clearer cytoplasm of the sucker and were identical to the complex patterns described for Phyllodistomum (page 242 illustration 241). The dorsal pattern above the oral sucker was also identical. These patterns were more difficult to discern than was the case for Phyllodistomum and this surprising similarity between the two genera was checked repeatedly and, as a result, many larvae died before full examination of the entire body could be made.

The stylet

This is identical to the cercarial structure but the larger numbers acquired increased the size range. There is a greater tendency for smaller larvae to produce shorter stylets which are secreted into a dorsal chamber occupying an identical position to that described for Phyllodistomum. The stylet is also similar to the latter, possessing two lateral wings, a forwardly directed spine, a keel and a rounded base. It is used and erected in a manner previously described, its position being altered by stylet muscles similar to those illustrated for Phyllodistomum on page 87 In the erect position the keel is directed posteriorly and when in use the cutting spine is held in a ventral position. The keel does not protrude laterally when the stylet

is viewed from above and possesses only one cutting edge and not two as is the case for Phyllodistomum. In the dorsal view the stylet base appears to be more pronounced than in the latter genus as the result of a deeper constriction behind the wings. The Gorgodera stylet is generally larger than the corresponding structure found in Phyllodistomum although in its lower size range the two overlap. The keel in Gorgodera compensates for the lack of a second cutting edge by being deeper and narrower than its Phyllodistome counterpart.

Penetration glands

A total of 12 gland cells arranged in clusters of 6 are situated on either side of the oesophagus. They are anterior to the position recorded for the cercaria lying either immediately anterior to the gut bifurcation or mid-way between the latter and the oral sucker. The gland contents vary. A few contain a clear transparent fluid which was seen on one occasion exuding from the terminal openings of the ducts in the form of hyaline droplets. The metacercaria on which this observation was made was artificially excysted but submitted to no coverslip pressure and was mounted in water during the period of fluid loss. The majority contain a finely granular precipitate which eventually takes neutral red stain. The position of the penetration glands in appearing to remain anterior to the gut bifurcation in the metacercaria differs from the Phyllodistome condition where there is more variability. Differential growth rates between the glands, their ducts and the anterior region occur in both genera.

The gut

This consists of a muscular, extensile oesophagus branching into two simple caecae at a point anterior to the position of the genital pore and acetabulum. The two caecae are usually of equal length and terminate a short distance from the posterior margin of the body. Slight local expansions occur immediately after the bifurcation and the entire system is identical to that described for Phyllodistomum.

Nervous system

Basically identical to the system described for Phyllodistomum. Two anterior largely eosinophyllic cerebral ganglia are linked by a transverse commissure, the entire ganglionic mass lying ventral to the oesophagus. The main nerves arise anteriorly, laterally and posteriorly from each ganglion. In Phyllodistomum three main nerves were described as arising from each side. The posterior nerve diverged immediately after the ganglion giving off a branch which served the acetabulum. In Gorgodera this internal branch appeared to arise directly from the nervous concentration.

Excretory system

This system was extremely difficult to follow in the living specimens and observations are incomplete. The main collecting ducts arise sub-terminally from the bladder and pass antero-laterally. One duct invariably crosses the ovarian rudiment. This condition is only rarely found in Phyllodistomum. Both ducts pass anteriorly and ^{may} divide into anterior and posterior collecting ducts at the level of the gut bifurcation. Only a few flame cells were seen and cell groupings could not be distinguished.

The bladder is distended apically and appears pear-shaped. It is filled with refractile concretions which are readily released either into the cystic fluid as a result of coverslip pressure or following excystment. The concretions appear to be crystalline laminated structures formed by the excretory cells lining the bladder. These structures are steadily produced throughout the period of encystment with the result that, whilst some had been released into the bladder cavity, others remained within the cells. The concretions averaged 0.016×0.0092 mm in size and were shed in company with fine neutral-red-staining particles which are also derived from the cells. When the bladder is empty it is narrower but retains an expanded apex unlike the form described for Phyllodistomum. It is, when empty, identical to the Gorgodera cercarial structure and occupies the same percentage of the posterior width. It takes up however less of the total length suggesting that the bladder is not keeping pace with the fast growing posterior region of the body.

Gland cells

The large eosinophilic gland cells visible in the cercaria appear to be absent from the metacercarial stage.

Reproductive system

This system shows a developmental advance upon the cercarial state. The genital pore is now formed and the male system differentiated. The uterine anlage extends from the genital pore immediately posterior to the gut bifurcation, passing dorsal to the acetabulum and coils repeatedly immediately posterior to the latter. The vitellaria appear

as two rounded bodies lying on either side of the uterine coils immediately posterior to the ventral sucker or are overlaid by its margin depending upon the degree of body contraction. The amphitypic ovary, spherical to ovoid in shape, is situated to one side of the ootype area. The testes are sub-divided into 4 to 5 lobes respectively. The number of portions into which either the anterior or posterior testis were divided varied from specimen to specimen. These ovoid subdivisions are either closely opposed or overlap one another. The system remains in a rudimental state and differs markedly from the well differentiated system found in Phyllodistomum. It is obvious that considerable development is necessary before these flukes will mature.

e) The Gorgodera juvenile and adult

The measurements for these specimens are given on pages 382, 389 & 394 and are illustrated on pages 381, 388 & 393.

Three experimental frogs were utilised in the infection procedures outlined on page 175. They were collected from a single locality on the 6th December, 1962 and were in a semi-hibernative state when brought into the laboratory. Although the room temperatures remained low over the next month (49° - 54°F) the frogs began to accept food from the 6th January, 1963. The staple diet of the frogs, when once they were feeding regularly, consisted of balls of Tubifex and laboratory-bred Diptera. Insect larvae were fed to them at intervals as they became available. The larvae were frequently bisected upon ingestion so that the head and thorax only were swallowed. This method

did not affect the potential success of the Gorgoderids which always encyst in the thorax but caused some loss in the numbers of Haematoloechus introduced into the frogs' system. The frogs' waste was largely deposited in small glass containers which were arranged in the frog tank to give the animals cover and in which they spent most of their time. Examination of this waste showed that in March insect larval remains were taking six days to pass completely through the gut. Even in May undigested material remained in the stomach 24 hours after ingestion.

e 1) Juvenile (1) (illustrated on page 38)

In experiment C a) page 15, 16 juvenile Gorgoderids were recovered from frog A. Until it was killed and examined on the 13th March, 1963, this frog had taken 14 insect larvae (potentially infected with Gorgoderids and derived from the experiments outlined upon page 42-43) and six weeks prior to its death, at least 6 Phyllodistome metacercariae were also ingested.

The four moribund Gorgoderid specimens recovered from the region of the seminal vesicles could not be identified as belonging to either trematode genus. It can be assumed that either Gorgodera juveniles do not survive when frequenting the seminal vesicle channels or that Phyllodistomes had remained alive within an unusual host for six weeks and had wandered into an abnormal situation and were dying. The single Gorgodera juvenile recovered from the rectum was progressing along the gut wall in the direction of the urinary openings and the cloaca. The host had accepted insect larvae five days previously and the rate of

migration parallels the digestion rate for this period. Upon entry into the urinary system the Gorgodera juveniles preferentially invade the ureters. They migrate to the distal regions remaining in the small side branches of tubes which permeate amongst the kidney tissue. The latter had to be teased carefully in order to find the flukes which, in all cases, remained in the urinary ducts and were not recovered from the kidney tissue itself. The juveniles from frog A (the measurements of which are given on page 382) possessed a heavily refractile, granular cytoplasm and rounded body which rendered the observation of excretory and reproductive systems extremely difficult. The flukes were cellular feeders browsing upon the uretal and bladder lining. The gut caecae are simple and usually, but not always, equal in length. They were active specimens executing a series of movements whilst in a watch-glass which were very similar to those described for Phyllodistomum. Their movements in situ were not seen since the majority occupied ducts which were shielded by opaque kidney tissue. The complete extension of the fluke produced an elongate cylindrical shape in which the acetabulum protruded in a manner not observed in Phyllodistomum, and this can be correlated to the greater acetabular size. The penetration glands were still visible and were situated uniformly anterior to the gut bifurcation. The excretory duct system was identical to that described for the metacercariae but the bladder was considerably narrower. The bladder cells were small and equivalent to Phyllodistomum in size measuring 0.0102×0.007 mm in the living state and containing finely granular contents uniformly distributed throughout the cell. The bladder had acquired a sinusoidal curve similar to the condition found in Phyllodistomum.

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GORGODERA - JUVENILE 1.

Ventral view of a juvenile recovered from
Frog A (13/3/63) drawn with the aid of a
projector.

Abbreviations:

FG = Penetration gland cell

GP = Gonopore

PA = Papilla

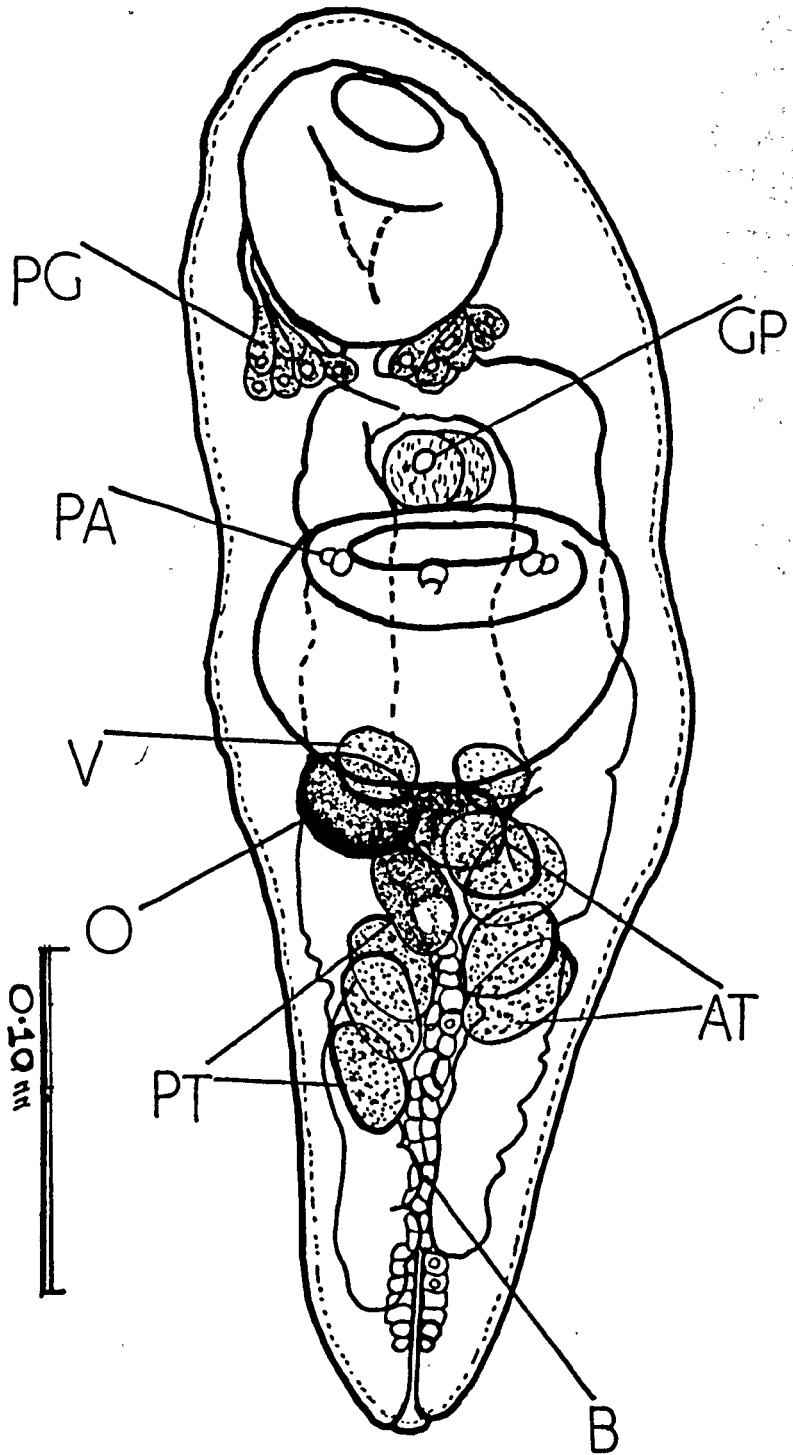
V = Vitellaria

O = Ovary

AT = Anterior testis - 5 lobes

PT = Posterior testis - 4 lobes

B = Bladder



GORGODERA = JUVENILE (1)

Gorgodera specimens from the definitive host (juveniles) (1)

Host - Rana temporaria - 13.3.1963.

1) Total length

Average length of 10 specimens (relaxed - extended state)
Maximum extensile range recorded for 1 individual from the fully contracted to the extended position
Minimum length recorded for a contracted specimen
Maximum length recorded for an extended specimen

a) Living material
= 0.777 mm (0.450 - 1.068 mm)
= 0.666 mm
= 0.293 mm
= 1.068 mm

Average length of 10 specimens (relaxed - extended state)

b) Mounted material
= 0.432 mm (0.330 - 0.612 mm)

2) Regional proportions

Measurement of the anterior region (-V/S) of 10 specimens (extended state)
Measurement of the posterior region (+V/S) of 10 specimens (extended state)

a) Living material
= 0.348 x 0.0800 mm (0.220-0.533 x 0.0445-0.191 mm)
= 0.414 x 0.0837 mm (0.275-0.578 x 0.0323-0.150 mm)
= 1:1.19
= 1:1.046

Ratio: Average anterior region: posterior region - length
width
Maximum extensile range recorded for 1 individual (each region being measured independently - anterior region
posterior region

= 0.445 mm
= 0.320 mm

Measurement of the anterior region (-V/S) of 10 specimens (relaxed - extended state)

b) Mounted material
= 0.156 x 0.110 mm (0.103-0.255 x 0.085-0.153 mm)

Measurement of the posterior region (+V/S) of 10 specimens (relaxed - extended state)

= 0.275 x 0.0913 mm (0.187-0.459 x 0.0765-0.117 mm)
= 1:1.763
= 1:0.830

Ratio: Average anterior region: posterior region - length
width

a) Living material

3) Sucker sizes

Measurement of the oral sucker of 10 specimens (relative to the organ)
Measurement of the ventral sucker of 10 specimens (relative to the organ)
Ratio: O/S: V/S Relative to B/A - length
Relative to B/A - width
Relative to organ - greatest diameter

= 0.0950 x 0.0747 mm (0.0734-0.110 x 0.0660-0.0917 mm)
= 0.115 x 0.110 mm (0.103-0.128 x 0.0917-0.128 mm)
= 1:1.211 (1:1.000 - 1:1.500)
= 1:1.473 (1:1.200 - 1:1.889)
= 1:1.211 (1:1.000 - 1:1.500)

Measurements of the oral sucker of 10 specimens (relative to the organ)
Measurements of the ventral sucker of 10 specimens (relative to the organ)
Ratio: O/S: V/S Relative to the B/A length
width
Relative to organ - greatest diameter

b) Mounted material
= 0.0854 x 0.0697 mm (0.0622-0.104 x 0.0561-0.0816 mm)
= 0.100 x 0.0872 mm (0.0847-0.124 x 0.0705-0.102 mm)
= 1:1.211 (1:1.013 - 1:1.456)
= 1:1.210 (1:1.212 - 1:1.491)
= 1:1.171 (1:1.147 - 1:1.361)

4) Excretory bladder size

Measurements of bladder from 5 specimens
Measurement of the posterior region of the 5 specimens above
Average percentage of the posterior width occupied by the bladder
Average percentage of the posterior length occupied by the bladder

a) Mounted material
= 0.155 x 0.0171 mm (0.139-0.165 x 0.0147-0.0183 mm)
= 0.266 x 0.100 mm (0.257-0.271 x 0.0917-0.117 mm)
= 17.1%
= 58.27%

5) Reproductive organs

Testis A - divided into 4. Mean measurement of each lobe
Testis B - divided into 5. Mean measurement of each lobe
Ovary
Amphitypic - ovary lies on the right in 60% of cases
Vitellaria - total mean
(Anterior Testis consisted of 5 lobes in 60% of cases (5 specimens)
(Posterior Testis " " 5 " " 40% " " " " " ")

a) Mounted material* (5 specimens)
= 0.0336 x 0.0251 (0.0306-0.0428 x 0.0204-0.0317 mm)
= 0.0363 x 0.0247 (0.0316-0.0428 x 0.0184-0.0326 mm)
= 0.0402 x 0.0300 (0.0326-0.0459 x 0.0255-0.0357 mm)
= 0.0340 x 0.0204 (0.0255-0.0357 x 0.0204-0.0214 mm)

Abbreviations:

O/S = oral sucker
V/S = ventral sucker
Rel. to B/A = measurements are taken relative the main body axes, disregarding those of the organ concerned.
All measurements are taken relative to the organ or regional axis except where stated otherwise.

N.B.* In the living material it was impossible to discern the complete reproductive system accurately. In the mounted state it took up little stain and the measurements, particularly the vitellaria, were made with difficulty.

