STUDIES ON THE BIOLOGY OF THE GYRODACTYLOIDEA (MONOGENEA).

a thesis submitted for the degree of

Ph. D.

by Philip David Harris

(Westfield College).
VARIABLE PRINT QUALITY IN THE ORIGINAL THESIS

TEXT ALSO CLOSE TO THE EDGE OF THE PAGE
This study has examined the interaction between the viviparous monogenean *Gyrodactylus* and sticklebacks. Six species of *Gyrodactylus* were collected from *Gasterosteus aculeatus* and *Pungitius pungitius* in Britain. Each was narrowly host specific, and was restricted to either the gills or the body surface of the fish. A difference in attachment mechanism was noted between gill parasites (e.g. *G. rarus*), in which the hamuli were more important, and skin parasites (e.g. *G. gasterosteii*), which relied upon the marginal hooks.

The observed growth rate of *G. gasterosteii* populations upon *Gasterosteus aculeatus* was considerably slower than the calculated potential rate. In the later stages of infestation the population declined, possibly as a result of an increase in the probability of the parasites becoming detached. This may have been related to an observed increase in the number of goblet mucus cells in the skin of infected fish. Although detached parasites were able to reinfect other fish, infection more frequently resulted from transmission during host-host contact. The abundance of *Gyrodactylus* spp. in the river Ver, Herts., England, was found to be limited by the annual life cycle of the hosts, which restricted transmission between adults and fry to a short period in midsummer.

A comparison was made with the biology of the related *Gyrodicotylus gallieni*, from the amphibian *Xenopus laevis*. This parasite, which has a suctorion attachment mechanism, inhabits the mouth of its host, entering this habitat via the nostrils. The slow population growth, and preponderance of older flukes in the population suggests that this parasite may be adapted for persistence in individual hosts. *G. gallieni* has a wider host specificity than *Gyrodactylus* spp., and has been recorded from five species and sub-species of *Xenopus*.

An oviparous monogenean, closely related to the viviparous genera, has been described from the catfish *Farlowella amazonum*. The origin and evolution of the gyrodactylids from a form similar to this parasite has been discussed.
ACKNOWLEDGEMENTS

I would like to thank Dr. R. C. Tinsley for his advice and supervision throughout this work. I am also grateful to the technicians (Celia, Jo, Graham, Roman and Dave) who helped with field work throughout three unpleasant winters, and to Geoff Joseph and Chris Walker, for their E. M. and photographic expertise. I would also like to thank Roman Sznöber, for supplying south american catfish.

The assistance of my parents in fetching, carrying, collating and photocopying has been invaluable, and this work could not have been completed without the help of Glynis Freeman, who in addition to her work on both figures and tables, was always tolerant of weekend collecting trips and of muddy buckets on her car seats.
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Ch. 1.   INTRODUCTION.
The external surfaces of fish are colonised by a wide range of organisms, including epibionts, utilising the host only for attachment, and ectoparasites, which also feed upon the host. The monogeneans, a class of ectoparasitic platyhelminths with a direct life cycle, form a characteristic element of the surface fauna of all teleosts. Most are oviparous, producing tanned eggs which hatch to give a ciliated, free swimming infective larva, the oncomiracidium. However, one family, the Gyrodactylidae Cobbold, 1864, contains only viviparous parasites which give birth to live young directly upon the surface of the host. In comparison with the remainder of the Monogenea, the viviparous forms show little morphological diversity, but they have a wide geographical and host range, increasing their importance as components of fish ectoparasite communities. The largest genus Gyrodactylus V. Nrdm., 1832, occurs on over 300 species of teleosts (Malmberg, 1970), and on some amphibians (Mizelle, Kritsky and Bury, 1968; Mizelle, Kritsky and Macdougal, 1969). Other gyrodactylids (Gyrodactyloides Bychowsky, 1948; Paragyrodactylus Gvosdev and Martechov 1953) also occur on teleost fish, and some genera have been described from primitive fish, anurans, cephalopod molluscs and parasitic crustaceans (de Beauchamp, 1912; Malmberg, 1956; Baugh, 1957; Vercammen-Grandjean, 1960; Shotter and Medaiyedu, 1978). They have been reported from fresh and salt water fish in Europe (Malmberg, 1956; 1970; Ergens, 1962), Asia (Yin and Sproston, 1948; Gussev, 1955) North America (Mizelle and Kritsky, 1964), South America (Szidat, 1973) and Africa (Paperna, 1979). Although the Monogenea of Australian fish have been examined in some detail (Johnston and Tiegs, 1922), no viviparous genera have been found, suggesting that this may be the only region in which this family is absent. In Europe and America, monogeneans are one of the most commonly encountered groups of fish ectoparasites (Hanek and Fernando, 1976). Within the Monogenea, the gyrodactylids form one of the largest groups, making up approximately one quarter of all species described from freshwater fish in Russia, America and Africa (Bychowskaya-Pavlovskaya, 1964; Hoffman, 1967; Paperna, 1979). Their importance is increased by their wide host range, as the other large families from freshwater, the Dactylogyridae and Ancyrocephalidae, are found on only a few host groups.

In addition to their widespread occurrence, the gyrodactylids are also important as pathogens of fish. Two species, Gyrodactylus medius and
G. elegans frequently cause disease epizootics amongst carp (Cyprinus carpio) in Russian fish farms, resulting in economically significant losses (Bychowskaya-Pavlovskaya 1964; Bauer, Musselius and Strelkov, 1973).

Halmberg (1972, 1976) indicated the importance of *Gyrodactylus* as a possible pathogen in trout and salmon hatcheries and Hoffman and Putz (1964), Mackenzie (1970) and Rawson and Rogers (1973) all considered the genus to be important as a disease organism of cultured fish. This genus has also been found to cause disease in natural populations of fish. Williams (1964) recorded *Gyrodactylus* sp. from dying roach, although he did not consider it directly responsible for the death of the fish. Shulman and Petrushevsky (1961) described a similar example, in which *Gyrodactylus arcuatus* and *G. bychowskyi*, in conjunction with the microsporidian *Gluca anomala* killed sticklebacks which had become isolated in tide pools. In both examples, the hosts were severely stressed, being exposed to high temperatures, overcrowding and concomitant infection with several ectoparasites. Johnsen (1978), however, demonstrated a natural disease epidemic amongst salmon in the Lakeseiva river, Northern Norway, which was probably directly due to *Gyrodactylus* infection. The importance of *Gyrodactylus* as a disease organism in other natural populations of fish has not been studied.

The *gyrodactylids* are the only truly viviparous monogeneans, although a number of genera in other families are ovoviviparous (Llewellyn, 1981b). The adult parasite contains a daughter fluke within its uterus, which becomes as large and as fully developed as its parent. This daughter contains a smaller embryo in utero, which may in turn contain a third generation embryo (Katherine, 1894; Braun, 1966; Khalil, 1970). This array of generations developing sequentially within each other is unique within the animal kingdom, and has attracted considerable attention. However, despite the importance of this phenomenon, it is still poorly understood.

The *gyrodactylids* have proved to be convenient organisms for experimental work, as they can be easily maintained and manipulated in the laboratory (Braun, 1966; Anthony, 1969; Lester and Adams, 1974a,b; Scott, 1982a,b). They breed on the surface of the host and have a high rate of reproduction, characteristics of 'microparasites' as defined by Anderson and May (1979a). They have therefore proved to be useful laboratory studies of microparasite population dynamics (Lester and Adams, 1974a,b; Scott, 1982a,b).
**Gyrodactylus** is of considerable importance as a subject for research because of its wide host range, economic significance as a pathogen, its complex and unique reproductive mechanism and its use as a model experimental system. However, little is known of the fundamental biology of the gyrodactylids. Their anatomy has been profoundly modified in association with viviparity, and in consequence the origin of the group within the Monogenea is obscure and controversial (Bychowsky, 1957; Lambert, 1979, 1980a, b; Llewellyn, 1981a; Harris, 1982c). Although the work of Malmberg (1956, 1964, 1970) has to some extent clarified the taxonomy of *Gyrodactylus*, the range of morphometric variation and host specificity of the individual species is still poorly known. The value of the work of Lester and Adams (1974a, b) and of Scott (1982a, b) on population dynamics has been reduced by this lack of basic research into the group. Finally, with the exception of *Macrogyrodactylus* Malmberg, 1956, which has been studied by Malmberg (1956a); Khalil (1964, 1970) Amirthalingam (1965) and Saoud and Mageed (1969), and *Gyrodactylus*, the biology of all other gyrodactylid genera has been completely neglected.

The present work set out to study the biology of *Gyrodactylus* in relation to its population dynamics, both in the laboratory and in the field. It has concentrated upon the interaction of *Gyrodactylus* species with the three spined stickleback, *Gasterosteus aculeatus*, also studied by Lester (1972) and Lester and Adams (1974a, b). The availability of material of *Gyrodactylus gallieni* Vercammen Grandjean, 1960, from the clawed toad, *Xenopus*, allowed a comparison of the biology of gyrodactylids from widely differing hosts. Finally, the fortuitous discovery of an oviparous monogenean, closely related to the viviparous genera, made possible a discussion of the origin of the Gyrodactylidae.

**The Morphology of Gyrodactylus**

A description of the anatomy of *Gyrodactylus* is included here to clarify aspects of its functional morphology which will be described later. The structure of all gyrodactylids is relatively conservative, and this account, although based on *Gyrodactylus*, can be applied with only minor differences to all other viviparous genera. The structure of the parasite is greatly modified by its adaptations for viviparity, being dominated by the uterus, which may contain a developing embryo. Although some specimens with small embryos may be seen, most individuals in natural populations usually contain a large, fully developed embryo, filling the uterus.
In life, Gyrodactylus is small (body length 0.4-1.2mm) transparent and cylindrical, with a distinct posterior haptor (Fig.1) which is armed with a single pair of ventral hamuli and sixteen articulated marginal hooks. A single ventral and dorsal bar are also present (Bychowsky 1957; Malmberg, 1956a,b, 1970). The exterior of the body bears two small cephalic lobes, each of which has a 'spike' sensilla at the tip (Lyons, 1969). Each spike sensilla consists of a bundle of fused, uniciliate sense organs, and is probably chemosensory (Lyons, 1973). The body of the gyrodactyliid bears large numbers of single, uniciliate sensillae (Lyons, 1969; Lambert, 1979) which are probably rheotactic (Lyons, 1973).

The tegument covering the body of Gyrodactylus is a syncytium, similar to that of other monogeneans (Lyons, 1970,1971,1972; Morris and Halton, 1971; Fournier, 1980). The outer distal cytoplasm is connected with nucleated regions in the deeper layers of the body wall by thin cytoplasmic connections (Lyons, 1973; Kritsky and Kruidenier, 1976). In the adult the outer syncytial layer lacks nuclei (Lyons, 1970), although these are abundant in the embryonic tegument (Kritsky and Kruidenier, 1976).

The gut and pharynx of Gyrodactylus resemble those of other monopisthocotylean monogeneans described by Bychowsky (1957). The ventral, subterminal pharynx is composed of an anterior chamber and a posterior glandular and muscular region (Fig.1). The anterior rim of the posterior part is lined by a series of forward pointing 'processes' (Malmberg, 1970), which are identical to the 'pyramidal cells' described by Bychowsky (1957). However, their relationship to the gland cells described by Kearn (1963a) from the pharynx of Entobdella soleae is not clear.

Immediately behind the pharynx, the gut divides into two unbranched blind crura, which extend the length of the body into the peduncle. These are lined by a single type of unpigmented cell, and a genito-intestinal canal is absent (Bychowsky, 1957).

The centre of the body, posterior to the pharynx and between the gut crura is dominated by the thin walled distensible uterus which opens to the exterior through a mid-ventral birth pore (Braun, 1966). The uterus frequently contains a large embryo, folded into a \( \cap \)-shape. Posteriorly, a transparent, globular sac abuts the uterus (Fig.1), separated from it by an irregular plug of cells (Braun,1966).
Fig. 1.: The morphology of Gyrodactylus sp.
This sac contains a large oocyte which, after the birth of a daughter, pushes through the plug of cells into the uterus, where it develops into the next embryo (Katheriner, 1904; Gille, 1914; Braun, 1966). The homologies of the sac containing the oocyte have been the subject of some controversy. Katheriner (1894) and Braun (1966) considered that it represented the ootype, which in other platyhelminths is responsible for moulding yolk cells, ovum and shell material into a single egg (Dawes, 1947; Smyth, 1966). Other workers, failing to observe the thin membraneous sac, have interpreted the structure as a naked ovum (Turnbull, 1956; Hoffman and Putz, 1964; Srivastrava and James, 1967; Mackenzie, 1970), whereas Malmberg (1956) considered the structure to function as a seminal receptacle. This latter hypothesis concerning the function of the sac is supported by the observation (Ch. 7) of a globular seminal receptacle placed between the ovary and uterus of the oviparous Oogyrudactylus farlowellae.

The maturing oocyte of Gyrodactylus, lying within the structure interpreted as a seminal receptacle, is very conspicuous. The ovary, however, in which oocytes arise, is not a prominent structure. Seven lobes of tissue which lie around the posterior ends of the gut crura were identified by Katheriner (1894) as the ovary, but other authors have interpreted these structures as vitellaria, and have been unable to locate a discrete germarium (Turnbull, 1956; Hoffman and Putz, 1964; Braun, 1966; Mackenzie, 1970). Srivastrava and James (1967) identified as the ovary a mass of cells lying between the seminal receptacle and the testis, but this structure has not been observed by any other workers. Braun (1966) discovered a small patch of ovarian tissue on the posterior wall of the seminal receptacle, an observation confirmed by the electron microscope study of Kritsky (1971). This patch of tissue was thought to be the true ovary of the parasite (Braun, 1966; Kritsky, 1971). The identity of the seven lobes of tissue surrounding the gut remains obscure, and they may represent vitellaria or some other organ of unknown function.

Mackenzie (1970) described two shell glands opening into the seminal receptacle of Gyrodactylus unicopula, but these have not been reported in any other anatomical account of Gyrodactylus, and may represent artifacts.
The male reproductive system is, in contrast to the female, simple and well understood. The crescent shaped testis occupies a position immediately posterior to, and partially surrounding the seminal receptacle. The vas deferens is indistinct and difficult to locate, but leads forward along one side of the body into the pharyngeal region, where it dilates into a sperm filled seminal vesicle (Hoffman and Putz, 1964; Braun, 1966; Kritsky, 1971) which opens into the spherical penis. The intromittent organ is frequently regarded as a cirrus (Turnbull, 1956; Braun, 1966; Srivastrava and James, 1967; Mackenzie, 1970). But, as pointed out by Bychowsky (1957), and confirmed in the case of Gyrodactylus by Braun (1966) and Kritsky (1971), in monogeneans this structure is protruded rather than everted, and should therefore be referred to as a penis. In Gyrodactylus, the penis has a flattened outer face armed with a single large hook and a number of small spines (Braun, 1966; Mackenzie, 1970). This structure exhibits some intergeneric variation (Ch. 6). Srivastrava and James (1967) described paired prostate glands on either side of the penis, although these have not been reported by other workers.

The excretory system has been studied by Malmberg (1956, 1970) and has been shown to be composed of two looped, longitudinal canals, one on either side of the body. Short collecting ducts, which may be expanded into small contractile bladders, open to the exterior on either side of the pharynx. Flame cells are located throughout the body, and drain into both anterior and posterior loops of the main canals (Fig. 4).

Reproduction in Gyrodactylus

The reproductive mechanism of Gyrodactylus is unusual in respect of (a) the maturation and fertilisation of oocytes, (b) the development of successive generations of embryos in utero and (c) the retention of embryos until an advanced stage of development.

(a) The maturation and fertilisation of oocytes.

The maturation of oocytes was studied by Gille (1914) and Braun (1966), both of whom found that the cells underwent a reduction division in the seminal receptacle before passing into the uterus. Katheriner (1904) had previously been unable to find any evidence of this division. Fertilisation of the oocyte has never been observed, although the presence of active sperm in the uterus and seminal receptacle suggests that it occurs in one of these sites.
(b) The development of oocytes within each other.

The cellular processes giving rise to the development of three or four embryos within each other have been adequately described by Kathériner (1904), Gille (1914) and Braun (1966). A single cell enters the uterus, where it may (Gille, 1914; Braun, 1966) or may not (Kathériner, 1904) undergo meiosis and fertilisation. It then divides into two equally large cells, each of which then divides unequally two or three times, producing small cells which lie between the two large cells.

One of the large cells then undergoes repeated division, forming a cell mass which grows around the other, quiescent cell. The cell mass derived from the dividing cell ultimately differentiates to form the F. embryo, whereas the quiescent cell gives rise to the two subsequent generations, by a similar pattern of division and asymmetrical growth. Because this process always gives rise to a quiescent cell with potential to produce two subsequent generations, it can apparently take place for an indefinite number of generations (Fig. 2). Braun (1966) has shown that an unbroken sequence of twenty daughter generations could arise in this way.

Bychowsky (1957), on the basis of the distribution of embryonated individuals within the parasite population, suggested an alternative mechanism of embryo development. He considered that a cell entering the uterus developed into an individual containing three embryos, which subsequently gave birth to a specimen containing two generations, which in turn gave birth to a parasite containing only one embryo. This individual gave birth to a daughter with an empty uterus, into which a cell passed and developed into a cluster of four embryos, starting the cycle anew (Fig. 3). This hypothesis requires a break in the sequence of development every four generations, during which a cell enters the uterus and develops into a cluster of embryos.

However, both Braun (1966) and Lester and Adams (1974) were able to culture Gyrodactylus for many generations without observing a break in the sequence of embryo development. It therefore seems likely that Bychowsky (1957) was mistaken in his interpretation, possibly because he confused the sequence of embryo development in the population with that in individuals, as first one, then two and finally three embryos differentiate from the cell mass in the uterus.

Kathériner (1904) suggested that polyembryony (the mitotic development of several embryos from a single oocyte) was responsible for the development of embryos within each other in Gyrodactylus, in a manner similar to the development of digenean larval stages (Wright, 1971). Braun (1966), however,
Fig. 2.: The reproductive sequence of Gyrodactylus, according to Braun.
Fig. 3.: The reproductive sequence of Gyrodactylus, according to Bychowsky.
interpreted the embryogenesis of *Gyrodactylus* in a different way. He observed meiotic configurations in the chromosomes of the single embryogenic cell, suggesting that this cell represented an oocyte. When the newly fertilised *Gyrodactylus* zygote divides, one cell undergoes rapid mitoses to form the $F_1$ generation soma, whereas the other cell represents an oocyte. This oocyte undergoes meiosis and is fertilised, becoming the $F_2$ generation zygote. The first division of this cell gives rise to the $F_2$ somatic cell and the $F_2$ ovum, which undergoes meiosis and fertilisation to form the $F_3$ zygote. This process is extreme paedogenesis rather than polyembryony, the development of an oocyte being confined to the first division of the zygote. An interesting parallel to this process has been observed in the Digenea, as Khalil and Cable (1969) observed meiotic elements in the chromosomes of the developing germ cells of rediae and sporocysts of *Philophthalmus*.

Braun's (1966) interpretation of the development of the *Gyrodactylus* embryo requires the presence of sperm in utero to effect the fertilisation of the embryonic oocytes as they appear. However, Lester and Adams (1974a) observed that *Gyrodactylus alexanderi* continued to give birth over many generations in the absence of any possibility of cross or self fertilisation. In order to account for these observations it becomes necessary to assume that the parasites store sperm, and are able to transfer it from mother to embryo for many generations. It is possible that the meiotic chromosomes in oocytes observed by Gille (1914) and Braun (1966) do not form part of a complete meiotic cycle. It has been shown in some parthenogenetic insects that although oocytes enter meiosis, the chromosomes subsequently return to their premeiotic configurations and the cells undergo mitosis (White, 1973). A process of this type may be involved in the reproduction of *Gyrodactylus*.

(c) The retention of embryos in utero.

In the oviparous monogeneans, the vitellaria are thought to produce, in addition to droplets of eggshell material (Smyth, 1966), sufficient energy reserves to support the larva through embryonic development and its short free living phase. The energy requirement of the oncomiracidium are probably small in comparison with those of the large embryo cluster of *Gyrodactylus*. However, the structures thought to be the vitellaria of *Gyrodactylus* are small and indistinct, and the source of nutrients for embryo growth and differentiation is not known. Braun (1966) observed material derived from organs which he considered to be vitellaria fusing with the oocyte in the seminal receptacle. It is possible also that nutrient uptake may take place across the larval tegument, which lies in close contact with the uterine wall of the parent.
It is of interest to note that Kritsky and Kruidenier (1976) observed large numbers of nuclei, ribosomes and golgi bodies in the larval tegument, which subsequently disappeared on maturation. This suggests that the larval tegument is metabolically active, and may be involved in nutrient uptake from the mother.

The relationships of the Gyrodactylidae

Thirteen genera of gyrodactylids have been described (Kritsky and Thatcher, 1977), of which twelve clearly show adaptations for viviparity. One of these thirteen genera, Phanerothecium Kritsky and Thatcher, 1977, was originally described as viviporous, but further examination of the type material (Ch.7) has shown it to be oviparous. The viviparous genera are all placed in the Gyrodactylidae, whereas Phanerothecium has now been transferred (Ch.7) to the Oogyrodactylidae Harris, alongside Oogyrodactylus farlowellae. One additional viviparous genus, Paragyrodactylus Szidat, 1973 was overlooked by Kritsky and Thatcher (1977) in their review of the Gyrodactylidae, and should be included in this family.

The Gyrodactylidae was divided into four sub-families by Kritsky and Thatcher (1977). All genera with two hamuli and ventral and dorsal bars (Gyrodactylus V. Nrdm., 1832, Gyrodactyloides Bychowsky, 1948, Paragyrodactylus Gvosdev and Martechov, 1953, Metagyrodactylus Yamaguti, 1963; Macrogoryrodactylus Malmberg, 1956, Archigyrolyactylus Mizelle and Kritsky, 1967, Swingleus Rogers, 1969, Fundulotrema Kritsky and Thatcher, 1977) were placed within the Gyrodactylinae Monticelli, 1892. The two genera lacking hamuli and bars (Isancistrum de Beauchamp, 1912 and Anacanthocotyle Kritsky and Fritts, 1970) were placed together in the Isanistrinae de Beauchamp, 1912. Gyrdicotylus Vercammen-Grandjean, 1960, with a suctorial attachment mechanism, and Polyclithrum Rogers, 1968, with numerous additional haptor sclerites were also placed in separate sub-families, the Gyrdicotylinae Vercammen-Grandjean, 1960 and the Polyclithrinae Rogers, 1968 respectively.

These subdivisions of the family have been based on the structure of the attachment mechanism, which has been considered to be highly variable and adaptive (Malmberg, 1970; Lambert, 1980a,b) and therefore not an ideal character for use in elucidating phylogenetic relationships. Malmberg (1974) considered Paragyrodactylus to be polyphyletic, and it is likely that several of the

** This name is invalid, due to preoccupation by Paragyrodactylus Gvosdev and Martechov, 1953.
described inter-relationships of the genera are unnatural. Only *Gyrodactylus* and *Macrogyrodactylus* have been studied in detail, and all of the genera require further examination, using a wide range of characters to determine their affinities. The classification of the Gyrodactylidae according to Kritsky and Thatcher (1977) is summarised in Table 1.

The relationships of the gyrodactylids to the oviparous monogeneans have been the subject of considerable controversy. The Monogenea can be sub-divided into eight distinct, natural groups (Llewellyn, 1963, 1971a):

(a) the scanchocotylids, distinguished by the possession of sixteen articulated marginal hooks (Nalmberg, 1982) and a pseudohaptor bearing ridges of sclerotised plates.

(b) the gyrodactylids, which also possess sixteen articulated marginal hooks, but which are additionally characterised by their hamuli and spike sensilla.

(c) the dactylogyrids, a large homogenous group with 14-16 inarticulate marginal hooks and an intricately coiled, sclerotised copulatory apparatus.

(d) the polyopiaothocotyleans, characterised by haematophagy and the presence of a genito-intestinal canal. Monogeneans belonging to this group have between 10 and 16 inarticulate marginal hooks.

(e) the capsalids, with 16 rigid marginal hooks (two of which are placed centrally), and an unarmed penis.

(f) the monocotylids, with 14 peripherally placed inarticulate marginal hooks. Despite a superficial resemblance to the capsalids, Lambert (1930a,6) has suggested that the two groups are not closely related.

(g) the microbothriids in which the haptor is reduced to an adhesive pad. Bychowsky (1957) did not consider that this group should be placed within the Monogenea, as no sclerites are present in the haptor. However, Kearns, (1965) showed that small spines are present in the embryonic haptor of *Leptocotyle* subsequently disappearing as the parasite develops, which indicate the monogenean affinities of the microbothriids.
the udonellids, a small group of platyhelminths hyperparasitic upon caligid copepods. Although regarded as monogeneans by Sproston (1946), Bychowsky (1957) removed them from the class because they lacked opisthaptor sclerites. Unlike the microbothrids, sclerites are also absent from the haptor of the unciliated larva (Sproston, 1946), making the affinities of this group uncertain.

The inter-relationships between these groups, and the evolutionary pathways between them, have not been adequately determined. The earliest sub-division of the Monogenea was that of Odhner (1912), who recognised the Monopisthocotylea, members of which lack a genito-intestinal canal and have a simple haptor, and the Polyopisthocotylea, representatives of which possess a genito-intestinal canal and have a complex haptor. All families of the Polyopisthocotylea are closely related, for, as pointed out by Llewellyn (1981a), the genito-intestinal canal is unlikely to have developed independently in different groups. However, the Monopisthocotylea probably does not represent a natural group, being divided by Sproston (1946) into the Gyrodactyloidea Johnston and Tiegs, 1922, containing the dactylogyrids and gyrodactylids, the Capsaloidea Price, 1936, accommodating the capsalids, microbothrids, monocotylids and udonellids, and the Acanthocotyloidea, containing only the acanthocotylids. This classification firmly linked the gyrodactylids and dactylogyrids, despite the differences in marginal hooks, penis, sense organs and reproductive mechanism in the two groups. It also linked the capsalids with the monocotylids and microbothrids, groups which are no longer thought to be closely related (Lambert 1980a, b).

Bychowsky (1957) devised a new classification of the Monogenea, based upon opisthaptor characters. He sub-divided the class into the Polyonchoinea with more than 12 marginal hooks, and the Oligonchoinea, with 12 hooks or fewer. All of the Monopisthocotylea, with the polystomatids (which have a genito-intestinal canal) were placed within the Polyonchoinea, whereas the Polyopisthocotylea, except the Polystomatidae, made up the Oligonchoinea. Bychowsky (loc. cit.) united the Gyrodactylidae and the Polystomatidae within the order Gyrodactylidea, because both families have sixteen marginal hooks. The description of Gyrodicotylus gallieni, a gyrodactylid with a suckorial attachment mechanism (Vercammen-Grandjean, 1960) lent support to Bychowsky's (1957) view of the relationships between gyrodactylids and polystomatids, although it has since been shown (Ch. 7) that this resemblance is superficial,
due to convergence. Lambert (1979, 1980a,b) also implied a similar relationship when he postulated that the gyrodactylids arose by neoteny from a polyopisthocotylean ancestor. However, Llewellyn (1963, 1965, 1981a) has stressed that Bychowsky (1957) artificially aggregated two groups which should remain separate, and the discovery of *Gyrodactylus farlowellae* has firmly established the monopisthocotylean affinities of the gyrodactylids (Ch. 7). No link between the gyrodactylids and any other group of monopisthocotyleans has been found, and for the present it seems most appropriate to adopt the suggestion of Llewellyn (1981a), that all of the major monopisthocotylean groups should be considered independent, without close inter-relationships between them.
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Ch. 2. TAXONOMY OF GYRODACTYLUS.
INTRODUCTION

The genus Gyrodactylus was erected by von Nordmann (1832) to accommodate the species G. lecanum, collected from the gills of Linnaeus's trimm.

Subsequently Wagener (1880) and Katheriner (1894) recorded what was thought to be this parasite from a range of freshwater fish. Other species described during the latter part of the 19th century were G. praemanius Levinsen, 1891, G. madlina Katheriner, 1894, and G. gracilis Katheriner, 1894. During this period, insufficient data on the morphometric variability and host specificity of Gyrodactylus species made it difficult to define new taxa.

In the 1930's, many new species were described from Russian fish, using the dimensions of the haptor sclerites as specific characters (Bychowsky, 1933). Although this approach introduced more consistency into the identification of Gyrodactylus species, confusion surrounding earlier descriptions persisted.

In addition many workers were less critical than the Russian school in identifying species of Gyrodactylus, and although Bychowsky (1933) greatly increased the range of characters included in taxonomic analysis, his work was still insufficiently rigorous to completely eliminate the possibilities of synonymies occurring. Thus Malaberge (1970) listed 29 synonyms of G. lecanum, from 7 host families.