The papillae were slightly clearer and identical systems to those described for the metacercariae were traced. In addition a few extra papillae from the body series could be discerned but observations remain incomplete. Dorsally on the anterior probe only one pair of papillae could be traced. These lay immediately behind the oral sucker. On the posterior region papillae lay close to the rounded body margin. On either side a line of five were seen but there may have been more. Their alignment did not appear to follow the marginals closely. Ventrally on the anterior probe two rows of four papillae alternating with the marginals and one another were seen to possess the same arrangement as in Phyllodistomum. Papillae in the ventral surface of the posterior region could not be traced.

Some of the active Gorgodera juveniles recovered from frog A may have reached the urinary system of their host some 8 weeks previously. The youngest juvenile recovered had not reached its correct habitat. All the juveniles were small and showed little increase in size even over the cercarial state. The sucker sizes and ranges of the juveniles and metacercariae overlap but remain smaller than the average metacercarial sizes recorded. The proportions of the suckers relative to one another are approximately equivalent to the cercarial condition. The anomaly between the sizes of the juveniles and metacercariae may be explained by the fact that frog A received larvae which had been collected and kept in the laboratory from 1 - 3 months during an extremely cold period. Data obtained from cysts recovered from Trichoptera which had been kept in the laboratory under these conditions

indicated that little growth could take place during this time. If the larvae which this frog ingested gained access to the intermediate host only a short period before they were collected or were experimentally introduced then the metacercariae would remain small. The lack of growth of the juveniles in the vertebrate host for a possible maximum of 9 weeks is in direct contrast to the Haplometra specimens already present in the lungs when the frog was captured and the Haematoloechus introduced with the Gorgoderid infection over a period of 8 weeks. Table 35 shows that the frog could not have been carrying any excessively small Haplometra upon introduction into the laboratory. The Haematoloechus larvae recovered from the Odonatan nymphs during routine examinations varied considerably in size according to their age and averaged between 2 and 3 mm in length. Those which remained for a maximum of two months in the definitive host increased considerably in length during this period. Possibly the Gorgoderids were more sensitive to the hibernative state, temperature or hormonal condition of the host than was the case for lung parasites. This agrees to some extent with the report given by Lees and Bass (1960) and Lees (1962) on the effects of hormones on the parasite burden of frogs. These workers found that Gorgoderina vitelliloba was more sensitive to high concentrations of female hormones than Haplometra cylindracea. In 1962 Lees suggested that the winter fall in parasite numbers may be related to the increase in amount of oestrogens and possibly androgens in the host at this time of the year. Perhaps such sensitivity on the part of the parasites is further reflected in their growth rates and

that those parasites most affected numerically by hormonal changes in the host may also be affected more severely as regards their rate of growth and even attainment of maturity (although the juveniles showed some reproductive differentiation in advance of the metacercaria - particularly in the male system).

The Gorgodera juveniles attained a high success rate of infection in this experiment. Although the exact numbers of metacercariae involved for either Gorgodera or Haematoloechus were not known faunal investigation indicated that the average number of flukes per larva would be much higher in the case of the lung parasites. The success of only 6 individuals from 14 insect larvae indicates a high rate of metacercarial loss following ingestion.

e 1) Juveniles (2) (illustrated on page 388)

In frog B a single fluke, considerably larger than those recovered from A but still a juvenile, was taken from the bladder. The living fluke could be seen through the transparent bladder wall attached by the acetabulum with the oral sucker plucking at the bladder lining. The posterior region was swung actively from side to side, sometimes opposing the swing of the anterior region and sometimes moving whilst the latter region remained motionless. The posterior region was thus utilised in a manner not encountered in Phyllodistomum and the active lashing movement made the fluke highly conspicuous. The posterior region was extended and contracted in a series of regular independent movements during which the posterior third of the region was flipped upwards at the end of the extensile phase; straightened and then the

Table 35

A) Examination of control frogs 6.12.1962. *Rana temporaria*

| <u>Region</u> | <u>Parasite</u> | <u>Male 1</u> | <u>Female 1</u> | <u>Female 2</u> |
|---|------------------------|---------------|-----------------|-----------------|
| Skin | - | - | - | - |
| Buccal cavity/under tongue/ Eustachian tubes | - | - | - | - |
| Oesophagus/Stomach | - | - | - | - |
| Duodenum | <u>Acanthocephalus</u> | P | - | - |
| | <u>Oswaldocruzia</u> | P | - | P |
| | <u>Dolichosaccus</u> | - | P | - |
| Ileum | <u>Oswaldocruzia</u> | P | P | P |
| | <u>Dolichosaccus</u> | - | P | - |
| Rectum | <u>Oswaldocruzia</u> | P | P | P |
| Abdominal cavity/walls | - | - | - | - |
| Liver/spleen/gall bladder | - | - | - | - |
| Lungs | <u>Haplometra</u> | - | - | P |
| | <u>Rhabdias</u> | P | - | P |
| Kidneys/ureters/bladder | - | - | - | - |
| Cloaca | - | - | - | - |

B) Examination of experimental frogs

| <u>Region</u> | <u>Parasite</u> | <u>A(13.3.63)</u> | <u>B(31.5.63)</u> | <u>C(31.5.63)</u> |
|---|----------------------------|-------------------|-------------------|-------------------|
| Skin | - | - | - | - |
| Buccal cavity/under tongue/ Eustachian tubes | - | - | - | - |
| Oesophagus/Stomach | - | - | - | - |
| Duodenum | <u>Oswaldocruzia</u> | - | P | P |
| Ileum | <u>Oswaldocruzia</u> | P | - | P |
| Rectum | <u>Oswaldocruzia</u> | P | - | - |
| | <u>Metacercarial cysts</u> | P | - | - |
| Abdominal cavity/walls | - | - | - | - |
| Liver/spleen/gall bladder | - | - | - | - |
| Lungs | <u>Haplometra</u> | P | - | P |
| | <u>Haematoloechus</u> | P | P | P |
| Kidneys | <u>Metacercarial cysts</u> | P | - | - |
| Ureters | <u>Gorgodera</u> | P | - | - |
| Bladder | <u>Gorgodera</u> | - | P | P |
| Cloaca | - | - | - | - |

N.B. A detailed examination of the protozoan fauna was not carried out. Kidney smears were examined for Myxosporidia which were found to be absent.

(Opalina, Nyctotherus, Balantidium were noted in the alimentary system.)

c) Haplometra:

| <u>Date</u> | <u>Parasitic Load</u> | <u>Size Range (fixed specimen)</u> | <u>Host</u> |
|-------------|-----------------------|------------------------------------|---|
| 6.12.62. | 4 | 2.5 mm - 8.5 mm in length | 1 control frog. |
| 13.3.63. | 3 | 7.0 mm - 10.0 mm in length | 1 experimental frog (A) 3 months in lab. |
| 31.5.63. | 2 | 6.5 mm - 7.0 mm in length | 1 experimental frog (C) 5 months in lab. |

D) Haematoloechus:

| <u>Date</u> | <u>Parasitic Load</u> | <u>Size Range (fixed specimen)</u> | <u>Host</u> |
|-------------|------------------------|------------------------------------|---|
| 6.12.62 | 0 | 0 | Control frogs |
| 13.3.63. | 6 | 4.0 - 6.5 mm in length | 1 experimental frog (A) 9 weeks feeding in lab. |
| 31.5.63. | ⁶ 18 12) | 2.5 - 7.5 mm in length | 2 experimental frogs (B)(C) 4 months feeding in lab. |

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GORGODERA - JUVENILE 2.

Ventral view of the mounted juvenile specimen
recovered from Frog B - drawn with aid of a
projector

Abbreviations:

GP = Gonopore

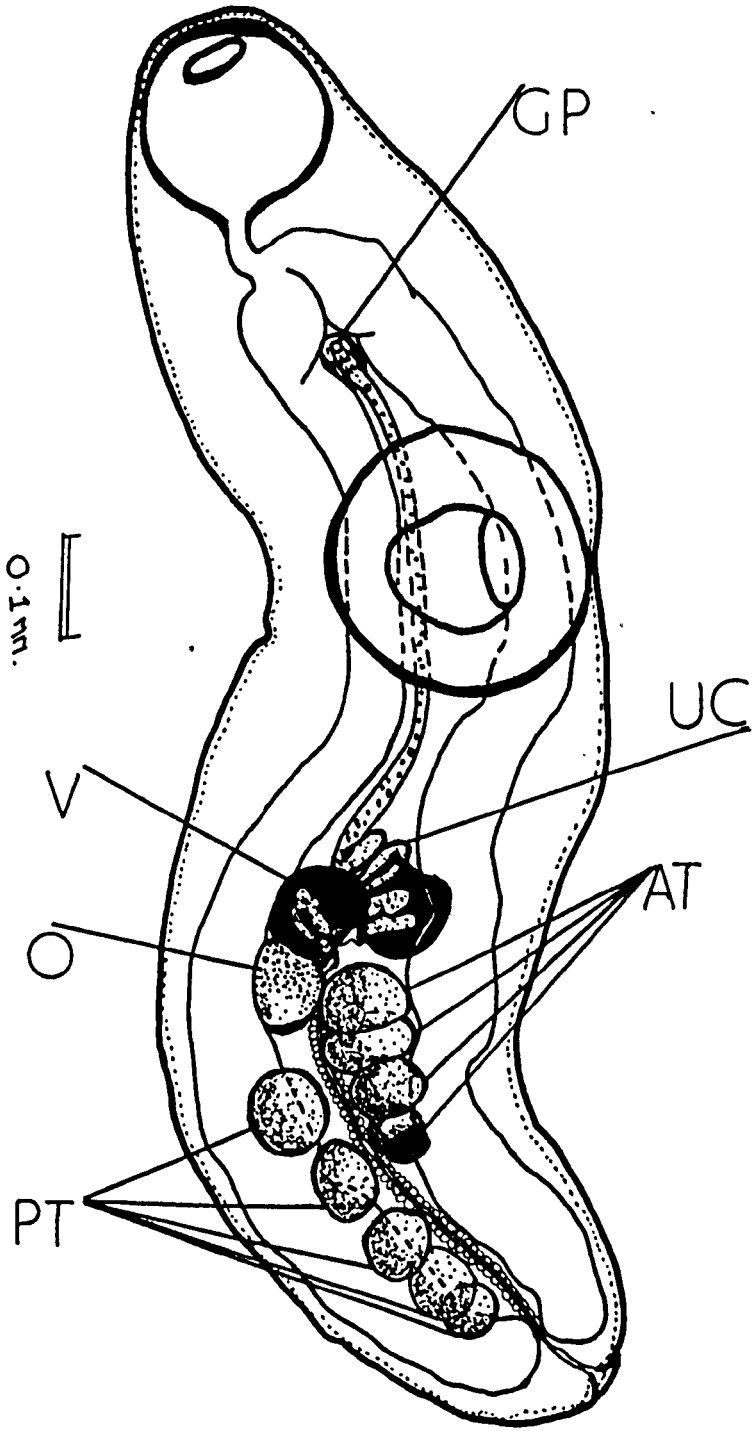
UC = Uterine coils

V = Vitellaria

O = Ovary

AT = Anterior testis - 4 lobes

PT = Posterior testis - 5 lobes



GORGODERA = JUVENILE (2)

Gorgodera recovered from the definitive hostHost - Rana temporariaOne juvenile specimen (2) of medium size recovered -
mounted material

| | | | |
|------------------------------------|---|--------------------|------------------------------------|
| Total length | = | 1.449 mm | |
| Anterior region (-V/S) | = | 0.455 x 0.350 mm | |
| Posterior region (+V/S) | = | 0.994 x 0.371 mm | |
| Ratio: anterior region: posterior | | | |
| region - length | = | 1:2.185 | |
| width | = | 1:1.060 | |
| Oral sucker | = | 0.186 x 0.184 mm | |
| Ventral sucker | = | 0.264 x 0.264 mm | |
| Ratio: O/S: V/S Rel. to B/A length | = | 1:1.419 | |
| Rel. to B/A width | = | 1:1.435 | |
| Rel. to organ - greatest diam. | = | 1:1.419 | |
| Reproductive organs: | | | |
| Anterior testis = 4 fragments - | | | |
| average measurement of each lobe | = | 0.0760 x 0.0570 mm | |
| | | | (0.0600-0.096 x 0.048-0.072 mm) |
| Posterior testis = 5 fragments - | | | |
| average measurement of each lobe | = | 0.0716 x 0.0612 mm | |
| | | | (0.054 - 0.084 x 0.054 - 0.080 mm) |
| Ovary - right side | = | 0.086 x 0.072 mm | |
| Vitellaria - right side | = | 0.080 x 0.080 mm | } average |
| - left side | = | 0.084 x 0.080 mm | |
| | | | 0.082 x 0.080 mm |
| Vesicula seminalis | = | 0.0735 x 0.0357 mm | |
| Excretory bladder | = | 0.453 x 0.0178 mm | |
| Percentage of posterior width | | | |
| occupied by bladder | = | 4.79% | |
| Percentage of posterior length | | | |
| occupied by bladder | = | 50.01% | |

entire region contracted. The suckers left deep impressions in the bladder tissues and the oral sucker dislodged considerable quantities of the transitional lining during the period of observation. The measurements of this fluke are given on page 389. These figures show that considerable growth has occurred since the metacercarial state particularly in the posterior region where the reproductive organs are now fully differentiated. The suckers are larger but can be considered to remain similar in proportion to one another if the metacercarial and previous juvenile ratio maxima are taken into account.

The marked increase in size and development of the Gorgoderid recovered from frog B may have occurred as a result of the rise in temperature, increase in activity and change in the hormonal state of the host. Six young Haematoloechus were recovered from this host graded in size from 4.0 - 6.0 mm.

e 2) Gorgodera adult (illustrated on page 393)

On examination of frog C eight large flukes were seen actively moving about in the bladder with one occupying the bladder channel to the opening into the cloaca. The youngest flukes were cream in colour, whilst the older specimens acquired a pale orange colouration tinged with pink. The gut contents were brown. The flukes actively lashed the posterior region back and forth in the host urine in a manner previously described as part of the juvenile behaviour. This action may have some respiratory importance preventing stagnation in the progressively concentrating urine of the host. The possession of a rounded body may necessitate such activity. The preference which

maturing flukes show for the bladder probably prevents excessive damage to the host. The presence of numerous cylindrical flukes in the ureters would have a greater tendency to block the ducts than similar numbers of flattened *Phyllodistomes*.

The measurements of the adult flukes are given on page 394

Apart from the great increase in size the flukes differ from the larger juvenile recovered from frog B in the following ways. The posterior region proportionally has increased both in length and width. The vitellaria are sub-divided into a total of 8 - 9 lobes. Previously these organs remained rounded and indentations were only noted on two occasions in one metacercaria and one juvenile. The uterus in the three youngest adults remained entirely inter-caecal and was composed of both an ascending and descending limb and is thus distinct from the single, characteristically coiled, ascending limb found in all juveniles and metacercariae. In the most advanced adults, the uterine coils overlaid the caecae and, where there was room, extended into an extra-caecal position. The peripheral situation of the gut branches, however, prevented pronounced extra-caecal development of a scale seen in *Phyllodistomum* and only a single row of eggs could be found in this position. The close packing of the uterine coils tended to obscure the reproductive organs and the growth sequence of the uterus could not be traced.

The flukes released eggs averaging 0.0568 x 0.0403 mm in the live state. The free-swimming pyriform miracidia were capable of extending from a size slightly smaller than the egg shell to 0.0732 mm

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GORGODERA - ADULT

The dorsal view of a mounted specimen
drawn with the aid of a projector

Abbreviations:

OS = Oral sucker

P = Prostatic glands

VES = Vesicular seminalis

GP = Gonopore

VS = Ventral sucker

U = Terminal widened portion of the uterus

E = Eggs packed in the uterine coils
posterior to the acetabulum

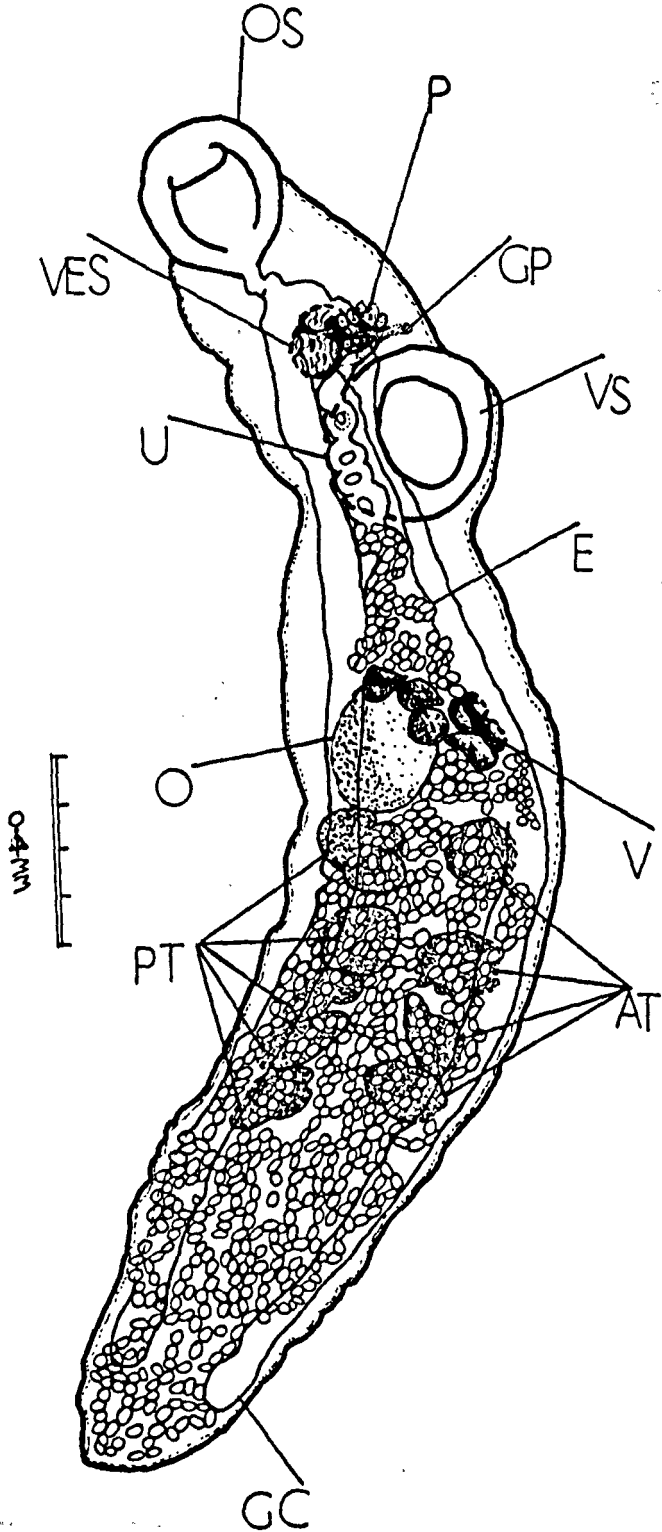
O = Ovary

V = Vitelline lobe

AT = Anterior testis

PT = Posterior testis

GC = Gut caecum.



GORGODERA = ADULT.

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GORGODERA - MIRACIDIUM

A diagrammatic representation of a miracidium
of Gorgodera in the form assumed during
locomotion.

Abbreviations:

M = Mouth

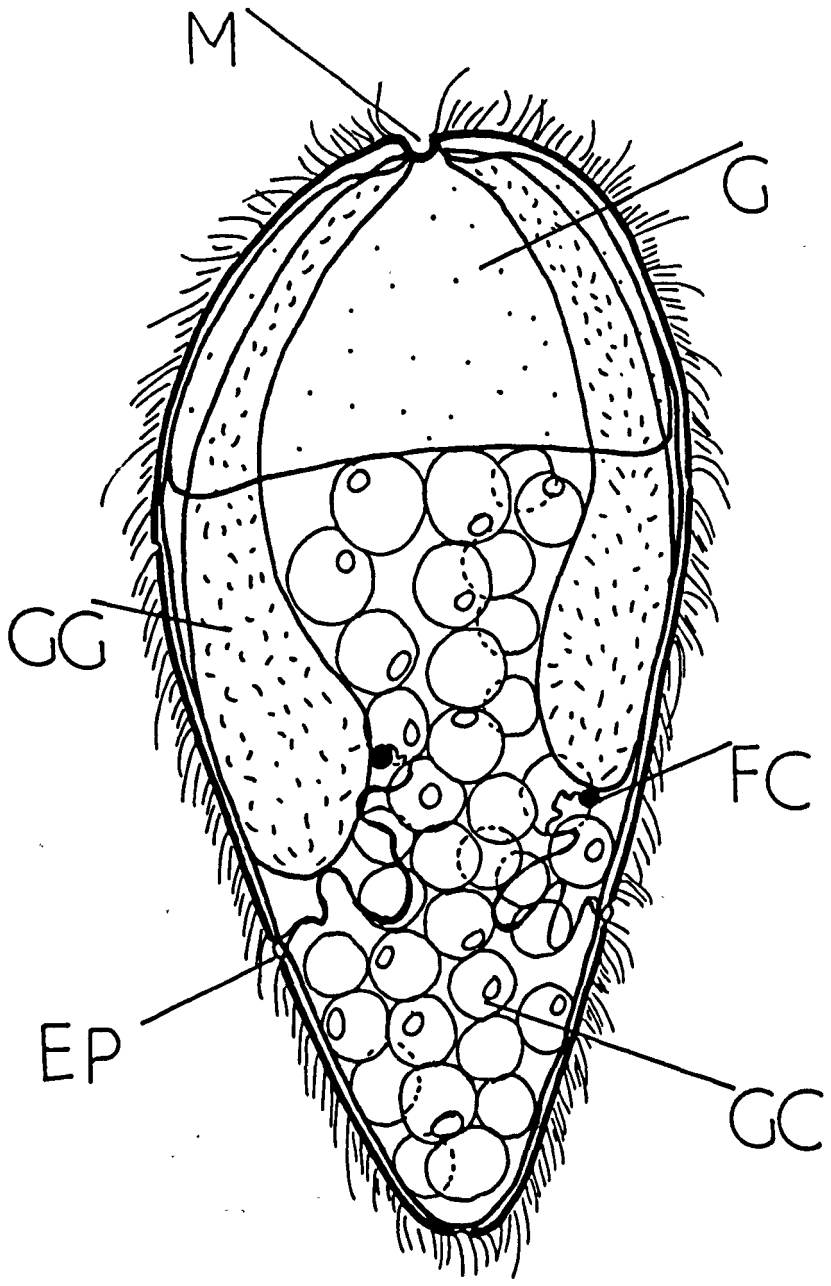
G = Gut

GG = Gland cell

FC = Flame cell

EP = Excretory pore

GC = Germinal material



GORGODERA ~

MIRACIDIUM.

in length. The egg and miracidial sizes thus overlap those of the considerably smaller Phyllodistomum. The miracidium (illustrated on page 396) possesses two flame cells and two lateral excretory pores, an anterior flask-shaped gut and a pair of what appeared to be equally sized gland cells, the granular contents of which did not readily take up neutral red stain. The epithelial coat could not be traced accurately but appeared to consist of from 3 - 4 rows of cells.

In frog C 12 Haematoloechus were recovered, ranging in size from those specimens which had just entered the lung (2.5 mm long) to a maximum length of 7.5 mm which represented at least more than 9 weeks' growth and may have resulted from 16 weeks occupation of the host. The Haplometra specimens must have been quite small when brought into the laboratory since they only attained a maximum length of 7.0 mm in 5 months. The largest Gorgoderids probably represented approximately 16 weeks' growth. Rapid growth must take place upon a rise in temperature and greater activity of the host coupled with a change in hormonal balance. The concentration of the sex hormones is greatest prior to the breeding season (Lees 1962) and, in this case, appeared to depress the growth rate of the specimens from frog A. Specimens from frogs B and C illustrate the sudden growth changes and increase in the rate of attainment of maturity as the hormonal concentrations in the host falls. This reaction probably ensures that the flukes release eggs during the period when the frogs are in water and that any trematodes ingested during late autumn do not mature until the following spring.

The percentage of Haematoloechus successfully invading the frogs' lungs was highest in host C. Considerable metacercarial losses are still indicated, however, and these may be related to the lack of a protective cyst. Many trematodes retain the cyst until safely through the stomach whilst Haematoloechus might be inactivated at this point. In all cases the success rate for Gorgodera metacercariae was probably high and the low numbers recovered in hosts B and C may be merely related to a diminishing number of infected insect nymphs available. This success is in contrast to Phyllodistomum and may be a consequence of the more powerful suckers possessed by Gorgodera at this phase.

Table 36a

a) Cercaria (Mounted Material)

| <u>Region Measured</u> | | <u>Phyllodistomum</u> | <u>Gorgodera</u> |
|--|--------|-----------------------------|-----------------------------|
| Body Length | | 0.391 (0.330 - 0.425) | 0.364 (0.282 - 0.513) |
| Anterior Region | L | 0.184 (0.147 - 0.205) | 0.139 (0.130 - 0.202) |
| (-V/S) | W | 0.084 (0.073 - 0.103) | 0.130 (0.114 - 0.147) |
| Posterior Region | L | 0.207 (0.179 - 0.227) | 0.225 (0.172 - 0.312) |
| (+V/S) | W | 0.081 (0.070 - 0.099) | 0.172 (0.093 - 0.132) |
| Ratio: Anterior:Posterior Region | L | 1:1.121 (1:1.058 - 1:1.258) | 1:1.613 - |
| | W | 1:0.967 (1:0.888 - 1:1.008) | 1:0.900 - |
| Oral sucker Rel/organ | L | 0.070 (0.061 - 0.082) | 0.082 (0.073 - 0.098) |
| | W | 0.057 (0.049 - 0.061) | 0.069 (0.062 - 0.077) |
| Ventral sucker Rel/organ | L | 0.073 (0.061 - 0.085) | 0.090 (0.076 - 0.102) |
| | W | 0.069 (0.057 - 0.079) | 0.080 (0.071 - 0.097) |
| Ratio: | | | |
| Oral: Ventral Sucker | B/A L | 1:1.007 (1:0.882 - 1:1.164) | 1:1.092 (1:1.013 - 1:1.202) |
| | B/A W | 1:1.244 (1:0.965 - 1:1.383) | 1:1.162 (1:1.029 - 1:1.301) |
| | G/R GD | 1:1.035 (1:0.950 - 1:1.375) | 1:1.092 (1:1.013 - 1:1.202) |
| Percentage of Posterior Region occupied by excretory bladder | L | 61.34% | 56.5% |
| | W | 57.29% | 54.09% |
| | | - (0.0208 - 0.0235) | - (0.0306 - 0.0326) |
| Stylet | L | | |
| <u>Live Material:</u> | | | |
| Tail - | L | 0.050 (0.035 - 0.070) | 0.089 (0.073 - 0.110) |
| Cuticular ring | T/A W | 0.121 (0.105 - 0.168) | 0.125 (0.092 - 0.143) |
| Chamber | T/A L | 0.263 (0.210 - 0.354) | 0.183 (0.154 - 0.257) |
| | W | 0.214 (0.182 - 0.301) | 0.202 (0.176 - 0.216) |
| Tail Stem | T/A L | 0.472 (0.385 - 0.560) | 3.075 (2.887 - 3.307) |
| | W | 0.079 (0.070 - 0.091) | 0.213 (0.183 - 0.257) |

Table 36b

b) Metacercaria (Mounted Material)

| <u>Region Measured</u> | | <u>Phyllodistomum</u> | | <u>Gorgodem</u> | |
|---|--------|-----------------------------|-----------------------------|-----------------|---|
| <u>Cyst (Live material)</u> | | | | | |
| Molluscan Host | L | 0.193 (0.165 - 0.257) | - | - | - |
| | W | 0.180 (0.147 - 0.220) | - | - | - |
| Insect Host | L | 0.216 (0.180 - 0.258) | 0.306 (0.202 - 0.436) | | |
| | W | 0.197 (0.176 - 0.230) | 0.284 (0.147 - 0.400) | | |
| Body Length | L | 0.347 (0.300 - 0.428) | 0.596 (0.323 - 0.774) | | |
| | W | 0.155 (0.128 - 0.204) | 0.236 (0.084 - 0.320) | | |
| Anterior region (-V/S) | L | 0.033 (0.033 - 0.126) | 0.148 (0.092 - 0.213) | | |
| | W | 0.193 (0.154 - 0.239) | 0.360 (0.238 - 0.453) | | |
| Posterior region (+V/S) | L | 0.080 (0.044 - 0.110) | 0.156 (0.103 - 0.231) | | |
| | W | | | | |
| <u>Ratio:</u> | | | | | |
| Anterior: Posterior region | L | 1:1.246 (1:0.839 - 1:1.713) | 1:1.524 | - | |
| | W | 1:0.964 (1:0.750 - 1:1.367) | 1:1.056 | - | |
| Oral sucker Rel/organ | L | 0.074 (0.063 - 0.086) | 0.108 (0.087 - 0.128) | | |
| | W | 0.055 (0.042 - 0.068) | 0.094 (0.066 - 0.117) | | |
| Ventral sucker Rel/organ | L | 0.075 (0.059 - 0.088) | 0.136 (0.095 - 0.165) | | |
| | W | 0.069 (0.057 - 0.076) | 0.127 (0.077 - 0.158) | | |
| <u>Ratio:</u> | | | | | |
| Oral: Ventral sucker | B/A L | 1:1.003 (1:0.774 - 1:1.134) | 1:1.256 (1:1.084 - 1:1.482) | | |
| | B/A W | 1:1.249 (1:1.000 - 1:1.548) | 1:1.341 (1:1.167 - 1:1.500) | | |
| | R/O GD | 1:1.003 (1:0.774 - 1:1.548) | 1:1.257 (1:1.084 - 1:1.482) | | |
| Percentage of Posterior Region occupied by excretory bladder | L | 55.61% | 45.46% | - | |
| | W | 26.21% | 55.09% | - | |
| <u>Stylet</u> | | | | | |
| Molluscan Host | L | 0.0241 (0.0204 - 0.0265) | - | - | |
| | W | 0.0249 (0.0224 - 0.0265) | 0.0309 (0.0255 - 0.0357) | | |
| Insect Host | L | 0.019 (0.014 - 0.036) | 0.036 (0.027 - 0.046) | | |
| | W | 0.013 (0.010 - 0.031) | 0.028 (0.021 - 0.031) | | |
| Vitellaria Rel/organ | L | 0.026 (0.020 - 0.036) | 0.037 (0.030 - 0.042) | | |
| | W | 0.023 (0.013 - 0.036) | 0.035 (0.031 - 0.038) | | |
| Ovary Rel/organ | L | 0.029 (0.020 - 0.041) | | | |
| | W | 0.024 (0.017 - 0.036) | | | |
| Anterior Testis Rel/organ | L | 0.032 (0.024 - 0.046) | | | |
| | W | 0.025 (0.016 - 0.036) | | | |
| Posterior Testis Rel/organ | L | | 0.023 (0.017 - 0.037) | | |
| | W | | 0.019 (0.015 - 0.025) | | |
| Testis divided into 4 lobes - each lobe | L | | 0.025 (0.015 - 0.041) | | |
| | W | | 0.020 (0.013 - 0.025) | | |
| Testis divided into 5 lobes - each lobe | L | 0.026 (0.021 - 0.031) | 0.040 (0.021 - 0.052) | | |
| | W | 0.020 (0.017 - 0.021) | 0.026 (0.013 - 0.038) | | |
| Vesicula Seminalis | L | | | | |
| | W | | | | |

Table 36c

c) Juveniles and Adults from the Definitive Host (Mounted Material)

| <u>Region Measured</u> | | <u>Phyllodistomum</u> | | <u>Goryodera</u> | |
|--|--------|-----------------------|---------------------|------------------|---------------------|
| Body Length | L | 0.898 | (0.483 - 1.890) | 1.517 | (0.330 - 3.535) |
| Anterior Region | L | 0.309 | (0.161 - 0.651) | 0.384 | (0.103 - 0.822) |
| (-V/S) | W | 0.164 | (0.070 - 0.294) | 0.265 | (0.085 - 0.612) |
| Posterior Region | L | 0.589 | (0.224 - 1.312) | 1.132 | (0.187 - 2.712) |
| (+V/S) | W | 0.307 | (0.063 - 0.672) | 0.355 | (0.076 - 0.770) |
| Ratio: | | | | | |
| Anterior: Posterior Region | L | 1:1.908 | (1:0.727 - 1:4.847) | 1:2.949 | - |
| | W | 1:1.881 | (1:0.846 - 1:3.700) | 1:1.340 | - |
| Oral Sucker Rel/organ | L | 0.144 | (0.074 - 0.232) | 0.188 | (0.062 - 0.343) |
| | W | 0.123 | (0.060 - 0.208) | 0.170 | (0.056 - 0.315) |
| Ventral Sucker Rel/organ | L | 0.184 | (0.086 - 0.296) | 0.228 | (0.847 - 0.420) |
| | W | 0.172 | (0.078 - 0.260) | 0.213 | (0.070 - 0.399) |
| Ratio: | | | | | |
| Oral: Ventral Sucker | B/A L | 1:1.282 | (1:0.877 - 1:2.000) | 1:1.234 | (1:1.013 - 1:1.456) |
| | B/A W | 1:1.403 | (1:1.000 - 1:2.138) | 1:1.224 | (1:1.200 - 1:1.491) |
| | R/O GD | 1:1.282 | (1:0.918 - 1:1.873) | 1:1.213 | (1:1.104 - 1:1.419) |
| Percentage of Posterior Region occupied by bladder | L | 51.9% | - | 39.8% | - |
| | W | 6.706% | - | 0.827% | - |
| Vitellaria | L | 0.072 | (0.010 - 0.144) | 0.070 | (0.025 - 0.270) |
| Rel/to entire organ | W | 0.047 | (0.010 - 0.092) | 0.045 | (0.020 - 0.120) |
| Per lobe | L | - | - | 0.086 | (0.070 - 0.110) |
| | W | - | - | 0.048 | (0.044 - 0.052) |
| Ovary Rel/organ | L | 0.107 | (0.036 - 0.224) | 0.117 | (0.033 - 0.304) |
| | W | 0.082 | (0.025 - 0.170) | 0.087 | (0.025 - 0.206) |
| Anterior Testis Rel/organ | L | 0.118 | (0.033 - 0.272) | - | - |
| | W | 0.083 | (0.020 - 0.184) | - | - |
| Posterior Testis Rel/organ | L | 0.129 | (0.035 - 0.270) | - | - |
| | W | 0.083 | (0.020 - 0.180) | - | - |
| Testis divided into 4 lobes - each lobe | L | | | 0.054 | (0.031 - 0.230) |
| | W | | | 0.047 | (0.020 - 0.204) |
| Testis divided into 5 lobes - each lobe | L | | | 0.056 | (0.032 - 0.150) |
| | W | | | 0.040 | (0.018 - 0.108) |

Abbreviations utilised in Sections a) b) c):-

L = Length

W = Width

V/S = Ventral Sucker

Rel/organ = R/O = Measurement is taken relative to the axes of the organ

B/A = Measurement is taken relative to the main body axes

All measurements are in millimeters.