Much confusion surrounded the taxonomy of Gyrodactylus until the work of Malaberge (1956, 1959, 1979), who introduced a system for the division of Gyrodactylus into sub-genera, based on characters of the secretory system. He further sub-divided the sub-genera into species groups on the basis of marginal hook morphology. Malaberge's (1956, 1979) work has allowed a more rational approach to the problems of Gyrodactylus taxonomy, and this author (Malaberge, 1964) has also critically re-examined and re-defined some of the more problematical species (G. lecanum, G. arcuatus Bychowsky, 1933, G. parva, Wagener, 1910), simplifying their identification considerably.

Gyrodactylus has recently been split into five component genera, Gyrodactylus, Postgyrodactylus, Mesogyrodactylus, Anguilladactylus, and Limosogyrodactylus by Clasee (1977), which correspond closely with the sub-genera of Malaberge (1979). However, the rigid formality of Clasee's (1977) designations is undesirable, because less than one third of the described species have been examined in sufficient detail to determine their affinities. This division of the genus has not gained wide acceptance, and both ecologists and taxonomists have retained the genus Gyrodactylus (Rasnag, 1989; Ogawa and Egusa, 1980; Dzhailov, 1981). Throughout the present work, the
nomenclature of sub-genera and species groups, introduced by Malmberg (1970) has been followed.

Very few studies of the ecology of Gyrodactylus have adequately identified the species under observation. This is particularly true of Britain, where although Gyrodactylus was first recorded in the mid 19th century (Bradley, 1861; Houghton, 1862; Cobbold, 1862), only eleven species from ten hosts were known over 100 years later (Kennedy, 1974). Two of these, G. elegans and G. medius, have been recorded from a wide range of hosts, records which, in view of Malmberg's (1964) redefinition of these taxa, are liable to refer to other species. No data exist concerning the infection of a further 25 species of fish (listed by Maitland, 1971) by Gyrodactylus. Because the confusion surrounding the identity of Gyrodactylus species has hampered the study of their ecology, it was considered necessary in the present work to precisely define the limits of the taxa infecting sticklebacks. In order to do this, the morphometric variation of the species collected from a range of freshwater fish was examined. In addition, the host specificity of Gyrodactylus gasterostei was studied, to determine the range of hosts infected by this species.

Species of Gyrodactylus from sticklebacks

Both the three and ten spined sticklebacks (Gasterosteus aculeatus and Pungitius pungitius) have a very wide geographical distribution, covering much of Eurasia and America between latitudes 35°N and 75°N, although they are seldom found far from the coasts (Wootton, 1976). When the ease of study, abundance and wide distribution of these fish are considered, it is not surprising that a large number of species of Gyrodactylus have been described from them. The earliest records are those of G. elegans from Gasterosteus aculeatus, by Bradley (1861), Houghton (1862), Cobbold (1862) and numerous other authors, listed by Sproston (1946). Subsequently, G. rarus Wegener, 1910 from Pungitius pungitius in Germany and G. arcuatus Bychowsky, 1933, from Gasterosteus aculeatus in Russia were described. Much confusion surrounded the identity of these three species, until resolved by Malmberg (1964). G. elegans sensu stricto is a parasite of the gills of bream, and is never found on sticklebacks (Malmberg, 1964, 1970). G. rarus and G. arcuatus, although confined to sticklebacks, have frequently been confused with other species from these hosts. More recently, G. alexanderi Mizelle and Kritsky, 1967, G. avaloniae Hanek and Threlfall, 1969, G. canadensis Hanek and Threlfall, 1969, G. lairdi Hanek and Threlfall, 1969, G. memorialis Hanek and Threlfall, 1969 and G. gasterostei Glaser, 1979 have been described from Gasterosteus aculeatus.
<table>
<thead>
<tr>
<th>Species from <em>Gasterosteus aculeatus</em></th>
<th>Locality</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. medius</em> Katheriner, 1895</td>
<td>N. Germany</td>
<td>Wegener, 1909</td>
</tr>
<tr>
<td><em>G. bychowskyi</em> Sproston, 1946</td>
<td>Baltic, White and Barents seas Amur river</td>
<td>Bychowsky and Polyansky 1953 Gussev, 1955</td>
</tr>
<tr>
<td><em>G. pungitii</em> Malmberg, 1956</td>
<td>Gt. Britain</td>
<td>Powell, 1966 Harris, 1980a</td>
</tr>
<tr>
<td><em>G. branchicus</em> Malmberg, 1970</td>
<td>Baltic</td>
<td>Malmberg, 1970</td>
</tr>
<tr>
<td><em>G. gasterosteoi</em> Glaser, 1974</td>
<td>N. Germany</td>
<td>Glaser, 1979</td>
</tr>
</tbody>
</table>
Table 2. (cont’d).

**Species from Rungitius pungitius**

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. bychowskyi</em> Sproston, 1946</td>
<td>Amur river</td>
<td>Gussev, 1955</td>
</tr>
<tr>
<td><em>G. pungitii</em> Malmberg, 1956</td>
<td>Sweden, N. Germany</td>
<td>Malmberg, 1970</td>
</tr>
</tbody>
</table>

**Species from Eucalia inconstans**

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Author</th>
</tr>
</thead>
</table>

**Species from Gasterosteus wheatlandi**

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Author</th>
</tr>
</thead>
</table>
In addition, G. puntitii Malaberg, 1956 from P. puntitius, G. eucaliace Ikezaki and Hoffman, 1957 from Eucalia inconstant, and G. terranovae Hance and Tryfall, 1959 from Gasteroacta wheatlandi have been described. The systematic position, site of infection and geographical distribution of these species are given in Table 2.

The host specificity of Gyrodactylus

Monogeneans in general are considered to be narrowly host specific, as a result of the long period of their coevolution with the teleost fish (Bychowsky, 1957; Llewellyn, 1957, 1982). On the basis of faunistic studies, Bychowsky (1957) considered the gyrodactylids to be the least specific group of monogeneans, and Dawes (1947) regarded the morphological variation between Gyrodactylus from different hosts to be insufficient to justify specific status for many taxa. On the other hand, anecdotal observations of individual Gyrodactylus species indicate a high degree of specificity (Parker, 1965; Hoffman and Putz, 1954; Malaberg, 1979; Glaser, 1974). Two extreme interpretations of the specificity of Gyrodactylus are therefore possible:

1. That Gyrodactylus species are narrowly host specific, each host supporting an assemblage of distinct parasite species.

2. That relatively few non-specific Gyrodactylus species exist, which show consistent small morphological variation when infecting different hosts. This variation is, however, only a reflection of their genetic adaptation to the host concerned.

The degree to which Gyrodactylus species are capable of hybridisation is impossible to estimate because insufficient is known of gyrodactylid reproduction. It must therefore be assumed that groups of individuals which can be separated on the basis of morphometric variation and host specificity represent different species. In order to determine the limits of Gyrodactylus taxa, a laboratory study of the host specificity of Gyrodactylus gasterostei was undertaken. In addition, other small fish occupying the same habitats as sticklebacks were surveyed to determine the structure of natural Gyrodactylus faunas.
Distribution, zoogeography and influence of the macromilieu on Gyrodactylus

Gyrodactylus has been described from marine and freshwater fish, and species have an almost worldwide distribution (Ch.1). However, individual Gyrodactylus species are more restricted in their ecological and geographical range. Malmberg (1956) noted some of the effects of macromilieu upon these parasites. One of the most important factors influencing their distribution was salinity. Species may be tolerant of either fresh or salt water, but very few (e.g. G. arcuatus and G. rarus) are euryhaline (Malmberg, 1956, 1970). Temperature may also be important in determining the distribution of individual species, and Malmberg (1956, 1970) found some evidence that water quality may effect the composition of a host's Gyrodactylus fauna.

In addition to their macromilieu requirements, the distribution of Gyrodactylus species may also be affected by the rate at which they colonise new habitats. As no resting stages are present in the life cycle, and parasites can only be transported long distances when attached to a host, the distribution of a Gyrodactylus species may reflect the colonising migrations of the host. This is particularly the case for those fish, including sticklebacks (Wootton, 1976) which are thought to have originated in the sea and to have migrated into freshwater. The bullheads (Cottidae) also have a present day distribution which suggests a marine origin. The majority of extant species are marine, but the genus Cottus is found in freshwater, and numerous endemic genera and species may be found in lake Baikal. One species which is normally marine, Myoxocephalus quadricornis is also found as a glacial relict in some freshwater lakes (Wheeler, 1978). It was thought that a consideration of the distribution of Gyrodactylus species on the Cottidae and Gasterosteidae might provide some evidence of the radiations of these families into freshwater.

MATERIALS AND METHODS

Hosts were caught by hand - or seine-netting from a range of habitats (Table 3) and placed in river water for return to the laboratory, where they were transferred to dechlorinated mains water and examined within 72 hours of capture. All fish were killed by cutting the spinal cord, small specimens being examined under a binocular dissecting microscope whereas, on larger individuals, fins, scales, skin scrapings and gills were removed and scanned separately. Individual Gyrodactylus were removed using insect pins, mounted in a drop of water on slides, flattened and examined under positive phase contrast illumination (Leitz Dialux). The excretory system was examined in living flukes, and after their death, they were flattened further (to the point of bursting) and fixed with one drop of ammonium-picrate glycerin (Malmberg, 1970).
### Table 3. Sites from which sticklebacks were collected.

<table>
<thead>
<tr>
<th>Site</th>
<th>Description</th>
<th>Species of <em>Gyrodactylus</em> found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drainage ditches, Cuxton and Allhallows, Kent.</td>
<td>Stagnant, brackish (3% and 7% salinity) water.</td>
<td><em>Gyrodactylus arcuatus</em></td>
</tr>
<tr>
<td>R. Medway, Kent, R. Yar, Isle of Wight; R. Rother, Sussex; R. Adur, Sussex.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walthamstow reservoirs, London.</td>
<td>Large, still water bodies, hard water.</td>
<td><em>G. arcuatus, G. gasterostei, G. rarus and G. pungitii</em></td>
</tr>
<tr>
<td>Sea school canal, Gravesend, Kent; R. Cam, Cambridge; Harting pond, Sussex; Brecon-Abergavenny canal; Griffin brook, Birmingham.</td>
<td>Slow flowing/stagnant water bodies, unpolluted</td>
<td></td>
</tr>
<tr>
<td>Grand Union Canal, London.</td>
<td>Slow flowing/stagnant heavily polluted.</td>
<td><em>G. arcuatus</em></td>
</tr>
<tr>
<td>Baildon Moor, Leeds.</td>
<td>Still water body, Oligotrophic.</td>
<td><em>G. arcuatus, G. gasterostei</em></td>
</tr>
<tr>
<td>Llyn Tegid North Wales</td>
<td>Still water body, Oligotrophic.</td>
<td><em>G. arcuatus, G. gasterostei</em></td>
</tr>
<tr>
<td>Ullswater, Cumbria.</td>
<td>Still water body, Oligotrophic.</td>
<td><em>G. arcuatus</em></td>
</tr>
<tr>
<td>Windermere, Cumbria.</td>
<td>Still water body, Meso/oligotrophic.</td>
<td><em>G. alexanderi, G. arcuatus</em></td>
</tr>
</tbody>
</table>

1) Specimens from Llyn Tegid collected by Chubb. See also Chubb (1964) and Powell (1966).

Both *P. pungitius* and *G. arcuatus* collected from habitats 2, 3 and 4.

Only *G. arcuatus* present in collections from all other habitats.
Several systems of measurement have been devised for Gyrodactylus, by Gasser (1972), Malinberg (1956, 1972), Mizelle and Kritsky (1967b) and Gasser (1974). Elements of all of these systems have been combined in the present work.

(a) Body length.

Body length is an unreliable taxonomic criterion, because living Gyrodactylus are highly contractile, and the final length is dependent of fixation technique. However, species have been broadly classified according to length in the following manner:

<table>
<thead>
<tr>
<th>Size</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Large</td>
<td>Body length greater than 1.0mm</td>
</tr>
<tr>
<td>Large</td>
<td>0.8-1.00mm</td>
</tr>
<tr>
<td>Medium</td>
<td>0.6-0.8mm</td>
</tr>
<tr>
<td>Small</td>
<td>0.4-0.6mm</td>
</tr>
<tr>
<td>Very small</td>
<td>Less than 0.4mm</td>
</tr>
</tbody>
</table>

In moderately contracted, unflattened individuals.

(b) Pharynx

This organ is divided into anterior and posterior chambers. The posterior bears pharyngeal processes (Malinberg, 1972), which extend forward into the anterior. The width of both chambers, and their combined length were measured (Fig. 4), and the type of process present (long or short) noted.

(c) The Penis

This spherical, bulbous organ, posterior to the pharynx (Fig. 1), is armed with a single large hook and a row of small spines (Fig. 4), the number and configuration of which is of some taxonomic importance. However, as the penis is present in only a proportion of the individuals in a population (Chs. 4 and 5), this character is of limited value.

(d) The excretory system

The pronephridial system is composed of two main longitudinal canals, linked by narrow ducts to flame cells in the parenchyma of the parasite (Fig. 4). A pair of short collecting ducts communicate with the exterior through small pores in the pharyngeal region. Contractile excretory bladders may be present upon this duct. The presence or absence of bladders, and of flame cells within the main canals, is of taxonomic importance.
Fig. 4. Dimensions of Gyrodactylus used in taxonomic analysis.

Pharynx and excretory System.

Key
ec- excretory canal; eb- excretory bladder; lf- lateral flame cell; ptl- pharynx total length; paw- pharynx anterior width; ppw- pharynx posterior width; htl- hamulus total length; hsl- shaft length; hrl- root length; ptl- point length; vbl- ventral bar length; tw- ventral bar width; pl- process length; ml- membrane length; dbl- dorsal bar length; mhtl- marginal hook total length; sl- shaft length; sil- sickle length; sidw- sickle distal width; sipw- sickle proximal width; h- penis hook; sp- penis spine.
(c) The opisthaptor sclerites.

The opisthaptor is armed with a single pair of hamuli, linked by dorsal and ventral bars, and with sixteen marginal hooks. Total length, root length, length, point length and the ratio of shaft length: root length of the hamuli were all measured (Fig. 4). The ventral bar is a narrow strip lying between the hamuli, expanded at each end into basal lobes. Two small processes, which articulate with the hamuli, may be present on the anterior of the bar. A broad, thin membrane extends posteriorly from the bar. Total length, total width, process length, basal width and membrane length were all measured (Fig. 4). The dorsal bar connecting the hamuli is much simpler than the ventral bar. Its total length, and the presence or absence of a central notch were noted.

(f) The marginal hooks

These are composed of a shaft and an articulated sickle (Fig. 4). The sickle has a curved point and an expanded base, which can be subdivided into heel and toe (Mizelle and Kritsky, 1967b). Total length, shaft length, sickle length, sickle proximal width and sickle distal width were measured (Fig. 4).

The host specificity of Oxydactylus gastrosaui

(1) Artificial cross infections.

Wild caught sticklebacks (Pungitius pungitius and Gasterosteus aculeatus) were cleansed of ectoparasites using the 1:4000 formalin bath recommended by Parker (1965) and Lester and Adams (1974a). After allowing fish to recover from this treatment (Ch. 4), they were infected artificially with 5 Oxydactylus gastrosaui, taken from wild caught G. aculeatus (Ch. 4). Experimental hosts were held for 2 weeks in 50 ml jars containing dechlorinated tap water, which was changed every 3 days. Fish were kept in the dark at 10°C, and were not fed during the experiment. At the end of the 2 week period, the fish were sacrificed by cutting through the spinal column. Any Oxydactylus present were counted and removed to slides for examination.

(4) Infections in mixed populations of host species.

Five Gasterosteus aculeatus, infected with Oxydactylus gastrosaui, were placed in 5L dechlorinated water in the dark at 10°C. 20 other fish, all of one host species, disinfected 5 days previously using a formalin bath, were added to the tank containing the 5 infected G. aculeatus, and maintained with them for 2 weeks.
The infected fish were marked with spine clips, and their Gyrodactylus burden counted at the start of the experiment. At the end of two weeks, all the fish were sacrificed, and their parasites counted, removed and mounted in ammonium picrate glycerin. The host species used were Leptorhynchus aculeatus (controls), Pungitius pungitius and Phoxinus phoxinus.

(c) Behavioural mechanisms of host transfer.

The response of parasites to a range of host-associated stimuli was examined. Flukes, both attached to glass and to dead fish, were maintained in petri dishes containing dechlorinated water. Test substances were placed in contact with the parasites for a duration of 1 second, 2 secs. or 5 secs. 1000 1 sec. contacts, 500 2 sec. contacts and 200 5 sec. contacts were performed. The number of parasites moving onto the test substrate was recorded. Test substrates used were stainless steel forceps, the fin of dead L. aculeatus and the fin of dead P. pungitius. All observations were carried out at 21°C, with sub-stage microscope illumination.

(4) The rate of transmission of G. gasterostei to an unnatural host.

Individual infected Gasterosteus aculeatus were maintained with uninfected L. aculeatus, P. pungitius and Phoxinus phoxinus in 50 ml. jars of dechlorinated water. After 24 hours at 19°C in a 12 hour Light : Dark photoperiod, both fish were sacrificed, and the number of Gyrodactylus gasterostei on each host noted.

(e) Morphometric variation of Gyrodactylus maintained on unnatural hosts.

All Gyrodactylus removed from unnatural hosts in this work were mounted in ammonium picrate glycerin and examined using positive phase contrast microscopy (Leitz Dialux). The dimensions of the sclerites of these parasites were measured in the manner described above.

RESULTS

Key to the subgenera and species groups of Gyrodactylus

The following key has been devised to facilitate rapid identification of Gyrodactylus, to the level of species groups, which are liable to be encountered in British freshwater. It includes all subgenera, but only those species groups so far encountered in European freshwater.
Key to sub-genera and species groups of Gyrodactylus:

1. Excretory bladders present (2)
   1. Excretory bladders absent (6)

2. Flame cells absent from main excretory canals. Pharyngeal processes long or short (3)
   2. Flame cells present in main excretory canals. Pharyngeal processes short, sub genus Mesonephrotus. Only the G. arcuatus species group is found in fresh water.

3. Excretory bladders large, without duct running through centre (4)
   3. Excretory bladders very small, pulsating. Duct running through centre to excretory pore. sub genus Neonephrotus. Contains only the G. anguillae species group.

4. Excretory bladders large, penis with several rows of small spines. Pharyngeal processes long. sub genus Paranephrotus, so far unrecorded from British freshwater.
   4. Excretory bladders small, penis with single row of spines, pharyngeal processes short. sub genus Metanephrotus (5)

   5. Marginal hook sickles with heels, Hamuli with straight shafts. G. eucaliae species group.

6. Flame cells present in main excretory canals. sub genus Gyrodactylus (7)
   6. Flame cells absent from main excretory canals. sub genus Limnonephrotus (8)

   7. Ventral bar with processes, central part of bar with boss, bar membrane broad, spatulate. G. phoxini species group.

8. Ventral bar processes long. G. katherineri species group.

Descriptions of Gyrodactylus species from sticklebacks
Sub genus Mesonephrotus, G. arcuatus species group

Gyrodactylus arcuatus Bychowsky, 1933.

HOST: Gasterosteus aculeatus

SITE OF INFECTION: Both skin and gills; usually found on gill arches and pharyngeal epithelium, but it may also occur on skin (see Ch.5).

DIAGNOSIS: Small species with short pharyngeal processes, penis with single row of six spines.

Hamuli with straight shafts and roots parallel with points, roots very short. Ventral bar long and stout, with prominent large processes and a large subrectangular membrane. Dorsal bar slightly curved, with prominent C shaped notch. Marginal hooks with large, semicircular heel, point slender, elongate. Toe pointed, small (Fig. 5 Table 4).
Fig. 5. Opisthaptor sclerites of Gyrodactylus arcuatus.
COMMENTS: This species is widespread on sticklebacks in Britain, occurring in a wide range of habitats (Table 3). It is the only member of its sub genus found in freshwater, and cannot be confused with any other species. It has been collected from British fish on three previous occasions: by Chubb (1964) and by Lyons and Turnbull, both cited in Malmberg (1970). Specimens collected by Chubb from sticklebacks in Llyn Tegid, Wales have been examined in the present work, and have been found to be identical to those collected from southern England. Drawings of specimens collected by Lyons, from Cambridge, and Turnbull, from Edinburgh, have also been examined (Malmberg, personal communication), and found to be identical. There is only one form of G. arcuatus present in British fresh and brackish water, although Malmberg (1970) has suggested that two forms of the species may exist. Bychowsky (1933) described a form with short (4 \( \mu \)m) ventral bar processes and small (3 \( \mu \)m) marginal hook sickles from G. aculeatus from the freshwater lake Konch in Karelia (USSR). In their redescription of G. arcuatus, Bychowsky and Polyansky (1953) noted long (8 \( \mu \)m) ventral bar processes and large (5 \( \mu \)m) marginal hook sickles in marine specimens collected in the Baltic and Barents seas. Malmberg (1970) distinguished between two separate forms of the species on the basis of these differences. He tentatively reinforced the separation using ecological criteria: The form with long processes (syn. G. aculeati Malmberg, 1956) occurred only in brackish and salt water, whereas the smaller form was recorded from the gills of G. aculeatus in freshwater. Malmberg's own specimens from the Baltic sea supported this distinction, but he noted that the form with long processes had been found in British freshwater by Lyons and Turnbull (Malmberg, 1970). All specimens of G. arcuatus collected during the present work have had long ventral bar processes, irrespective of site of infection or habitat salinity. In addition it has been shown that the site specificity of G. arcuatus is influenced by the presence of the freshwater G. gasterostei (Ch.5). This suggests that Malmberg's (1970) distinction between two forms of G. arcuatus does not have a fundamental genetic basis. It is possible that a different form of G. arcuatus does exist in the Karelian lake district (further collection would be necessary to determine this). However, Bychowsky (1933) did not publish a detailed description of G. arcuatus, and the type specimens are no longer available, for examination. It is probable therefore, that the confusion over two forms of this species has arisen out of inaccuracies in the original description.
Sub genus Metanephrotus, *G. rarus* species group

*Gyrodactylus rarus* Wegener, 1910

SITE OF INFECTION: Gills.

HOST: *P. pungitius*, but can occasionally infect *G. aculeatus*.

DIAGNOSIS: Small species, with short pharyngeal processes, penis with single row of small spines.

Hamuli crescent shaped, with strongly curved shafts and long, diverging roots. Dorsal bar slightly curved, with a median semicircular notch. Ventral bar large, with short processes and a rectangular membrane.

Marginal hooks of this species group characteristic: sickles large, lacking well defined heel, point not reflexed (Fig. 6; Table 4).

COMMENTS: *G. rarus* has previously been recorded from British freshwater by Chappell (1969), from the skin of *Gasterosteus aculeatus*. Bychowsky and Polyansky (1953), Gussev (1955), Bychowskaya-Pavlovskaya (1964) and Chappell (1969) all considered this to be the normal host and site of infection of this species. Malmberg (1964), after reconsidering Wegener's (1910) description of *G. rarus*, concluded that this species is specific to the gills of *Pungitius pungitius*, as has been found in the present work. There is therefore a dichotomy of opinion over the identity of this species. It seems likely that an error originally occurred in the account of Bychowsky and Polyansky (1953), who described a skin parasitic form with straight, parallel-shafted hamuli and clawed marginal hooks as *G. rarus*. This species was probably a member of the *G. wageneri* species group. These authors did not find *G. rarus* sensu stricto in their work, but they identified a closely related form, with crescent shaped hamuli and unheeled marginal hook sickles, from *Gasterosteus aculeatus* as *G. bychowskyi* Sproston, 1946. Gussev (1955) and Bychowskaya-Pavlovskaya (1964) both repeated the view that *G. rarus* is a species with straight, parallel-shafted hamuli and clawed marginal hooks, from the skin of its host, and Chappell (1969) based his identification upon these Russian papers. It can therefore be concluded that Chappell ([loc. cit.]) worked with a *G. wageneri* type species (probably *G. gasterosteii*) from the skin of *Gasterosteus aculeatus*, rather than with *G. rarus* sensu stricto. During the course of the present study, in March 1980, sticklebacks from Baildon Moor pond, Yorkshire, the locality where Chappell obtained his material, were collected and found to be infected with *G. aculeatus* and *G. gasterosteii*.
Fig. 6. Opisthaptor sclerites of Gyrodactylus rarus.
Table 4. Sclerite dimensions of G. arcuatus, G. rarus and G. branchicus.

<table>
<thead>
<tr>
<th>Species</th>
<th>G. arcuatus</th>
<th>G. rarus</th>
<th>G. branchicus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hamulus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total length</td>
<td>38.8±1.8</td>
<td>52.7±2.5</td>
<td>50.4</td>
</tr>
<tr>
<td></td>
<td>(35-43)</td>
<td>(49-56)</td>
<td></td>
</tr>
<tr>
<td>Shaft length</td>
<td>30.6±1.7</td>
<td>41.9±2.1</td>
<td>42.0</td>
</tr>
<tr>
<td></td>
<td>(28-34)</td>
<td>(38-45)</td>
<td></td>
</tr>
<tr>
<td>Point length</td>
<td>15.5±1.0</td>
<td>17.6±1.6</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>(14-17)</td>
<td>(15-21)</td>
<td></td>
</tr>
<tr>
<td>Root length</td>
<td>8.2±0.7</td>
<td>18.5±1.9</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>(7-10)</td>
<td>(17-21)</td>
<td></td>
</tr>
<tr>
<td><strong>Ventral Bar</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>21.0±1.2</td>
<td>18.2±2.0</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td>(19-23)</td>
<td>(15-21)</td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td>13.1±0.9</td>
<td>20.9±1.5</td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td>(17-20)</td>
<td>(19-23)</td>
<td></td>
</tr>
<tr>
<td>Process length</td>
<td>7.7±0.7</td>
<td>1.4±0.1</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(5.6-9.4)</td>
<td>(1.0-1.7)</td>
<td></td>
</tr>
<tr>
<td>Membrane length</td>
<td>3.3±0.3</td>
<td>9.7±0.8</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>(7-10)</td>
<td>(3-11)</td>
<td></td>
</tr>
<tr>
<td><strong>Marginal hook</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total length</td>
<td>22.2±1.4</td>
<td>34.8±1.3</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>(19-25)</td>
<td>(33-36)</td>
<td></td>
</tr>
<tr>
<td>Shaft length</td>
<td>17.2±1.2</td>
<td>26.5±1.3</td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td>(14-18)</td>
<td>(25-28)</td>
<td></td>
</tr>
<tr>
<td>Sickle length</td>
<td>4.5±0.2</td>
<td>8.1±0.6</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>(4.0-5.5)</td>
<td>(7.7-8.5)</td>
<td></td>
</tr>
<tr>
<td>Sickle distal width</td>
<td>2.9±0.2</td>
<td>5.8±0.6</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>(2.5-3.5)</td>
<td>(5-7)</td>
<td></td>
</tr>
<tr>
<td>Sickle proximal width</td>
<td>3.3±0.3</td>
<td>6.3±0.6</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>(3.0-4.2)</td>
<td>(5-7)</td>
<td></td>
</tr>
<tr>
<td>No. measured</td>
<td>30</td>
<td>20</td>
<td>1</td>
</tr>
</tbody>
</table>

All measurements in \( \mu \text{m} \).

Expressed as mean, ±1 standard deviation. Range of all specimens in parentheses.

Only one specimen of G. branchicus available for measurement.
Wootten (1977) recorded *Gyrodactylus rarus* from both skin and gills of *Pungitius pungitius*. In view of the site specificity of *G. rarus* (see Wegener, 1910; Malmberg, 1964, 1970; Ch.5), this record was probably based on a mixture of both *G. pungitii* and *G. rarus*.


**HOST:** *Gasterosteus aculeatus*

**SITE OF INFECTION:** Gills

**DIAGNOSIS:** Small species, pharynx with short processes, penis not present in few specimens encountered.

Hamuli strongly curving with diverging roots, similar to *G. rarus* but slightly larger. Ventral bar large with stout rectangular membrane and rounded basal lobes. Very small ventral bar processes present. Dorsal bar slightly curved, with median C-shaped notch.

Marginal hooks large, sickles proportionally very large, of similar overall shape to *G. rarus*. However, toe of sickle blunter and thicker than in *G. rarus*, without 'ledge' on upper edge, just beyond tip. Heel of sickle poorly developed (Fig. 7, Table 4).

**COMMENTS:** This is the first record of *G. branchicus* in England. It has previously been recorded from the Baltic by Malmberg (1970), and, as the synonym *G. bychowskyi*, from the Baltic, Barents and Japanese seas (Bychowsky and Polyansky, 1953). It is possible that *G. canadensis*, described by Hanek and Threlfall (1969) from Newfoundland, is also a synonym of this species (Malmberg, 1970).