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Footnote to Tables 36 a-c

A comparison of the measurements of Phyllodistomum and Gorgodera at all stages of their life cycles illustrates clearly the different proportional changes taking place in the respective growth sequences. Two of the most striking concern the body and the suckers. The body proportions in both Trematodes change radically only after the definitive host has been entered. In Gorgodera the sucker sizes however increase at the metacercarial stage and attain the proportions (both in the average and maximum range measurements) which are found in the adult. Phyllodistomum, in contrast, possesses suckers which increase in size only after the animal has invaded the definitive host or which progressively change in proportion as the fluke ages.

The Identification of Gorgodera

Preliminary investigation of Pigulevsky's account of this genus (1953) reveals a general similarity with the adult G. pagenstecheri Ssnitzin, 1905 and G. dollfusi Pigulevsky, 1945 but the larval stages of the former species do not coincide with the specimens studied here. Most of the descriptions given in Pigulevsky's paper are based upon fully adult Gorgodera which reach considerably larger sizes than were dealt with in this instance. The differences in the measurements are so marked that it is difficult to extend the records and retain any certainty that such data will be accurate. The literature concerning the genus has not been fully investigated to date and it is possible that older specimens of this species may have been described elsewhere and that future work will combine the two accounts. It is for this reason that I am leaving the species described in this report as an unidentified Gorgodera sp.

Section 10 - Taxonomy

a) Natural and Artificial Variation:

a(1) The Use of Relaxants:

These substances have not been commonly employed by workers dealing with Phyllodistomes and only five techniques have been referred to in the literature. Four of these were tested on flukes of varying ages but the fifth, and the most modern, involved the use of 0.4% Chloretone (in 0.75% saline) and this substance could not be obtained from the manufacturers during the period of investigation. The employment of a diluted solution of high grade alcohol to a concentration of 10% gave the most rapid and satisfactory results out of all four techniques investigated. In one hour adult flukes were immobilised and had adopted either a pronounced elongate position or were to be found in a moderately extended and flattened state. Apart from this variation in the degree of extension there was also a tendency for the anterior region to arch dorsally in some specimens. Immobilisation took considerably longer when using sodium bicarbonate solution and at the end of one hour, although the adult trematodes were still capable of movement, they were predominantly in a contracted state. Indeed Slusarski (1958) found that P. folium remained alive longer in 0.6% sodium bicarbonate than in isotonic sodium chloride solution. Two other relaxant techniques have been utilised which involve either leaving the trematodes in water for several hours without any treatment or repeating the same procedure after slowly heating the water containing the flukes to a temperature of 35°C. Neither technique proved particularly satisfactory for this species.

When their resistance is not reduced by heat treatment adult flukes kept in water are capable of remaining viable for several days and of retaining their mobility throughout this period. They tend to remain immobile when undisturbed but become active upon stimulation such as the movement of the container. They do not enter a long period of relaxation prior to death and are still capable of weak movements after visible degenerative changes such as the localised expansion of the cuticle have taken place. The use of heat merely hastens the time when such degenerative features appear and if the trematodes are not fixed before this point is reached the mounted specimen possesses an abnormal cytoplasmic structure and appears rounded in cross section which is totally unlike the living condition. The time taken for the correct end point to be reached varies with the age of the parasite and the temperature of the water. Younger flukes tend to die more quickly than the larger adults and extremely small juveniles have to be handled with greater care than the two latter techniques practicably allow. Slusarski (1958) reported that specimens of P. folium left dying in cold water attained accidental shapes and became twisted (and shrunken(?)). He killed his trematodes in heated tapwater but even then had to select straightened specimens from organisms deformed by this treatment.

a (2) Fixatives:

The diagnostic descriptions of Phyllodistomes are based on specimens which were fixed in a variety of ways. In order to assess the effects of these varying methods and to check for possible distortion, a comparison was made of the results obtained following the

use of twelve of the most common fixation techniques employed by previous workers in this field. The solutions involved are listed on page 46 where their effects upon the eggs are discussed.

Shaking methods of fixation as favoured by Looss could not be relied upon to produce extended specimens in which both regions remained in the same plane and which did not possess an anterior region which was reflexed over the posterior section of the body. In techniques where the trematodes were plunged directly into the fixative provided that the flukes were in the desired contractile state when placed in the solution, they would retain this position because the size and shape allows immediate penetration. The advantages of the coverslip method in maintaining a flattened specimen may be outweighed by a reduction in penetration time and the artificial expansion caused by pressure effects if care is not taken. Goodchild (1948) found, contrary to general beliefs, that Looss's shaking method allowed more contraction to take place in specimens of Gorgodera amplivava than the coverglass pressure method which he employed. When working with more cylindrical and larger Gorgoderids, therefore, the latter technique may be of greater use than for dorso-ventrally flattened smaller species. Slusarski (1958), for example, considered that artificial flattening of P. folium led to deformation. When fixing active specimens it is practically impossible to ensure that each trematode attains an identical regional contractile state. The use of a relaxant is preferable in order to attain some uniformity or a large collection should be made to cover the numbers of

variations which will occur. Regardless of the technique employed the shock of fixation when relaxants had not been used had the effect of extending the flukes beyond the average relaxed length attained during life (i.e. that length lying between extreme contraction and extreme extension). If heat was utilised this extension was greater in most cases whilst shaking techniques produced only moderate results. Techniques intended to produce relaxed specimens frequently resulted in trematodes acquiring a posterior region which was rounded in outline and dissimilar from any position attained during active life.

The maximum sucker diameters alter in life from length to breadth independently according to the contractile phase of the region and the attachment or detachment of the organ. When fixed, specimens exhibit various diameter changes when compared with their live state which may be independent of the position in which the body was maintained during the fixation process.

All fixatives cause shrinkage and the extent to which this occurs depends upon the concentration and composition of the fluid. P. lohrenzi was recorded by Venard (1940) as differing by as much as half its size from the live to the fixed state.

a (3) Dehydration and Mounting:

Dehydration of trematodes following the use of the aquatic and alcoholic fixatives discussed previously caused a further decrease in length ranging from 0.06 - 0.58 mm. from the fixed state. Total shrinkage from the living to the mounted state averaged approximately 20% of the total length of the trematode. Together with contractions therefore, variation from the live state can be considerable. Finally,

if a minimal quantity of mountant is used there is the possibility, even at this stage, of causing considerable distortion, particularly in the case of the suckers. If a large quantity of mountant is used, however, the method is perfectly adequate but it still suffers from certain disadvantages. Slusarski (1958), for example, considered the permanent mounting of specimens as an outmoded and antiquated technique and preferred to be able to examine both surfaces of the flukes in a thorough examination of cleared specimens floating in a cavity slide.

a (4) Contraction Ranges of the Living Fluke:

The extensibility of these organisms introduces many problems concerning their measurement and it was considered that it might be desirable if the total contractile ranges of the flukes at all ages were known. A total of 70 specimens from the cercarial to the adult phase were measured whilst fully extended and again when completely contracted. When assembling the results obtained it was noted that a percentage of trematodes could not have exhibited maximum extensibility during examination but had kept well within their apparent capabilities. The difference between the maxima attained by flukes situated in the same size grouping and being of moderate activity with those exhibiting complete extension approximated an average 0.2mm. This also applied to the noticeably sluggish metacercarial specimens obtained. In Table 37 given below only true maxima are recorded and the metacercarial results are adjusted by the addition of 0.2mm to the recorded contractile range to account for inactivity.

Table 37

| | Total length at maximum ex- tension (mm) | Total contraction from the fully extended - fully contracted position (mm) | Contractability (calculated per 0.1mm total length) |
|------------------|--|--|--|
| Cercariae | 0.40 - 0.60 | 0.24 - 0.37 | 0.067 |
| (Cercariae | 0.60 - 0.80 | 0.37 - 0.48 | 0.052 |
| (Metacercariae | | | |
| (Metacercariae - | | | |
| (juveniles - | 0.80 - 1.20 | 0.48 - 0.68 | 0.050 |
| (young adults | | | |
| adults | 1.20 - 1.40 | 0.68 - 0.76 | 0.040 |
| | 1.40 - 1.60 | 0.76 - 0.83 | 0.035 |
| | 1.60 - 2.20 | 0.83 - 0.99 | 0.027 |
| | 2.20 - 2.40 | 0.99 - 1.04 | 0.022 |
| | 2.40 - 2.80 | 1.04 - 1.12 | 0.020 |
| | 2.80 - 3.20 | 1.12 - 1.18 | 0.012 |

The figures indicate that although in the larger flukes there is a greater measureable difference between the two states there is a progressive decrease in the actual extensibility (calculated per 0.1mm of their length) as the flukes increase in size. This is probably correlated with the steady increase of relatively incompressible structures growing and accumulating in particular in the posterior region.

(b) Discussion: The validity of characters used in the classification of the Phyllodistominae.

Several authors have attempted to produce keys in order to separate the Phyllodistomum species recovered from definitive hosts. Pearse (1924) gave a short diagnostic table for United States fauna; Holl (1929) produced a key which was later modified by Van Cleave and Mueller (1932) for North American forms; Nyebelin (1926) formulated a similar list for European species and Lewis (1935) and Bhalerao (1937) both attempted generalised keys for the world fauna known at that period; Kaw (1950) and Gupta (1953) separated the Indian species. One of the most recent generalised accounts was produced by Pigulevsky (1953) who also classified the Family Gorgoderidae Looss, 1901 and erected a number of new subfamilies and subgenera in the process. The sections of Pigulevsky's classification which are relevant to this study can be summarised as follows:

Family: Gorgoderidae Looss, 1901

Subfamily: Gorgoderinae
Looss, 1901

Subfamily: Phyllodistominae
Pigulevsky, 1953

Genera: Gorgotrema Dayal, 1938
Gorgoderina Looss, 1899

Genera: Dendrorchis Travassos, 1926
Phyllodistomum Braun, 1899
Xystretum Linton, 1910
Phyllochorus Dayal, 1938
Gorgoderina Looss, 1902

Subgenera: G. Gorgoderina Looss, 1899
Antoderina Pigulevsky, 1952
Extremoderina Pigulevsky,
1952
Medioderina Pigulevsky, 1952
Postoderina Pigulevsky, 1952
Gorgoderina (Looss, 1899)

Subgenera: G. Phyllodistomum
Braun, 1899
Catoptroides (Odhner, 1902)
Phyllodistomum (Braun, 1899)
Vitellarinus (Zmeev, 1936)
Microlecithus (Ozaki, 1926)

Subgenera: G. Gorgoderina Looss, 1902
Gorgoderina (Looss, 1902)
Gorgorimma Pigulevsky,
1952

Yamaguti (1958) did not adopt this system but retained a more traditional approach. The scheme put forward by Pigulevsky, (1953) retains many characters which have been criticised by previous workers as unreliable and which in relation to the present work appear to possess doubtful specificity. In the following discussion all the criteria which have been utilised in the past are considered, in addition to characters retained by the above author.

The overall body size of the trematode has to be regarded against the background of possible differing host effects and crowding, the age of the specimen and the degree of contraction. Goodchild (1943) was of the opinion that trematode sizes would vary according to their geographical location in such a manner that the differing length of the seasons across large continents would allow some specimens a longer period in which to grow and mature.

Bodily proportions and shape can vary with growth. Posterior expansion in a transverse and longitudinal direction accommodates for uterine development and the increasing size of the reproductive organs. A dorso-ventrally flattened foliate posterior region has been regarded as a characteristic of the Phyllodistomum genus whilst a lanceolate cylindrical area which is not distinctly divided off from the anterior region is generally ascribed to Gorgoderina. There is also a tendency for the former genus to parasitise Fish whilst the latter infects mainly Amphibia. The form of the body is the only character which has been retained by recent authors (including Pigulevsky (1953)) to separate the two genera. This difference in body outline and shape in cross

section need not apply throughout the life cycle since larval and juvenile stages can assume both forms during certain contractile phases. The shape change taking place during the life cycle is illustrated for *P. (P.) folium* (page 271) and *P. (P.) simile* (page 335) by Pigulevsky (1953) and this is supported for the latter genus by Thomas (1958). The difference is primarily an adult feature extenuated by uterine and reproductive development. In the literature the two characteristics can be found merging together in a manner which obscures the clear division of the two genera and creates doubt as to the validity of the criteria used. For example, *P. singulare* Lynch, 1936, and *P. (Distomum) patellare* Sturges, 1897 (considered as synonymous to *P. enterocolpium* Holl, 1930 by Dollfus (1931); Lynch (1936); Dawes (1946); Pigulevsky (1953)) parasitise Amphibia (Urodela). They are laterally expanded posteriorly and dorso-ventrally flattened at the fully adult stage (a feature clearly shown in section by Holl (1930)). *P. patellare* was described as cylindrical but drawn as if flattened by Sturges, (1897). *P. solidum* according to Rankin (1937) was non-spatulate and not sharply divided into two regions. Goodchild (1943) illustrated the transition in the shape of the living adult *P. solidum* from the *Gorgoderina* to the *Phyllodistomum* type, whilst Groves (1945) commented on the loss of the bodily division on mounting in what he considered to be the same species. Goodchild (1943) also illustrated how the lateral expansion of the body increased with age. From the illustrations from Rankin (1937), Goodchild (1943) and Groves (1945) the body of this parasite appears less dorso-ventrally flattened than the previous

examples given for Urodeles, but the subject is not discussed in the literature and sections were not made. Another Urodele parasite P. rhyacosiredonis Bravo-Hollis, 1943 was illustrated in a form characteristic of Gorgoderina but was described as possessing a wide posterior region in life whose shape was lost upon fixation. The extent of the dorso-ventral flattening was not mentioned. Similarly the illustrations of Anuran parasites:- P. shandrai Bhalerao, 1937; P. (Microlecithus) kajika Ozaki, 1926; P. coatneyi Meserve, 1943; P. bufonis Frandsen, 1957; all possess body outlines and proportions which can be equated with Gorgoderina tenua Rankin, 1937 and G. bilobata Rankin, 1937 from Urodeles and outlines, but not proportions, which are similar to G. translucida (Stafford, 1902) and G. megalorchis Bravo-Hollis, 1948 from Anura. P. shandrai was described as flat or elliptical in cross section; P. (M.) kajika, P. coatneyi and P. bufonis were described as flattened forms. G. translucida from Anura is separated from G. bilobata, G. tenua and G. intermedia recovered from Urodeles (according to Olsen (1937)) by its flattened posterior region (which is one-third that of its width in thickness) while the latter species were diagnosed as subcylindrical with thick opaque posterior regions. Osborn (1903), when describing P. americanum from Urodeles, found that younger specimens were slender and not spatulate and gained the impression that older specimens would all become spatulate eventually. The shape in cross section was not discussed. P. frequentum was described by Kaw (1950) from Anura as subcylindrical with no well-differentiated body regions but was

classified as a Phyllodistomum species because the author regarded Gorgoderina as synonymous to that former genus.

In Fish parasites the absence of a regionally divided body or spatulate condition was noted many times, e.g. P. conostomum Olsen, 1876; P. almorii Pande, 1937; P. nocomis Fischthal, 1942; P. carangis Manter, 1947; P. leilae Nagaty, 1956; P. pacificum Yamaguti, 1951 and P. marinae Bravo-Hollis and Manter, 1957 - the two latter being recovered from marine hosts. Fish parasites have never been described as cylindrical however. The question involving dorso-ventral flattening is further complicated by the fact that many authors only described mounted material and that pressure was utilised in some cases and scrupulously avoided in others. At no time was the criterion of dorso-ventral flattening defined numerically.