*G. branchicus* is very similar to *G. rarus*, but can be distinguished by its slightly larger opisthaptor sclerites, and by the form of the marginal hook sickle. In *G. rarus* the toe of the sickle tapers abruptly to a point (Fig. 6), and has a slight ledge just behind the apex. In *G. branchicus*, the toe of the sickle is much broader, tapers less abruptly and does not have a ledge on upper edge. These species are so similar that they are easily confused, especially when few specimens are available for comparison. The danger of confusion is increased because *G. rarus* can sometimes infect *Gasterosteus aculeatus*. The description of *G. branchicus* in this account is based on specimens obtained from sticklebacks from a brackish drainage ditch at Cuxton, Kent.
Fig. 7. Opisthaptor sclerites of Gyrodactylus branchicus.
As no P. pungitius were collected in this sample, it is thought that confusion with G. rarus is unlikely, as observations of other species (Malmberg, 1970; Glaser, 1974) suggest that temporary infections are rapidly lost when the normal host is not available to maintain the infection.

G. eucaliae species group


HOST: Gasterosteus aculeatus.

SITE OF INFECTION: Skin and fins.

DIAGNOSIS: Large species, with short pharyngeal processes, penis with single row of 4 spines.

Sclerites large and robust, hamuli with straight shafts and long, parallel roots. Dorsal bar curved, with small central notch. Small projections present on either side of notch, increasing its apparent depth. Ventral bar with short processes, membrane rectangular, with prominent longitudinal striations.

Marginal hook sickles very large, toe pointed, heel globular, point not recurved. Hook shafts have prominent 'shank ligament' (Table 5, Fig. 8).

COMMENTS: G. alexanderi was described by Mizelle and Kritsky (1967a) from sticklebacks in California, and was subsequently recorded by Lester (1974) from Vancouver. Only one previous record of this species in North West Europe exists (Glaser, 1979), from northern Germany. The form of the marginal hook sickles of British specimens correspond closely with those of American specimens, and there is a close resemblance between individuals from the two areas. Despite their geographical separation, it is considered that English specimens are identical to those from American sticklebacks. This is the first record of this species in Britain.

Sub genus Limnonephrotus, G. mageneri species group.

Gyrodactylus pungitii Malmberg, 1956.

HOST: Pungitius pungitius.
Fig. 3. Opisthaptor sclerites of Gyrodactylus alexanderi.
Table 5. Sclerite dimensions of *G. alexanderi*, *G. pungitii* and *G. gasterostei*

<table>
<thead>
<tr>
<th>Species</th>
<th><em>G. alexanderi</em></th>
<th><em>G. gasterostei</em></th>
<th><em>G. pungitii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hamulus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total length</td>
<td>79.3±3.2</td>
<td>53.0±1.7</td>
<td>66.1±3.3</td>
</tr>
<tr>
<td>(77-83)</td>
<td>(55-61)</td>
<td>(59-73)</td>
<td></td>
</tr>
<tr>
<td>Shaft length</td>
<td>54.0±2.0</td>
<td>41.6±1.8</td>
<td>44.0±1.9</td>
</tr>
<tr>
<td>(52-56)</td>
<td>(39-45)</td>
<td>(42-48)</td>
<td></td>
</tr>
<tr>
<td>Point length</td>
<td>31.7±2.5</td>
<td>28.4±1.0</td>
<td>23.2±1.3</td>
</tr>
<tr>
<td>(29-34)</td>
<td>(26-31)</td>
<td>(26-31)</td>
<td></td>
</tr>
<tr>
<td>Root length</td>
<td>24.6±2.5</td>
<td>16.6±1.5</td>
<td>21.9±3.0</td>
</tr>
<tr>
<td>(22-27)</td>
<td>(14-20)</td>
<td>(16-28)</td>
<td></td>
</tr>
<tr>
<td><strong>Ventral Bar</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>34.0±2.0</td>
<td>24.7±0.9</td>
<td>22.9±1.3</td>
</tr>
<tr>
<td>(32-36)</td>
<td>(23-27)</td>
<td>(21-25)</td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td>28.7±0.6</td>
<td>22.9±1.5</td>
<td>27.2±1.2</td>
</tr>
<tr>
<td>(23-29)</td>
<td>(21-25)</td>
<td>(23-28)</td>
<td></td>
</tr>
<tr>
<td>Process length</td>
<td>4.8±1.5</td>
<td>3.4±0.6</td>
<td>2.8±0.2</td>
</tr>
<tr>
<td>(3-6)</td>
<td>(3-4)</td>
<td>(2-3)</td>
<td></td>
</tr>
<tr>
<td>Membrane length</td>
<td>18.0±1.7</td>
<td>11.8±1.0</td>
<td>12.9±1.4</td>
</tr>
<tr>
<td>(17-20)</td>
<td>(10-14)</td>
<td>(10-14)</td>
<td></td>
</tr>
<tr>
<td><strong>Marginal Hook</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total length</td>
<td>45.5±1.6</td>
<td>32.4±1.1</td>
<td>35.2±1.2</td>
</tr>
<tr>
<td>(43-46)</td>
<td>(30-34)</td>
<td>(33-35)</td>
<td></td>
</tr>
<tr>
<td>Shaft length</td>
<td>35.5±0.6</td>
<td>26.7±1.2</td>
<td>29.4±1.3</td>
</tr>
<tr>
<td>(35-36)</td>
<td>(23-28)</td>
<td>(23-31)</td>
<td></td>
</tr>
<tr>
<td>Sickle length</td>
<td>9.5±0.8</td>
<td>5.3±0.5</td>
<td>5.9±0.6</td>
</tr>
<tr>
<td>(8-10)</td>
<td>(5-6)</td>
<td>(5-7)</td>
<td></td>
</tr>
<tr>
<td>Sickle distal</td>
<td>6.7±0.8</td>
<td>4.0±0.5</td>
<td>4.1±0.3</td>
</tr>
<tr>
<td>width</td>
<td>(5-7)</td>
<td>(3-5)</td>
<td>(3-5)</td>
</tr>
<tr>
<td>Sickle proximal</td>
<td>6.7±0.5</td>
<td>3.9±0.5</td>
<td>4.1±0.3</td>
</tr>
<tr>
<td>width</td>
<td>(5-7)</td>
<td>(3-5)</td>
<td>(3-5)</td>
</tr>
<tr>
<td>No. measured:</td>
<td>5</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

All measurements in µm.

Expressed as mean, ±1 standard deviation. The range of all specimens in parentheses.
SITE OF INFECTION: Skin and fins.
DIAGNOSIS: A large species with long pharyngeal processes, penis with
single row of six or seven spines.

Hamuli with straight shafts and long, parallel roots. Dorsal bar strongly
curved, lacking notch, ventral bar with triangular membrane and short processes.
Marginal hook sickles claw shaped, with well developed semi-circular heel
(Fig. 9, Table 5).

COMMENTS: As with G. rarus, this species has only been recorded from
Gasterosteus aculeatus in Britain, although Malmberg (1970) considered it to
be specific to Pungitius pungitius. It was recorded by Powell (1966) from
Llyn Padarn on the basis of specimens described as Gyrodactylus sp. by Chubb
(1964). Re-examination of these specimens during the present work has shown
that this record is incorrect, and that they are in fact G. gasterostei.
G. pungitii was probably also encountered by Dartnall (1973), who described
it as Gyrodactylus sp., and by Wootten (1977), who recorded it as G. rarus.


HOST: Gasterosteus aculeatus.

SITE OF INFECTION: Skin and fins.

DIAGNOSIS: A large species with long pharyngeal processes penis with single
row of six to eight small spines.

Hamuli with straight shafts and parallel roots. Ventral bar long, with small
processes and slender triangular membrane. Marginal hook shafts long, sickles
claw shaped, heel rounded, toe tapering to a rounded tip. Point of sickle
does not extend beyond toe (Fig. 10, Table 5).

COMMENTS: This species is very similar to G. pungitii, but it may be
distinguished by its smaller overall size and shorter hamulus roots.
G. gasterostei was first formally described by Glaser (1974), but it had been
encountered by many previous workers, who referred to it as Gyrodactylus
rarus, G. pungitii, or simply as Gyrodactylus sp. In Britain, Chappell (1969)
confused this species with G. rarus, and Powell (1966) misidentified it as
G. pungitii. Numerous other references to Gyrodactylus sp. concern this
species, e.g. those of Hopkins (1959), Madan (1965), Arme and Owen (1967),
Dartnall, Lewis and Walkey (1972) and Shillcock (1972).
Fig. 9. Opisthaptor sclerites of Gyrodactylus pungitii.
Fig. 10. Opisthaptor sclerites of *Gyrodactylus gasterostei*. 

20 \mu m
SUMMARY

6 species of *Gyrodactylus*, *G. arcuatus*, *G. gasterostei*, *G. branchicus* and *G. alexanderi* from *Gasterosteus aculeatus* and *G. pungitii* and *G. rarus* from *P. pungitius*, have been recorded from sticklebacks in this study. The previous records of *Gyrodactylus* from British sticklebacks, and their probable synonymies are listed in Table 6.

The host specificity of *Gyrodactylus gasterostei*

(a) Artificial cross infections.

In all replicates, the five *G. gasterostei* placed upon each *Pungitius pungitius* failed to persist until the end of the 14 day experimental period and, in all, only 3 parasites were recovered from 30 hosts of this species. On *Gasterosteus aculeatus* however, the populations of *G. gasterostei* persisted and increased slowly, up to an average intensity of 7.2 parasites per fish. The growth of *G. gasterostei* populations on *P. pungitius* is significantly less (*P < 0.001*) than on *G. aculeatus* (Mann-Whitney U-test). These results are summarised in Table 7.

(b) Infections in mixed populations of host species.

In all cases where uninfected hosts were maintained with infected fish, allowing transmission to occur unhindered, the results corroborated those obtained from artificial infections. Populations of *G. gasterostei* spread rapidly onto uninfected *Gasterosteus aculeatus* and increased in size considerably. However, when using *P. pungitius* or *Phoxinus phoxinus*, most fish remained uninfected and the parasite populations failed to increase (Table 8). The growth of the parasite populations was significantly different on all three species of fish (*P < 0.01*, Quenouille test). This analysis is complicated by differences in population growth on the donor fish. Although these tended to increase when the experimental host was *Gasterosteus aculeatus*, they frequently declined when *Phoxinus phoxinus* or *Pungitius pungitius* were used. These results, when taken in conjunction with data from artificially infected hosts, indicate that *Gyrodactylus gasterostei* is narrowly host specific to *Gasterosteus aculeatus*, failing in most cases to infect either *Pungitius pungitius* or *Phoxinus phoxinus*.

(c) The rate of transmission of *G. gasterostei* to unnatural hosts.
Table 6. Records of *Gyrodactylus* species from British sticklebacks.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Author</th>
<th>Probable Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. elegans</em></td>
<td><em>G. aculeatus</em></td>
<td>Houghton, 1862</td>
<td><em>G. gasterosteii</em> and/or <em>G. arcuatus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bradley, 1861</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cobbold, 1862</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sproston, 1946</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dawes, 1947</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treasurer, 1974</td>
<td><em>G. arcuatus</em> (1).</td>
</tr>
<tr>
<td><em>P. pungitius</em></td>
<td></td>
<td>Dawes, 1947</td>
<td><em>G. pungitii</em> and/or <em>G. rarus</em></td>
</tr>
<tr>
<td><em>G. rarus</em></td>
<td><em>G. aculeatus</em></td>
<td>Chappell, 1969</td>
<td><em>G. arcuatus</em> and/or <em>G. gasterosteii</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P. pungitius</em></td>
<td>Wootten, 1973</td>
<td><em>G. pungitii</em> and <em>G. rarus</em></td>
</tr>
<tr>
<td><em>G. arcuatus</em></td>
<td><em>G. aculeatus</em></td>
<td>Chubb, 1964</td>
<td><em>G. arcuatus</em> (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lyons and Turnbull, cited in Malmberg, 1970</td>
<td></td>
</tr>
<tr>
<td><em>G. pungitii</em></td>
<td><em>G. aculeatus</em></td>
<td>Powell, 1966</td>
<td><em>G. gasterosteii</em> (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chubb, 1970</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Harris, 1980a</td>
<td></td>
</tr>
<tr>
<td><em>Gyrodactylus</em></td>
<td><em>G. aculeatus</em></td>
<td>Hopkins, 1959</td>
<td><em>G. arcuatus</em> and/or <em>G. gasterosteii</em></td>
</tr>
<tr>
<td>sp. indet.</td>
<td></td>
<td>Arme and Owen, 1967</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vickers, 1951</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Madan, 1965</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chubb, 1964</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Lyons, 1969</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dartnall, Lewis and Walkey, 1972</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shillcock, 1972</td>
<td></td>
</tr>
<tr>
<td><em>Gyrodactylus</em></td>
<td><em>P. pungitius</em></td>
<td>Dartnall, 1973</td>
<td><em>G. pungitii</em> and/or <em>G. rarus</em></td>
</tr>
<tr>
<td>sp. indet.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(1) Identity of record confirmed.
Table 7. The success of artificial cross infections of *G. gasterosteii* on *Pungitius pungitius*

<table>
<thead>
<tr>
<th>Parasites infecting:</th>
<th><em>G. aculeatus</em></th>
<th><em>P. pungitius</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial infection</strong></td>
<td>X 5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>α 0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>n 30</td>
<td>30</td>
</tr>
<tr>
<td><strong>Final infection</strong></td>
<td>X 7.6</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>α 6.6</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>n 30</td>
<td>30</td>
</tr>
<tr>
<td><strong>Total number of parasites recovered</strong></td>
<td>220</td>
<td>3</td>
</tr>
</tbody>
</table>

Experiments conducted at 10°C in 50mls of water. Infected fish kept for 14 days before dissection.

Mean increase of parasite population on *P. pungitius* significantly less (*P* < 0.001) than on *G. aculeatus* (Mann-Whitney U-test).

**Legend**

- **X**: arithmetic mean of all replicates
- **α**: standard deviation of all replicates
- **n**: number of replicates
Table 8. The population growth of Gyrodactylus gasterosteii on previously uninfected hosts when maintained with 5 infected G. aculeatus.

Initial population size: Donors- 5 parasites per fish (5 fish)  
Test hosts- 0 parasites per fish (20 fish)

<table>
<thead>
<tr>
<th></th>
<th>Gasterosteus aculeatus</th>
<th>Pungitius pungitius</th>
<th>Phoxinus phoxinus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate 1</td>
<td>21.9</td>
<td>0.055</td>
<td>0</td>
</tr>
<tr>
<td>x</td>
<td>50.6</td>
<td>0.23</td>
<td>0</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Replicate 2</td>
<td>62.2</td>
<td>0.57</td>
<td>0</td>
</tr>
<tr>
<td>x</td>
<td>34.0</td>
<td>0.96</td>
<td>0</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Replicate 3</td>
<td>100.0</td>
<td>0.32</td>
<td>0</td>
</tr>
<tr>
<td>x</td>
<td>41.0</td>
<td>0.33</td>
<td>0</td>
</tr>
<tr>
<td>n</td>
<td>19</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Replicate 4</td>
<td>11.1</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td>x</td>
<td>3.8</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Replicate 5</td>
<td>18.4</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>x</td>
<td>9.4</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Mean of replicates</td>
<td>42.7</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td>x</td>
<td>37.7</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Mean population growth differs significantly on P. pungitius and P. phoxinus in comparison with that on G. aculeatus (P<0.01, Quenouille test).

All hosts maintained in 4l of water at 10°C for 2 weeks.

**Legend:**
- x: arithmetic mean of all replicates
- σ: standard deviation of all replicates
- n: number of replicates
Table 9 indicates that a significantly larger proportion of parasites transfer from *Gasterosteus aculeatus* onto conspecific hosts than onto either *P. pungitius* or *P. phoxinus* in a 24 hour period. This is independent of population growth, and suggests a behavioural specificity in the transfer of parasites from one host to another.

(d) The behavioural response to different test substrates.

The response of *G. gasterosteoi* to contact with different tissues is dependent on the duration of the contact. One second brushes were too brief to allow parasites to complete their movement onto the test substrate, but a significantly higher proportion completed their transfer with 2 or 5 s contacts. 2 s contacts were therefore used to test the response of *G. gasterosteoi* to different tissues. The response is also dependent upon the substrate to which the flukes are attached. They were much more easily transferred to a test substrate when attached to glass than when attached to dead *Gasterosteus aculeatus*, implying a perception of the substrate to which they are attached.

The parasites show a significant difference in their response to different test substrates. They move readily onto *G. aculeatus*, but not onto *P. pungitius* tissue. A significantly larger proportion of parasites (*P < 0.05, Mann-Whitney U-test*) attached to tweezers than to *P. pungitius* (Table 10), implying that a mechanism inhibiting movement onto this host is present in addition to an attraction to the normal host, *Gasterosteus aculeatus*.

(e) Morphometric variation in parasites maintained on *G. aculeatus* and *P. pungitius*.

*Gyrodactylus gasterosteoi* recovered from *Pungitius pungitius* after up to 2 weeks of infection showed no significant morphological differences from specimens maintained on the normal host, *Gasterosteus aculeatus* (Table 11). This suggests that specimens maintained on different hosts do not develop variations in haptor morphology on which descriptions of new taxa might be erroneously based.

Species of *Gyrodactylus* from other hosts in small freshwater streams

A considerable number of *Gyrodactylus* species were obtained from several fish species captured in the same habitat as sticklebacks. These species, (listed in Table 12) were all morphologically distinct, and were only associated with one host. Species of the *G. wageneri* species group, many
Table 9. The proportion of *G. gasterosteii* transferring from *G. aculeatus* to uninfected fish of other species in a 24 hour period.

<table>
<thead>
<tr>
<th>Uninfected host species</th>
<th><em>G. aculeatus</em></th>
<th><em>P. pungitius</em></th>
<th><em>P. phoxinus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{x} )</td>
<td>( s )</td>
<td>( n )</td>
</tr>
<tr>
<td><em>G. aculeatus</em></td>
<td>10.1</td>
<td>0.19</td>
<td>1.2</td>
</tr>
<tr>
<td>% transferring in 24hrs</td>
<td>15.3</td>
<td>0.49</td>
<td>3.8</td>
</tr>
<tr>
<td><em>P. pungitius</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% transferring in 24hrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. phoxinus</em></td>
<td>1.2</td>
<td>3.8</td>
<td>10</td>
</tr>
</tbody>
</table>

Fish kept in 50ml of water in 12h :12h L:D period at 10°C.

Legend:
- \( \bar{x} \) arithmetic mean
- \( a \) standard deviation
- \( n \) number of replicates

\% transferring onto *G. aculeatus* significantly greater (\( P < 0.05 \), Mann-Whitney U test) than onto either *P. pungitius* or *P. phoxinus*. 
Table 10. The behavioural response of Gyrodactylus gasterostei to different substrates.

Data expressed as No. of parasites moving onto test substrate per 33 2s. contacts.

<table>
<thead>
<tr>
<th>Test Substrate</th>
<th>G. aculeatus fin</th>
<th>P. pungitius fin</th>
<th>Forceps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasites attached to:</td>
<td>( \bar{x} )</td>
<td>( \alpha )</td>
<td>( n )</td>
</tr>
<tr>
<td>Glass</td>
<td>31.6</td>
<td>3.1</td>
<td>10</td>
</tr>
<tr>
<td>Dead G. aculeatus</td>
<td>1.1</td>
<td>0.9</td>
<td>10</td>
</tr>
</tbody>
</table>

All means compared using Mann-Whitney U-test.

Proportion of *G. gasterostei* moving onto *G. aculeatus* significantly higher \((P<0.05)\) than proportion moving onto *P. pungitius* or forceps.

Proportion of *G. gasterostei* moving onto tweezers significantly greater than proportion moving onto *P. pungitius* \((P<0.05)\).

Proportion moving onto *G. aculeatus* significantly greater \((P<0.05)\) than proportion moving onto *P. pungitius*.

Proportion of *G. gasterostei* moving onto forceps significantly higher \((P<0.05)\) than proportion moving onto *P. pungitius*.

**Legend**
- \( \bar{x} \): arithmetic mean
- \( \alpha \): standard deviation
- \( n \): number of replicates
Table 11. The dimensions of *G. gasterostei* maintained on *Gasterosteus aculeatus* and *Pungitius pungitius*.

<table>
<thead>
<tr>
<th>Dimension</th>
<th>From <em>Gasterosteus aculeatus</em></th>
<th>From <em>Pungitius pungitius</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamulus</td>
<td>x α n</td>
<td>x α n</td>
</tr>
<tr>
<td>Total length</td>
<td>58.0 1.7 30</td>
<td>57.8 1.2 10</td>
</tr>
<tr>
<td>Root length</td>
<td>16.2 1.4 30</td>
<td>16.6 1.0 10</td>
</tr>
<tr>
<td>Shaft length</td>
<td>41.6 1.8 30</td>
<td>41.9 1.4 10</td>
</tr>
<tr>
<td>Point length</td>
<td>28.4 1.0 30</td>
<td>26.7 1.4 10</td>
</tr>
<tr>
<td>Root length</td>
<td>0.4 0.03 30</td>
<td>0.39 0.03 10</td>
</tr>
<tr>
<td>Shaft length</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dorsal bar</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>23.5 1.9 30</td>
<td>23.6 1.7 10</td>
</tr>
<tr>
<td>Median width</td>
<td>1.5 0.2 30</td>
<td>1.7 0.4 10</td>
</tr>
<tr>
<td><strong>Ventral Bar</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>24.7 0.9 30</td>
<td>25.4 1.0 10</td>
</tr>
<tr>
<td>Distance between processes</td>
<td>25.6 1.5 30</td>
<td>25.6 0.9 10</td>
</tr>
<tr>
<td>Width</td>
<td>22.9 1.5 30</td>
<td>22.5 1.2 10</td>
</tr>
<tr>
<td>Process length</td>
<td>3.4 0.6 30</td>
<td>2.9 0.2 10</td>
</tr>
<tr>
<td>Membrane length</td>
<td>11.8 1.0 30</td>
<td>10.8 1.1 10</td>
</tr>
<tr>
<td><strong>Marginal Hook</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total length</td>
<td>32.4 1.1 30</td>
<td>32.6 1.3 10</td>
</tr>
<tr>
<td>Sickle length</td>
<td>5.4 0.4 30</td>
<td>5.4 0.5 10</td>
</tr>
<tr>
<td>Shaft length</td>
<td>26.7 1.2 30</td>
<td>27.0 1.2 10</td>
</tr>
</tbody>
</table>

All dimensions in μm. All parasites maintained in dechlorinated water at 10°C for 2 weeks before measurement.

Legend:

- x: arithmetic mean
- α: standard deviation
- n: number of replicates
of which were very similar, were particularly abundant. These could be distinguished by a consideration of marginal hook and hamulus length (Fig. 11). The species described here were collected from the River Rother, Rogate, the River Ouse, Newport Pagnell, the River Chess, Rickmansworth and the River Ver, St. Albans. They probably represent only a small proportion of the total Gyrodactylus fauna of British freshwater fish.

Gyrodactylus wageneri species group.

Gyrodactylus rogatensis n.sp.

HOST: Cottus gobio.

SITE OF INFECTION: Skin and fins.

SPECIFIC DESCRIPTION: Body fusiform, bearing two anterior cephalic lobes with spike sensilla. Length of fixed, flattened holotype 0.26mm. In life, body length varying between 0.37 (0.35-0.39)mm contracted and 0.76 (0.43-0.63)mm extended. Opisthaptor elongate, 98 (56 to 150) μm long, 126 (36 to 147) μm wide. Pharynx sub terminal, ventral, 38 (34 to 47) μm long, anterior width 20 (17 to 30) μm, posterior width 43 (42 to 35) μm. Penis absent in holotype, when present 16 μm diameter, bearing single row of 6-8 spines.

Excretory system of Limnonephrotus type, lacking excretory bladders and flame cells in main canals. Morphology of marginal hook sickles and hamuli characteristic of G. wageneri group.

Hamuli 50.2 (56 to 63) μm total length, shafts straight, 39.2 (37.8 to 43.4) μm long, roots 22.4 (16.3 to 22.4) μm, and points 29.4 (23 to 33.6) μm. Dorsal bar strongly curved, lacking notch. Ventral bar with small processes and a triangular or slightly rounded membrane. Marginal hooks 35-37.3 μm long. Sickles with rounded heels, toe tapering to blunt tip (Fig. 12, Table 13).

COMMENTS: This species was originally discovered on Cottus gobio from the river Rother, Rogate. It was initially thought that specimens found on the skin of this host were individuals of G. gasterosteii, which had transferred to the bullhead after capture. However, it has since been found in abundance on Cottus gobio from the river Chess, although sticklebacks were absent from the collection. Although G. rogatensis is very similar to G. gasterosteii.

* Dimensions refer to holotype. Parentheses give range of dimensions in a sample of 27 paratypes.
Table 12. Species of Gyrodactylus from hosts found in the same habitats as sticklebacks.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Locality</th>
<th>Gyrodactylus species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phoxinus phoxinus</td>
<td>R. Ver, R. Chess, R. Lea, Herts.;</td>
<td>G. aphyae, G. macronychus</td>
</tr>
<tr>
<td></td>
<td>R. Rother, Sussex;</td>
<td>G. limneus, G. laevis, G. minimus</td>
</tr>
<tr>
<td></td>
<td>R. Ouse, Newport Pagnell, Bucks.; Ullswater, Cumbria.</td>
<td></td>
</tr>
<tr>
<td>Cottus gobio</td>
<td>R. Rother, Sussex; R. Chess, Herts.</td>
<td>G. rogatensis</td>
</tr>
<tr>
<td>Noemacheilus barbatulus</td>
<td>R. Colne, Herts.; R. Rother, Sussex.</td>
<td>G. ravlovskyi, G. sedelnikowi</td>
</tr>
<tr>
<td>Cobitis taenia</td>
<td>R. Ouse, Newport Pagnell, Bucks.</td>
<td>G. cobitis</td>
</tr>
<tr>
<td>Esox lucius</td>
<td>R. Ouse, Newport Pagnell, Bucks.</td>
<td>G. lucii</td>
</tr>
<tr>
<td>Abramis brama</td>
<td>R. Ouse, Newport Pagnell, Bucks.</td>
<td>G. elegans</td>
</tr>
<tr>
<td>Salmo trutta</td>
<td>R. Chess, Herts.</td>
<td>G. truttae</td>
</tr>
</tbody>
</table>
Fig. 11. Variation in marginal hook and hamulus length in species of the G. wageneri species group.
Fig. 12. Opisthaptor sclerites of Gyrodactylus rogatensis.
G. puncticulatus, G. aphyae and other G. wageneri group species, its marginal hooks are longer in relation to hamulus length than in any other species of this group (Fig. 11).

This is the first species of the G. wageneri group to be recorded from Cottus gobio, and can be easily separated from G. hrabei Ergens, 1961 and G. cotti Roman, 1956, also from this host, on the structure of the hamuli and marginal hooks.

Gyrodactylus aphyae Malmberg, 1956.

HOST: Phoxinus phoxinus.

SITE OF INFECTION: Skin and fins.

DIAGNOSIS: A large species with long pharyngeal processes, penis with a single row of 7-9 spines.

Hamuli with straight shafts and long parallel roots. Ventral bar with triangular membrane and short processes; dorsal bar thin, strongly curved, lacking notch. Marginal hook sickles claw shaped, heel rounded, toe gently curved on upper surface (Fig. 11, Table 1).

COMMENTS: This species is similar to G. gasterostei although the opisthaptor sclerites are considerably smaller (Fig. 11). Specificity observations have shown that Gyrodactylus gasterostei will not infect the minnow (Glaser, 1974; vide supra), and it is therefore thought that the two species are distinct, being restricted to separate hosts. This difference in host specificity is confirmed by the slight differences in morphology which may be observed. G. pannonicus Molnar, 1968, from Phoxinus phoxinus is very similar to G. aphyae, but is slightly smaller overall. Although present on the continent, this species was not encountered on British minnows. G. aphyae has previously been recorded from Britain by Chubb (1964) and Powell (1966).

Gyrodactylus macronychus Malmberg, 1956.

HOST: Phoxinus phoxinus.

SITE OF INFECTION: Fins and skin.

DIAGNOSIS: Very large species, long pharyngeal processes, penis with seven or eight spines in a single row.
Fig. 13. Opisthaptor sclerites of Gyrodactylus aphyae.
Table 13. Sclerite dimensions of *G. rogatensis*, *G. aphyae*, *G. macronychus* and *G. pavlovskyi*.