The literature indicates that Fish support only dorso-ventrally flattened specimens exhibiting a range in body shape from an elongate undivided form to a pronounced spatulate condition. The Urodela and Anura act as hosts to trematodes with an identical range in form, but are parasitised in addition by specimens which are thickened posteriorly. The cylindrical trematodes of the genus Gorgodera are predominantly Anuran parasites occasionally being found or experimentally introduced into Urodeles (Gorgodera cygnoides (?) Pigulevsky (1953); G. amplicava (Goodchild, 1948)). There is thus no obvious point of transition between the main parasitic forms. The attainment of a cylindrical body may demonstrate a change in the metabolic efficiency of the trematode and this feature, and not the

body form, may warrant the retention of the taxonomic division of the Gorgoderina and Phyllodistomum genera. On morphological studies alone it is difficult to retain such a distinction because this single character appears too variable and insignificant to be used at the level of generic distinction. The life cycles and cercarial types do not separate into two but merge (see Fischthal (1951); Coil (1954); Thomas (1958)). Considerably more knowledge is required before the position of either group can be considered dogmatically, and although Kaw (1950) agreed with Pande (1937) in combining Gorgoderina with the more comprehensive genus Phyllodistomum, Pigulevsky (1953) and Yamaguti, (1958) retained both genera. The lanceolate or attenuated species diagnosed as belonging to the Phyllodistomum genus and derived from Fish, Urodeles and Anurans were placed by Pigulevsky into the subgenera Microlecithus (Ozaki, 1926) and Vitellarinus (Zmeev, 1936)

The use of criteria such as the presence or absence of flutes (Lewis (1935); Bhalerao, (1937); Steen (1938); etc.), a crinkled body margin (Van Cleave & Mueller (1932); etc.) or a posterior notch (Lewis, (1935); Holl (1929); etc.) has been repeatedly criticised in the literature as they depend solely upon the degree of contraction at the time of fixation. Similarly, the shape of P. angulatum Linstow, 1907 or P. elongatum Nyebelin, 1926 must be considered against the background of general variability exhibited by this contractile genus. Although Pigulevsky (1953) indicates that in extended specimens of both species the body form remains constant this must remain as a criterion of minimal importance until the full contractile range is

recorded for both species.

Several authors have described marginal thickening in the posterior region but none have shown this phenomenon in section. P. lowisi Srivastava, 1938 was considered synonymous, by Pigulevsky (1953) to the unidentified species described by Bhalerao (1937). What Srivastava referred to as regular, feebly-muscular, puckering Bhalerao merely termed a crinkled margin. The difference could lie in the age of the specimens since Bhalerao's material was considerably younger, but the diagrams given by both authors of relaxed material shows three pairs of semi-circular thickenings posteriorly. Rai (1964) described another Indian form, P. srivastavi, as possessing four pairs of muscular depressions after it had remained for 15 days in the definitive host but did not indicate their presence in the metacercaria. An identical number of peripheral thickenings were reported to occur along the posterior margins of P. marinae described by Bravo-Hollis & Manter (1957) and a non-localised, marginally thickened hind-body was reported for P. symmetrorchis by Thomas (1958). In all cases it is not clear whether such thickenings are a feature of the living animal or appear solely in preserved material.

The sucker ratio has been mentioned by many authors as a valuable character in diagnostic descriptions. The figures obtained during sucker ratio measurements, however, require careful interpretation as has already been shown in Section 7. Past workers have described measurements based on transverse diameters, whilst others have recorded the longitudinal dimensions. Other authors have relied upon a com-

parison based upon the greatest diameter of both organs regardless of their orientation. This latter measurement does not always coincide with the longitudinal axes due to contractile effects but, on average, the two are approximately equivalent. In the species studied during this investigation the ratios alter during the life cycle. At all ages from the cercaria to the adult population the two suckers can range from approximate equality to a point where the ventral sucker is roughly from $1\frac{1}{2}$ to 2x the size of the oral at least in one dimension. The maximum differences are attained in the oldest age group, indicating that the acetabular growth rate gradually increases over that of the corresponding structure. There is little information on this point so that the wide range of ratios at all ages may either be a species character or may merely serve to decrease the value of this feature for taxonomic purposes. (Luhe (1909) may have discovered a similar phenomenon in P. macrocotyle when he recorded a variation in the sucker ratio during development but identical ratios from both mature and immature forms under a certain size.) Goodchild (1943) found that the sucker ratio could be caused to vary by pressure effects in mounted specimens. Luhe (1909) reported that the sucker ratio of mature P. macrocotyle increased with the size of the trematode while recording that the two suckers of P. folium differed in small specimens but reached a similar size in larger flukes. Thomas (1958) demonstrated that the sucker ratios of P. simile varied only slightly throughout the life cycle. For this character to be of specific value a record of all stages of the life cycle is desirable and large numbers of each age group taken

from the definitive host should be measured to include as much of the range as possible. The value of mean measurements may increase as more information is obtained on each species.

The main characters utilised in the separation of *Phyllodistome* species are usually based upon some aspect of the reproductive structure. Pigulevsky (1953) primarily separates the genera Phyllodistomum and Dendrororchis from Xystretum and Phyllochorus on the spatial arrangement of the reproductive organs within the posterior region. In the former grouping the organs are diffusely orientated between the acetabulum and the posterior margin and in the latter they are restricted to a compact area close to the ventral sucker. In the division of the subgenus Catoptroides (genus Phyllodistomum) Pigulevsky uses the same characteristic to separate similar species. He figures Odhner's illustration (1902) depicting P. (C.) spatulaeforme (page 411) which shows an identical reproductive arrangement to that illustrated for Xystretum solidum Linton, 1910 (page 555) and yet, despite his key, places the former under the genus Phyllodistomum subgenus Catoptroides. In the species of Phyllodistomum studied during this investigation the position of the reproductive organs was found to vary and, according to the illustration on page can occupy the anterior half, two-thirds or three-quarters of the posterior region. The spatial distribution depends upon the age of the specimen and the contractile phase. One feature to which there was found only one minor exception was the relationship between the gonads and the gut caecae. All the gonadial organs touched or overlapped one of the caecae at all stages

of contraction but never were completely extra-caecal in position. This intercaecal position of the reproductive organs is characteristic of all Phyllodistome species described except for P. lesteri where the occasional extra-caecal position of the ovary and vitellaria was however considered to be a fixation effect (Wu, 1938).

The position of the reproductive organs relative to one another is used by Pigulevsky in the separation of many species in the Phyllodistomum subgenera. In the species studied here the ovary is always situated on the opposite side of the anterior testis and occupies a position which ranges from a point parallel to the testis to where it lies completely anterior to it. The anterior margin of the ovary may lie posterior to that of the testis but the entire organ is never placed completely posterior to the male structure. The ovary frequently overlaps the vitellarium but never protrudes anteriorly beyond it. In the Phyllodistomum genus the ovary is never completely posterior to the anterior testis but nearly attains this state occasionally in P. solidum according to Groves (1945). The ovary commonly lies posterior or, at most, parallel to the vitellaria throughout this genus but there are marked exceptions in P. spatula Odhner, 1902 and P. symmetrorchis Thomas, 1958 where the ovary lies principally anterior to these organs. Whether this position is maintained throughout the life cycle or is a variable character in the adult is unknown. Lateral displacement of the ovary and anterior testis so that the vitellaria lie between them was noted for P. ghanense, P. symmetrorchis (Thomas, 1958), P. spatula and P. spatulaeforme (Odhner, 1902) and a number of other species including

P. (P.) dogieli Pigulevsky, 1953 where according to the figures the condition either changes with increasing age of the specimens or appears variable. The position of the testes, regardless of the contractile phase assumed, is so variable in the specimens studied in this investigation that if this is the case for many other species it would not appear that their orientation either relative to one another, to the other organs or their position in the posterior region can possess much specific value. For example, a similar range in testis position has already been noted in P. almorii by Kaw (1950).

Gonadal lobation cannot be used as a diagnostic criterion. There is a tendency, but it is by no means a rule, in the species studied in this report, for the organs to lack marked lobation whilst juvenile and to develop slight to deep marginal indentation as the fluke ages. Choquette (1947) noted the reverse occurrence in P. lachancei. Lobation proceeds at different rates in different individuals and between the reproductive organs of one specimen. (The main variations have been discussed in Section 7 and a few are illustrated on page 26.) A pair of vitellaria may not even attain an equivalent degree of lobation in a single specimen or may remain smooth in older flukes possessing well-developed extra-caecal uterine coiling. The organs of this species paralleled Pigulevsky's descriptions dividing the subgenera Phyllodistomum, Catoptroides, Vitellarinus and Microlecithus in ranging from a rounded or ovoid state to an entire, weakly or deeply lobed, clustered structure. They

never assumed a branched form comparable to that described for P.(C.) pawlovskii (Zmeev, 1936); P.(C.) singulare (Lynch, 1936) or P.(C.) acceptum (Looss, 1901). The vitellaria in relaxed specimens of the species studied during this investigation generally lie orientated transversely but may be longitudinally elongated upon extension of the posterior region. Also included in the collection, however, are relaxed specimens with vitellaria longitudinally elongate similar to the state illustrated for P.(P.) megalorchis by Pigulevsky (1953). Vitelline orientation therefore does not appear to be a reliable character.

Proportionally the vitellaria are the smallest reproductive organs in most Phyllodistomum adults. P.(C.) zachvatkini Pigulevsky, 1953 is described as the exception where the vitellaria narrowly exceed the size of the ovary but the accompanying diagram (page 423) does not support this contention. In Looss's drawing of the adult P. acceptum (1901) the ovary was substantially smaller than the deeply lobed vitellaria. In a description and diagram given by Pigulevsky (1953) (page 353) this is not the case. These discrepancies seem to indicate that the size relationship between the ovary and vitellaria is variable amongst adult specimens of some species. In the specimens forming the basis for this report the vitellaria are always smaller than the ovary during the adult phase although the measurements frequently overlap in one dimension. In the metacercaria this condition is generally maintained but there are a few exceptions where these organs do exceed the size of the ovary. A similar change in proportion is

noted by Pigulevsky (1953) for P. pseudofolium Nyebelin, 1926 and P. dogieli Pigulevsky, 1953 where in the younger larvae and juveniles the vitellaria are recorded as exceeding the size of the ovary.

Pigulevsky (1953) considered that the overall size of the main reproductive organs relative to the size of the fluke could be used as a specific criterion. While this conclusion, when used in conjunction with other factors and related to age, might prove to be correct, it is doubtful whether it can be employed at the present time in the manner utilised by Pigulevsky. He attempted the final separation of species solely upon this one criterion basing his key on available data which he did not supplement and which, in the cases he chose, remained totally inadequate for his purpose. For example, he separated P.(C.) lacustri (Loewen, 1929) from P.(C.) staffordi (Pearse, 1924) and P.(C.) spatula (Odhner, 1902) from P.(C.) spatulaeforme (Odhner, 1902) on the single consideration that the ovary and testes are either large or small respectively. The body sizes of the former pair overlap in respect to length (3.33 - 4.76 mm and 4.1 mm) and closely approximate one another as regards width (2.11 - 2.55 mm as compared with 2.98 mm in mounted material). In P.(C.) lacustri the testes measured 0.54 - 0.77 x 0.42 - 0.51 mm and the ovary 0.34 - 0.38 x 0.22 - 0.28 mm. In P.(C.) staffordi the testes were recorded as measuring 0.42 mm in diameter and the ovary as 0.25 mm. Pearse (1924) examined only 18 specimens and yet gave no size ranges. It is also not clear upon how many specimens his measurements were based. The position concerning P.(C.) spatula and P.(C.) spatulaeforme

is even less satisfactory. In the accounts given by Odhner, (1902, 1911) although he discussed a size difference between the reproductive systems he only recorded one measurement, that of the ovary of P.(C.) spatula. In addition, he stated that he recovered a total of 2 - (4?) specimens of P.(C.) spatula and 3 specimens of P.(C.) spatulaeforme. The significance of reproductive size variations in such a small sample is considerably reduced.

Pigulevsky (1953) utilised the proportions of the ovary relative to the testes as a specific criterion. In the species studied during this investigation the ovary is generally smaller than either testis. However, in some juveniles, metacercariae and adults the ovary can exceed the size of the anterior male organ. In adult flukes if the greatest diameter of the organs is the only factor considered (and several authors do record only one dimension) the ovary appears occasionally to exceed the testis size and of course this is also the case for atropheid specimens. The ovary is not generally submitted, due to its more usual anterior position, to such pronounced elongation during movement as are the male organs and this is reflected in the respective size ranges. The posterior testis is on average the largest reproductive organ but metacercariae, juveniles and large adults have been found where this does not apply. In many Phyllodistomum species the ovary is generally the smallest main reproductive organ and Pigulevsky (1953) indicates that this proportional difference is maintained throughout the life cycle of at least P. simile and P. dogieli. Variability is not discussed however and

more information is required concerning the stability of this character in those species where the relative proportions appear distinctive before the usefulness of the character can be evaluated. Kaw(1950)found considerable variation in P. almorii, and at the present time, therefore, too much weight cannot be placed upon this feature.

In no case in the genus Phyllodistomum does the uterus extend extra-caecally to a point further forward than the acetabulum. The only specimen possessing peripheral uterine looping, similar to the Phyllodistome condition but which extends to the oral sucker, is retained in a separate genus - Dendrorchis Travassos, 1926 by Pigulevsky, (1953). The specimens were also described as possessing a cirrus. In Phyllodistomum the uterus increases from the inter- to the extra-caecal position as the trematode ages. The rate at which the latter condition is attained will depend as much upon the position of the reproductive organs as the sequence of uterine development. In all species the distance between the caecae and the body margin is sufficient to allow uterine extension extra-caecally. The space between the vitellaria and the acetabulum appears insufficient to allow for pronounced uterine coiling at this point of the type found in many species of Gorgoderina and Gorgodera, although slight coiling of this nature was noted in several specimens of the species studied here. The sequence of uterine development has received little attention in the literature. A markedly different pattern from that outlined in this report was described by Osborn(1903) for P. americanum parasitising Urodeles. In

addition, Osborn, on examination of Stafford's specimens of Gorgoderina translucida (Stafford, 1902) from Anura, stated that the sequence appeared to be somewhat similar in both forms. A review of the diagrams for Phyllodistome species indicates that the sequence in many cases may be similar to that described in this account. The diagrams for P.(C.) hunteri Arnold, 1934; P.(C.) lacustri Loewen, 1929 (given by Arnold (1934)) and P.(P.) wiskowskyi Pigulevsky, 1953 (after Wu, (1937)) exhibit a peripheral coiling which is unlike most species. How characteristic and constant the sequence of uterine coiling remains in different species is not known. In the species studied in this investigation there was little variation.

The position of the gonopore, on the present information, cannot be used in specific diagnosis. In all Phyllodistomes it is situated between the gut bifurcation and the acetabulum. Its exact position relative to these two structures varies according to the contractile phase and its position within the anterior region can vary with the differential growth rates during the life cycle as several of the illustrations given by Pigulevsky (1953) demonstrate. The pore is commonly situated at the extreme anterior margin of the vesicula seminis and most variations figured could be regarded as pressure effects. However, Looss (1894) and Wu (1938) showed respectively in section and whole mount that the gonopore lay clearly posterior to the vesicle in D. folium and P. lesteri. Bravo-Hollis & Manter (1957) found a similar condition occurred in the two specimens of P. marinae which they recovered. Information concerning the structure and

orientation of the vesicle is sparse. In the majority of species where it is described the structure assumes a bipartite form in which the posterior chamber is the larger. The vesicle is definitely recorded as a single chamber in 3 species. The illustration of Phyllochorus macronius given by Dayal(1938) shows a single chamber and no further expansions to the system, whereas Ozaki(1926)depicted a swollen pars prostatica anterior to the single vesicular chamber of P. kajika. In a redescription of P. carangis Manter, 1947 - Winter (1957) recorded a globular seminal vesicle which alternated in its position relative to the gonopore from an anterior to a posterior situation. The orientation of the vesicle does not offer a reliable character for diagnostic purposes because in practically all species it lies parallel to the longitudinal body axis and never transversely. The position of the chamber relative to the pore would appear to be either a variable feature or too vulnerable to pressure and fixation effects.

The presence of a receptaculum seminis was used by Dayal(1938) as an important criterion for separating Phyllochorus macronius from the Phyllodistomum genus and species. This is another character however upon which information is limited and it is difficult to determine its taxonomic value. The receptaculum is present in the species studied in this investigation and was also found in P. brevicecum Steen, 1938; drawn by Looss(1894)for D. folium and recorded as present by Goodchild (1943) and absent by Groves (1945) for P. solidum. Ozaki(1926)and Gupta(1951)stated that a receptaculum seminis associated with the oviduct was absent in P. kajika

and P. singhiai but the latter noted that a receptaculum seminis uterinum was present instead. Similar structures of both types were recorded as absent in P. enterocolpium Holl, 1930; P. coatneyi Meserve, 1943 and P. frequentum Kaw, 1950. There seems to be some discrepancy associated with interpretation when referring to this chamber and a localised expansion of the oviduct is not always recorded as a receptaculum, (e.g. Gupta (1951) fig. 3 P. singhiai). In a large percentage of the species described the presence or absence of such a chamber is not noted and the female reproductive ducts are not fully elucidated. The presence of a receptaculum, at least in some species, in the genus Gorgoderina (e.g. G. tanneri Olsen, 1937) suggests that this feature holds little taxonomic potential value for the future. Laurer's canal is an associated duct which will probably be found to be universally present in the genus following further work.

A cirrus is absent from Phyllodistomes. Holl (1930) misinterpreted the seminal vesicle as being a cirrus sac. Osborn (1903) described the presence of a cirrus but did not indicate its presence in his diagram and the observation is generally regarded as inaccurate. The genital pouch seen in P. vitatussi by Dayal (1949) may be a swollen pars prostatica.

The egg size has been used to separate species (Slusarskii (1958)) but the present situation requires clarification before the true value of this criterion can be established. A characteristic of the family is the progressive growth of the eggs in the uterus until the stage where an active miracidium is produced which hatches shortly after

laying. Provided a large number of fully-developed eggs are measured preferably both in the living and mounted state, this character when used in conjunction with others may aid the separation of some species. However, examination of British species indicates that the size ranges overlap to such a degree that the value of this character is substantially reduced or negligible. Operculate eggs have been recorded for P. singhiai by Gupta (1951) and in Phyllochorus macronius by Dayal (1938). This is an unusual feature because the majority of the Phyllodistome species possess nonoperculate eggs but an operculum is recorded in the pharyngeate genus Plesiochorus by Looss (1902) placed in the family Gorgoderidae by Pigulevsky (1953).

Byrd et al. (1940), in a discussion upon the validity of the excretory arrangement as a taxonomic criterion, considered that the system should conform to a well described pattern in species comprising a single genus and that this pattern, with or without modification, should remain constant among closely related genera such as those assigned to a natural subfamily or even family. They supported their contention by examining the excretory systems of Phyllodistomum (Catoptroides) lacustri, P. lohrenzi, Gorgodera amplicava and Gorgoderina tanneri and demonstrated that the total numbers of accessory and capillary tubules and flame cells were uniform and constant in all four cases. They then proceeded to distinguish the genera Phyllodistomum, Catoptroides, Gorgodera and Gorgoderina primarily on the length of the common collecting ducts and the position of their bifurcation and the manner in which the accessory tubules originated. Fischthal (1951)

and Thomas (1958) criticised the conclusions drawn by these workers and listed many exceptions and anomalies existing in the literature concerning the position of the bifurcation site in the family. Komiya (1961) considered that although the flame cell number could not be utilised to separate or include species in one genus, the grouping of the flame cells was important and that if variation was noted this indicated that the genus was not a natural assembly. He added that obviously differing forms can possess identical systems due to convergence. Within the Gorgoderidae there is considerable variation in the flame cell groupings. Thomas (1958) arranged the diverse cercarial forms into six groups and included the flame cell arrangement as a characteristic of some of the sections. For the macrocercous forms he included the 4 grouping as a characteristic feature. The macrocercous C. steelmani Baker (1943) however was recorded as possessing the cells in groups of 3; in C. raiacauda Steelman (1938) the arrangement varied and groups of 3 and 6 were indicated; Vickers (1941) gave the incomplete C. macrocerca formula as $2[(3+3) + (3+9)]$ and C. coelocerca Steelman (1939) was recorded as $2[(3+3+3) + (5+4)]$. In the Rhopalocercariae Thomas also gave the characteristic of flame cell grouping in fours but included in the list following this description, the form C. pyriformoides, which was shown by Coil (1954) to possess a formula given as $2[(5+7+6) + (7+7+7)]$. The total number of flame cells present is of no taxonomic value because it has been shown that at least three genera possess species with the same total number (Byrd et al, 1940) and that the number may vary in

a single individual (Looss(1894); Sturges(1897)). The tubular arrangement described for the species of Phyllodistomum studied in this investigation is identical to that recorded for Gorgodera amplicava by Byrd et al (1940). The flame cell pattern $2 \left[(4+5) + (5+5+4+4) \right]$ is similar to that described in the only other record for a British species (P. simile) in that there are two groupings anteriorly and four posteriorly, but the tubular system is only approximately equivalent. In specimens recovered in the present investigation, the shape assumed by the bladder at the junction with the common ducts appears to be a feature shared by many Phyllodistomum and Gorgodera. Considerably more information is required before the value of the excretory system can be elucidated either at the family or at the generic level. The system would not appear to have any specific value however.(?)

An unusual feature concerning the excretory system has been described for two Indian species from Lucknow. Dayal(1949) and Gupta, (1951) recorded the existence of three lateral branches arising from the excretory bladder in P. vachius and P. singhiai respectively. Gupta stated that his specimens were identical to those previously described (but not identified) by Bhalerao(1937) from Poona while Pigulevsky, (1953) considered Bhalerao's specimens to be identical to the larger P. lewisi Srivastava, 1938 from Allahabad. Bhalerao did not describe the excretory system while Srivastava merely stated that the bladder of P. lewisi was characteristic of the genus. The Indian species possess several unusual characteristics. P. vittatusi Gupta, 1953

(1 specimen only recovered) and P. singhiai Gupta, 1951 (6 specimens collected) were both taken from the intestine of the definitive Fish host in a gravid state. P. vachius, P. lewisi (12 specimens recovered) were taken from the urinary bladder while Bhalerao's immature specimens were obtained from the stomach and intestine as migrating stages.

The cuticle in most Phyllodistomes is aspinose but usually striated or lightly patterned in some way. A spinose cuticle has however been recorded for some adult species (P. sinipercae Long and Wai, 1958 and P. singulare Lynch, 1936) and is indicated in the diagram of P. marinum reproduced by Lewis (1935). Wu (1938) reported that the progenetic P. lesteri possessed cuticular spines as did P. cotti (but this species was apparently never described). Tokata (1922) recorded a similar condition in an unidentified Phyllodistome metacercaria. Wu wondered whether the spinose state found in larvae would be lost in the adult and referred to the record by Dollfus (1929) for Ratzia parva where this condition changed with age. There are also reports of localised projections in the suckers (P. simile (Thomas, 1958), C. macrocerca Vickers, 1940) and the occasional appearance of scale-like projections on P. carassai by Long and Wai (1958) suggesting that the condition is of a transient nature in this case.

As more information accumulates concerning papillary patterning it does not appear that it will provide such a useful specific character as was originally hoped. Early reports indicated that the patterns remained constant from their appearance in the cercaria through to the

adult as is the case for the species studied in this investigation. A few minor variations in the exact numbers (particularly on the body) have been noticed but recently major differences during the life cycle were recorded by Dechtiar (1966). Papillae on the acetabulum of young P. coregoni although arranged in three concentric rings as in the adult condition differ from the latter in the numbers of papillae constituting each ring and their exact location. From the figures it would appear that the oral sucker papillary numbers also differ and Dechtiar states that papillary arrangement anteriorly is irregular. Thomas (1958) described the papillary pattern for P. simile as being divided into marginals and sucker papillae noting no dorsal or ventral series. His observation that the marginals were irregularly arranged and varied from 18-24 pairs may indicate the existence of such a series or merely reflect a wider variation in the marginals than was noted for the species studied during this investigation. The acetabular papillary pattern of the latter is identical with P. simile and the ring of 6 double papillae common to both species is also a feature of many Gorgoderids. The oral sucker pattern described for the Phyllodistomum and Gorgodera studied in this investigation were identical and, in addition, appear closely similar to P. simile. One of the main differences appears to lie in interpretation and a comparison of the diagrams is perhaps more helpful. Papillae situated close together have been considered in this study as being double whereas Thomas counted them singly. The close similarities between these sucker patterns and the identical parallel seen in Gorgodera for this region

suggests that papillary patterns might offer little more than evidence of convergent evolution. A comparison of all the diagrams and descriptions given on this topic indicates that a general pattern exists for the genus. Marginally there are 8 - 26 pairs of papillae, 6 - 10 of which occur along the anterior region. Maximum variation in numbers along the margins appears to be 6 but commonly only equals 2. A ring of 6 papillae around the acetabular opening has been recorded for C. steelmani, Baker, 1943; C.P. solidum (C. conica) Goodchild, 1939; C. donecerca and C. coelocerca, Goodchild, 1939; C. raiacauda Steelman, 1938; C. Gorgodera amplicava Krull, 1935; P. brevicecum Steen, 1938 and P. simile Thomas, 1958. In seven cases these papillae were double and in C. coelocerca Steelman, 1939 they are said to be double on occasions. A 6 + 4 sequence was recovered from P. simile Thomas, 1958; C. coelocerca Steelman, 1938 and in P. brevicecum Steen, 1938. An underlying 6 + 4 pattern can be traced in the heavily papillate Rhopalocercariae described by Fischthal (1951) and Coil (1954) (C. pyriformoides). 1 - 2 pairs of rows of papillae are distributed dorsally and ventrally along the dorsal and ventral surface of the body. Fischthal (1951) stated that the papillae found on the dorsal surface of Rhopalocercariae were constant but that those found ventrally and marginally in the posterior region were inconstant and variable in position. He described cercariae as possessing identical sucker papillary patterning:- C. honeyi, C. micromyae, C. pyriformis, C. filicauda, C. catatonki for the oral sucker; C. catatonki, C. honeyi, C. micromyae, C. pyriformis for the ventral sucker

and, in addition, C. micromyae and C. honeyi with identical body papillary patterns. The oral sucker possesses in many cases a ring of six papillae around the mouth and 1 - 2 rings both anterior and posterior to this area with the possibility of a few papillae nearer the posterior margin. 2 - 3 pairs are usually found on either side of the stylet position. P. undulans, P. brevicecum and P. lesteri possess oral papillae arranged in a ring of four around the mouth, a pattern which is repeated around the acetabulum of P. lesteri. The Rhopalocercariae oral sucker patterns diverge more sharply from the general patterns in C. eriensis Coil, 1953; C. anodontae Coil, 1954 and C. lamsilae Coil, 1954 where the papillae are clustered in groups of threes at the lateral edges of the mouth. It would appear from the evidence above that the attempts by Fischthal (1951) and Dunagan (1957) to divide cercarial forms using papillary numbers and arrangements is a little premature. C. eriensis and the species of Phyllodistome studied in this investigation both possess a total of 26 papillae on the oral sucker, while C. anodontae and the five Rhopalocercariae described by Fischthal (1951) all possess 33. The latter author noted that although the number of these papillae was constant, the size of the projections varied.

Papillary structure varies from single or double rounded refractile projections to the sporadic or regular occurrence of single to many setae per structure. The latter were noted both in Rhopalocercariae and P. undulans Steen, 1938. A rounded papillary form was shown in section by Ssinitzin (1904) for P. folium, while Sturges (1897) noted

extremely short setae in sections of D. patellare papillae. Certainly in the genus and probably in many individuals the sensory receptors will vary widely in structure according to their location and function. Rohde (1966) has illustrated a wide variation in Multicotyle purvisi Dawes, 1941 and there seems every likelihood that an equivalent range of structures may be present in Phyllodistomes.

The presence or absence of a lip above the oral sucker cannot (as suggested by Dechtiar(1966)) be regarded as specifically important. Certain contractile positions tend to cause the lip to become pronounced and it can be easily missed if the material is not examined live. Its prominence may change during the life cycle and this may account for its inclusion in Pigulevsky's drawings (1953) of younger stages of P.(P.) folium and P.(P.) angulatum and its apparent absence in the adult. The lip is of importance in the feeding activities of all stages of the species studied in this investigation.

There is little variation in the basic structure of the gut throughout the genus. A pharynx is absent. The rudimentary pharynx described by Bravo-Hollis and Manter(1957) for P. marinae approximates to a swelling recorded for P. pacificum by Yamaguti(1951) but which he did not refer to as a pharynx and which appeared, from the wording of the account, to be transitory. Both these flukes were recovered from marine fish - 2 P. marinae from the bladder and 4 gravid P. pacificum from the small intestine(!) In neither case was the material sectioned and it would appear that a local dilation of an extensile muscular tube may have been mistaken for a pharyngeal

structure in the case of P. marinae.

The distance between the termination of the caecae and the posterior region varies within a single species and also according to the contractile phase. In the majority of species the caecae extend almost to the posterior margin with the notable exception of P. brevicecum where in 30 specimens the gut branches rarely extended beyond the posterior testis and terminated about a third of the body length from the posterior end. Shortened caecae were of sporadic occurrence in the species studied during this investigation and usually involved only a single structure. This, therefore, appears to be a valid and unique constant character for P. brevicecum. The caecae are never interconnected in Phyllodistomum as shown for Xystretum and remain as blind-ending simple sacs. The narrow or voluminous nature of the 2 branches will vary according to the contents, phase of digestion and possibly the age of the individual. The length of the oesophagus is a variable character because it is such a contractile structure and has to accommodate for anterior regional extension. Its length as regards that of the oral sucker varies during the life cycle as the bodily proportions alter.

The study of chromosome number has not yielded information to date which is of generic value. Walton (1959) recorded that in the Gorgoderidae chromosomal number varied from 6 - 8. 7 chromosomes were counted in Gorgoderina attenuata (Willey & Koulish, 1950) and 8 chromosomes were found in both Gorgodera amplicava (Britt, 1947) and Phyllodistomum spatula (Dhingra, 1954). 11 other families have been

shown to contain members with chromosome numbers in this range.

The distinctive cercarial characters do not appear to assist species separation at the adult level. Thomas (1958) demonstrated this point adequately and suggested that they may be caenogenetic adaptive characters and bear little or no relation to phylogeny. Completion of further life cycles however will undoubtedly aid the separation of species. Miracidial structure appears too simple to be of assistance.

Host specificity during the adult phase has been shown to be lacking in numerous Gorgoderina species where Urodele and Anuran hosts are equally acceptable (Mitchell, 1966). Gorgodera amplicava was shown to successfully invade several Anuran forms and some Urodeles (Goodchild, 1954 & 1955). Phyllodistomum species have been recorded from both Amphibia and Fish but no single species has been reported to occur in both these groups simultaneously. Some Phyllodistomes do not appear to be specific even to the extent of parasitising fish belonging to a single family. Specificity at the level of the first intermediate host is also not marked in many cases and apparent specificity may mainly relate to lack of information. The outstanding example is that of Sphaerium corneum which is utilised by P. simile and the species studied in this investigation and C. macrocerca according to Vickers (1941); C. Gorgodera pagenstecheri, C.G. varsoviensis and C.G. vitelliloba according to Ssinitzin (1905) as well as other cercariae of doubtful affinity. Ssinitzin (1905) also recorded C.G. pagenstecheri as being capable of infecting 2

Sphaeriid hosts and one Pisidium species and C.G. vitelliloba of occupying the same two Sphaeriids as the former trematode.

Conclusions:

There would appear to be few characters visible in the adult phase which are easily recognisable and specific. The only means at the present time of distinguishing the various Phyllodistome species is by taking measurements of large numbers of trematodes (living and mounted) at all phases of growth and from different hosts if possible, and then fitting the size ranges obtained into equivalent age groups. Unfortunately the extent of uterine inter- and extra-caecal extension can only give an approximation of the trematodes age in a mixed population, so a large population must be studied to offset this variation. Egg sizes should also be used if the full range can be obtained. Completion of the life cycle would greatly aid identification. The only alternative to this laborious method might be the biochemical analysis of adult stages. It is obvious that the metacercarial stages differ metabolically by the different form which their waste products assume. Comparative electrophoresis of proteins may provide an answer although there is the problem of parallel and convergent evolution and the possibility that trematodes which have for so long inhabited a similar environment may only differ extremely slightly. A greater difference between the flukes living in Amphibia and those in Fish is to be expected

Classification and the division of the species described on the basis of available information is largely impossible. Pigulevsky (1953) is the only author to attempt to record the sizes of trematodes at various stages in their life cycle but his classification scheme is, of necessity, based primarily upon the many inadequate accounts which are

to be found in this field. It incorporates too many criteria which are either variable or totally invalid and his scheme must be regarded as a temporary system which should be altered pending future information.

The role of statistics or numerical taxonomy in this field would appear to relate to the future rather than the present situation. There are too many variables and too little information at present to allow the successful application of these systems in order to attain valuable results. Coil (1955) collected 103 specimens of P. lacustri from 13 channel fish and reduced the number of variations relating to size by studying uncrowded, relaxed mounted trematodes all taken from hosts of the same species and size. He concluded that the trematode size differences would be due at most to genotype, age and developmental history of the flukes. He stated that differences due to genotype and developmental history would appear as single variations and would be inseparable on mere observation. His conclusions parallel those made in this investigation in that he found considerable variation in the position of the testes relative to one another; the posterior notch was an unreliable character; the ratio of the oral to the ventral sucker changed with the growth of the worm; similar structures grew at a more similar rate (e.g. suckers) than dissimilar structures and the body increased at a faster rate than the acetabulum. The body length increased at slightly more than 2x the rate of the width and he concluded that the uterus alone was not responsible for this length increase. This conclusion supports the views given by Thomas (1965) concerning the postero-antero growth gradients in

trematodes and relates particularly to the paper by Dawes (1962) concerning growth rates in Fasciola. Work concerning growth rates on a time-graded series or changing proportions and allometry tests and the statistical analysis of the data obtained may well provide additional characteristics which can be used in specific separation. Standardisation of the fixation technique is not sufficient basis for this work and fully relaxed specimens should be first obtained by anaesthesia. The interpretation of the results will require considerable care particularly if wild populations are utilised and the use of experimentally established forms is probably more advisable. The criterion separating Gorgoderina and Phyllodistomum if the two genera are valid may be expressed at some future date in terms of the growth rate of the posterior region relative to the transverse and vertical width, or simply on biochemical analysis.