<table>
<thead>
<tr>
<th>Species</th>
<th>G. rogatensis</th>
<th>G. aphyae</th>
<th>G. macronychus</th>
<th>G. pavlovskyi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>Cottus gelio</td>
<td>Phoxinus phoxinus</td>
<td>Phoxinus phoxinus</td>
<td>Noemachilus barbatulus</td>
</tr>
<tr>
<td>Hamulus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total length</td>
<td>60.0 (57-63)</td>
<td>54.4 (49-57)</td>
<td>72.4 (71-76)</td>
<td>48.7 (46-52)</td>
</tr>
<tr>
<td>Shaft length</td>
<td>41.7 (37-46)</td>
<td>40.1 (37-42)</td>
<td>51.1 (50-52)</td>
<td>37.8 (36-39)</td>
</tr>
<tr>
<td>Point length</td>
<td>29.9 (23-34)</td>
<td>27.5 (24-30)</td>
<td>30.1 (29-31)</td>
<td>27.7 (26-28)</td>
</tr>
</tbody>
</table>

Ventral bar

| Length | 21.3 (19-23) | 25.4 (23-28) | - | - |
| Width | 25.3 (21-28) | 21.7 (21-25) | - | - |
| Process length | 1.7 (1.4-2.8) | 1.8 (1.0-1.5) | - | - |
| Membrane length | 11.3 (11-14) | 9.9 (3-11) | - | - |

Marginal hook

| Total length | 35.3 (33-38) | 28.4 (28-32) | 35.7 (35-37) | 28.3 (28-30) |
| Shaft length | 29.4 (26-31) | 23.3 (22-25) | 25.9 (24-27) | 21.6 (21-23) |
| Sickle length | 5.7 (5-7) | 5.6 (5-6) | 9.6 (9-10) | 6.9 (6-7) |
| Sickle distal width | 4.2 (3-5) | 4.5 (4-6) | 10.1 (10-11) | 5.9 (5-7) |
| Sickle proximal width | 4.1 (4-5) | 4.4 (4-5) | 5.6 | 4.8 (4-6) |

Number examined | 23 | 8 | 4 | 5 |

All measurements in μm. Expressed as mean ± standard deviation (when more than five specimens present in sample). Range of dimensions in parentheses.
Hamuli very long, shafts straight, with long, parallel roots. Ventral bar with short processes and a long triangular membrane; dorsal bar curved, lacking notch. Marginal hooks characteristic, with very large sickles, the point of which extends beyond the toe (Fig. 14, Table 13).

COMMENTS: The size and shape of the marginal hook sickles are distinctive, and no other species can be confused with *G. macronychus*. Although placed in the *G. wageneri* species group by Malmberg (1970), the marginal hooks differ from those of the majority of species, and further research may indicate that it should be placed in a separate species group, with *G. lagowskii* Ergens, 1980. *G. macronychus* has previously been recorded in Britain by Powell (1966).

*Gyrodactylus pavlovskyi* Ergens and Bychowsky, 1967.

HOST: *Nemacheilus barbatus*.

SITE OF INFECTION: Skin and fins.

DIAGNOSIS: Medium sized species, pharynx with long processes, penis with single row of spines.

Hamuli large, shafts parallel, roots folded over onto shafts giving impression of a very short, stout root. Dorsal bar straight, thickened, lacking notch; ventral bar stout, with short processes and a rectangular membrane. Marginal hooks large, heel small and truncated, sickles with strongly curving points extending beyond downturned toe. Sickle distal width greater than proximal width (Fig. 15, Table 13).

COMMENTS: This species is dissimilar to other *G. wageneri* group species in sclerite form, although its excretory system is identical. It can be separated from this group by the form of the folded hamulus roots. The marginal hook sickles are similar to those of *G. macronychus*, though much smaller. *G. pavlovskyi* has an excretory system of the *Limnonephrotus* type, and on the basis of marginal hook and ventral bar form, it is placed within the *G. wageneri* group. This species was separated, with *G. jiroyeci* Ergens and Bychowsky, 1967 and *G. papernae* Ergens and Bychowsky, 1967, from *G. nemacheili* Bychowsky, 1936. This is the first record of *G. pavlovskyi* from Britain.

*G. elegans* species group


HOST: *Phoxinus phoxinus*. 
Fig. 14. Opisthaptor sclerites of Gyrodactylus macronychus.
Fig. 15. Opisthaptor sclerites of Gyrodactylus pavlovskyi.
SITE OF INFECTION: Gills.

DIAGNOSIS: Small species, pharynx with short processes. Penis with single row of spines.

Hamuli short, shafts curved, greatest width across roots, which are very short and diverging. Ventral bar lacking processes, basal lobes rounded, membrane composed of a thin spine of sclerotised material. Dorsal bar straight, without notch, triangular in cross-section.

Marginal hooks short, sickles relatively large but slender, heel and toe small (Fig. 16, Table 14).

COMMENTS: Members of this species group from the minnow can be recognised by their curving hamulus shafts and narrow ventral bar membrane. *G. minimus* Malmberg, 1956 also occurs on the minnow in Britain, but is much smaller than *G. laevis*, and is unlikely to be confused. *G. paralaevis* Ergens, 1966 and *G. malmbergensis* Prost, 1974, also occur on continental minnows (Prost, 1974). Both of these species are smaller than *G. laevis*, which has previously been recorded in Britain by Powell (1966).

*Gyrodactylus minimus* Malmberg, 1956

HOST: *Phoxinus phoxinus*.

SITE OF INFECTION: Gills.

DIAGNOSIS: Very small species with short pharyngeal processes and a single row of spines on the penis.

Hamuli short, with curving shafts and very short diverging roots. Ventral bar lacking processes, basal lobes rounded, membrane a thin spine. Marginal hooks very small, sickles with a slightly elongate point (Table 14).

COMMENTS: This species is very similar in shape to *G. laevis*, but is much smaller. It is conspecific with the larger species on the gills of the minnow, but is much rarer. Only two individuals have been collected (one from Rogate, one from Newport Pagnell), despite the large number of minnows examined. This is the first record of this species from Britain.
Table 14. Sclerite dimensions of *G. laevis*, *G. minimus*, *G. limneus* and *G. sedelnikowi*.

<table>
<thead>
<tr>
<th>Species</th>
<th><em>G. laevis</em></th>
<th><em>G. minimus</em></th>
<th><em>G. limneus</em></th>
<th><em>G. sedelnikowi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Host Hamulus</td>
<td><em>P. phoxinus</em></td>
<td><em>P. phoxinus</em></td>
<td><em>P. phoxinus</em></td>
<td><em>Neomachiulus koratalus</em></td>
</tr>
<tr>
<td><strong>Total length</strong></td>
<td>39.9±2.1 (35-43)</td>
<td>28 (35-58)</td>
<td>- (35-39)</td>
<td>36.6±1.5 (35-39)</td>
</tr>
<tr>
<td><strong>Shaft length</strong></td>
<td>31.7±2.2 (28-34)</td>
<td>21 (35-39)</td>
<td>- (28-32)</td>
<td>29.5±1.3 (28-32)</td>
</tr>
<tr>
<td><strong>Point length</strong></td>
<td>15.5±1.0 (14-17)</td>
<td>14 (21-27)</td>
<td>- (17-21)</td>
<td>19.6±1.4 (17-21)</td>
</tr>
<tr>
<td><strong>Root length</strong></td>
<td>3.2±0.7 (7-10)</td>
<td>7 (14-21)</td>
<td>- (7-10)</td>
<td>8.2±0.9 (7-10)</td>
</tr>
<tr>
<td><strong>Ventral bar</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>13.3±1.2 (12-16)</td>
<td>11.2 (15-18)</td>
<td>13.2±1.1 (11-14)</td>
<td></td>
</tr>
<tr>
<td>Width</td>
<td>16.3±1.4 (14-18)</td>
<td>9.3 (21-27)</td>
<td>17.4±1.4 (15-20)</td>
<td></td>
</tr>
<tr>
<td>Process length</td>
<td>- (0.2-0.7)</td>
<td>- (0.3-0.7)</td>
<td>0.4±0.2 (0.3-0.7)</td>
<td></td>
</tr>
<tr>
<td>Membrane length</td>
<td>9.8±0.9 (9-11)</td>
<td>7 (12-18)</td>
<td>11±1.3 (9-13)</td>
<td></td>
</tr>
<tr>
<td><strong>Marginal hook</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total length</td>
<td>19.4±1.5 (16-21)</td>
<td>14 (21-23)</td>
<td>19.8±0.9 (18-21)</td>
<td></td>
</tr>
<tr>
<td>Shaft length</td>
<td>13.6±1.1 (12-16)</td>
<td>11.2 (14-16)</td>
<td>14.4±1.1 (12-15)</td>
<td></td>
</tr>
<tr>
<td>Sickle length</td>
<td>5.6±0</td>
<td>2.3</td>
<td>5.6 (3.5-5.6)</td>
<td>4.5±0.7 (3.5-5.6)</td>
</tr>
<tr>
<td>Sickle distal width</td>
<td>2.8±0</td>
<td>1.4</td>
<td>1.4 (2.1-2.8)</td>
<td>2.5±0.4 (2.1-2.8)</td>
</tr>
<tr>
<td>Sickle proximal width</td>
<td>2.8±0</td>
<td>1.4</td>
<td>1.4 (2.1-2.8)</td>
<td>2.5±0.4 (2.1-2.8)</td>
</tr>
<tr>
<td><strong>No. measured</strong></td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

All measurements in μm. Expressed as mean± standard deviation (when more than five specimens present in sample). Range of dimensions in parentheses.

1) Only one specimen available for measurement.
Fig. 16. Opisthaptor sclerites of Gyrodactylius laevis.
Gyrodactylus phoxini species group

Gyrodactylus limneus Malmberg, 1964

HOST: Phoxinus phoxinus

SITE OF INFECTION: Skin and fins.

DIAGNOSIS: Medium sized species with short pharyngeal processes and ten to thirteen spines arranged in a double row on the penis.

Hamuli straight shafted, roots parallel. Dorsal bar straight, lacking notch, broadest at centre. Ventral bar with rectangular based lobes, small processes and a broad, spatulate membrane, characteristic of this species group. A small triangular boss is present in the centre of the bar (Fig. 17, Table 14).

Marginal hooks small, shafts short, sickles slender with a long tapering toe, rounded heel and elongate point.

COMMENTS: This species is found on the skin of Phoxinus in association with G. aphyae and G. macronychus. It can be distinguished from these species by the short pharyngeal processes, the triangular boss on the ventral bar and the shape of the ventral bar membrane. Only one other species of this group (G. sedelnikowi Gvosdev, 1950) has been encountered in Britain, but this differs in the form of the hamulus roots and is unlikely to be confused.

G. limneus has previously been recorded in Britain by Powell (1966).

Gyrodactylus sedelnikowi Gvosdev, 1950

HOST: Noemacheilus barbatulus

SITE OF INFECTION: Skin and fins.

DIAGNOSIS: Medium sized species, pharynx with short processes, penis with double row of short spines.

Hamuli short, with parallel or slightly curved shafts and very short, clubbed roots which are rounded at their tips. Ventral bar with short processes, a long narrow membrane and a triangular boss in the centre. Dorsal bar straight, triangular in cross section, lacking a notch. Marginal hooks small, sickles slender with square truncated heels and pointed toes (Fig. 18, Table 14).
Fig. 17. Opisthaptor sclerites of Gyrodactylus limneus.
Fig. 18. Opisthaptor sclerites of Gyrodactylus sedelnikowi.
COMMENTS: This species can be separated from *G. limneus* by the rounded, clubbed tips of the hamulus roots. In other respects it closely resembles *G. limneus* and other species of the *G. phoxini* group, particularly in the form of the marginal hooks and the ventral bar. It can be differentiated from *G. pavloowskyi* by the form of the hamulus roots and marginal hooks. *G. sedelnikowi* was originally described from European Russia (Gvosdev, 1950). Specimens collected in the present work agree closely with the published account of the species (Gvosdev, 1950; Bychowskaya-Pavlovskaya, 1964). This is the first record of *G. sedelnikowi* for Britain.

DISCUSSION

A range of *Gyrodactylus* species occurs on British sticklebacks. In southern England, *Gasterosteus aculeatus* is typically infected by *Gyrodactylus arcuatus* (gills) and *G. gasterosteii* (skin and fins), whereas *Pungitius pungitius* is host to *G. rarus* (gills) and *G. pungitii* (skin and fins). In the presence of *G. gasterosteii*, *G. arcuatus* is normally found only on the gills, but when present as a monospecific infection (R.Yar, Whitwell, Isle of Wight and Grand Union Canal, London), it commonly occurs on the body surface. This species has a more generalised attachment strategy than *G. gasterosteii* (Ch.3), and can utilise both skin and gills. Its restriction to the gills in the presence of *G. gasterosteii* may therefore be due to an interspecific interaction (Ch.5). On *Pungitius pungitius*, both *G. rarus* and *G. pungitii* are highly site specific, and are unlikely to interact directly.

A similar distribution of *Gyrodactylus* on sticklebacks has been observed elsewhere in Europe. Roman (1960) recorded *G. rarus* (a synonym of a *G. wageneri* group species) and *G. arcuatus* upon *Gasterosteus aculeatus* and *Pungitius platygarster* in Rumania. Bychowsky and Polyansky (1953) also observed *G. rarus* from *G. aculeatus*, *P. pungitius* and *P. platygarster* from a variety of Russian localities. They also (loc. cit) reported *G. arcuatus* from the skin and gills of *G. aculeatus* and *P. pungitius*. Malmberg (1970) recorded *G. rarus* and *G. pungitii* from *Pungitius pungitius* and *G. arcuatus* from *Gasterosteus aculeatus* in Sweden and Northern Germany. He also collected, but failed to describe, a *G. wageneri* form (probably *G. gasterosteii*) from *G. aculeatus* (personal communication). In Britain, Lyons observed *G. arcuatus* and *G. gasterosteii* on *G. aculeatus* in Cambridge, and Turnbull found *G. arcuatus* in Edinburgh (Malmberg, personal communication).
Although in many cases the specific identity cannot be reliably determined, in Eurasian freshwater sticklebacks are infected by both a *G. arcuatus* or a *G. rarus* group species on the gills, and a *G. wageneri* group species on the skin.

In Britain, *G. gasterosteus* has not been found in brackish (Cuxton and Allhallows, Kent) or marine (Treasurer, 1974) habitats. *G. arcuatus*, however, occurs at all salinities, Bychowsky and Polyansky, 1953; Malmberg, 1970; Treasurer, 1974). Malmberg (1970) recorded *G. pungitii* from brackish habitats in the Baltic, up to a salinity of 5-6°/oo, although he indicated that the brackish water form might differ from the freshwater form. He found only the *G. wageneri* form from *Gasterosteus aculeatus* in freshwater (Malmberg, personal communication), and was unable to transfer *G. pungitii* from brackish to sea water (Malmberg, 1970).

These observations suggest that *G. wageneri* group species are intolerant of high salinities. Bychowsky and Polyansky (1953) report a species of this group on sticklebacks in the Caspian sea, with a salinity of 12.5°/oo (Zenkevitch, 1963) which although higher in total salinity, approximates to freshwater in its ionic composition (Zenkevitch, loc. cit.). Bychowsky and Polyansky (1953) also reported a *G. wageneri* group species from both *Gasterosteus aculeatus* and *Pungitius pungitius* sinensis from the Japanese sea, which is similar in salinity to oceanic water (Zenkevitch, 1963). Although this suggests that a *G. wageneri* species can osmo-regulate in full strength sea water, Bychowsky and Polyansky (loc. cit.) collected from the South Kuril shallows and from South West Sakhalin, both sites in which salinity may be reduced by freshwater runoffs.

In brackish and sea water, *G. branchicus* infects the gills of *Gasterosteus aculeatus*, and Malmberg (1970) considered the species to be restricted to areas of high salinity. In the present work this species was found only at Cuxton, Kent, in a salinity of 7°/oo. As *G. branchicus* is a specialist gill parasite, closely related to *G. rarus*, it may interact with *G. arcuatus* when this species is present in the gills. This may explain the observation that on the fish infected with *G. branchicus* in the present work, *G. arcuatus* was restricted to the skin.

The collection of *G. alexanderi* from Lake Windermere during the present work is of great interest. This species occurs on sticklebacks on the west coast of America (Mizelle and Kritsky, 1967a; Lester, 1974), but was not found in
In Europe, this species has been recorded from northern Germany, by Glaser (1974), but not from Scandinavia (Malmberg, 1956b, 1970) or European Russia (Bychowsky and Polyansky, 1953; Bychowskaya-Pavlovskaya, 1964). In Britain, it appears to be very local, and although further collecting in the north and west is needed, it seems to be absent from south east England.

*G. alexanderi* may have been introduced into Europe with its host by man. Although unlikely to be deliberately introduced, the stickleback may have been accidentally released with a consignment of North American fish, for example rainbow trout (*Salmo gairdneri*) or brook charr (*Salvelinus alpinus*). Numerous releases of these species have been made in Britain (Wheeler, 1974) and north Germany (Wheeler, 1978). Alternatively, *G. alexanderi* may have a natural distribution spanning both Eurasia and America, although it occurs only sporadically in Europe. The *G. eucaliæ* species group is very abundant in America (Malmberg, 1970), but *G. alexanderi* is the only species of the group found in Europe, which probably arose in America, spreading onto the initially marine sticklebacks (Wootton, 1976) when they started to colonise freshwater. If *G. alexanderi* is a genuine member of the Eurasian *Gyrodactylus* fauna, it would indicate that sticklebacks originated in America, subsequently radiating to Eurasia. This hypothesis would require that *G. alexanderi* be euryhaline. Although this species group is principally found in freshwater, Lester (1974) reported *G. alexanderi* from brackish habitats near Vancouver.

In Europe, cyprinids are infected by *G. wageneri* group species (Malmberg, 1970), which have also radiated onto some originally marine host families, including the Gasterosteidae and Cottidae, after their entry into freshwater. The salinity tolerance of these parasites is probably limited, and they are unlikely to survive marine conditions. *G. gasterosteï* and *G. pungitii* probably infected sticklebacks which colonised Britain after the most recent glaciation from the Rhine basin, which was at that time in direct contact with the Thames (Wheeler, 1974). A migration of this type, by the more southerly *leirus* race of *Gasterosteus aculeatus*, was postulated by Munzing (1963). The distribution of sub-species of *Pungitius pungitius* suggests that this species also colonised S.E. England by a similar route (Wootton, 1976). Rivers of North West England were always in direct communication with the Atlantic, and were unavailable for colonisation by European freshwater fish (Wheeler, 1974). The more northerly races of sticklebacks probably colonised these habitats from the sea (Wootton, 1976). It is possible that *Gyrodactylus alexanderi* was originally found on the northern *trachurus* race of *G. aculeatus*.
whereas *G. gasterosteii* was found on the more southerly *leiurus* race. In areas where the two races have come into contact, characterised by the hybrid *semi-armatus*, *G. gasterosteii* may have replaced *G. alexanderi*. In Britain, *G. alexanderi* may have survived where *G. gasterosteii*, infecting *Gasterosteus aculeatus leiurus*, failed to penetrate the north and west, in river draining directly into the sea. However, the influence of man (deliberate and accidental fish introductions, canal building) may have allowed fish from west and east to mix freely (Wheeler, 1974), culminating in the replacement of *G. alexanderi* by *G. gasterosteii*.

The occurrence of *G. alexanderi* in northern Germany at first sight refutes this hypothesis. However, *Gasterosteus aculeatus trachurus* is abundant in fresh water between the Elbe and Oder rivers, having spread into this area from a glacial refugium in the Black Sea (Munzing, 1972). *G. alexanderi* may have survived here because *G. gasterosteii* in neighbouring drainage systems would be unable to survive the marine migration of hosts into this area.

*Gyrodactylus arcuatus* group species are widely distributed on *Gasterosteus aculeatus* in the Pacific coast of America (Malmberg, 1970), Newfoundland (Hanek and Threlfall, 1969) Iceland (personal observation), North West Europe (Bychowsky, 1933; Bychowsky and Polyansky, 1953; Malmberg, 1970; Glaser, 1974; present study) and Pacific Eurasia (Bychowsky and Polyansky, 1953). *G. arcuatus* belongs to a predominantly marine species group (other species occur on gadids), and probably colonised sticklebacks during a marine phase of their evolution. Its general occurrence throughout the host range is a reflection of its euryhalinity, demonstrated by Malmberg (1970). The *G. rarus* species group are probably also of marine origin, as Malmberg (1970) recorded that *G. branchicus* is only found in salt water, and that a third species of this group, *G. cyclopteri* Scyborskaya, 1948, occurs on the marine lump sucker, *Cyclopterus lumpus*. This species group probably colonised sticklebacks in the sea, spreading with them into freshwater. The present day distribution of *Gyrodactylus* on sticklebacks is shown in Fig 19. Further evidence for the pattern of colonisation of sticklebacks by *Gyrodactylus* could be obtained by a study of these hosts from Greenland, Iceland, Spitsbergen and the Faeroes, where the influence of man of fish distribution has been negligible, and which have always been isolated from the freshwater systems of America and Eurasia.

With the exception of salinity, which influenced the distribution of *Gyrodactylus gasterosteii* and *G. branchicus*, no relationship was observed between water body...
Fig. 10. World distribution of Gyrodactylus species from sticklebacks.
and the *Gyrodactylus* species found, which were similar throughout the range of habitats examined (Table 3).

The host specificity of *Gyrodactylus gasterostei*

*Gyrodactylus gasterostei* was usually unable to infect either *Pungitius pungitius* or *Phoxinus phoxinus*. The failure of infections of *G. gasterostei* on individually infected *P. pungitius* may have been due to a different response to stress in this host. Handling stress has been shown (Pickering and Macey, 1977) to affect goblet mucus cell density in charr (*Salvelinus alpinus*), and it has been demonstrated (Ch. 3) that mucus cells are involved in the response of *Gasterosteus aculeatus* against *Gyrodactylus gasterostei*. Wootton (1976) described the different behaviour of *G. aculeatus* and *P. pungitius* to a predator: *G. aculeatus* remains motionless, relying on its spines for protection, whereas *P. pungitius* takes refuge in flight. It may therefore be suggested that the two species respond differently to the handling stress imposed when infected with *G. gasterostei*, accounting for the observed differences in specificity. However, when *P. pungitius* and *Phoxinus phoxinus* were maintained in aquaria with infected *Gasterosteus aculeatus*, *G. gasterostei* still failed to infect them. This indicates that a difference in attraction of these hosts for *G. gasterostei* exists, irrespective of the stress placed upon them.

Two hypotheses may account for the restriction of *G. gasterostei* to *Gasterosteus aculeatus*. The parasites may infect other hosts, but due to physiological maladaptation, rapidly die. Alternatively, they may actively select a suitable host, using specific stimuli to identify it. Llewellyn (1982) considered that in most monogeneans active larval selection of the host takes place, as was shown by Kearn (1967a) for *Entobdella soleae*. *G. gasterostei* infects unsuitable hosts more slowly than *G. aculeatus* (Table 9), and parasites placed upon *P. pungitius* rapidly moved off the skin. These observations suggest a behavioural basis for specificity, preceding any physiological adaptation to the particular host. Further evidence for this was obtained by measuring the rate of movement of parasites onto different substrates (Table 10). The difference in rate of movement of *G. gasterostei*, from glass and dead fish onto tweezers, *G. aculeatus* and *P. pungitius* show that this parasite can perceive both the nature of the substrate to which it is attached, and that to which it is moving. It is of interest to note that the rate of movement onto tweezers was significantly higher than that onto *P. pungitius* tissue, suggesting that, not only does the
parasite react positively to *G. aculeatus* tissue, but that it is also inhibited from moving onto *P. pungitius*.

The response of *G. gasterosteii* to a host is similar to that of *Entobdella soleae*, in which Kearn (1967a) demonstrated a perception of specific identity of hosts, and to that of dactylogrid larvae, studied by Molnar (1971). Llewellyn (1982) considers such a response typical of these strictly host specific monogeneans. However, the specificity of *Gyrodicotrema gallieni* is not of this type (Ch. 6). This parasite does not respond during infection to stimuli identifying the host, although a degree of physiological specificity exists, as the parasite survives suboptimally in unusual hosts. Although host recognition during invasion is very important in determining monogenean specificity (Llewellyn, 1982), it is not a universal phenomenon.

The sensory basis for the identification of suitable hosts by gyrodactyldids has not been examined. Single cillum receptors, which occur all over the body (Lyons, 1973) and compound uniciliate 'spike' sensilla (Lyons, 1969) sited upon the cephalic lobes, may be important in host recognition. Although the function of these organs has not been experimentally determined, Lyons (1973) concluded that from their structure, single cillum sense organs were tango-or probably rheo-receptors, and that spike sensilla were chemosensory. In all other monogeneans, host location is carried out by the oncomiracidium, and spike sensilla are absent. The swimming larvae are still capable of chemosensory distinction between potential fish hosts (Kearn, 1967a) an ability attributed to the presence of a group of decidual dorsal sensilla in all oncomiracidia (Lambert, 1981). Spike sensilla are probably used for chemoreception by gyrodactyldids, being frequently placed in contact with surfaces to which the parasite is about to move.

The chemical composition of the skin is known to contain sufficient intra-and inter-specific variation to be used by parasitic larvae in host identification. Kearn (1967a) showed that the swimming larvae of *Entobdella soleae* were able to recognise a specific chemical factor produced by the epidermis of the normal host, *Solea solea*, which was absent from the other teleosts tested. This substance was probably produced by the goblet epidermal mucus cells, as it was not present in corneal tissue from which these cells are absent (Kearn, loc. cit.).

Barry and O'Hourke (1959) found that it was possible to separate mucus from different fish using chromatography, and Bardach and Todd (1970) showed that the mucus of different fish differed sufficiently to allow recognition by conspecifics.
The morphology of \textit{G. gasterostei} recovered from \textit{Pungitius pungitius} did not differ significantly from that of parasites cultured on normal hosts (Table II). This species does not vary morphometrically on different hosts, making it unlikely that morphologically distinct gyrodactylids found on separate hosts are only varieties of the same species, capable of interchange between hosts.

The behavioural mechanisms maintaining the separation of \textit{Gyrodactylus} from different hosts form a very rigid barrier to hybridisation between their populations. Isolation of this nature, associated with close physiological adaptation to a single host, is thought to have led to the development of strains of parasites on different hosts, as in the case of \textit{Echinococcus granulosus} (see Smyth, 1969). Llewellyn, Macdonald and Green (1980) described the colonisation of a novel host, \textit{Trisopterus minutus}, by \textit{Diclidophora esmarkii}, and Llewellyn (1982) has suggested that the parasite stock on this host may ultimately become differentiated from that on the normal host, \textit{Trisopterus esmarkii}. This process of colonisation of new hosts and subsequent close adaptation and speciation may be rarer in gyrodactylids because of their rigid behavioural specificity. Some individuals, however, possibly with genetically determined differences in chemosensory ability, may sometimes infect other host species. The rapid reproduction and limited genetic recombination thought to occur in \textit{Gyrodactylus} may then lead to rapid speciation. \textit{Gyrodactylus} has apparently speciated by the radiation of species groups onto phylogenetically or ecologically related hosts (Table 15). It implies that the chemosensory abilities of the parasite break down at some point, allowing radiation onto new hosts before new chemosensory responses develop and speciation occurs. The observations of Malmberg (1970), concerning \textit{Gyrodactylus errabundus} are of interest, as this species, normally found on \textit{Zoarces viviparus}, can transfer to a wide range of other hosts. This suggests that \textit{G.errabundus} could in favourable circumstances, permanently colonise new hosts, and that it may be undergoing the first stages of a new radiation, which would ultimately give rise to a new species group.

Other species of \textit{Gyrodactylus} present in small rivers

As \textit{Gyrodactylus gasterostei} is narrowly specific to \textit{Gasterosteus aculeatus}, possessing behavioural mechanisms to maintain it on this host, the morphological variants found on different hosts are best regarded as different species. The degree of specificity shown by \textit{G.gasterostei} makes it unlikely that, as suggested by Dawes (1947), \textit{Gyrodactylus} contains relatively few species, showing morphological variation on different hosts.
Table 15. Phylogenetic and ecological distribution of hosts of Gyrodactylus species groups.

<table>
<thead>
<tr>
<th>Species group</th>
<th>Host families (in order of importance)</th>
<th>Ecological distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. elegans</td>
<td>Cyprinidae</td>
<td>Freshwater (principally European)</td>
</tr>
<tr>
<td></td>
<td>Cobitidae</td>
<td></td>
</tr>
<tr>
<td>G. phoxini</td>
<td>Cyprinidae</td>
<td>Freshwater (principally European)</td>
</tr>
<tr>
<td></td>
<td>Cobitidae</td>
<td></td>
</tr>
<tr>
<td>G. afghanensis</td>
<td>Cobitidae</td>
<td>Freshwater (Central Asia)</td>
</tr>
<tr>
<td>G. arcuatus</td>
<td>Gadidae</td>
<td>Marine</td>
</tr>
<tr>
<td></td>
<td>Gasterosteidae</td>
<td></td>
</tr>
<tr>
<td>G. eucaliae</td>
<td>Gasterosteidae</td>
<td>Freshwater (principally American)</td>
</tr>
<tr>
<td></td>
<td>Poeciliidae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cyprinodontidae</td>
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<tr>
<td></td>
<td>Centrarchidae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anuran tadpoles</td>
<td></td>
</tr>
<tr>
<td>G. marinus</td>
<td>Gadidae</td>
<td>Marine</td>
</tr>
<tr>
<td>G. rarus</td>
<td>Gasterosteidae</td>
<td>Marine and brackish, European</td>
</tr>
<tr>
<td></td>
<td>Cyclopteridae</td>
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<td>G. harengi</td>
<td>Clupeidae</td>
<td>Marine</td>
</tr>
<tr>
<td>G. emembranatus</td>
<td>Gadidae</td>
<td>Marine</td>
</tr>
<tr>
<td>G. lotae</td>
<td>Gadidae</td>
<td>Freshwater (Europe)</td>
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<tr>
<td>G. flesi</td>
<td>Pleuronectidae</td>
<td>Marine</td>
</tr>
<tr>
<td>G. unicopula</td>
<td>Pleuronectidae</td>
<td>Marine</td>
</tr>
<tr>
<td>G. perluckidas</td>
<td>Zoarcidae</td>
<td>Marine</td>
</tr>
<tr>
<td>Species group</td>
<td>Host families</td>
<td>Ecological distribution</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td><em>G. rugiensis</em></td>
<td>Gobiidae</td>
<td>Marine and brackish</td>
</tr>
<tr>
<td><em>G. anguillae</em></td>
<td>Anguillidae</td>
<td>Freshwater</td>
</tr>
<tr>
<td><em>G. katherineri</em></td>
<td>Cyprinidae</td>
<td>Freshwater (Europe)</td>
</tr>
<tr>
<td><em>G. wageneri</em></td>
<td>Cyprinidae, Salmonidae, Esocidae, Gasterosteidae, Cottidae</td>
<td>Freshwater (principally European)</td>
</tr>
<tr>
<td><em>G. cobitis</em></td>
<td>Cobitidae</td>
<td>Freshwater (Europe)</td>
</tr>
<tr>
<td><em>G. nemacheili</em></td>
<td>Cobitidae</td>
<td>Freshwater (Europe)</td>
</tr>
</tbody>
</table>
Many other species of *Gyrodactylus* can be found on fish in the same habitats as *Gasterosteus aculeatus* and *Pungitius pungitius*. As this study investigated only the smaller, more easily captured hosts, the number of *Gyrodactylus* species in these environments may be substantially underestimated. Nevertheless, eleven species have been identified from only five host taxa (Table 12). None was found on more than one host, further suggesting that *Gyrodactylus* species are strictly host specific.

All of the species found show a predilection for a particular site of infection, either skin, fins or gills. In some cases this specificity is inherent to the parasite, in others it may be modified by the environment. For example, the site specificity of *G. arcuatus* is dependent upon the presence of *G. gasterosteii* on the host (Ch.5). On the other hand, the site specificity of *G. gasterosteii* cannot be modified by changes in the environment (Ch.3).

Although strict site specificity does exist, some species still coexist with other closely related species in the same habitat (*G. laevis* and *G. minimus*; *G. macronychus* and *G. aphyae*). Price (1978) has argued that competition between parasites is rare, because their unstable population dynamics prevent them utilising fully the host's resources, reducing niche overlap. However, in the case of gyrodactylids, closely related species often coexist on the same host, a situation in which it is possible that competition takes place between them. It is not clear how site segregation reduces interspecific competition, and the mechanisms allowing coexistence of more than one species are unknown.

The large and variable number of *Gyrodactylus* species per host is a product of their pattern of speciation, involving separate radiations by different species groups. These radiations have sometimes occurred amongst phylogenetically related hosts (e.g. the *G. elegans* species group is restricted to cyprinids and loaches), and in other cases amongst species with a similar ecology. In this way, the *G. wageneri* group, principally parasites of cyprinids, have come to radiate onto salmonids, percids, sticklebacks and pike. Successive radiations of this type have taken place, until the characteristically complex community of *Gyrodactylus* species on a single host has built up.

An interesting example of this sequential colonisation is shown by the gyrodactylids of the bullheads (Cottidae). The current distribution of
bullheads suggests that these fish are of marine origin (Wheeler, 1978). The marine bullheads of the genus *Myoxocephalus* are infected by species of the *G. gronlandicus* species group (Levinsen, 1881; Zhukov, 1960).

*Gyrodactylus hrabei* from *Cottus gobio*, *Gyrodactylus* sp. from *Cottus poecilopus* and *G. bairdi* from *Cottus bairdi* are freshwater species (Malmberg, 1974b) which are probably derived from marine forms similar to the *G. gronlandicus* group, when their hosts entered freshwater. Malmberg (1970) indicated that *G. cotti* Roman, 1956 *sensu* Gussev, 1967 may also be derived in a similar fashion, from the sub-genus *Metanephrotus*. On the other hand, some species may have radiated onto the bullheads after their entry into freshwater, from other freshwater fish. *G. rogatensis* n.sp is an example of this type of colonisation, being closely related to other *G. wageneri* group species found on cyprinids. The three *Gyrodactylus* species described by Bogolepova (1949) from endemic lake Baikal cottids and cottocomephorids may also have originated in this manner, as suggested (loc. cit.) for *Dactylogyrus colonus* Bogolepova, 1949.

This account indicates the considerable complexity of *Gyrodactylus* communities on freshwater fish. All of the hosts considered occur in the same habitats, and often shoal together (Glaser, 1974). However, the *Gyrodactylus* communities of individual host species remain distinct because of the behavioural mechanisms maintaining the specificity of individual hosts. The strict host specificity of *G. gasterosteai* suggests that the taxonomic separation of *Gyrodactylus* into many species from different hosts is justified. The co-existence of several species of *Gyrodactylus* on an individual host is also normal, greatly increasing the diversity of the genus. Little is known of the mechanisms allowing co-existence of several parasite species on the same host, or indeed whether the separate species do interact. The complexity of the community of gyrodactylids on a host has developed from the discontinuous pattern of their evolution.
Ch. 3. THE INTERACTION OF CYRODACTYLUS WITH THE HOST.
INTRODUCTION

The demography of several species of Gyrodactylus has been studied experimentally (Anthony, 1969; Lester and Adams, 1974a, b; Harris, 1980a; Scott, 1982a,b) and in nature (Srivastrava and James, 1967; Chappell, 1969; Rawson and Rogers, 1973; Barkman and James, 1979; Harris, 1982a). However, in order to understand the nature of the factors influencing the population dynamics of the parasites, it is necessary to have a knowledge of the biology of the host-parasite interaction. In the case of most fish ectoparasites, the population size is determined not only by factors acting upon the adults, but also by those acting upon free living larval stages. Although Paperna (1964) has shown that, in the case of Dactylogyrus vastator upon carp, the biology of the adult parasite is important in limiting population size, in the case of most oviparous monogeneans, the probability of infection by the free living oncomiracidia is probably the most important constraint (Bychowsky, 1957; Anderson, 1981). In the viviparous gyrodactyliods, reproduction in situ upon the host increases the relative importance of factors influencing the adult parasites, including feeding and attachment, their effect upon the host, and the response of the host to infection and inter-and intra-specific interactions with other epibionts. Although no obligate free-living phase is present in their life-cycle, gyrodactyliods can live for some time away from the host, and factors affecting their survival during this period may also influence their population size.

Monogeneans attach to the gills, body surface or internal cavities (nasal fossae, acoustic-lateralis system, cloaca, coelomic cavity) of fish using the haptor, which is armed with a diverse complement of marginal hooks, hamuli, suckers and clamps. The functional morphology of the attachment apparatus of some monogenean gill parasites has been thoroughly examined. Llewellyn (1956) studied a range of polyopisthocotyleans which use clamps, finding that in all cases that the attachment was very firm, and that with the exception of Cyclocotyla, the parasites were relatively immobile. Kearn (1968a, 1971) observed that the monopisthocotylean dactylogyrids, ancyrocephalids, tetraonchids and diplectanids pierced the gill tissue deeply with their hamuli. Although polyopisthocotyleans inflict relatively little damage upon the epithelium of the secondary gill lamellae using clamps, monopisthocotyleans can stimulate pathogenic responses in the host because of the severity of the wounds which they cause. Thus Paperna (1964) observed a proliferation of the gill epithelium against Dactylogyrus vastator on carp, leading to the death of the parasites.

The less numerous, but more diverse, groups of skin parasitic monogeneans have been less adequately studied. Skin parasitism is seen in the Microbothridae, Monocotylidae, Caposalidae and Gyrodactylidae.
The microbothrids and monocotylids are restricted to elasmobranch hosts (Sproston, 1946), the skin surface of which differs considerably from that of teleosts. Acanthocotylids are normally also parasites of elasmobranchs, but Malmberg and Fernholm (1982) have reported the discovery of some new species which infect hagfish, the skin of which is more similar to that of teleosts than elasmobranchs (Blackstad, 1963). These parasites are relatively weakly attached by the marginal hooks, using the pseudohaptor as a pressure pad which does not pierce the host epidermis (Malmberg and Fernholm, loc.cit.). The very small monogenean, *Enoplocotyle minima*, also attaches to the host using only the marginal hooks (Tagliani, 1912; Kearn, 1976). The attachment mechanism of two species of capsalid has been studied, that of *Entobdella soleae* by Kearn (1964) and that of *Pseudobenedenia nototheniae* by Williams, Ellis and Spaull (1973). In both cases, despite minor differences, the haptor functions as a weak sucker, the prominent hamuli supporting the roof of the sucker while the marginal hooks pin down the marginal valve. The larvae of most gill parasitic *monopisthocotyleans* undergo a migration across the skin to the gill chamber (Prost, 1963; Lambert, 1980a), during which they are attached solely by the marginal hooks (Cone and Burt, 1981).

These studies have shown that most skin parasitic monogeneans are relatively weakly attached, relying on suckorial mechanisms, pressure pads or marginal hooks. At first sight the gyroactylids are an exception to this principle, as they possess large ventral hamuli which form the most conspicuous part of the attachment apparatus. However, in *Gyrodactylus alexanderi*, the only species of the genus in which attachment has been studied, the hamuli are not used to gaff tissue, but merely brace the marginal hooks (Lester, 1972). *Gyrodactylus* is a very large genus, showing considerable variation in site of attachment and hamulus morphology. Because of this, Lester's (1972) observations on *G. alexanderi* may not be representative of the entire group. The strength of the attachment, which may be dependent upon the mechanism involved, would be of paramount importance in determining the probability of parasites becoming detached from the host.

The quantity and quality of food ingested by parasites may also have an important effect upon the dynamics of the host-parasite system. Consumption of a rapidly regenerated, nutrient rich resource, such as blood, allows a higher parasite fecundity and causes less harm to the host than consumption of a nutrient poor, slowly regenerated tissue such as dermis. Three sources of food are available to fish epibionts and ectoparasites. They may consume other epibionts and organic particles adhering to the surface of the fish as shown
for the peritrich ciliates *Aplospora* and *Epistylium* by Lom (1973a). Organic particles may also be ingested by *Trichodina*, a peritrich more conventionally regarded as parasitic (Sleigh, 1973). The second resource available to ectoparasites is mucus and epithelial cells, which, because of the presence of goblet cells in the epidermis, are ingested together. Several monopisthocotylean monogeneans (*Entobdella soleae, Acanthocotyle lobianchi, Leptocotyle minor, Dendromonocotyle kuhlii*) have been shown to feed exclusively upon host epithelium, without damaging the dermis (Kearn, 1963a, 1965, 1979). Many other fish ectoparasites utilise this resource, including the copepods *Ergasilus* and *Caligus* (see Einszporn, 1965; Shotter, 1971) and the digenean *Transversotrema patiallense* (see Mills, 1979). The third food source used is blood, which although consumed almost exclusively by polyopisthocotyleans most (Llewellyn, 1954), is not important for monopisthocotyleans. Young (1967) considered that the monopisthocotylean *Dendromonocotyle lines* were haematophagous, but Kearn (1979) showed that *Dendromonocotyle kuhlii* normally feeds upon epithelial cells, and that the pigment within its gut is of epidermal origin. Markov (1958) suggested that larger dactylogryids and cestoids may facultatively feed upon blood, and Fournier (1980) showed that this resource is utilised by the unusual monopisthocotylean *Euzetrema knobpflneri*, from the bladder of *Euproctus montanus*. Amongst the gyrodactylds, Lester (1972) and Kearn (1976) have suggested that epithelial cells may form the bulk of the diet, although Khalii (1970) observed that *Macrogryrodactylus polypteri* might ingest some blood.

Periods of activity increase the risk of dislodgement of monogeneans, because they rely upon the weak protractor for attachment during locomotion. Many gill parasitic polyopisthocotyleans remain almost immobile throughout life, some having so many attachment organs that they lack the co-ordination necessary for movement (Bychowsky, 1957). On the other hand, skin parasitic monogeneans are thought to be relatively mobile: Kearn (1962, 1963a, 1970) has observed respiratory feeding and reproductive movements in detached *Entobdella soleae*, which are thought to occur also on the surface of the living fish. It may also be presumed that, in those monogeneans in which spermatophores are deposited, for example *Acanthocotyle greeni*, the adult flukes are active enough to locate and assimilate these objects (Macdonald and Llewellyn, 1980). Likewise, in the absence of aggregations of parasites for mating purposes (Rohde, 1979), skin parasitic monogeneans must search for partners with which to copulate.
Unfortunately, because of the relatively large size of the hosts, the behaviour of skin parasitic monogeneans is difficult to observe in vivo, and has either been inferred from morphological observations (Macdonald and Llewellyn, 1980) or from the behaviour of detached parasites in vitro (Kearn, 1962, 1963a, 1970). In the case of Gyrodactylus, no observations concerning their behaviour (including feeding, copulation and movement) upon living hosts have been made.

The outer integument of fish is composed of a delicate epidermis and a fibrous dermis (Whitear, 1977). The epidermis is made up of metabolically active epithelial cells, capable of division throughout the layer (Henrikson and Matoltsy, 1968a, b; Bullock, Marks and Roberts, 1978). These cells are normally unkeratinised, although Mittal and Whitear (1979) have found evidence of keratinisation in the skin of the catfish Bagarius bagarius. In the absence of keratin, which in addition to its waterproofing qualities also confers protection against ectoparasites (Whitfield, 1979; Tinsley and Whitear, 1980) and a degree of physical strength, the fish epidermis is extremely fragile. It is protected by a layer of mucus, produced from the surface of the epithelial cells (Whitear, 1970) and from goblet mucus cells (Pickering, 1974; Harris, Watson and Hunt, 1973). The epidermis may also contain specialised chloride cells (Whitear, 1971), free nerve endings (Whitear, 1977), eosinophilic granule cells and macrophages (Roberts, Young and Milne, 1972).

The functions of the epidermis include protection against physical damage and biotic attack. When damaged, the epidermis heals rapidly by cell migration (Kearn, 1971) and division (Bullock, Marks and Roberts, 1978), but the healing rate of the dermis is, by contrast, much slower (Finn and Nielson, 1971a, b). The thickness of the epidermal mucus is important in protecting the host against attack. Lubbock (1981) has shown that the mucus layer is much thicker in those species of Amphiprion which are regularly associated with sea anemones than in species which do not enter into the symbiosis. It is thought that the additional thickness of mucus confers protection against the coelenterate nematocysts. The continuous production of mucus by the epidermis probably prevents epibionts settling on the fish (Van Oosten, 1957). It has been suggested that mucus production can be varied by the fish in response to the magnitude of epibiont burdens. Lester (1972) described a response by Gasterosteus aculeatus against Gyrodactylus alexanderi, in which sheets of mucus were sloughed off at high parasite densities removing the parasites, a phenomenon which has attracted considerable interest.
Whitear (1970) considered that the general mucus layer covering fish skin was thin, and was produced from the surface epithelial cells, but that when the fish is stressed or harmed, this layer of mucus may be dislodged by the discharge of the goblet mucus cells. Pickering and Macey (1977) and Pickering, Bottinger, and Christie (1982) have shown that the density (number of cells per unit area) of goblet mucus cells increases greatly when trout (Salmo trutta) and char (Salvelinus alpinus) are stressed. This phenomenon, which may be related to shedding of the mucus 'cuticle', may also occur in response to parasite infection. In this context Pickering and Christie (1980) have shown that male brown trout carry significantly higher burdens of ectoparasites than females. This may be related to a decline in goblet cell density in the male epidermis during the breeding season. However, as this difference in ectoparasite burdens is maintained outside the breeding season, when the epidermis of males and females is similar, some other factor unrelated to mucus cell density may be involved (Pickering and Macey, loc. cit).

The epidermis and mucus may also have a chemical resistance against invasion. Fish mucus has been shown (Fletcher and White, 1973) to contain lysozyme, a non-specific enzyme which disrupts bacterial cell walls. Several authors (Fletcher and Grant, 1969a; Di Conza and Halliday, 1971; Smith, 1977) have recorded specific immunoglobulins in fish mucus, and Goven, Dave and Gratzek (1980) achieved immunisation of channel catfish (Ictalurus punctatus) against Icthyophthirius multifilis. On the other hand, some authors (e.g. Cottrell, 1977) have failed to detect antibodies in fish mucus. The disappearance of Epibdella melleni from a range of coral reef fish after a period of infection has been attributed (Nigrelli and Breder, 1974; Nigrelli, 1975a,b,c,1977) to an immune reaction by the host. However, although Icthyophthirius multifilis can stimulate a host response (Buschiel, 1710; Bauer 1973), it does so by burrowing under the epithelial cells, presenting a more intense antigenic stimulus than is the case for a true ectoparasite such as Epibdella. It is not known how ectoparasitic monogeneans could stimulate a host response involving specific antibodies, nor is it known how much a response would affect the parasites.

Although the most important factors influencing ectoparasite population dynamics act at the host-parasite interface, the survival of the parasites away from the host may also be important. Unlike the oncomiracidia of oviparous monogeneans, which have a maximum life span of 24 hours (Llewellyn, 1972), detached gyroactylids can live for a few days (Khalil, 1970; Lester and Adams, 1974a). This, probably a consequence of the increased body size of the gyroactylids, increasing their available food reserves when detached. Anderson (1976) has developed models for detached parasites, in which instantaneous death rate increases...
exponentially with time, as food reserves are exhausted. This pattern of increasing mortality may, however, be modified by the behaviour of the parasites. Many digenean cercariae (Smyth, 1966) and monogenean oncomiracidia (Kearn, 1981) alternate bouts of swimming with periods of inactivity, an adaptation increasing the longevity of the larva. The survival of the larva may also be dependent upon environmental factors, including temperature and oxygen tension (Smyth, 1966).

In the present work, an analysis of the biology of Gyrodactylus gasterostei has been undertaken in order to determine those aspects which are important in determining the population dynamics of the parasite. This has involved a consideration of the attachment mechanisms of a range of gyrodacltylids, and a study of the behaviour of G. gasterostei when attached to a host and when detached. The effect of G. gasterostei upon the host (Gasterosteus aculeatus) has also been examined.

MATERIALS AND METHODS

Individual fish were kept and infected with G. gasterostei according to the techniques outlined in Ch.4. Observations of parasites upon living fish were made by placing a single infected host in a 50ml beaker containing dechlorinated tap water, which was held on the stage of a binocular dissecting microscope. Individual parasites were observed at up to X40 magnification and classified according to the degree of development of their contained embryos. The number of individual movements made by the parasite during a 15-minute observation period were recorded. During this period the fish were illuminated from below by a single bench lamp, allowing the parasites to be seen in transmitted light. All observations were made in a 15°C constant temperature room with a water bath placed between lamp and microscope to avoid overheating.

Parasites were allowed to move freely from dead hosts in petri dishes containing dechlorinated water, then transferred in groups of five to watch glasses. Their survival was assessed at 5°C, 10°C and 15°C in dechlorinated water, and at 15°C in oxygenated and deoxygenated pond water obtained by removing water from the deoxygenated lower layer of a stagnant pond in a sealed vessel. This water was divided into two, one half being aerated and illuminated at 15°C for 24 hours, while the other was kept in a sealed vessel in darkness. The deoxygenated water was placed in a watch glass, parasites added and a glass cover placed upon the watch glass in such a way as to exclude air. Vaseline was used to seal the edge of the glass cover to the
watch glass. Parasites were examined every few hours upon the stage of a binocular microscope. Each watch glass was subjected to an equivalent degree of disturbance before observation began. To assess activity, each movement of the individual parasites was scored over three one minute observation periods.

The attachment mechanisms and the effect of feeding were examined using scanning electron microscopy. Fish were killed by cutting through the spinal cord and segments of the caudal peduncle were immediately removed and fixed in 2% osmium tetroxide in 0.2M cacodylate buffer (pH 7.2). After one hour in fixative, specimens were washed in 0.2M cacodylate and distilled water (one hour each), dehydrated in acetone and critical point dried (Polaron 3000). They were mounted on aluminium stubs, coated with gold (Polaron 5000) and examined in a Cambridge 600 scanning electron microscope at 7.5 KV accelerating voltage.

After preliminary observation with SEM, individual parasites were removed from the specimens using insect pins, attached to stubs with double sided tape and orientated so that the inside of the haptor could be examined. Both parasite and host specimens were recoated with gold before further examination of the attachment organs and of the wounds inflicted upon the host.

For light microscopy, feeding wounds were fixed in 10% formol-saline, dehydrated in alcohol and embedded in wax. 5-7 μm sections were stained with haematoxylin and eosin.

To determine the effect of parasite infection upon the structure of the host skin, wild caught Gasterosteus aculeatus were maintained individually in 41 of dechlorinated water for two weeks at 10°C in an alternating light regime of 12 hours light followed by 12 hours darkness (12 h L :D). The fish were fed ad libitum on living Tubifex, and received a 50% water change every 5 days. The number of parasites upon the fish were counted directly (Ch.2.4) before and after the two week experimental period. At the end of the period fish were killed by cutting the spinal cord and placed in 10% formol-saline fixative.

Segments of fixed caudal peduncle and fins were removed from the fish, dehydrated in alcohol and embedded in paraffin wax. 5-7 μm sections were stained with haematoxylin and eosin, haematoxylin and alcian blue pH1 and pH3 (Harris, Watson and Hunt, 1971) and toluidene blue. These specimens were used to describe the structure and histochemistry of stickleback skin. In order to examine the distribution and density of goblet mucus cells, the whole fish was immersed in alcian blue (pH3) as described by Pickering (1974). In the trout
these authors found that this technique stained only those goblet cells open to
the skin surface. However, the stickleback has a very thin epidermis, and in
this host, all cells were stained. An index of the number of goblet cells present
per unit area of fish skin was obtained in the following way. Using an eyepiece
micrometer at X100 magnification, all goblet cells touching a line 0.25 mm long
were counted. Ten replicates on each fish were performed, using lines orientated
randomly in the peduncle region. The thickness of the epidermis was estimated
after embedding formalin-fixed pieces of the caudal peduncle in Epon 812 epoxy
resin after dehydration in acetone. 2 μm sections were cut on an LKB-Huxley
MK II ultramicrotome and stained with toluidine blue. Using an eyepiece
micrometer at X1000 magnification, the thickness of the epidermis was measured.
Measurements were taken with the micrometer at right angles to the epidermal
surface, from the epidermal surface to the basement membrane. Ten thicknesses
were measured randomly on each section, avoiding areas of intense dermal folding.

RESULTS

Observations on attachment

The attachment mechanisms of the skin parasites *G. bullatarudis* and *G. gasterostei*,
the gill parasite *G. rarus*, and the unspecialised *G. arcuatus* were examined.

a) *Gyrodactylus arcuatus*

This species may be attached to skin, fins or gills. It is unlikely to be influenced
by the gross structure of the gills, as individual parasites were found to be
small relative to the gill primary lamellae (Plate 1). The tips of the hamuli
remained embedded in the host when the parasite was removed for examination
(Plate 3), suggesting that they are used to pierce the host epidermis. However,
they did not leave a conspicuous wound, and could not be distinguished from
the numerous small marginal hook wounds in the surface of the host (Plate 2).
The ventral bar processes were conspicuous through the haptor tegument (Plate 3),
and may have been pressed against the host skin, acting as a pressure pad.

b) *Gyrodactylus bullatarudis*

Marginal hook wounds were inconspicuous in this species (Plate 5). However,
the host epidermal cells were pulled up into points where gripped by marginal
hooks (Plate 4). Although this appearance was probably an artefact, caused
by the shrinkage of epidermis away from the parasite, it shows that the marginal
hooks had a relatively important role in attachment. On removing the parasite
from the host, the hamulus tips were not left embedded in the epidermis (Plate
5 and 6), suggesting that they did not penetrate the host. However, the
attachment wound showed two deep depressions, caused by the hamulus shafts,
within which the surface topography of the epithelial cells was pressed flat.
This suggests that, although not used to pierce the host epidermis, the hamuli
were pressed against it with some force.
Plate 1. Gyrodactylus arcuatus, attached to the gills of the host.
Plate 2. Wound of Gyrodactylus arcuatus in the skin of the host.

mw: wound inflicted by marginal hook.

wounds of hamuli not visible.
Plate 3. Ventral face of haptor of G. arcuatus.

ha; tips of hamuli, broken during removal from host
vbp; ventral bar process
mh; marginal hook
Plate 4. Gyrodactylus bullatarudis, attached to skin of guppy.

Note marginal hooks (mh) pulling up host epidermis.
Plate 5. Wound of G. bullatarudis in skin of guppy.

mhw - marginal hook wound
hd - depression on epidermis caused by hamuli

Tips of hamuli are not left in the wound after removal of the parasite.
Plate 6. Ventral surface of haptor of G. bullataramis.

pt intact point of hamulus
The hamuli of *G. gasterostei* remained intact when the parasite was removed from the host, suggesting that they are not used to pierce the epidermis (Plate 9). This was corroborated by the observation that the tips of the hamuli were blunt (Plate 9), and apparently covered with tegument (cf. Plates 12 and 24, showing species in which the tips of the hamuli are free of tegument). The hamuli left no mark upon the epidermis, although the marginal hooks inflicted a semi-circular row of large, conspicuous pits (Plate 8). Although the epidermis shown in Plate 8 was damaged, with the dermis visible through scattered patches of epidermal cells, the wound of *G. gasterostei* appeared similar in fish with a normal epidermis. The marginal hook wounds of *G. gasterostei* were larger than those of *G. arcuatus* or *G. bullatarudis*, and may have reflected differences in the depth to which these sclerites penetrated. The hamuli did not appear to be involved in attachment in the case of *G. gasterostei*.

*(d) Gyrodactylus rarus*

*Gyrodactylus rarus* was usually attached to the gill filaments (Plate 10), although they were also found on the gill arches and rakers. On removal of the parasite from the gill, the hamuli snapped, and most of the points remained in the wound (Plate 11 and 12). Deep pits could be seen surrounding the hamuli (Plate 11), suggesting that they penetrated a considerable distance into the gill epithelium. The hamuli were free of tegument for a considerable portion of their length (Plate 12). Although the marginal hooks did not leave conspicuous wounds, and although most pulled free from the host when the parasite was removed (Plate 12), one remained embedded in gill tissue (Plate 11), suggesting that they may be of some importance in attachment.

**Observations on the activity of parasites on living hosts**

Four patterns of activity can be discerned in *Gyrodactylus gasterostei*:

(a) Looping movements in which the cephalic lobes are attached to the host and the haptor is moved forward to meet it in a 'leech' - like movement. This is the principal means of movement about the host.

(b) Reaching movements, in which the body is extended considerably, with cephalic lobes spread, although the anterior of the body does not actually attach to a substrate. The cephalic lobes may be touched to the host skin, to other objects touching the fish, or merely directed away from the host into the water. This movement is thought to be exploratory.

(c) Feeding movements, in which the body is extended considerably, and the cephalic lobes are attached to the host skin. The pharynx is extruded onto the skin, and the pharyngeal processes placed in contact with the epithelial cells (Plate 13). The pharynx contracts rhythmically during feeding, which lasts for two or three minutes. The cephalic lobes are then released, and the anterior of the parasite contracts, forcing the ingested material into the gut crura. The feeding wound is a small crater, 20-30 μm in diameter, in the fish epidermis (Plate 14; Fig. 20). The depth of the wound is difficult to
Plate 7. *G. gasterostei* attached to skin of stickleback.
Plate 8. Wound of G. gasterosteii upon skin of stickleback.

mhw - marginal hook wounds.

Heavy infection has severely damaged epidermis. Dermis visible through scattered epithelial cells (ec)

Note hamulus tips (ht) are shielded in tegument, and relatively blunt (cf. Plates 12 and 24).
Plate 10. *G. rarus*, attached to gill of *Pungitius pungitius*. 