(c) The Taxonomic Position of the Species under Investigation in relation to other records for the British Isles, etc.:

c(1) Historical survey:

Nicoll (1924) published a species list for trematode parasites of British freshwater fishes. The sections relative to the Phyllodistominae are reproduced below:

| | | |
|------------------------------|------------------------------|------------------------------------|
| <u>P. folium</u> | <u>P. conostomum</u> | <u>Catoptroides macrocotyle</u> |
| <u>Cottus gobio</u> | <u>Coregonus oxyrhynchus</u> | <u>Carassius carassius</u> |
| <u>Acerina cernua</u> | | <u>Barbus barbus</u> |
| <u>Esox lucius</u> | | <u>Gobio Gobio</u> |
| <u>Salvelinus salvelinus</u> | | <u>Rutilus rutilus</u> |
| <u>Thymallus thymallus</u> | | <u>Scardinius erythrophthalmus</u> |
| | | <u>Leuciscus cephalus</u> |
| | | <u>Abramis brama</u> |
| | | <u>Blicca björkna</u> |

Rawson (1952) pointed out that Nicoll had included in this list parasites which had then only been recorded from the Continent, concluding that the same species of parasites would be found in identical hosts in both Britain and Europe. Baylis (1939) published a species list for parasitic worms from British Vertebrates and included P. folium (v. Olfers, 1816) from Gasterosteus aculeatus in Cambridgeshire. Dawes (1947) reviewed both European and British species and suggested that the European P. pseudofolium Nyebel, 1926 was synonymous to P. folium (v. Olfers, 1817) Braun, 1899, which at that time had been reported from Britain, Sweden and Canada. He also considered that the Swedish P. conostomum (Olsson, 1876) and P. acceptum Looss, 1901 (from Trieste) would prove identical to the latter genus. P. macrocotyle (Lühe, 1909) Odhner, 1911 was considered by Dawes (1947) to include P. folium of Ssinitzin (1905) nec Olfers (1817); Catoptroides macrocotyle Lühe, 1909

and possibly also P. angulatum Linstow, 1907 and Catontroides angulatus (Linstow), Lühe, 1909. Rawson (1952) reviewed the literature concerning ecological studies based on trematodes of freshwater Fish throughout the world and also reported the existence of P. folium from the urinary bladder of Sticklebacks (Gasterosteus aculeatus) collected from Lake Windermere and from the same host location in immature Trout (Salmo trutta) from the River Severn. The only detailed description of a British Phyllodistome adult phase was given by Thomas (1958). This author described a species he assigned to P. simile which had not previously been reported in Britain. He collected his specimens from Salmo trutta in Wales. Dawes (1947) considered P. megalorchis Nyebelin, 1926 as a synonym of P. simile Nyebelin, 1926 and suggested that in the ultimate analysis P. simile was likely to prove identical with P. folium. He also concluded that P. elongatum Nyebelin, 1926 was almost certainly identical with P. simile and that it might eventually turn out to be simply an extended form of P. folium.

(2) Classification of the Species studied during this Investigation:

In the following section, for the sake of clarity, the species studied in this report will be referred to as 'X'.

A detailed comparison with the British record for P. simile at all stages of the life cycle indicates that X is distinct from the former species as it is defined by Thomas (1958). The two species utilise an identical primary intermediate host which is Sphaerium corneum. The sporocysts are similar structures differing mainly in the position of the birth pore while internally the older developmental stages of X

cercariae are slightly smaller than the equivalent phases of P. simile. This size difference is accentuated in the fully developed cercariae although the lower size ranges of P. simile overlap most X measurements. Similarly the stylet size ranges coincide but the form of the two structures differs. The excretory pattern and the width of the bladder in comparison with that of the posterior region is also at variance and the papillary patterns and presence or absence of sucker cuticular spines are other points where the species do not coincide. X remains smaller at the metacercarial stage also, with the suckers of P. simile almost totally exceeding its equivalent measurements. A detailed comparison between the figures given by Thomas for specimens recovered from the definitive host and the measurements similarly obtained from 200 mounted specimens of X is given in Table 38, page 443. It can be concluded from the literature that, due to a considerable lack of important information, it may have been customary to compare data presented in this form. If such a comparison was to be attempted in this case the two sets of figures would appear to demonstrate that a considerable degree of agreement existed between the two species. P. simile, occupying a larger host, would appear only to have extended the size range given for the smaller trematode X and this size difference could be used to explain the greater size of the reproductive organs in the Salmonid parasite. The dimensions of the eggs given in the two records coincide and the maximum average sucker ratio record given by Thomas is identical to the average figure obtained for X in the longitudinal and greatest diameter axis reading. If the description

Table 38

| Region/Organ Measured | | Average Measurements for 200 mounted specimens of <i>Phyllodistomum</i> recovered from the definitive host in this investigation | <i>P. simile</i> (Thomas, 1958) Specimens recovered from the Definitive Host. Mounted Material. Numbers of Trematodes involved - unknown. |
|---|--------|--|--|
| Total length | | 0.898 (0.483 - 1.890) | - (1.04 - 3.64) |
| Anterior region (-V/S) | L | 0.309 (0.161 - 0.651) | - (0.360 - 0.760) |
| | W | 0.164 (0.070 - 0.294) | - *(0.350 - 0.450)(d) |
| Posterior region (V/S) | L | 0.589 (0.224 - 1.312) | - (0.770 - 2.670) |
| | W | 0.307 (0.063 - 0.672) | - (0.180 - 2.120) |
| Ratio: | | | |
| Anterior: Posterior Region | L | 1:1.908 (1:0.727 - 1:4.847) | - *(1:2.139 - 1:3.511) Max./Min. |
| | W | 1:1.881 (1:0.846 - 1:3.700) | ? |
| Oral Sucker Rel/organ | L | 0.144 (0.074 - 0.232) | - (0.140 - 0.320) B/A |
| | W | 0.123 (0.060 - 0.208) | - (0.140 - 0.300) B/A |
| Ventral Sucker Rel/organ | L | 0.184 (0.086 - 0.296) | - (0.16 - 0.33) B/A |
| | W | 0.172 (0.078 - 0.260) | - (0.18 - 0.35) B/A |
| Sucker Ratio - Oral:Ventral Sucker | B/A L | 1:1.282 (1:0.877 - 1:2.000) | - 1:1.09 - 1:1.28 B/A Diagnosis |
| | B/A W | 1:1.403 (1:1.000 - 1:2.138) | - *(1:1.031 - 1:1.143) Max./Min. B/A |
| | R/O CD | 1:1.282 (1:0.918 - 1:1.873) | - *(1:1.167 - 1:1.286) Max./Min. B/A |
| Vitellaria Rel/organ | RVL | 0.073 (0.0102 - 0.144) | - R+L(0.08 - 0.21) Diagnosis |
| | RVW | 0.048 (0.0102 - 0.086) | - R+L(0.07 - 0.18) |
| | LVL | 0.072 (0.0112 - 0.130) | - R+L(0.11 - 0.25) Table 3 |
| | LWW | 0.047 (0.0102 - 0.092) | - (0.06 - 0.15) |
| Ovary Rel/organ | L | 0.107 (0.0357 - 0.224) | - (0.14 - 0.39) B/A |
| | W | 0.082 (0.0255 - 0.170) | - (0.18 - 0.39) B/A |
| Amphitypic | %R | 52% | Amphitypic ? |
| | %L | 48% | ? |
| Anterior Testis Rel/organ | L | 0.118 (0.0326 - 0.272) | - (0.22 - 0.82) B/A? |
| | W | 0.083 (0.0204 - 0.184) | - (0.18 - 0.76) B/A? |
| Posterior Testis Rel/organ | L | 0.129 (0.0347 - 0.270) | - (0.28 - 1.09) B/A? |
| | W | 0.083 (0.0204 - 0.180) | - (0.24 - 0.72) B/A? |
| Vesicula seminalis (Av. 10 specimens) (immature to old specimens) | L | 0.0647 (0.0357 - 0.0840) | - *(0.0875 - 0.0714)(d) (young-older specimen) |
| | W | 0.0395 (0.0210 - 0.0735) | - *(0.0562 - 0.0357)(d) |
| Excretory bladder (Av. 10 specimens) (immature to old specimens) | L | 0.270 (0.165 - 0.440) | - |
| | W | 0.0154 (0.007 - 0.022) | - |
| Percentage of PR occupied by bladder | L | 51.9% | - |
| | W | 6.706% | - |
| Eggs - Living material (155 measured) | L | 0.0593 (0.0541 - 0.0724) | |
| | W | 0.0382 (0.0330 - 0.0459) | |
| Eggs - Mounted material (150 measured) | L | 0.0362 (0.0306 - 0.0469) | - (0.0300 - 0.0325) Diagnosis |
| | W | 0.0229 (0.0163 - 0.0316) | - (0.0250 - 0.0275) Table 3 |
| | | | - (0.030 - 0.037) |
| | | | - (0.020 - 0.022) |

Abbreviations and Symbols:

- L = Length
- W = Width
- RV = Right vitellarium
- LV = Left vitellarium
- B/A = Measurement is relative to main Body Axes.
- Rel/organ = R/O = Measurement is relative to organ's axes.
- V/S = Ventral Sucker
- * Signifies that the figures have been calculated from the data provided and do not appear in the account.
- (d) Signifies that the measurements were obtained from the diagrams given in the 1958 article.
- Max./Min. Signifies that the figures were calculated from the maximum and minimum readings given in the account and may not relate to the condition in individual trematodes.

All measurements are in millimeters.

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for each of these two trematodes was limited, as in the majority of Phyllodistomum species, to reports based upon mounted collections where neither the life cycle nor excretory system, etc. had been determined there would appear to be every justification at first sight for including X as a member of the P. simile species which had remained in a smaller state as a result of host effects. Life cycle studies have already indicated in this case that such a conclusion would be totally inaccurate. A comparison of the adult data in this way, without reference to the age of the trematodes, suppresses proportional differences evident at different stages and camouflages the results of differential growth rates which might reflect specific characteristics.

A more accurate numerical diagnosis for X is given in Table 39 , page 445 . The scheme is based upon the age of the trematode as indicated by uterine development. This unfortunately can only give an approximation of the flukes' age but it appears to be the only readily recognisable feature available. Provided the range and the number of specimens is large enough, discrepancies associated with a variation in the timing of the first appearance of uterine extra-caecal extensions can be adequately accounted for. Thomas (1958) recorded the measurements of an unknown number of mature flukes taken from the definitive host. From his diagrams it would appear that P. simile possesses an inter-caecal uterus when reaching the lowest size range which Thomas records, i.e. from 1.04mm in length. If the smallest figures in each size range are taken to refer to flukes approximating 1+mm in length and these are then compared with the figures given for

Table 39

Numerical Analysis of the Phyllodistomum species studied in this investigation (Mounted Material)

| Region/Organ <u>Measured</u> | | <u>Juveniles</u> | <u>Young Adults</u> | <u>Adults</u> | <u>Adults</u> |
|---------------------------------|--------|--------------------------------|---|---------------------------------|------------------------------|
| | | No eggs; intercaecal uterus | Few eggs; intercaecal uterus-basal coiling | Extra-caecal uterine coiling | Exhibiting Testal Atrophy |
| Total length | | 0.448 - 0.850 | 0.553 - 1.162 | 0.525 - 1.890 | 0.728 - 1.358 |
| Anterior Region (-V/S) | W | 0.070 - 0.144 | 0.084 - 0.238 | 0.084 - 0.294 | 0.140 - 0.266 |
| Posterior Region | L | 0.224 - 0.646 | 0.315 - 0.777 | 0.336 - 1.312 | 0.469 - 0.994 |
| (V/S) | W | 0.060 - 0.182 | 0.140 - 0.441 | 0.112 - 0.672 | 0.259 - 0.672 |
| Ratio: Anterior: | L | 1:0.800 - 1:1.956 | 1:1.205 - 1:3.080 | 1:1.443 - 1:4.847 | 1:1.063 - 1:2.869 |
| Posterior Region | W | 1:0.917 - 1:2.000 | 1:1.000 - 1:2.424 | 1:1.208 - 1:3.700 | 1:1.425 - 1:3.000 |
| Oral Sucker | L | 0.080 - 0.136 | 0.084 - 0.184 | 0.090 - 0.232 | 0.120 - 0.232 |
| Rel/organ | W | 0.060 - 0.118 | 0.076 - 0.164 | 0.080 - 0.208 | 0.120 - 0.208 |
| Ventral Sucker | L | 0.092 - 0.158 | 0.090 - 0.296 | 0.011 - 0.296 | 0.176 - 0.296 |
| Rel/organ) | W | 0.080 - 0.150 | 0.086 - 0.240 | 0.106 - 0.260 | 0.160 - 0.258 |
| Ratio: Oral: | B/A L | 1:1.021 - 1:1.535 | 1:0.877 - 1:1.786 | 1:1.000 - 1:2.000 | 1:1.024 - 1:1.629 |
| Ventral Sucker | B/A W | 1:1.000 - 1:1.634 | 1:1.038 - 1:2.134 | 1:1.016 - 1:2.138 | 1:1.287 - 1:1.667 |
| | R/O GD | 1:1.021 - 1:1.535 | 1:0.918 - 1:1.849 | 1:1.000 - 1:1.873 | 1:1.072 - 1:1.534 |
| Vitellaria | L | 0.011 - 0.070 | 0.020 - 0.100 | 0.042 - 0.144 | 0.040 - 0.144 |
| Rel/organ | W | 0.010 - 0.046 | 0.020 - 0.066 | 0.030 - 0.092 | 0.030 - 0.086 |
| Ovary | L | 0.036 - 0.100 | 0.056 - 0.120 | 0.064 - 0.224 | 0.070 - 0.224 |
| Rel/organ | W | 0.025 - 0.072 | 0.044 - 0.094 | 0.040 - 0.170 | 0.064 - 0.170 |
| Anterior Testis | L | 0.033 - 0.126 | 0.070 - 0.146 | 0.080 - 0.272 | 0.070 - 0.150 |
| Rel/organ | W | 0.020 - 0.092 | 0.044 - 0.120 | 0.044 - 0.184 | 0.020 - 0.100 |
| Posterior Testis | L | 0.035 - 0.130 | 0.072 - 0.172 | 0.086 - 0.270 | 0.072 - 0.208 |
| Rel/organ | W | 0.020 - 0.104 | 0.048 - 0.120 | 0.040 - 0.180 | 0.080 - 0.150 |
| Eggs | L | | | 0.0306 - 0.0469 | |
| | W | | | 0.0163 - 0.0316 | |

Abbreviations:

L = Length

W = Width

V/S = Ventral sucker

B/A = Measurement taken relative to body axis

Rel/organ = R/O = Measurement taken relative to axis of the organ

All measurements are in millimeters.

young X adults at an equivalent size and stage of development, proportional discrepancies are immediately evident. The sucker sizes of P. simile at this stage are considerably smaller than the equivalent structures in X. The main reproductive organs, however, are much larger. The bodily proportions, at least in respect of the length of the anterior and posterior regional ratio, remain closely similar in both cases. Unfortunately, Thomas only recorded a general sucker ratio range (presumably based upon the longitudinal axes) and additional data shown in Table 38 was calculated from the maximum and minimum readings and may not relate to the condition in individual trematodes. The only conclusion which can be made therefore concerning the proportional difference maintained between the suckers of the two trematode species in this age group (particularly as regards the wide range exhibited by X) is that in both cases the acetabulum exceeds the oral sucker size.

Although the ultimate body size and proportionally larger reproductive organs of P. simile adults might be discussed on the basis of host effect, the sucker sizes, excretory and papillary patterns could not be considered in this light. When, however, any attempt is made to compare X with other species ascribed to the genus Phyllodistomum considerable difficulty is experienced. Pigulevsky (1953) is the only author to have attempted to define adult trematode species by measurements taken at various ages. He has managed to supplement or supply original data for at least 10 species but it is often not evident whether the figures represent average or single measurements or

upon how many trematodes the size ranges are based. (It should be noted, however, that my translation of Pigulevsky's work is incomplete, but as far as I am aware, this does not affect the above statement.) It is unfortunate that Pigulevsky deals with so few size ranges because the studies based on X strongly infer that wide fluctuation in values may be a common feature of these trematodes. It is of vital importance that all adult species should be defined adequately according to their age because the life cycles, excretory systems, papillary patterns and growth changes taking place in the definitive host as well as host effects are totally unknown in most Phyllodistome species. The doubtful taxonomic value of practically all morphological features means that identification must be basically at a numerical level.

Pigulevsky recorded the following measurements (in millimeters) for specimens which he assigned to P. folium from Esox lucius.

| | <u>Col.(1)</u> | <u>Col.(2)</u> | <u>Col.(3)</u> | <u>Col.(4)</u> |
|----------------------|----------------|----------------|----------------|----------------|
| Total body length | 0.72 | 1.06 | 1.72 | 1.0 - 2.0 |
| Anterior region (L) | 0.44 | 0.58 | - | - |
| Posterior region (L) | 0.28 | 0.48 | - | - |
| Maximum width | 0.20 | 0.50 | 0.58 | 0.68 - 0.80 |
| Oral sucker | 0.12 | 0.16 | *0.18 | *0.18 - 0.20 |
| | x 0.10 | x 0.14 | x 0.18 | *x 0.16 - 0.18 |
| Ventral sucker | 0.14 | *0.18 | 0.27 | 0.16 - 0.30 |
| | x 0.14 | x 0.16 | x 0.27 | x 0.25 - 0.26 |
| Vitellaria | 0.04 | 0.06-0.08 | 0.12 | 0.12 - 0.14 |
| | x 0.02 | x 0.04 | x 0.06 | x 0.06 - 0.08 |
| Ovary | 0.04 | 0.10 | *0.14 | ? 0.16 |
| | x 0.04 | x 0.08 | x 0.14 | ? *x 0.12 |
| Anterior testis | 0.06 | 0.12 | 0.20 | 0.26 - 0.34 |
| | x 0.04 | x 0.08 | x 0.12 | x 0.16 - 0.22 |
| Posterior testis | 0.08 | 0.14 | 0.26 | *0.36 - 0.44 |
| | x 0.06 | x 0.10 | x 0.12 | x 0.16 - 0.24 |

(* signifies a discrepancy on comparison with X at an equivalent age)
 (- if the above figures relate to single specimens)
 ((L) = length).