- t- tips of hamuli embedded in wound.
- b- bacterial colony, indicating the possibility of secondary infection through attachment wound.

Deep pits (p) surround the hamuli where host tissue has shrunk away from hamuli during fixation. These pits indicate the depth of penetration of the hamuli.

A broken marginal hook (mh) is in view at extreme right of plate.
Plate 12. Ventral surface of haptor of *G. rarus*.

- *t*- broken tip of hamulus point (tip can be seen embedded in wound in Pl. 12.
- *mh*- marginal hook sickle.

Hamulus point appears to be free of tegument back as far as arrow.

ph - pharynx placed against host epithelium.
Plate 14. Possible feeding wound of G. gasterostei in host epidermis.
Fig. 20. Feeding wounds of *G. gasterostei* in the host epidermis.

a) X 100

b) X 1000.
estimate, as true wounds were difficult to distinguish from incidental damage in sections. The bottom of the wound failed to stain with alum carmine, a basophilic stain, and appeared acellular. This suggests that the wound extended to the dermis. Epidermal cells of *G. aculeatus* have a diameter of approximately 4-5µm, suggesting that a parasite would remove 25-40 cells in each feeding bout.

(d) Parasites were also observed giving birth. This process took place very rapidly, the daughter emerged head-first from the mid-ventral birth pore of a parent fluke, whereupon it grasped the host epidermis and pulled its opisthaptor clear. The parent parasite remained extended for a short period, before contracting strongly.

These activity patterns are summarised in Table 16. The parasites are relatively inactive during life (Table 17), although they spontaneously perform reaching movements, the most frequently observed form of activity. The activity of the parasites was correlated with the stage of embryo development: parasites without embryos were significantly more active (P < 0.01, Mann-Whitney U test) than those with small or large embryos (Table 17). Activity also decreased significantly (P < 0.01, Mann-Whitney U-test) after each feeding bout, which occurred approximately every 15 minutes. Copulation was not observed amongst parasites attached to living hosts.

The behaviour and survival of *G. gasterosteii* after host death

After the parasites became detached, their activity increased dramatically (Fig. 21). This increase was made up of reaching and looping movements, serving to both disperse the parasites throughout the habitat and to increase their probability of attachment. When first detached, parasites showed spontaneous movement, but after several hours, they remained inactive, responding only to vibration and water currents with vigorous reaching and looping. The survival of parasites was dependent upon temperature (Fig. 22a), but activity remained relatively constant across the range of temperatures used (Fig. 22c). The mortality rate of parasites increased in a manner proportional to the period for which the parasites were detached. At high temperatures (10-20°C) mortality probably increased exponentially with the period detached (Fig. 22a), but at low temperatures it remained constant for much of the free-living period.

The effect of *G. gasterosteii* upon the skin surface of *Gasterosteus aculeatus*

The epidermis of *G. aculeatus* is 20.7 (± 3.4 µm) thick, composed of 5-8 layers of epithelial cells, which are columnar in the deeper layers, becoming squamous at the
Table 16. Characteristics of parasite behaviour patterns.

A) Reaching behaviour.
   1) Body extends to 1.5-2X resting length.
   2) Cephalic lobes spread.
   3) Probable sensory function.

B) Looping behaviour.
   1) Body extended, cephalic lobes spread.
   2) Cephalic lobes attached to skin.
   3) Opisthaptor released, moved to reattach behind head.
   4) Head released.

C) Feeding behaviour.
   1) With body fully extended, cephalic lobes attached to skin.
   2) Pharynx extruded.
   3) Body held away from fish.
   4) Head released, followed by strong contraction.
   5) Anterior third contracts rhythmically, posterior remains motionless.

D) Giving birth.
   1) Birth pore of mother held over surface of fish.
   2) Daughter forced through birth pore by mother.
   3) Daughter grasps fish and pulls clear of mother.
   4) Mother remains motionless for a short period, before contracting strongly.
Table 17. The influence of embryo development upon activity.

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<th>Feeding</th>
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Table 17B. The influence of feeding upon parasite activity.

<table>
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<th>B 0-5 minutes before feeding</th>
<th>C 0-5 minutes after feeding</th>
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<td>3.7</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>13</td>
</tr>
</tbody>
</table>

Significance of differences:
- A vs B Not significant
- A vs C Significant (P<0.05)
- B vs C " Significant (P<0.01)
Fig. 21. The increase in looping activity of G. gasterostei after host death.

mean number of looping movements per minute, ± 1 standard deviation.

Dashed line represents point of host death. Mean value for activity before death compounded from data presented in Table 17, and does not represent activity at a specific time before host death.
Fig. 22. The survival and activity of detached G. gasterostei in relation to environmental temperature and oxygen tension.

Relationship between survival and time (A) suggests that instantaneous mortality rate increased exponentially throughout period detached at 10, 15 and 20°C.
Numerous goblet mucus cells occur throughout the epidermis. These have a characteristic distribution on the body (Fig. 24), being sparsely distributed on the fins, and most abundant on the penduncle flanks and throat. Goblet cells stain positively with alcian blue (pH 1 and pH 3), and with PAS, and show a greenish blue metachromasia with toluidine blue. This suggests that they contain both sulphated and acidic mucopolysaccharides.

Fish which were heavily infected with *Gyrodactylus gasterostei* showed a reduction in the thickness of the epidermis (Plate 15). In fish carrying increasing infections of *G. gasterostei*, the density of mucus cells showed a weak negative correlation \( r = -0.43 \) with parasite density (Fig. 25). In fish carrying increasing populations of *G. gasterostei*, however, the density of mucus cells was significantly higher than in declining or static infections (Table 18), or in uninfected fish.

**DISCUSSION**

All of the haptor sclerites of *Gyrodactylus* are involved in the attachment of this parasite to its host, but the relative importance of marginal hooks, hamuli, ventral bar and dorsal bar in the different species groups varies. In *G. farlowellae*, which is the most primitive gyrodactyliid known, the hamuli have the most important role in attachment (Harris, 1982c), penetrating the host epithelium to a considerable depth (Ch. 7, Plate 23). The marginal hooks are very small in the adult, serving merely to pin the edges of the opisthaptor, preventing the parasite twisting about the longitudinal axis of the hamuli. The marginal hooks, aided by the small, dumbbell shaped ventral bar, which is pressed into the host epidermis, prevent water currents from lifting the anterior of the haptor, an action which would tear the hamulus points from the host skin. The dorsal bar holds the hamuli close to each other, preventing them from splaying.

*Gyrodactylus arcuatus*, which can attach to both skin and gill tissue, has a similar attachment apparatus to *G. farlowellae*. The hamuli do not penetrate the host epidermis as deeply, although the marginal hooks are larger, and have a more important role in attachment. The suppression of the dorsal root of the hamuli in the gyrodactylids may be associated with the reduction in the importance of the hamuli in gaffing the host epidermis. In *Gyrodactylus*, the roots of the hamuli contribute to the anterior pressure pad which is formed of the ventral bar. In *G. arcuatus*, the ventral bar has a large membrane,
Fig. 23. The structure of the epidermis of Gasterosteus aculeatus.

g- goblet mucus cell; ep-epidermis; d-dermis; m- melanocyte; ec-epithelial cell.
Fig. 24. The distribution of goblet mucus cells on the body of Gasterosteus aculeatus.
Plate 15. Changes in the epidermal thickness of sticklebacks infected with Gyrodactylus gasterosteii.

A). Uninfected host.

B). Fish infected with 950 G. gasterosteii.

e—epidermis; d—fibrous dermis; g—goblet mucous cell.
Fig. 25. The relationship between mucus cell density and parasite burden in fish carrying increasing infections of G. gasterostei.
Table 18. Density of goblet mucus cells in sticklebacks infected with Gyrodactylus gasterosteii.

<table>
<thead>
<tr>
<th></th>
<th>Uninfected or static infections</th>
<th>Decreasing infections</th>
<th>Increasing infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mucus cells</td>
<td>$\bar{x}$ 13.4</td>
<td>17.5</td>
<td>25.9</td>
</tr>
<tr>
<td>per 0.25mm strip</td>
<td>$\sigma$ 3.9</td>
<td>5.2</td>
<td>9.3</td>
</tr>
<tr>
<td>$n$</td>
<td>6</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

**Legend**

- $\bar{x}$ arithmetic mean
- $\sigma$ standard deviation
- $n$ number of replicates
creasing its surface area, and large forward facing processes. These lie among the hamulus shafts and are pressed into the host epidermis by the roots of the hamuli.

The specialist skin parasite, *Gyrodactylus bullatarudis*, shows a further stage in the use of the hamulus roots as a pressure pad. In this species, the principal organs of attachment are the marginal hooks, and the hamuli fail to pierce the host epithelium, although they indent it deeply. The ventral bar is large, and although it has smaller processes than *G. arcuatus*, it probably functions as a pressure pad. This attachment mechanism is similar to that observed by Lester (1972) in *Gyrodactylus alexanderi*, which is also a species of the *G. eucaliace* group. It seems probable that the marginal hooks grip on the host pulls the dorsal lobe of the haptor down onto the ventral hamuli and bars, holding these sclerites in position against the surface of the host.

*Gyrodactylus gasterosteai* shows the penultimate stage in the trend of attachment by marginal hooks. In this group the hamuli do not penetrate the host epidermis, and as they do not leave an imprint, it seems unlikely that they exert much pressure upon it. The ventral bar is small, and probably does not act as a significant pressure pad. Instead, the marginal hooks penetrate the host epidermis relatively deeply, holding the parasite firmly in place. The marginal hooks inflict more damage upon the host in this group than in any other. The trend towards a reduction in the role of the hamuli and bars in skin parasitic *Gyrodactylus* culminates in *Isancistrum*, from cephalopod molluscs, and *Anacanthocotyle*, from teleosts. In both genera, hamuli and bars are not present (de Beauchamp, 1912; Fritsky and Fritts, 1970), and attachment depends solely upon the marginal hooks. It is not known whether these genera are related, or whether their resemblance is due to convergence (Malmberg, 1970).

In several genera of skin parasitic gyroactylids, the haptor has probably been modified to form a weak sucker. In *Macrogyrodactylus*, the marginal hooks are grouped at the posterior of the haptor, and the tegument has become expanded to form a suckorial disc (Malmberg, 1956; Khalil, 1970). The hamuli and bars are reinforced by several accessory sclerites, which probably act to raise the roof of the sucker, creating suction. In *Polyclithrum* and *Swingleus*, numerous additional sclerites radiate from the ventral bar to the margin of the disc-like lower lobe of the haptor (Rogers, 1969). The marginal hooks are again distributed at the front and back of the haptor. The entire organ probably functions as a sucker, the additional sclerites strengthening the roof of the disc.
Fig. 26. Attachment strategies and hamulus morphology of Gymnodactylus species groups.

GILL PARASITES - curved hamuli
- G. elegans
- G. marinus
- G. rarus
- G. emembratus
- G. perlucidus
- G. anguillae
- G. unicoopula

SKIN PARASITES - straight hamuli
- G. phoxini
- G. eucaliae
- G. flesi
- G. katherineri
- G. wageneri
- G. arcuatus
- G. latae

GILL AND SKIN PARASITES
The aggregation of marginal hooks at the posterior and anterior of the haptor increases the strength of attachment at these points, where the parasite is most likely to become detached. This is analogous to the haptor of *Entobdella soleae*, in which longitudinal movement is prevented by the posterior hamuli (Kearn, 1964).

Amongst gill parasitic gyrodactylids, however, an increase in the role of the hamuli during attachment can be seen. In the case of *Gyrodactylus rarus*, although the hamuli have short dorsal roots, their strong curvature, probably increases the power with which they can be embedded into gill tissue. In addition to the importance of the hamuli, the marginal hooks are also very large, and play an active role in the attachment mechanism. From a consideration of sclerite morphology, it is probable that members of the *G. elegans*, *G. harrowi*, *G. emabrmanatus*, *G. perlucidus*, *G. marinus* and *G. anguillae* species groups have a similar mechanism (Fig. 26), as in all cases, their hamuli are strongly curved and their roots diverging. In contrast, the skin parasitic *G. phoxini*, *G. eucaliae*, *G. flesi*, *G. atherineri* and *G. wageneri* species groups have straight shafted hamuli with long parallel roots. Species of the gill parasitic *G. unicopula* species group, and the skin parasitic *Gyrodactylus nemacheili* have hamuli with roots which are folded inwards onto the shafts (Fig. 26). Although capable of attachment to both gills and skin, it is not clear how this attachment mechanism functions.

Species of *Gyrodactyloides*, from the gills of marine salmonids, gadids and clupeids, also have strongly curved hamuli, and appear to have an attachment mechanism similar to that of *Gyrodactylus rarus* (Fig. 27). The ventral bar of this genus is elaborated (Bychowsky, 1949), and may have an important role in attachment. Three species of *Macrogyrodactylus* are found on the gills of the host (Paperna, 1977), although it has been argued that species of this genus have an attachment mechanism adapted for skin parasitism (*vide supra*). It is possible that these species have secondarily colonised the gills of the host, in the way that some capsalids are found in the gill chamber (Sproston, 1946), although they are principally adapted for skin parasitism (Kearn, 1964; Williams, Ellis and Spauld, 1976). The attachment mechanism of other gill parasitic gyrodactylids, such as *Archigyrodactylus*, has not been studied.

The firmest attachment mechanism of any gyrodactylid is that of *Gyrodicotylus pallieni* which has the haptor modified to form two partially separated suckers (Ch. 6). This parasite infects the oral epithelium of *Xenopus*, attaching to a substrate which is considerably thicker and which may be more easily deformed than
Fig. 27. Attachment strategy and haptor morphology within the Gyrodactyloidea.
fish skin. Although the oral epithelium of *Amphiporus* is more similar to fish skin than to gill tissue, the habitat of *Gyrodactylus* resembles the gill chamber of a fish in its degree of enclosure, preventing the fortuitous transmission of parasites. The trend towards firm attachment in gill parasites and weak attachment in skin parasites may be in response to differences in the structure of the substrates which affect the function of the haptor. The irregular surface of the secondary gill lamellae may prevent the use of the hamulus roots and ventral bar as a pressure pad, and may require that the parasite actively gaff the tissue with the hamuli. However, *Gyrodactylus* from the gills are so small that irregularities in surface topography would be unlikely to affect their attachment (Plate 10). Also, unlike polyplisthocotyleans, which show a marked preference for a particular microhabitat within the gill chamber (Llewellyn, 1956), gyrodactylids may frequently be found throughout the gills. Thus, *G. marinus* from *Pungitius pungitius*, may be found attached to the gill arches, the rakers, primary lamellae and secondary lamellae. The differences in microtopography of these habitats make it unlikely that the surface structure of the fish skin could influence the attachment strategy of its gyrodactylids. Finally, *Gyrodactylus gallieni* gaffs the host with its hamuli, although its substrate resembles teleost skin more closely than teleost gill.

Alternatively, the difference in attachment strategy of skin and gill parasitic gyrodactylids may be related to the occurrence of a host response against these parasites. Such a response has been shown to cause the disappearance of *Gyrodactylus alexanderi* (see Lester, 1972; Lester and Adams, 1974) and *G. asterostai* (Harris, 1980, Ch. 4) from the skin of sticklebacks. Stott (personal communication) has suggested that it may also occur against *G. bullatarudis* on guppies (*Lobistes reticulatus*). A host reaction has also been inferred to take place against the capsidal *Epibella melleni*, a skin parasite (Nigrelli, 1935a, b, c, 1937), and Paperna (1954) demonstrated a host reaction against the gill parasite *Dactylogyrus vastator*, mediated through the proliferation of hyperplastic gill tissue. However, Paperna (loc. cit.) also showed that this response was not stimulated by the presence of the smaller *D. extensus* or *D. anchoratus*, and it is probable that the majority of small ancyrocephalids, tetraonchids and dactylogyrids do not normally invoke a host reaction. If the gills of teleosts are less sensitive to the presence of monogeneans than the skin, they may be able to tolerate more damage inflicted during the attachment of gill parasites. However, the host reaction against *Gyrodactylus* has been demonstrated in only one teleost, the 3-spined stickleback...
Further work is necessary to determine the range of teleost families which are capable of mounting such a response, and to investigate the reaction of teleosts to gill parasitic gyroactylids.

*Gyrodactylus gasterosteii* feeds in the same manner as *Acanthocotyle lobianchi*, *Entobdella soleae*, *Leptocotyle minor* and *Dendromocotyle Kuhlii* studied by Kearn (1963a, 1965, 1979b) and the digenean *Transversotrema patialense* (see Mills, 1979). The epidermal cells are digested extra-corporeally within the chamber of the anterior pharynx, and the resulting cell lysate is forced into the intestine by the muscular posterior chamber of the pharynx. Kearn (1963) found that in *Entobdella soleae*, digestive enzymes were produced by cells in the posterior pharynx which were passed forwards during feeding to touch the skin. The pharyngeal processes of *Gyrodactylus* may function in a similar manner to these cells during feeding, although it is not known whether these structures produce digestive enzymes. Several *Gyrodactylus* species groups have short pharyngeal processes, unlike the long processes of the *G. kageneri* group, and it is possible that the feeding mechanism differs in these groups.

*Gyrodactylus gasterosteii* does not breach the host dermis, although the feeding wound extends as far as this layer. This is probably also the case for other gyroactylids, although Harris (1982c) found that, in heavy infections, *Gyrodactylus farlowellae* breached the dermis and fed on blood, and Khalil (1970) observed that *Macrogryrodactylus polperti* facultatively fed upon blood. The epidermis of *Polypterus*, the host of *Macrogryrodactylus polperti*, is unusually thin, overlaying very thick ganoid scales. The ingestion of blood from vessels between the scales may therefore be a normal occurrence in the feeding of this parasite.

There are two probable reasons for the restriction of the feeding of monopisthocotyleans to the epidermis. Firstly, this layer regenerates much more rapidly than the dermis (Kearn, 1971; Finn and Nielsen, 1971a, b), which is composed of collagen fibres. Consumption of the dermis would expose the fish to the risk of secondary infection through slowly healing wounds, while providing relatively small quantities of nutrients for the parasite.

As gyroactylids lack a specific infection phase in their life cycle, populations of parasites on individual hosts can rapidly increase to the point
where they may become pathogenic to the host. *Gyrodactylus gasterosteii* feeds approximately every 30-60 minutes at 15°C, removing 20-30 epidermal cells on each occasion. A wound of this size restricted to the epidermis would heal rapidly (Lester and Adams, 1974a), but even so, in heavy infections this cell layer becomes badly damaged (Plate 15). A strong selective pressure probably acts to reduce the damage done to the host, during both attachment and feeding of *gyrodactyliids*.

When moving about on the host, *Gyrodactylus gasterosteii* is temporarily attached by the adhesive glands of the cephalic lobes, which are much weaker than the opisthaptor. This increases the parasite's chance of dislodgement, and its inactivity upon the living host is probably an adaptation reducing this risk. The parasites do, however, show some periods of activity when attached to the host. The rate of movement of the parasite increases considerably shortly after giving birth, when the uterus is empty (Table 17). Such a period of activity may increase the probability of accidental dislodgement, a hypothesis which is supported by the pattern of age-specific mortality observed in this parasite (Ch. 4). Moreover, as *G. gasterosteii* is protogynous, dislodgement at this stage of the life cycle reduces the number of parasites in the population with a functional male reproductive system. This period of activity disperses the parasites upon the host, may prevent the formation of local concentrations of parasites. Reaching activity is seen throughout the life of *G. gasterosteii*, and may increase the probability of transmission to a host when fish touch together, for example when shoaling.

The dispersal behaviour of *Gyrodactylus* has not been observed amongst any other groups of fish ectoparasites. However, it is probably a reflection of the 'microparasitic' reproductive strategy of *Gyrodactylus*, which Anderson and May (1979a,b) have shown can generate an unstable host-parasite interaction.

This instability is created by the ability of the parasites to grow unchecked until the host dies, without undergoing a phase of transmission. The dispersal activity of *G. gasterosteii* reduces the stress placed upon one host or part of the host, and reduces the proportion of hosts carrying very heavy parasite burdens. The dispersal of local concentrations of the parasites may prevent the triggering of a host reaction against them at low absolute population densities. Several protozoan parasites (*Trichodina, Apionema, Chilodonella*) also have a
microparasitic strategy, but these have not been examined in sufficient detail
to reveal dispersal mechanisms.

The failure to observe copulation in Gyrodactylus gasterostei is of considerable
interest. Malmborg (1956a) observed copulation in Macrogryrodactylus polypteri,
as did Malmborg (personal communication) and Braun (1966), in several
Gyrodactylus species groups. However, the reproductive mechanism of
Gyrodactylus is imperfectly understood (Katheriner, 1994; Gille, 1914; Braun,
1966), and the role of sexual processes is unclear. Observations of G.
alexanderi (see Lester and Adams, 1974), G. gasterostei (Ch.4) and a G. wageneri
species from goldfish (Braun, 1966) have shown that these parasites continue to
give birth to daughters over many generations when isolated individually on
fish. As the parasites are protogynous, the male system failing to develop until
after a parasite has given birth for the first time, it is impossible for the
mother fluke to copulate with the daughter, thereby precluding any possibility
of cross fertilisation occurring. Moreover, the timing of the development of
the male system of G. gasterostei is such that the ovum of the second daughter
of a parasite has started development before the male reproductive system
becomes functional, thereby preventing self-fertilisation. The role of sexual
reproduction in these species is therefore debatable, although Braun (1966)
considered that sperm was necessary for the development of an ovum. The age
structure of the population of G. gasterostei being biased in favour of young
flukes, further reduces the opportunities for cross fertilisation. It is, however,
possible that in other species groups with different attachment strategies,
copulation is more important to the reproductive biology of the parasites. In
the protandrous G. longus, copulation takes place frequently between younger flukes (Ch 7),
and normal cross-insemination probably occurs.

The behaviour of Gyrodactylus gasterostei changes significantly after detachment
as the frequency of both looping and reaching movements increase. As pointed
out by Kearn (1976) and Llewellyn (1981b), adult gyrodactylids cannot swim,
and detached parasites will sink to the river or lake bed before resuming active
movement. If a host dies and sinks, the activity of the parasites will serve
to disperse them away from the body, which will rapidly become anoxic as it
begins to decay. Furthermore, the reaching activity may lead to the parasites
being transmitted to other fish feeding upon the corpse. G. gasterostei has a
greatly curtailed survival period in deoxygenated water (Fig. 22b), so the
adaptive significance of dispersal away from a dead host is considerable.
Malsberg (1970) observed species specific differences in parasite behaviour after host death, and found that although G. rarus dispersed away from the host, G. pungitii did not. The increase in activity of G. gasterostei after host death may be in response to the cessation of water currents over the fish, as it occurs before post-mortem changes in the structure of the skin have taken place.

G. gasterostei survives for 2-3 days at low temperature, but for only 20-40 hours at 15°C. After an active period when first dislodged, reaching and looping behaviour is seen only after the parasite has been disturbed by vibration or water current, reducing the energy expenditure when the probability of contacting a potential host is small. Similar behaviour is also observed in Pisciola geometrica, a leech with a similar transmission strategy (personal observation). At 10°C, 15°C, and 20°C, the instantaneous death rate of G. gasterostei increases with the period detached, in a similar manner to that shown by Anderson and Whitfield (1976) for cercariae of Transversotrema patialense, and considered typical of free-living stages of parasites by Anderson (1976). This pattern of mortality may be generated by the progressive exhaustion of the energy reserves of the parasites, which are unable to feed while detached. The reduced survival period at higher temperatures is probably due to the increased metabolic rate, exhausting the energy reserves of the parasite more rapidly. At 25°C, the mortality of the parasites does not show a simple relationship with the period of detachment, but is initially high, subsequently undergoing a period of reduced mortality before finally increasing rapidly with time after 50 hours. The phase of initially high mortality (the first 10 hours of detachment) may be equivalent to the period when the parasites are spontaneously active. After this time, the parasites respond only to external stimuli, reducing their energy expenditure and hence their rate of mortality. The period of infectivity of detached G. gasterostei has not been determined, but, as has been shown for Transversotrema patialense by Anderson and Whitfield (1976), it is unlikely to be equivalent to the total survival period. However, G. gasterostei is able to perform looping and reaching movements until near the end of its free living life, and is probably capable of infecting fish as long as this capacity for coordinated movement is retained.

Adult gyrodictyliids survive for a much longer period than the oncomiracidia of oviparous monogeneans, most of which survive for less than 24 hours (Llewellyn, 1972). The oncomiracidium, however, can utilise only the
remaining energy reserves from its yolk cells, whereas the gyrodactyldis
have tissue reserves accumulated from feeding, and the remnants of the most
recent meal within the gut at the time of detachment. The survival period may
be related to the size of the parasite, as Khalil (1964) found that the large
Macrogryrodactylus polypteri could survive for up to 9 days after becoming
detached. The survival of adult oviparous monogeneans when detached from
the host has been poorly studied. However, Kearn (1967a) found that
Entobdella soleae could survive for 2-6 days away from the host at 14-17°C.
Adult oviparous monogeneans are unable to reinfect a host after being detached,
and their food supply is limitless, they have little need for an energy store,
but it is not known whether adult gyrodactyldis have additional energy reserves,
allowing them to survive after the exhaustion of cellular energy sources and
the gut contents. The nature of monogenean energy reserves is unknown, but
may be glycogen, as in the case of digeneans (Smyth, 1966; Erasmus, 1975).

Infection with Gyrodactylus gasterosteoi has two effects upon the skin of the
host. At very high parasite densities, the thickness of the epidermis is
reduced, but when the parasite burden is smaller, if the infection is increasing
in size, the density of goblet mucus cells increases. Lester (1972)
described a host 'shothing' reaction in stickleback infected with Gyrodactylus
gasterosteoi in which sheets of mucus, with attached healthy parasites,
become dislodged from the skin of the infected host. The consequences of
this reaction upon the parasite population are discussed in Ch.4, and the
observations presented here on the histological changes in stickleback skin
during infection are probably related to it.

The increase in goblet mucus cells during the increasing phase of infection
may be interpreted as a stress reaction by the fish to the presence of
parasites. Pickering and Macey (1977) and Pickering, Pottinger and Christie
(1982) showed an increase in goblet mucus cell density in stressed salmonids.
In sticklebacks, the increase was observed at levels of infection which are
so low that it seems unlikely that it could have been related to the host's
parasite burden. However, although no other studies of aquatic hosts are
available for comparison, it is known that terrestrial vertebrates respond
to extremely low ectoparasite burdens. For example, Mellanby (1944) showed
that man responded to infections of the itch mite, Sarcoptes scabei, such
that an intense tissue reaction occurred when less than 20 parasites were
present on the host. In view of this, the low threshold for response to
Gyrodactylus observed (an increase in goblet cell density was observed in fish
with 20 parasites) is less remarkable.
In the declining phase of infection, the goblet cell density is reduced. This is not related to absolute parasite density, since it is reduced in fish which have lost most of their parasites, and in those which still carry a considerable number. It is possible that the reduction in density is due to the discharge of the goblet mucus cells onto the surface of the fish. This was suggested by Whitear (1970) as a response to stress by fish: the sudden discharge of large numbers of goblet cells would lift off the thin, normal mucus cuticle, produced from the epithelial cells. This is thought to greatly increase the slipperiness of fish under pursuit from a predator (Whitear, 1970), but would also remove the epibionts attached to the normal mucus. This response may form the basis for the host reaction observed by Lister (1972). In this context, the observations of Pascoe and Woodworth (1930) are of interest. These authors noted that in the case of fish exposed to several sources of stress, including heavy metal poisoning, starvation and parasite infection, Gyrodactylus rapidly disappeared from the fish. This may have been due to the heavy metals, or to the production of excess mucus by the fish in response to heavy metal poisoning, as noted by Jones (1947). However, there is no direct evidence of goblet cell discharge lifting off the normal mucus layer (and in fact there is some debate as to the difference between mucus from epithelial cells and goblet cells). In addition, in the present work, fish were rarely seen shedding mucus, although the decline in parasite numbers frequently took place (Ch.4). It is possible therefore, that the goblet cells may have some other role in the host reaction against Gyrodactylus. Harris, Watson and Hunt (1973) and Pickering (1974) considered that goblet mucus was composed principally of acid mucopolysaccharides, particularly N-acetyl neuraminic acid. The present work has shown a similar composition (on the basis of histochemical criteria) of the goblet cell mucus of Gasterosteus aculeatus. Acid mucopolysaccharides probably have no antibiotic role, but merely contribute to the lubricative properties of the mucus. However, the mucus may also contain traces of other substances with an antiparasitic function. It is known that mucus contains non-specific lysozyme (Fletcher and White, 1973) and specific antibodies (Di Conza and Halliday, 1971; Smith, 1977; Fletcher and Grant, 1969a,b), but as detached parasites are healthy, they are unlikely to be implicated in the response against Gyrodactylus.

It is possible that the goblet cell mucus contains a substance which renders the host unattractive to Gyrodactylus, as has been implicated in host specificity
to this parasite (Ch.2). However, parasites attach with equal readiness to both uninfected hosts and those which are losing an infection, so such a change in the attraction of the fish is unlikely. The increase in goblet cells may influence the frequency of parasite feeding. It was observed that activity increased shortly before feeding commenced, and that some of this activity was probably directed at finding a suitable area of epidermis. If the presence of large numbers of goblet cells renders the epidermis unsuitable for feeding, the parasites may become more active, and more exposed to the risk of dislodgement.

The reduction in goblet cell density and epidermal thickness as infection levels increase may be due to the grazing of the epidermis by the parasites. As the goblet cells may be involved in the host reaction, this may explain the observation of Lester and Adams (1974a), and of the present work (Ch.4), that if a host reaction does not take place, the infection may increase and kill the host.

The biology of Gyrodactylus gasterostei differs considerably from that of oviparous monogeneans, in ways which may have an important effect upon the population dynamics of the parasites. The most important differences are in the viviparous reproduction and in their ability to survive away from the host.

Viviparity greatly reduces the importance of parasite immigration onto the fish in determining the final population size. Instead, factors influencing the parasites in situ upon the host are most significant in controlling abundance. Gyrodactylus upon the skin of the host may generate a host skin reaction, leading to their becoming dislodged. Skin parasitic species show adaptations reducing the strength of attachment, and hence the damage inflicted upon the host, which may reduce the probability of invoking a host response. However, this reduction in the strength of attachment may also increase the probability of accidental dislodgement, the consequences of which are explored more fully elsewhere (Ch.4). Gill parasitic species do not appear to be exposed to this selective pressure, as they actively gaff the gill epithelium. Thus, the attachment mechanism of the parasite is an important factor influencing its ecological strategy. The survival of parasites away from the host, and their ability to reinfect other hosts is also of significance. In the case of Gyrodactylus species, reproduction is not automatically linked with transmission. The size of the sub-population of detached parasites will not be closely linked with the reproductive output of the population on the fish, but will be influenced also by factors affecting mortality and detachment. As the survival of detached parasites may, in some cases, be considerable, they cannot be ignored in a consideration of population dynamics. The survival of these parasites may also reduce the importance of the host reaction and host death in regulating the G. gasterostei population.
Ch. 4. LABORATORY STUDIES OF THE POPULATION DYNAMICS OF *GYRODACTYLUS GASTEROSTEII*. 
INTRODUCTION

The interaction of an organism with its environment is ultimately reflected in the numerical behaviour of its population. In the case of parasites, two environments affect the organism, that provided by the host (the micromilieu, Dogiel, 1964) and that of the habitat surrounding the host (the macromilieu). The relative importance of these varies with the degree of intimacy of the host-parasite relationship, but both can interact with the biological properties of the parasite to generate an observed pattern of population change. In the case of *Gyrodactylus*, the external site of infection exposes the parasites to the macromilieu, and it is reasonable to suspect that they are strongly influenced by this (Malmberg, 1956b; Chubb, 1977). A section of the present work (Ch.5) has examined population changes of *Gyrodactylus* in nature, in which both macro- and micromilieu are free to vary. In order to obtain maximum information from this study it was necessary to have a knowledge of the important parameters of the host-parasite interaction, and to examine the effect of changes in micromilieu under laboratory conditions of controlled macromilieu. This chapter considers these two aspects of *Gyrodactylus* population dynamics.

Although the population dynamics of free living organisms are well studied, the complexity of parasitic interactions has hindered research into their population biology (Anderson, 1976; Whitfield, 1979). Amongst free living animals, most attention has focused on the growth and regulation of populations within single habitat patches, which are usually large and widely spaced relative to the size and powers of dispersal of the organism. However, this approach is not suitable in an analysis of parasite-host population dynamics. The basic unit of the habitat is the host, which is usually capable of supporting only a relatively small parasite population, and which has a finite life, necessitating the dispersal of parasites between hosts. The parasite population biology therefore requires a consideration, not only of population growth and regulation within a single host (habitat patch), but also of the distribution of parasites throughout large numbers of hosts, and of their movements between these hosts. Parasites can also influence their habitats, by stimulating a host reaction or by killing the host, and the importance of these in the regulation of the parasite 'supra-population' (Esch, Hazen and Aho, 1975) must also be considered.

In the case of most parasites, reproduction and transmission are associated such that the offspring of one parasite generation undergo a phase
of obligatory dispersal before colonising another host. In this case, the parasite population on a single host is increased only by immigration (Anderson and May, 1978, 1979a,b; May and Anderson, 1978). In Gyrodactylus, however, reproduction in situ upon the host takes place, a life cycle more typical of microparasites (Anderson and May, 1979a) such as viruses, bacteria and protozoans. Population growth within the host can be estimated from a knowledge of reproductive rate, and of the rate of loss of the parasites from the host. These parameters are perhaps the easier to determine in a parasite host interaction, in comparison with those regulating the movements of individuals between hosts. In the case of Gyrodactylus, the fecundity and maximum potential rate of reproduction have been determined for G. furus, G. bullatarudis, G. alexanderi and G. gasterosteii, by a technique of direct observation (Turnbull, 1956; Bychowsky, 1957; Lester and Adams, 1974a; Harris, 1980a; Scott, 1982b). However, the measurement of actual population growth upon the host and parasite mortality has been less adequately studied. It has been attempted only for G. alexanderi by Lester (1972) and Lester and Adams (1974a,b) and for G. bullatarudis (see Scott, 1982b). Population growth of G. alexanderi upon Gasterosteus aculeatus showed a pattern of increase and subsequent decline, which Lester and Adams (loc. cit) attributed to a host response, mediated through shedding of living parasites attached to mucus flakes (Ch.3). Scott (personal communication) observed a similar decline in G. bullatarudis, which was obscured overall by the large variation in individual population growth rates on different fish (Scott, 1982b). However, neither Lester and Adams (1974a,b) nor Scott (1982b) took account of loss of parasites from the host other than those associated with natural mortality, and in both cases, the experimental techniques used (use of anaesthetics, constant handling of fish when determining age specific mortality) make interpretation of their observations on Gyrodactylus mortality difficult.

The observations on Gyrodactylus population dynamics of Lester and Adams (1974a), Harris (1980a) and Scott (1982b) have shown that rapid reproduction is the major source of population increase upon the host, and that in comparison with other parasites immigration and transmission are less important. However, unlike most monogeneans, transmission of Gyrodactylus can take place throughout the cycle, and a knowledge of the effect of this aspect of its biology is essential in understanding of the overall dynamics of the parasite supra-population.
Transmission of *Gyrodactylus* can take place in two ways:

(1) by contact between hosts, allowing parasites to move between them, and

(2) by movement of detached parasites onto the skin of a fish. The free-living parasites may have originally become detached by accidental dislodgement from a living host, or they may have moved from the surface of a dead host (Ch.3). Malmberg (1970), on the basis of barrier experiments, considered that movement of parasites between touching hosts was the only route of transmission used, but Hoffman and Putz (1964) and Parker (1965) both indicated that transmission by detached parasites was also important. Few other fish ectoparasites are comparable with gyrodactylids in this respect, because once the parasite has become established upon a host, it is committed to infecting it, and will not move from it at a later stage of the infection. Although the branchiuran *Argulus* is transmitted by swimming between hosts (personal observation), the route of transmission of other crustaceans with a similar life cycle (e.g. cymothoids and the amphipod *Cyamus*) has not been studied. Amongst arthropods with a similar life cycle which are parasitic upon terrestrial vertebrates (including fleas, lice and pupiparans), transmission both by host-host contact and by detached parasites may take place (Smart, 1942; Mead-Briggs, 1964; Marshall, 1976). However, except in the case of fleas, which are transmitted principally by detached parasites (Buckle and Harris, 1980), the relative importance of these routes of infection has not been determined.

The importance of transmission for parasite population dynamics resides in its dependence upon host density. Transmission by host-host contact is restricted to periods when hosts touch each other deliberately (sexual reproduction, giving birth), or accidentally, during shoaling. Transmission over greater distances is only possible if the parasite leaves one host and becomes free living. In a shoaling organism, transmission by detached parasites might be expected to be dependent upon host density, whereas transmission during host contact might be related to the behaviour of the hosts (Bychowsky, 1957; Llewellyn, cited in Kearn, 1976).

The distribution of parasites within the host population has attracted considerable attention (Crofton, 1971a, b; Anderson, 1976; Anderson and May, 1978; May and Anderson, 1978). As with free living organisms, populations of which can be modelled by a negative binomial distribution (Southwood, 1966), parasite populations are normally overdispersed within the host population (Williams, 1944, 1964; Southwood, 1966; Crofton, 1971a). However, the function best describing this overdispersion is not the negative binomial, and has been
the subject of considerable debate. Williams (1944, 1964) considered that the logarithmic distribution (excluding the proportion of uninfected hosts in the population) to be most appropriate for modelling the distribution of ectoparasitic arthropods. However, Crofton (1971a) suggested that the truncated negative binomial function modelled parasite distributions more accurately, the truncation being due to the death of heavily infected hosts as a result of parasite infection. This led Crofton (1971b) to develop a model of parasite population regulation in which the death of heavily infected hosts removed sufficient parasites from the population to regulate their reproductive output. A host reaction may also act in this manner, being the equivalent of host death in its effect upon the parasite population.

Anderson and May (1978) have shown that the precise nature of the parasite distribution within the host population is unimportant for regulation to occur. However, the Gyrodactylus-host interaction cannot be modelled according to the assumptions of a parasite life cycle used by May and Anderson (1978) for two reasons. In the first place, parasite reproduction in situ destabilises the host-parasite interaction (Anderson and May, 1978, 1979a, b; Anderson, 1980, 1981), preventing regulation by host death or a host reaction, and is capable of re-infecting a new host. All predictions concerning the regulation of parasite populations have been made on the assumption that parasites are killed after host death or host reaction (Anderson and May, 1978, 1979a, b; May and Anderson, 1978). Thus, the regulation of Gyrodactylus populations by host death and host reaction is liable to be complicated by the survival of detached parasites, especially at high host densities, when the probability of these reattaching to the host is increased.

The regulation of Gyrodactylus populations must, therefore, occur at two levels: on the individual host, where stochastic fluctuations in birth and death rates (Bradley, 1972) and density dependent (including host response) processes may limit population size, and in the population as a whole, where the interaction of the distribution of parasites within the host population, parasite transmission and host death are important in determining the size of the parasite supra-population.

Processes affecting the population on an individual fish and within the host population as a whole may be influenced by environmental factors, of which the most important are temperature and host density, modified in nature by reproduction and shoaling behaviour. Temperature affects the reproductive rate of organisms in a complex manner (Wieser, 1973), and Hutchinson (1978) has
pointed out that an increase in temperature may reduce the carrying capacity of an environment, because of the increase in the metabolic rate of the organisms under consideration. Host density may also be an important factor in parasite population dynamics because the transmission rates of the parasites may vary in a non-linear fashion with density. The present work set out therefore to develop an understanding of the factors regulating both infra- and supra-populations of *Gyrodactylus gasterosteii* on sticklebacks, and to examine the effect of temperature and host density upon the overall size of the parasite population.

**MATERIALS AND METHODS**

**Sources of fish and parasites**

Sticklebacks (*G. aculeatus* var. *semi-armatus*) were collected from Walthamstow reservoirs (O.S. TQ 364957), the River Brent (O.S. TQ 240890), the Grand Union Canal, Wembley (O.S.TQ 192838) and from the river Colne, Colney Heath (O.S. TL 200061). All fish were considered genetically homogenous.

Fish were maintained in dechlorinated tap water in rectangular plastic buckets (Addis). Soon after capture, they were immersed for one hour in 1:4000 formalin solution in water, to remove ectoparasites (Davis, 1961; Lester and Adams, 1974a). Subsequent examination of these fish failed to reveal living parasites. After treatment, fish were kept for 1 week before use in experiments.

To obtain large numbers of *Gyrodactylus gasterosteii*, uninfected hosts were maintained at high density (5-10 fish per litre) with infected hosts from the sites listed above. After 2-3 weeks at 10°C, the fish were killed and parasites on their skin surface identified and used in experiments. (*G. arcuatus*, not used in experiments, has conspicuous excretory bladders which can be seen using x 25 magnification. These bladders are not present in *G. gasterosteii*).

**Infection of experimental hosts**

Infected hosts were killed by cutting the spinal cord and placed in petri dishes containing dechlorinated water. They were kept for 4 hours in a 10°C constant temperature room, in which time most parasites moved from the host onto the glass dish, where they were more easily manipulated.

Uninfected experimental hosts were held individually in 50 ml dechlorinated water immediately prior to infection. The petri dish containing living
parasites was placed on the stage of a binocular dissecting microscope, allowing direct observation. The uninfected stickleback was then grasped about the pectoral girdle with forceps (Lester and Adams, 1974a), and the tail placed in contact with a detached parasite, which usually moved onto the host. No anaesthetics were used upon either parasites or hosts. After infection with a single parasite, the host was returned to the 50ml beaker to recover before being infected with a second parasite. Although Gyrodactylus is susceptible to desiccation (Braun, 1966), sufficient water was retained in the fin folds of the fish to maintain the parasite when it was held out of water. After infection of the host was completed, it was immediately transferred to experimental conditions.

The distribution of Gyrodactylus in the host population

Two large samples of sticklebacks were collected from Walthamstow reservoir (1980) and from the river Brent (February, 1981). The number of Gyrodactylus present on the surface of each fish was noted, and arranged into a frequency distribution. The observed frequency distribution of Gyrodactylus upon these hosts was tested for goodness of fit against predicted negative binomial (Elliot, 1977), poisson (Elliot, 1977) and logarithmic (Williams, 1964) distributions using the $\chi^2$ test (Elliot, 1977). The distribution of parasites upon their hosts in small samples (10-15 fish) from laboratory and natural infections was tested for its similarity to a negative binomial using U and T tests (Anscombe, 1950; Elliot, 1977).

The reproductive rate of Gyrodactylus gasterostei

Fish were infected with a single parasite and maintained individually in 50 ml jars containing dechlorinated tap water. They were kept in darkness at 5°C, 10°C, and 15°C in constant temperature rooms, without feeding, and examined daily, when the water was changed. During examination the body surface was scanned until the parasite was located. When it had given birth, it was killed, and the fate of the daughter followed. At each subsequent birth, either mother or daughter was killed or removed to another fish. From these experiments, data on the frequency of births were compounded into a dendrogram of the type described by Lester and Adams (1974a) from which reproductive rate was calculated.

The pattern of population growth

Groups of 10 uninfected fish were each infected with 5 Gyrodactylus gasterostei (see Ch.2) and placed in 4 L of dechlorinated water. These fish were maintained for 2, 4 and 6 weeks at 5, 10 and 15°C in the dark without feeding. A 50% water change was carried out every 3 days.
At the end of the experimental periods, the fish were sacrificed and their *Gyrodactylus* infections counted. All parasites were removed from the host and examined to determine age. At interim periods, every 3-5 days, 5 fish were removed from each replicate at random. They were gripped in forceps, held in a crystallising dish containing dechlorinated water and scanned for *Gyrodactylus*. The effect of this treatment upon the host was probably slight (Lester and Adams, 1974a; Ch.2). Although some increase in the rate of detachment of parasites might be expected, preliminary experiments using fish handled daily showed that this was not significant. After counting, the fish were returned to the experimental containers. At the same time, three 10ml samples of water and sediment were pipetted from the aquarium and examined for *Gyrodactylus*. Five replicates were performed at each combination of temperature and experiment duration used.

**Determination of age in *Gyrodactylus gasterosteii***

Individual *G. gasterosteii* were maintained upon sticklebacks kept isolated in 50 ml water at 5°C, 10°C and 15°C. Parasites of known age were examined microscopically to determine the correlation between age and the stage of development of the male reproductive system (presence or absence of the penis), and the size of the embryo mass. The ageing system derived from this is shown in Fig. 34.

**The transmission of parasites**

The transmission rate at 10°C was determined at a range of host densities. Two fish, one heavily infected with *G. gasterosteii*, the other uninfected, were placed in dechlorinated water. The containers used were (a) 50ml beakers, (b) 150ml crystallising dishes, (c) 250ml glass jars, (d) 11 glass jars and (e) 4 4 plastic buckets. The internal dimensions of these receptacles are indicated in Table 19. The two fish were maintained without feeding in the container for 24 hours, in a 12:12 h LD regime, then sacrificed and the number of parasites on each counted.

To assess the reattachment rate of detached parasites, large numbers of *Gyrodactylus* were placed in 50ml beakers or 150ml crystallising dishes containing dechlorinated water. A single, uninfected fish was introduced and the container was maintained in a 12:12 h LD regime without feeding at 10°C. After 24 hours the fish was sacrificed, and the number of *Gyrodactylus* attached to the fish and free in the dish were recorded.

To determine the importance of host-host contact in transmission, a barrier was placed in a 150ml crystallising dish, such that the parasites could pass
Table 19. Dimensions of containers used in transmission experiments.

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<th>Volume</th>
<th>Description</th>
<th>Dimensions</th>
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<tr>
<td>41.</td>
<td>Rectangular plastic bucket.</td>
<td>29cm X 21cm X 3cm.</td>
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<td>11.</td>
<td>Glass jar, circular base.</td>
<td>10cm diameter X</td>
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<td>12cm.</td>
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<tr>
<td>250ml.</td>
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<td>5cm.</td>
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<td>150ml.</td>
<td>Circular glass crystallising dish.</td>
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<td>3cm.</td>
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<td>50ml.</td>
<td>Glass jar, circular base.</td>
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through but hosts could not make contact. The barrier consisted of 2 layers of 2mm mesh, separated by a 3mm gap. A 2mm gap existed between the bottom of the barrier and the glass floor of the dish (Fig. 28).

The rate of movement of parasites through the barrier was determined by placing a known number of detached parasites on one side of the barrier, with an uninfected fish on the other side. The dish was left for 24 hours at 10°C in a 12:12h LD photoperiod, after which the number of parasites on the fish and on each side of the barrier was counted.

To determine whether this route was important in the transmission of attached parasites between fish, an infected host was placed on one side of the barrier, with an uninfected fish on the other. After 24 hours, the number of parasites present on each host was counted.

The effect of Gyrodactylus on host mortality
Wild caught infected hosts were scanned for Gyrodactylus and their initial parasite burden counted. They were placed individually in 4l dechlorinated tap water, and then maintained in a 12:12 hr LD regime at 10°C for 2 weeks and fed ad libitum. The death of hosts during the 14 day period was noted, and the parasite burden of the survivors counted.

RESULTS

The distribution of Gyrodactylus amongst the host population
The observed distribution of Gyrodactylus gasterosteus on sticklebacks for Walthamstow and the river Brent is shown in Fig. 29. The negative binomial distribution was fitted to both sets of data according to the technique of Elliot (1977) and tested using the $X^2$ test. No significant difference from the negative binomial distribution was observed in either sample ($P>0.05$).

In the case of small samples from laboratory and natural infections (Table 20, Ch. 5), a negative binomial distribution usually modelled the observed distribution adequately. At the start of experimental infections, and in samples of newly infected fry (Ch. 5), the distribution of parasites on the hosts sometimes approximated a poisson distribution rather than a negative binomial. In the laboratory, this may have been due to the initially uniform infection levels of the fish, which are subsequently modified by birth and detachment. In these cases, the infections have not yet attained the negative binomial distribution. In natural populations, the poisson distribution is probably generated by the initially
Fig. 28. Diagram of barrier used to separate hosts in transmission experiments.

Constructed from 150ml crystallising dish.
Fig. 29. Distribution of Gyrodactylus within large samples of sticklebacks from natural populations.

a) R. BRENT

observed distribution
fitted negative binomial
\( k = 2.64 \)

b) WALTHAMSTOW

observed distribution
fitted negative binomial
\( k = 1.11 \)
Table 20. Mean, variance and fitted distribution in laboratory populations of Gyrodactylus gasterostei.

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NB — Negative Binomial distribution.
P — Poisson distribution.

V/W — Variance/mean ratio (approximates to 1 in Poisson distribution).

random wave of infection experienced by the fry, before parasite reproduction significantly alters the size of the populations on individual fish.

The reproductive rate of *G. gasterosteii*

At 15°C, *G. gasterosteii* gives birth when 1 and 5 days old, at 10°C when 4 and 12 days old and at 5°C when 8 and 37 days old (Table 21). Little variation was observed in these periods. No parasites survived to give birth more than twice, although the presence of small embryos in utero suggests that those parasites which do survive the second birth could go on to give birth on a third occasion. The maximum intrinsic growth rate of the parasite population can be estimated from fecundity data. Lester and Adam (1974a) applied the equation

\[ R_0 = e^{rt} \]  

(1)

to obtain reproductive rate, where \( R_0 \) is equal to the net reproductive rate of the parasites, and \( T \) is the generation time. This equation is derived from the exponential growth equation

\[ N_t = N_0 e^{rt} \]  

(2)

(where \( N_t \) = the number of offspring produced after time \( t \), and \( e^r \) = the intrinsic rate of reproduction), in the special case where \( N_0 = 1 \), and \( N_t = R_0 \).

However, when generations overlap to the extent seen in *Gyrodactylus*, generation time becomes difficult to define and the use of equations (1) or (2) introduces considerable error into the estimation of the instantaneous population growth rate (Cole, 1954). The graphical estimation of Bychowsky (1957) is more accurate in this respect, and has been used to estimate the intrinsic growth rate of *Gyrodactylus gasterosteii* (Table 22). The reproductive rate of *G. gasterosteii* is strongly dependent upon temperature, increasing fourfold with a 5°C increase in temperature.

The growth of parasite populations

Populations of *G. gasterosteii* increased to a peak on their hosts at 10°C and 15°C, subsequently declining to a low level of infection (Fig. 30&31). At 15°C, this decline occurred after 10-12 days (Fig. 31), and was accompanied by the presence of detached parasites in the experimental tank. At this temperature, all of the parasites subsequently disappeared from the fish. At 10°C, the decline occurred after 35-40 days (Fig. 31), and some parasites remained on the fish until the end of the experiment. Detached parasites were recovered from the tank while the parasite population was still increasing at this temperature.

At 5°C, the decline in parasite abundance was not observed.
Table 21. The Fecundity of *Gyrodactylus gasterostei*.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>5°C</th>
<th>10°C</th>
<th>15°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at first birth (days⁻¹)</td>
<td>10</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>No. of births observed</td>
<td>7</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>Age at second birth (days⁻¹)</td>
<td>37</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>No. of births observed</td>
<td>1</td>
<td>9</td>
<td>13</td>
</tr>
</tbody>
</table>
Table 22. Maximum potential and observed population growth rates of *Gyrodactylus gasterostei*.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Maximum growth rate ((e^r)) calculated after Lester and Adams (1974a)</th>
<th>Maximum growth rate ((e^r)) calculated after Bychowsky (1957)</th>
<th>Observed maximum growth rate ((e^r)) calculated from regression of population growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.038</td>
<td>1.05</td>
<td>1.014</td>
</tr>
<tr>
<td>5</td>
<td>1.122</td>
<td>1.12</td>
<td>1.047</td>
</tr>
<tr>
<td>10</td>
<td>1.411</td>
<td>1.39</td>
<td>1.047</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td>1.18</td>
</tr>
</tbody>
</table>
Fig. 30. Population growth of Gyrodactylus gasterostei on sticklebacks after 2, 4 and 6 weeks.
Fig. 31. Population Growth of Gyrodactylus gasterosteii on sticklebacks over a 6 week period, sampled non-destructively.
During the period when parasite populations on the fish were declining, numbers of detached, healthy parasites, which were capable of reinfecting other hosts, were recovered from the sediment at the bottom of the aquarium. This suggests that the decline in population is mediated through an increase in the rate at which parasites become detached from the host.

The population growth rate during the initial phase of the parasite infection was determined by fitting a linear regression to the observed data, the slope of which was used to estimate the growth of the parasite population (Table 22). At each temperature, the maximum population growth rate attained prior to the decline in parasite infection was 30% of the maximum possible, estimated from their fecundity. This 70% reduction in growth rate was probably due to accidental dislodgement, although it may have been due to density dependent factors affecting fecundity.

The age-structure and age-specific detachment rate of Gyrodactylus gasterostei populations

Age-specific detachment of *G. gasterostei* was determined from the age structure of detached parasites, after the method of Deevey (1947). This technique requires that the population has attained a stable age structure, which in the case of *G. gasterostei* will occur after 5 days at 15°C and 12 days at 10°C. The development of stability could be delayed if the age structure of the parasite population used to infect the fish was biased in favour of older flukes. As natural populations of *G. gasterostei* from which these parasites were taken, are normally biased in favour of younger flukes (Ch.5), the stable age structures were probably attained even more rapidly than suggested here, and at the time of sampling, most populations would have attained stability.

Deevey's (1947) method also requires that the growth rate of the population concerned should be known. In the case of *G. gasterostei*, the average growth rate is known, but is affected by large, day to day stochastic variations. However, little effect was noted when the age-specific detachment rates were calculated for average population growth rate, maximum potential growth rate and zero growth rate, the largest range of variation expected in nature (Fig. 32).

At both 15°C and 10°C, the probability of detachment is greatest shortly after giving birth for the first time. This detachment was not associated with the birth itself but with a period shortly after a new oocyte has entered the uterus.
Fig. 32. Age specific detachment rates and survivorship of Gyrodactylus gasterosteii.
A similar increase in age specific detachment rate occurred after parasites had given birth for a second time.

The age structures of *G. gasterosteii* populations after 2, 4, and 6 weeks at 5°C, 10°C and 15°C are shown in Fig. 33. They are biased in favour of younger parasites, indicating rapid recruitment and significant detachment of older flukes. As the parasite populations start to decline, the older age classes (pre-and-post-second birth) and the newborn flukes become a less important proportion of the parasite population. This truncation is more apparent at high temperatures.

**The transmission of *G. gasterosteii***

*G. gasterosteii* was transmitted from infected to uninfected hosts when a barrier to host contact was present (Table 23), although the rate of transmission was significantly higher when the barrier was removed. Detached parasites infected hosts at a significantly higher rate than parasites which were initially attached to another host. This suggests that although uptake of detached parasites is very efficient, and can take place in nature, host-host contact is the more important route of transmission.

The rate of transmission is inversely dependent upon host density (Fig. 35), although the relationship between these parameter was non-linear. Transmission rate is not simply related to host density, and stabilises at 1-1.5% of the total parasite burden transmitted in 24 hours at low host densities. At higher host densities (40 fish l⁻¹), up to 10% of the total parasite burden may be transmitted in this period.

Transmission rate is independent of the size of the parasite population upon the fish over a wide range of parasite densities (Fig. 36). There is no evidence of an increase in transmission rate at higher parasite densities.

**The effect of Gyrodactylus upon host mortality**

The proportion of hosts dying during the two week experimental period was directly related to the initial parasite burden of the fish (Table 24). The difference in mortality rates in the classes of parasite burdens was highly significant (P < 0.005, χ² test).
Fig. 33. Age structure of laboratory populations of Gyrodactylus.
Fig. 34. The system used in ageing individual *Gyrodactylus gasterostei*.

Newly born *G. gasterostei* contain a large *F*₁ embryo and a smaller undifferentiated *F*₂ embryo. This Figure indicates the increase in size of the embryonic hamuli as the parent becomes older. The penis is only present in individuals which have given birth once. Vertical lines represent births, after which embryo development starts again.

Age scales at the base of the figure are adjusted to show development rates at 5, 10 and 15°C.
Table 23. The transmission of Gyrodactylus gasterosteii in the presence of a barrier.

<table>
<thead>
<tr>
<th>Route of transmission</th>
<th>Fish:fish transmission</th>
<th>Detached parasites: fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No barrier</td>
<td>barrier</td>
</tr>
<tr>
<td></td>
<td>( \bar{x} )</td>
<td>16.5</td>
</tr>
<tr>
<td>Percentage transmission</td>
<td>( \alpha )</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>6</td>
</tr>
</tbody>
</table>

Fish:fish transmission significantly higher \((P<0.05)\) when barriers absent than when present (Mann-Whitney U-test). No significant difference in rate of transmission of detached parasites.

Legend

- \( \bar{x} \) arithmetic mean
- \( \alpha \) standard deviation
- n number of replicates
Fig. 35. The effect of host density upon transmission rate in *Gyrodactylus gasterosteii.*
Fig. 36. The effect of parasite density upon transmission rate in Gyrodactylus Gasterostei.
Table 24. The effect of Gyrodactylus gasterostei infection upon host mortality.