Pigulevsky's measurements for P. folium closely approximate those given for X at all ages. (This may indicate that host effects upon Phyllodistome growth rates, at least in the early stages up to maturity, are negligible, but experimental data is required before this can be proved for older specimens.) There are, however, some discrepancies in the series of measurements. Minor differences are to be found in the reproductive system where the posterior testis (column (4)) is recorded as exceeding the respective X dimensions beyond the point where the slightly larger size of P. folium specimens could account for the difference. This is only in one dimension however. The ovarian measurement is not given as a size range (columns (3) and (4)) and the measurements appear small. It is doubtful whether any significance, however, can be attached to such minor differences when they are presented in this form. The variation in the sucker dimensions alternates from an identical situation in the two species at an early stage (column (1)) to an acetabular difference (in one dimension - column (2)) to an oral sucker discrepancy (in one dimension - column (3) and in both dimensions in column (4)). A comparison of the measurements for flukes reaching 1.06 and 1.72mm long with the ranges given for trematodes measuring from 1.0 to 2.0mm presents an apparently anomalous picture. The egg sizes are recorded as 0.023 - 0.033 x 0.016 - 0.023mm and are possibly extended to 0.033 - 0.035mm. They therefore overlap the dimensions given for mounted material in X. The scale to Pigulevsky's diagrams has been omitted (page 271) but the specimens appear to be morphologically similar to X in all the stages

illustrated. The cluster of cells below the oral sucker depicted in the youngest juvenile may represent the nervous concentration, interstitial cells and/or the remains of part of the penetration gland system.

Pigulevsky considered that his specimens P.(P.) folium (Olfers, 1816) were synonymous with Distoma folium Olfers, 1816, nec D. folium Rud., 1819 and Phyllodistomum folium (Olfers, 1816) Braun, 1899. All the above authors recovered trematodes from Esox lucius and gave minimal descriptions which cannot possibly be thoroughly checked. The only way in which these early specimens can be classified is to follow Pigulevsky's scheme and associate them with other flukes from the same hosts. Pigulevsky's description of P.(P.) folium is original and not an assemblage of synonymised material. He did not record any details concerning the life cycle. Until further information is obtained concerning the Phyllodistomes infecting Esox lucius and the true ranges for P. folium in all its hosts are elucidated, it is impossible to dogmatically assign the specimens studied in this investigation (X) to the same species. It can only be said that the two appear approximately equivalent on the evidence available. In view of the lack of many valid taxonomic characters, any differences which may become apparent in the future are likely to be extremely small and may be more obvious in this case in the larval phases. It would not serve any purpose in the present uncertain and chaotic position to erect yet another species simply to contain X. I am therefore tentatively placing this species into Phyllodistomum folium as defined at present by Pigulevsky (1953)

whilst noting that discrepancies do exist in the two descriptions and that there is a marked difference in the definitive host.

Many other accounts referring specimens directly to P. folium differ in some respect from the figures either given for Pigulevsky's material and/or for the species studied in this investigation. A brief review of these forms and their main associated synonyms has been attempted in the following pages but such a comparison, being premature and based on inadequate records, cannot be satisfactory and the conclusions can at the very most be regarded as only tentative.

Slusarski (1958) reported that he obtained specimens of P. folium from Salmo sp. but only recorded the dimensions of the body (1.93 - 3.11 x 0.75 - 1.37mm); the sucker ratios (1:1.1 - 1.4 and 1:1.38 - 1.53) and the egg size (0.037 - 0.038 x 0.020 - 0.022mm). The maximum overall size exceeds that recorded by Pigulevsky although the egg sizes overlap the record given in this study. Slusarski illustrated his account with a single scale diagram (Fig. 101) and a series of three diagrams (not to scale) illustrating the topography and shape of the reproductive organs. Measurements from Fig. 101 indicate that the specimen reaches 3.13mm in length and although exceeding the sizes given by Pigulevsky or recovered during this investigation, appears to be a natural extension of the latter maintaining the proportions established in the smaller specimens. Pigulevsky, on including von Olfers' specimens as synonyms, indicated that he thought that his specimens were capable of attaining up to at least the 3mm recorded by the latter author. Rudolphi stated Olfers' material was smaller than his so the large size recorded by

Slusarski is adequately covered by these observations and as far as can be gathered he was correct in his diagnosis.

Slusarski considered that his P. folium specimens were synonymous amongst other species with Distomum folium Looss, 1894. Looss reported in his material that the suckers were almost equal in size, with the acetabulum slightly larger in certain contractile states. His diagrams indicate that from the juvenile to the adult state the suckers maintain only a fractional difference between them. In this investigation the above condition can be paralleled but the sucker differences are generally more marked. Looss was apparently dealing with abundant material but upon how many specimens he based his description is not clear. He also worked on living material and presumably gave the egg size in this state when he recorded that the largest eggs to be recovered measured 0.053 x 0.031mm. He remarked that these were not ripe and this coincides with findings of the present report. In Looss's material the reproductive organs are separated from the gut caecae and the excretory system, although not fully determined, involves the division of the common ducts at the posterior acetabular rim. These specimens described by Looss do not appear synonymous with the P. folium material as defined by Pigulevsky or as studied in this account.

Slusarski (1958) also suggested that P.(P.) bychowskii Pigulevsky, 1953 was a synonym of P.(P.) folium. P.(P.) bychowskii host list is recorded by Pigulevsky as including Alburnus alburnus; Lota lota; Thymallus thymallus; Salmo trutta and Salvelinus alpinus. The trematode attains a greater length than the specimens recovered in this study,

reaching from 2 - 2.3mm in length when mounted. The measurements of all organs just overlap the maxima described for this species which was recorded at a mounted maximum of 1.89mm and thus appears to extend the figures obtained during this investigation. The egg sizes (0.023 x 0.033 - 0.036mm) overlap the present size range. Pigulevsky did not illustrate his description which, as it stands, indicates that Slusarski may have been correct in placing this trematode as a synonym of P. folium. The characters which Pigulevsky uses to separate the latter species from P.(P.) bychowskii (egg size, sucker ratio (1:1.5 - as compared with 1:1.2 - 1.5); and the extent of the lobing of the testes and vitellaria are invalid. Pigulevsky considered that P.(P.) bychowskii possessed the synonyms Distoma folium Rudolphi, 1819 nec Distoma folium Olfers, 1816; Phyllodistomum folium (?) (Russian worker, 1936).

Zandt (1924) collected P. folium from Leuciscus rutilus and L. leuciscus. His brief description indicates that his specimens were probably identical to the flukes described in this study. The egg size (given as 0.058 - 0.06 x 0.031 - 0.035mm) presumably refers to living material. The account was not illustrated.

Nyebelin (1926) also described specimens which he assigned to P. folium. It appears from his diagrams and description that either he was dealing with two species or a different form from that described either by Pigulevsky or in the present account. The smaller specimens drawn by Nyebelin (Fig. 2) and collected from Esox in the "Königsberg area (Braun's material), described as measuring 1.55 - 1.75mm, coincide in bodily proportions with the present species X; the larger

flukes drawn in Fig. 1 and collected from Esox in the Upsala area do not readily extrapolate the dimensions shown by the Königsberg material since the oral sucker, anterior testis and ovarian measurements are too small in proportion to the body length. Nyebelin did not recover fully developed eggs and recorded the maximum sizes as 0.033 - 0.040 x 0.018 - 0.022mm. Whether this was in the live state or only from mounted material is not known. If the latter is the case, the species is not identical either to Pigulevsky's material or to X. If Nyebelin was discussing one species it suggests that some trematodes may acquire a close proportional relationship to other species in the juvenile and in younger adult stages and only digress at a later adult phase, making specific separation impossible on the basis of an incomplete age series.

Stafford (1904) assigned Phyllodistomes he recovered from Esox lucius in Montreal to P. folium. His description (based on a single specimen) was extremely brief and no diagram was given. When Miller (1941) reviewed this report he redescribed the single Stafford specimen giving different measurements from those recorded by the original author. Lewis (1935) was the first worker to illustrate the structure of this P. folium, obtaining the specimen from Stafford's collection. Measurements of this diagram differ from the previous two accounts. However, all the descriptions and the illustration indicate that the sucker sizes do not coincide with those found in trematodes of equivalent size in the present collection. However, as the records are based on a single specimen in which the oral sucker is recurved, the validity of such a separation is questionable. The egg sizes as given by Miller (1941)

(0.032 x 0.016mm) overlap the dimensions recorded for X. On present information these specimens cannot be assigned to any species with certainty.

"Luhe (1909) described trematodes from Salmo lacustris, S. salvelinus and Acerina cernua which he termed P. folium but which do not appear to coincide with the X even after accounting for the greater length of Luhe's specimens. The sucker sizes are too small and the difference between the two organs is supposed to diminish with age. The egg sizes (0.060 x 0.035mm) coincide if the measurement was made upon live material.

Pigulevsky (1953) erected P.(P.) dogieli and placed P. folium Ssinitzin, 1905 nec P. folium (Olfers, 1816); Catoptroides macrocotyle Luhe, 1909 as the synonyms. He gave a host list which included 17 genera and species of Cyprinid fish. In Luhe's original account of C. macrocotyle he described specimens in which the width of the posterior region decreased proportionally with age and which therefore is distinct in this respect from Pigulevsky's material for both P. folium and P. dogieli. If Luhe measured living egg sizes the record of 0.054 x 0.036mm overlaps the dimensions given for X. From the remainder of Luhe's description (which was not illustrated) there is too little information supplied on which to classify this species. Luhe followed Ssinitzin's (1905) work in assuming that the cercaria was a stumpy-tailed variety developing in Dreissena polymorpha and that the cercariae encysted inside the sporocyst. Pigulevsky repeats Ssinitzin's diagrams concerning the life cycle. The measurement given by Pigulevsky for juveniles and adults of P. dogieli are distinct from X and P. folium (Pigulevsky, 1953). The

juvenile sucker sizes are considerably larger, although the dimensions of the reproductive organs overlap in a body size considerably longer than that attained by X at an equivalent stage. In the adults all measurements exceed those given for X but the dimensions of the eggs (0.018 x 0.024 - 0.039 - 0.040mm) are equivalent. Pigulevsky's illustrations indicate that the uterine sequence of development differs markedly from the species described in this investigation. P. dogieli as recorded by Pigulevsky appears to be distinct from his description of P. folium. Whether C. macrocotyle and Ssinitzin's P. folium are synonyms of P. dogieli is not clear as insufficient information is available. The postulated life cycle is not experimentally linked with a definite adult form. Nyebelin (1926) assigned P.(C.) macrocotyle as a synonym to his P. folium specimens which may, in turn, have belonged to two species. It is not known whether Nyebelin was correct in his assumption. Because Zandt's specimens are referable to P. folium the latter author's suggestion of synonymy of his material with P. macrocotyle is probably incorrect. Slusarski (1958), however, states that he considers P. dogieli as the synonym of P. macrocotyle (Luhe, 1909).

Pigulevsky placed among the synonyms of P.(P.) pseudofolium Nyebelin, 1926 Distomum folium Looss, 1894, nec Distomum folium Olfers, 1816; Spathidium folium (Olfers, 1816) Looss, 1899; P. conostomum Odhner, 1911 pro parte; nec P. pseudofolium Gnedina & Sawina, 1930. Nyebelin's original account referred to one specimen supplemented by Looss's article in 1894. The few details recorded do not fit the description given in this account for X. Pigulevsky (1953) supplemented

the report still further and gave a series of measurements which, although overlapping those recorded in this study, do not coincide so closely as the latter measurements with Pigulevsky's record for P. folium. Pigulevsky gives Looss's diagram (1894) (as did Nyebelin (1926)) in addition to a series from the juvenile to the adult. In the latter the main reproductive organs are drawn separated from the gut caecae as in Looss's material. The egg sizes are recorded as measuring 0.035 x 0.018mm (presumably when mounted) and Pigulevsky added that the life cycle included the Sphaeriidae.

P. conostomum, as described by Nyebelin (1926); Pigulevsky (1953) and Petrushevskii (1957) appears to be a single species and to be distinct from P. pseudofolium and the P. folium studied in this present investigation. The flukes are proportionally distinct especially when younger. Olsson (1876) and Looss (1902) described the species but briefly and, according to Nyebelin (1926) Olsson's specimens were poorly preserved.

P. acceptum, when described by the original author Looss, 1901 is, as far as can be estimated from the brief description given, distinct from P. folium and X species discussed here. It shows a close similarity to P.(C) pawlovskii Zmeev, 1936 although Pigulevsky retains both species after comparing them.

P. angulatum, as described by the original author Linstow (1907) and subsequently by Lühe (1909); Loewen (1929); Nyebelin (1926) and Pigulevsky (1953) is apparently distinct from the P. folium of this study. The egg sizes overlap but the bodily proportions do not

coincide.

Dawes (1947) suggested that P. elongatum may possibly be nothing more than an extended form of P. folium. The original author Nyebelin (1926) and Pigulevsky (1953) described specimens which are distinct from P. folium. According to Pigulevsky (1953) and X. In the extremely young stage described by Pigulevsky the two species appear equivalent but diverge as the flukes age. The egg size is only recorded for P. elongatum by Nyebelin. He gives the measurement 0.055 x 0.033m remarking that these eggs did not enclose a fully formed miracidium. This differs from some of the specimens recovered during this investigation if the former measurement was in the live state. Dawes (1947) considered P. elongatum identical with P. simile which was also erected by Nyebelin in 1926. He also considered that P. megalorchis Nyebelin, (1926) was a synonym of P. simile and Slusarski (1958) was in agreement. The descriptions given by Nyebelin (1926) for the two former species indicate that they are distinct and Pigulevsky's supplementation to these descriptions agrees with the original separation. In the very early stages the two species are closely similar numerically but diverge as they age. P. simile and P. megalorchis, when described by Nyebelin (1926); Pigulevsky (1953); Thomas (1956) appear to exhibit only a size difference with the latter species attaining the greater dimensions. The hosts from which the two forms have been recovered are in some cases identical. Until the respective life cycles have been compared and growth in various hosts checked, it does not seem possible to state whether the two species are synonymous. So far

Nyebelin's report on finding metacercarial cysts of P. megalorchis in Phoxinus remains unique. Thomas (1958) noted that P. simile was also similar to P. lachancei Choquette, 1947 which was not listed by Pigulevsky (1953). The differences in the egg sizes between these two may simply relate to Choquette examining living material dissected from the fluke and Thomas measuring mounted material. The two species P. simile and P. lachancei cannot be satisfactorily separated on the evidence available.

A comparison of Pigulevsky's and Nyebelin's measurements for P. simile and those given by Thomas exhibit discrepancies at various stages concerning particularly the sucker sizes and testes. Slusarski (1958) described P. simile from Salmonids in Poland and gave the egg size as 0.035 - 0.05 mm x 0.025 x 0.042mm. Slusarski did not give detailed measurements but, from his diagram, it would appear that the proportional sizes of the organs relative to the overall size do not closely fit any previous description of P. simile. These measurements are from a single specimen and perhaps sizes ranges would show greater compatibility. The position of P. simile and its synonyms is by no means clear at the present time.

The present position of the type species P. folium as discussed in this report may be summarised as follows:

| | <u>Author</u> | <u>Host (British & European Fauna)</u> |
|-----------------------|------------------|--|
| <u>Phyllodistomum</u> | Pigulevsky, 1953 | <u>Esox lucius</u> |
| | Slusarski, 1958 | <u>Salmo sp.</u> |
| | Zandt, 1924 | <u>Leuciscus rutilus</u> |
| <u>folium</u> | | <u>Leuciscus leuciscus</u> |
| | Johnston, 1966 | <u>Gasterosteus aculeatus</u> |
| | Nyebelin, 1926 | <u>Esox lucius</u> |
| pro parte | | |

Synonym : P. bychowskii Pigulevsky, 1953 Alburnus alburnus
Lota lota
Thymallus thymallus
Salmo trutta
Salvelinus alpinus

Synonyms:

| | | |
|--------------------------------|----------------------------|--------------------|
| ? <u>Distoma folium</u> | Olfers, 1816 | <u>Esox lucius</u> |
| ? <u>Distoma folium</u> | Rudolphi, 1819 | <u>Esox lucius</u> |
| ? <u>Phyllodistomum folium</u> | (Olfers, 1816) Braun, 1899 | <u>Esox lucius</u> |

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