<table>
<thead>
<tr>
<th>Parasite burden</th>
<th>0-50</th>
<th>50-100</th>
<th>100-150</th>
<th>150-200</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of fish examined</td>
<td>89</td>
<td>20</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>No. of fish dying</td>
<td>6</td>
<td>4</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Percentage of fish dying</td>
<td>6.7</td>
<td>20.0</td>
<td>82.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>

All fish maintained individually in 4l of dechlorinated tapwater for 14 days at 10°C. They were fed ad libitum on Tubifex and kept in a 12h:12h LD photoperiod.
DISCUSSION

The distribution of *Gyrodactylus* within the host population is highly overdispersed in both laboratory and natural populations, and can be represented by a negative binomial distribution. This is similar to the distribution of most free living (Southwood, 1966) and parasitic (Pennycooke, 1971; Boxshall, 1974; Whitfield, 1979) organisms, and because of the flexibility of the negative binomial model (Boxshall, 1974), reveals little of significance of the biology of the host parasite interaction. A negative binomial distribution may be generated by the exposure of the host to numerous waves of infection, by the clumping of infective stages of the parasite or by changes in the probability of infection caused by a previous infestation (Crofton, 1971a). In the case of *Gyrodactylus*, *a priori* consideration of the life cycle suggests that an alternative model may be more appropriate for describing its distribution within the host population. This parasite reproduces in situ upon the host, some individuals subsequently dispersing to infect other hosts. This could be modelled by a Poly-Aeppli distribution, originally developed to describe the distribution of random clumps of individuals, when each clump is growing geometrically (Elliot, 1977). The Poly-Aeppli is less skewed than the negative binomial (Anscombe, 1950; Evans, 1953), resulting in a large proportion of hosts being infected with an average parasite burden. Despite the theoretical preference for a Poly-Aeppli model, the negative binomial describes the observed distribution of *Gyrodactylus gasterosteii* more closely than any other (Fig. 29; Table 20).

A similar distribution has been observed for *G. medius* from natural populations of *Onos muscula* by Srivastra and James (1967) and in experimental populations of *G. bullatarudis* on guppies by Scott (1982a). The greater overdispersion shown by *Gyrodactylus* populations in comparison to the predicted Poly-Aeppli distribution may be due to stochastic variation in population growth rate on individual fish, which Bailey (1964) considered capable of generating a negative binomial distribution. Alternatively, it may be due to a heterogeneity in the susceptibility of the fish to infection, which may be genetic, or due to a host reaction against the parasites. The Poly-Aeppli may also be inappropriate because of the high rate of transmission of *G. gasterosteii* between fish, which masks the effect of population growth on individual hosts. It may be a more suitable model of the parasite distribution when the transmission rate is low relative to the population growth rate within individual hosts, as, for example, in species of *Eupolystoma* and *Metapolyplstoma* (see Combes, 1981).
The birth rate of *G. gasterostei* at different temperatures shows little individual variation: parasites give birth to only two daughters, although Turnbull (1956), Bychowsky (1957) and Scott (1982b) found that more than two offspring could be produced. The restriction of fecundity in *G. alexanderi* (see Lester and Adams, 1974a) and *G. gasterostei* may have been due to experimental stress upon the fish. Constant handling can stress the fish, leading to the accidental dislodgement of most parasites after only two births. Parasites which had given birth twice had a small embryo in utero, indicating that they were capable of giving birth on a third occasion. However, the very small ovary in comparison with other monogeneans (Braun, 1966, Kritsky, 1971) suggests that the maximum potential fecundity of *Gyrodactylus* is relatively low. This potential underestimation of fecundity, caused by the failure to observe parasites giving birth more than twice is unlikely to introduce a serious error into the calculation of reproductive rate, which is principally determined by the age at first birth (Cole, 1954).

The fecundity of parasitic organisms is in general very high, many being amongst the most fecund organisms in the animal Kingdom (Anderson, 1976; Whitfield, 1979). Such fecundity is usually assumed to be an adaptation to the difficulties of transmission faced by parasitic organisms. In comparison with other parasites, *Gyrodactylus* has a very low fecundity, probably giving birth to less than five daughters in its life span under optimum conditions. This could be taken as indicative of a very low mortality during transmission or of a limit to the reproductive output and extreme selection for persistence in a single host (a K-selected strategy, Macarthur and Wilson, 1967). However, reproductive rate depends not only upon fecundity, but also upon the age of the organism when it begins to reproduce (Cole, 1954). In *Gyrodactylus*, embryo clustering reduces the age of a fluke when reproduction begins to between $\frac{1}{4}$ and $\frac{1}{2}$ of the necessary development time of the first born embryo (Turnbull, 1956; Lester and Adams, 1974a; Harris, 1980a). Generations of *Gyrodactylus* overlap to such an extent that calculations of reproductive rate based upon mean generation time (Ricklefs, 1973), as were determined for *G. alexanderi* by Lester and Adams (1974a), seriously underestimate this parameter, which can best be calculated using the graphical technique of Bychowsky (1957) or by the Soper-Thompson technique, described by Cole (1954). Table 21 shows that, contrary to the impression created by fecundity measurements, the reproductive rate of *Gyrodactylus* is far greater than that of other monogeneans, which live longer and produce more eggs. A similar observation was made by Bychowsky (1957), comparing the reproductive rate of *G. rarus* with that of *Dactylogyrus*.
The reproductive rate of *Gyrodactyulus gasterostei* is temperature dependent (Fig. 37), increasing fourfold with a 5°C increase in temperature, a relationship also seen in other species of skin parasitic gyrodactylids (Turnbull, 1956; Bychowsky, 1957; Lester and Adams, 1974a). Gill parasitic species have not been studied, due to the experimental difficulties in observation, but the slow population growth rate of *G. rarum* in nature suggests a slow reproductive rate. *Gyridicotylus gallieni* from the mouth of *Xenopus*, has a much slower rate of reproduction than the temperate *G. gasterostei*. The distribution of birth throughout the life of this parasite may be similar to that of *Gyrodactyulus*, but the lifespan and hence the pre-reproductive period, is probably considerably longer (Ch. 6).

The observed rate of increase of *G. gasterostei* populations is considerably calculated less than the maximum possible rate in the absence of pre-reproductive mortality. The growth rate observed in the early stages of infection on fish at 5°C, 10°C and 15°C was approximately 30% of that predicted from the potential rate of increase of the parasites. In addition, considerable variation in parasite population growth rates between individual fish was noted, although the maximum rate of reproduction showed little variation. This reduction is caused by a high rate of accidental dislodgement of parasites, rather than death, as moribund flukes were never observed. The variation in accidental dislodgement from fish to fish probably generated the observed variation in individual growth rates, and ultimately the highly overdispersed distribution of parasites within the host population.

After an initial period of growth, laboratory populations of *G. gasterostei* undergo a period of decline. At 15°C this occurred 10-13 days after the initial infection, and the parasites subsequently disappeared from the fish. At 10°C, the populations grew for 30-38 days before declining, whereas at 5°C they continued to grow throughout the experimental period of 42 days. Some parasites persisted upon the hosts after the phase of decline at 10°C. During the decline, detached parasites were frequently found in sediment removed from the aquarium. This phenomenon was also observed by Lester (1972) and Lester and Adams (1974a) in the case of *Gyrodactyulus alexanderi* on *Gasterosteus aculeatus*. These authors attributed the decline in parasite populations to a host reaction against *G. alexanderi*, resulting in large numbers of parasites being shed from the host, attached to flakes of mucus. In the present work, although epidermal changes associated with the presence of *G. gasterostei* were noted (Ch. 3), the shedding of parasites, attached to mucus was rarely observed. Changes in the epidermis probably increase the detachment rate of *G. gasterostei*, without acting in the manner discribed by
Fig. 37. The effect of temperature upon reproductive rate in Gyrodactylus species.

3). *G. gasterosteii*, present study.

Number of parasites produced after 20 days exponential growth from one parasite, calculated after the graphical method of Bychowsky (1957).
Lester (1972). This may be a consequence of the different attachment strategies of G. gasterostei, which utilises only the marginal hooks in attachment, and G. alexanderi, which depresses the host epidermis with the hamuli (Lester, 1972; Harris, 1982b; Ch. 3). As pointed out previously, a true immune response is unlikely to be involved, as detached parasites are healthy and able to reinfect other hosts. Sticklebacks reacted against G. gasterostei at a level of 20 parasites per fish, which is similar to the intensity of infection at which changes in the structure of the epidermis can be discerned (Ch. 3). In the case of G. alexanderi, a host response was not manifested until populations had grown to an average size of 50 parasites per fish (Lester and Adams, 1974b). However, these workers initially infected each fish with 20 parasites, and the final populations may reflect the maximum attained in the two weeks necessary for the host to mount an efficient response. It is not known whether the host responds to the size of the parasite infection, or to its duration. This may be important in natural populations, as immigration of flukes from other hosts may lead to sudden elevations in parasite burden without an accompanying period of gradual population growth.

The duration of the reacting phase in individual fish has been found to be short (Lester and Adams, 1974b; Scott, personal communication), and hosts can be reinfected shortly after this period ends. During the present work, Gasterosteus aculeatus could be infected with Gyrodactylus gasterostei at all times during an infection, but the success rate of parasites infecting a host during the shedding phase of the infestation may be low. It appears that an absolute refractory period does not exist, but that for a few days the detachment rate of G. gasterostei is elevated and that host susceptibility to infection increases once more after the initial infection has been lost. This was also observed in the case of G. alexanderi upon Gasterosteus aculeatus by Lester and Adams (1974b).

The observed decline in parasite infestations may have been due to host stress rather than to a host reaction. Fish are particularly susceptible to stress, which may be induced by handling, changes in water quality or temperature shock (Barton and Peter, 1982). Stress stimulates changes in the host epidermis (Pickering and Macey, 1977; Pickering, Pottinger and Christie, 1982) which may in turn influence their susceptibility to parasite infection (Pickering and Christie, 1981). This potential link between host stress and octoparasite population density suggests that the experimental procedure used to demonstrate a host reaction against Gyrodactylus (fish kept in dark, without feeding) may
have influenced the observed response. However, experiments examining host mortality and skin changes in response to parasitic infection (Ch.3) and vide infra were designed to avoid this: fish were handled only at the start of the two week experimental period and were maintained at low density under a natural photoperiod. Parasite infections also declined in these experiments and significant skin changes, correlated with Gyrodactylus burdens, were observed (Ch.3). The decline in Gyrodactylus abundance is therefore thought to be due to a specific response against the parasites by the host, and is not an artifact of the experimental conditions used.

The absence of a host response against G. gasterosteoi at 5°C may have been due to the failure of infections to reach a threshold density in the experimental period, or the response may have been suppressed by low temperatures. Alternatively a host reaction may have taken place, but been masked by stochastic variation on individual fish. During the phase of population growth, individual variations in parasite detachment rates generate considerable variation in the size of the parasite population on individual fish, which in turn causes variation in the timing of the onset of the host reaction. This would be masked at high temperatures by the rapid population growth rates of the parasites, accounting for the short period of response at 15°C and the subsequent total disappearance of parasites. At 10°C the individual variation extends the shedding reaction over a greater period. Some fish then finished reacting against their parasite burdens before others have started, and some susceptibles are always present, acting as reservoir of Gyrodactylus infection. At 5°C, the heterogeneity of population growth rates on individual hosts may be sufficient to mask the shedding response completely.

If, as suggested by these data, the size of G. gasterosteoi populations is limited by accidental dislodgement of parasites, the pattern of age specific detachment is of considerable importance in determining population growth rate. In G. alexanderi and G. bullatarudis, a pattern of increasing 'mortality' was calculated from the survival of a cohort of individuals examined daily throughout their life span (Lester and Adams, 1974a; Scott, 1982b). Constant handling of fish, necessary in the analysis of these workers, may directly affect parasite survival upon the host, and it is reasonable to assign a constant daily detachment rate to this experimental technique. This could generate the observed pattern of 'mortality' whilst obscuring subtler, age dependent variations in the probability of detachment. In the present work, age-dependent detachment was studied using free living parasites, avoiding stress to the host. The use of this cross-sectional technique has disadvantages
in comparison to that of following the fate of a single cohort (Caughley, 1977) because it requires the assumption of a stable age structure and a knowledge of population growth rates. The rapid reproduction of Gyrodactylus ensures that the parasite population quickly attains a stable age structure (within 5-7 days at 15°C) and the reproductive rate during the initial part of the infection has already been estimated. Variations in the population growth rate (from zero population growth up to the maximum potential rate) do not significantly alter the observed pattern of age specific detachment, which can therefore be estimated from cross-sectional techniques.

The pattern of age specific detachment shown by Gyrodactylus gasterostei differs significantly from that of G. alexanderi or G. bullatarudis, studied by Lester and Adams (1974a) and Scott (1982b). Instead of 'mortality' (detachment) increasing with age, two distinct periods of increased detachment, after 1.5 and 4.5 days at 15°C and 5 and 13 days at 10°C can be seen (Fig. 32). These correspond with the periods immediately after the parasites have given birth, when their activity increases and they wander extensively upon the surface of the host (Harris, 1980b, Ch.3). Accidental dislodgement is most likely to occur during movement, when the parasites are momentarily attached only by their anterior adhesive glands. The effect of the shedding cycle of the host upon age specific detachment is not clear, as data collected before, during and after the period of population decline were combined to provide sufficient information. However, the pattern of age specific detachment is probably not qualitatively different during the host reaction, unless epidermal changes cause an increase in activity at other stages of the life cycle (Ch.3), for example immediately before feeding.

The age structure of the parasite population upon the host is normally biased in favour of younger flukes (Fig. 33), as would be predicted from the high birth and death rates of G. gasterostei. In this respect G. gasterostei differs from Gyrodactylus gallieni (Ch.6), which has a population age structure biased in favour of older parasites. The age structure of G. gasterostei becomes slightly truncated during and after the host reaction, as the parasite population then contains fewer newborn and very old flukes. This is to be expected if the rate of detachment during post reproductive activity increases during the declining phase of population growth, as it would reduce the proportion of parasites surviving to give birth twice, in turn reducing the proportion of new-born flukes in the population.
In the case of *Gyrodactylus* detached parasites do not die immediately, but may be capable of infecting other hosts. The survival of detached parasites at a range of temperatures has been shown to be significant (Ch.3), allowing parasites a few days to reinfect another host. The reattachment of detached parasites is extremely efficient (Table 23) at the host densities used in laboratory infections. These densities (10 fish per litre) are between 10 and 100 times greater than the densities of sticklebacks observed in nature (Wootton, 1976). Transmission of attached parasites also takes place at a significant rate, with up to 10% of the parasite burden transferred to another host in a 24 hour period at high densities (Fig. 35). This rate of transmission declines from this level to approximately 2% per 24 hours at lower host densities (similar to those observed for natural populations by Wootton, 1976). Transmission by detached parasites is probably the more important route in nature but, contrary to the observations of Almberg (1970), transmission by detached parasites may also take place at a significant rate. Although the rate of transmission is inversely related to host density, the relationship is complicated by host shoaling. At high densities, fish maintain a constant spacing, reducing the probability of contact, whereas at low densities they shoal, increasing the probability of contact (Symons, 1971). The role of shoaling in facilitating transmission in nature is unclear. Adult sticklebacks tend to avoid contact in nature (Symons, 1971), although they may move close together when feeding or shoaling (Wootton, 1976). During the breeding season, males occupy territories, and transmission between them is unlikely. However, the probability of transmission between breeding partners during spawning must be high. The behaviour of sticklebacks at night is not known, but guppies (*Lebistes reticulatus*) rest upon the substrate, an activity which Kearn (1976) thought important for transmission. Scott (personal communication) has suggested that *G. bullatarudis* may transfer from the anal region of the mother guppy onto the fry as they are born, and Llewellyn (1979) considered that *Isanctistrum* may be transmitted during copulation of squid.

Transmission of *Gyrodactylus* shows a relationship with parasite density upon the host. In view of the density dependence of the host shedding response this is surprising, as it might be expected that transmission rate would increase at high parasite densities. The failure of this work to demonstrate such a response may be due to the heterogeneity of the hosts used, which were all wild-caught and at different stages in the cycle of abundance of their *Gyrodactylus* populations. This may have masked any density dependence in their transmission rate.
At levels of infection in excess of those stimulating a host response, *Gyrodactylus gasterostei* increased the mortality of its host. This may be due directly to *Gyrodactylus*, as shown by Lester and Adams (1974a), or to a secondary infection of pathogens which have entered the host through *Gyrodactylus* feeding and attachment wounds. A curious feature of heavy *Gyrodactylus* infections is that, whereas in some cases the infection declines and disappears, in others it increases until the host is killed. This has been observed in the present work, and was also noted by Lester and Adams (1974a) in *G. alexanderi*. These authors attributed the response to 'mucus stasis', the failure of the mucus layer to be shed in flakes (Nigrelli, Jakowski and Padnos, 1955). However, in the present work, the shedding of mucus flakes by sticklebacks was observed only infrequently, even upon fish with declining infections. It is therefore more probable that host mortality occurred when *G. gasterostei* 'overwhelmed' the host, the parasite population growing at a rate in excess of that at which parasites could be rejected. Damage to the host skin by large numbers of parasites, an effect aggravated as the *Gyrodactylus* population increases until the host is killed. The initial increase of the parasite population above the threshold level which is liable to result in host death may be due to stochastic varieties in population growth rates on different hosts, or because of the sudden immigration of *Gyrodactylus* onto the fish. As detached parasites are not killed, the host reaction against *Gyrodactylus* does not automatically reduce the parasite suprapopulation size, only acting in this way if host density is low, when most detached parasites die without locating a new host. In cases of intensive fish culture, the host reaction may be rendered ineffectual because a large proportion of the detached parasites may reinfect other fish. Under these conditions the *Gyrodactylus* infection may assume the dimensions of an epizootic, killing large numbers of hosts (Bychowskaya-Pavlovskaya, 1963; Bauer, Musselius and Strelkov, 1973).

The life cycle of *Gyrodactylus* involves reproduction in situ upon the host, with no obligate relationship between transmission and reproduction. This microparasitic strategy is intrinsically unstable (May and Anderson, 1973), because the size of the parasite population on an individual host is determined by reproduction as well as immigration. The model on which this prediction is based forms one of a cluster (Anderson and May 1978, 1979a, b; May and Anderson 1978) developed by these authors to describe a host-parasite interaction. They make the assumption that the parasite population upon the host is increased by immigration (and in microparasites, reproduction), and diminished by death.
Fig. 37. A model of the Gyrodactylus-host interaction.
Fig. 38. The predicted effect of host density and environmental temperature upon the size and regulation of the Gyrodactylus suprapopulation.
However, Gyrodactylus populations upon the host are not affected by death, but by detachment. Their dynamics might therefore be predicted by an immigration-reproduction detachment model, summarised in Fig. 37.

Gyrodactylus populations may be regulated in three ways, by stochastic variation in the rate of parasite detachment from the host, by a host reaction, and by host death. The role of these regulatory mechanisms is dependent upon environmental temperature and host density. At low density and temperature, stochastic variations in detachment rate can offset the reproductive output of the parasites. Few detached parasites are likely to reinfect another host, because the probability of contacting a fish is small. At higher temperatures, the reproductive rate of the parasites exceeds the rate of accidental dislodgement, and populations increase to a threshold density (approximately 20 parasites per fish in the case of Gyrodactylus gasterosteii) at which a host response occurs. On individual fish, the parasite infra-population may then show a cyclic pattern of growth and decline. These cycles may become more synchronous on all hosts as temperature increases, until they are also reflected in the behaviour of the supra-population. If host density remains low, the host reaction is sufficient to limit the parasite population. Moreover, the synchrony which hosts will exhibit in rejecting parasites at high temperature will reduce the supra-population to a very small size for much of the cycle. At lower temperatures the supra-population may attain a higher average size, and be less subject to cyclical fluctuations in abundance.

If host density is high, however, the effectiveness of the host reaction is limited because the proportion of detached parasites successfully reinfecting other hosts is increased. Then, the parasite supra-population may increase in size until hosts start to die of gyrodactyliasis. Because host death does not kill the Gyrodactylus, this does not automatically limit the size of the parasite population. However, a period of host deaths will reduce the host population to a size at which few detached parasites successfully reinfect other fish, and the host reaction can once more limit Gyrodactylus abundance. The probable effect of the interaction of temperature and density upon Gyrodactylus abundance is summarised in Fig. 38.
Ch. 5. SEASONAL VARIATION IN ECTOPARASITE ABUNDANCE IN NATURAL POPULATIONS OF STICKLEBACKS.
INTRODUCTION

Numerous studies of the seasonal variation of fish parasite populations exist, but, as pointed out by Kennedy (1977), little is known of the factors responsible for the observed seasonal cycles, or of the mechanisms regulating the overall size of the parasite population. Chubb (1977, 1979, 1980), in an extensive review of this topic, concluded that water temperature was the most important factor influencing parasite population dynamics. Although a simple relationship apparently exists between temperature and seasonal variation in monogeneans, which have a direct life cycle (Chubb, 1977), this relationship is obscured in digeneans (Chubb, 1979) and cestodes and nematodes (Chubb, 1980) by the complexity of the interaction of temperature with each stage of the life cycle.

In monogeneans, the seasonal variation in abundance is dependent in part upon the life cycle strategy of the parasites. The larger polyopisthocotylean monogeneans from freshwater fish, including Discocotyle sagittata and Diplozoon paradoxum live for at least one year, and have a single pulse of reproduction during summer (Paling, 1965; Bovet, 1967). This pattern of reproduction generates a series of discrete generations affected only by most mortality. Conversely, small dactylogyrids and gyrodactylids reproduce throughout the year, producing a continuum of overlapping generations (Bychowsky, 1957; Crane and Mizelle, 1968). The population dynamics of these parasites are affected by changes in both mortality and fecundity, and the short generation time would allow tracking of short term environmental fluctuations. Between these two extremes, a spectrum of life cycle strategies exists, modified by the effect of environment upon reproduction. Low temperature reduces the turnover of generations of smaller monogeneans in winter (Bychowsky 1957), and can disrupt reproduction entirely. When this occurs, life cycle strategy becomes intermediate between that of pulsed and continuously reproducing forms. For example, Molnar (1971) has shown that reproduction in Dactylogyrus fallax and D. lamellatus ceases in winter, due to low temperatures. Laboratory studies (Bychowsky, 1957; Lester and Adams, 1974a; Harris 1980a; Ch.4) have shown that reproduction in Gyrodactylus continues at low temperatures, albeit at a greatly reduced rate. Chappell (1969) observed populations of G. rarus (syn. G. gasterostei) in August in which none of the individuals was embryonated. Although this could be interpreted as evidence of a check in parasite reproduction, the observation was based on a very small sample, and considerable care is necessary in interpreting data of this type in the absence of supporting observations on fecundity and mortality (Ch.4). Laboratory studies suggest that in general,
gyrodactylids reproduce continuously, population size on individual hosts being determined by immigration, birth and emigration processes (Ch.4) throughout the year.

Amongst the monogeneans with continuous reproduction and overlapping generations, a range of seasonal patterns of abundance has been recorded. Chubb (1977) formulated a hypothesis accounting for this seasonal variation, suggesting that each monogenean species has an optimum environmental temperature at which its population is of maximum size. Below this temperature, the population size is limited by a reduction in reproductive rate, whereas above it, mortality limits abundance. Each monogenean has a characteristic optimum temperature, which determines its response to seasonal variations in the environment (Chubb, loc. cit). This hypothesis has been used to account for the observations of Rawson and Rogers (1972), who found that of the five species of ancyrocephaline studied on *Lepomis macrochirus*, two, *Anchoradiscus triangularis* and *Clavunculus bifurcatus* were most abundant in autumn, and were at a nadir in midsummer, whereas the remaining three *Cleidodiscus robustus* *Urocleidus acer* and *U. dispar* were all most abundant in summer.

All studies of the seasonal abundance of gyrodactylids support Chubb's (1977) hypothesis with species falling into two groups, depending on the pattern of variation:

(1) Species with a maximum of abundance in summer; Malmberg (1970) observed this pattern in *Gyrodactylus arcuatus*, *G. pungitii* and *G. anguillae*, and Barkman and James (1979) observed a midsummer peak of abundance of monogeneans on *Fundulus heteroclitus*. Unfortunately these authors did not distinguish between the dynamics of the three species present on this host, *Fundulotrema (Gyrodactylus) prolongis*, *G. stephanus* and *Urocleidus angularis*. Dickinson and Breelfall (1975) also observed a peak of abundance of *F. (G.) prolongis* in summer, at a locality considerably further north than that studied by Barkman and James (1979). A number of species of *Gyrodactylus* have been recorded from their hosts only during summer. These include *G. rarus* and *G. bychowskyi* from *Gasterosteus aculeatus* in a Leningrad reservoir (Banina and Isakov, 1971), *G. lucii* from *Esox lucius* in Lithuania (Rautskis, 1970) and *G. laevis* and *G. elegans* from break in Czechoslovakia (Wiersbicka, 1974). *Gyrodactylus* lacks overwintering stages, and shows narrow host specificity
which suggests that the failure of these authors to find the parasites in winter was due to a low prevalence of infestation in the host population at this time of year. A similar phenomenon was observed in G. rarus on Gasterosteus aculeatus by Chappell (1969), who found that, after a peak of abundance in mid summer, this species almost disappeared in July and August, being found on only a small proportion of the hosts examined.

(2) Species most abundant during winter: These include Gyrodactylus stephanus on Fundulotrema heteroclitus, studied by Dickinson and Threlfall (1975), and G. medius from Onos mustela (Srivastrava and James, 1967). In addition, Bychowskaya-Pavlovskaya (1963) recorded that G. medius and G. elegans most frequently cause epizootics in carp ponds in winter.

However, certain seasonal studies cannot be adequately explained by Chubb's (1977) hypothesis. For example, G. macrochiri from Lepomis macrochirus and Micropterus salmoides shows a peak of abundance in spring (Rawson and Rogers, 1974) at an intermediate temperature. This observation could have been predicted from the work of Hoffman and Putz (1964), who estimated the optimum temperature for G. macrochiri to be 12°C. However, it does not show a similar peak during autumn, when water temperature is again close to the optimum (Rawson and Rogers, 1973). The seasonal cycles of abundance observed in Gyrodactylus species are listed in Table 25.

In addition to temperature, many other environmental factors exhibit seasonal variations, which were not considered by Chubb (1977) to influence monogenean populations. These include host migrations and breeding cycles, and the interactions of parasite populations with each other, all of which may impose a seasonality on parasite population dynamics which is unrelated to temperature.

Host migration may be important in determining infection, especially in the case of gyrodactylids of euryhaline fish such as Anguilla or Fundulus (Malmberg, 1970; Dickinson and Threlfall, 1975). The best documented example of the effect of migration upon parasite infection is that of the interaction between Dactylogyrus ivanowi and its euryhaline host, Leuciscus brandtii, described by Bychowsky (1957). This parasite infects spawning hosts in freshwater, matures during the marine phase and reproduces during the subsequent breeding migration, on the return of the fish to freshwater. Other host migrations involving less dramatic changes in habitat, may also affect parasite abundance.
Table 25. Previous studies of the seasonality of Gyrodactylus populations.

a) Species abundant in colder part of year.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Locality</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>medius, G.</td>
<td></td>
<td>Russia</td>
<td></td>
</tr>
<tr>
<td>elegans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>heteroclitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. medius</td>
<td>Onos mustela</td>
<td>Wales</td>
<td>Srivastava and James, 1967.</td>
</tr>
<tr>
<td>Gyrodactylus spp.</td>
<td>Fundulus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>heteroclitus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b) Species most abundant in summer

<table>
<thead>
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<th>Species</th>
<th>Host</th>
<th>Locality</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aculeatus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pungitius</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>anguilla</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gyrodactylus</td>
<td>Fundulus</td>
<td>Connecticut,</td>
<td>Barkman and James, 1979.</td>
</tr>
<tr>
<td>spp.</td>
<td>heteroclitus</td>
<td>U.S.A.</td>
<td></td>
</tr>
<tr>
<td>G. prolongis</td>
<td>Fundulus</td>
<td>Newfoundland</td>
<td>Dickinson and James, 1975.</td>
</tr>
<tr>
<td></td>
<td>heteroclitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. rarus</td>
<td>Gasterosteus</td>
<td>European</td>
<td>Banina and Isakov, 1972.</td>
</tr>
<tr>
<td>G. bychowskyi</td>
<td>aculeatus</td>
<td>Russia</td>
<td></td>
</tr>
<tr>
<td>G. laevis</td>
<td>Abramis brama</td>
<td></td>
<td>Czechoslovakia</td>
</tr>
<tr>
<td></td>
<td>Blicca bjoerkna</td>
<td></td>
<td>Wiersbicka, 1974.</td>
</tr>
</tbody>
</table>
Table 25. Previous studies of the seasonality of Gyrodactylus populations....(cont'd).

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Locality</th>
<th>Author</th>
</tr>
</thead>
</table>

(c) Species most abundant in spring and autumn.
Paling (1965) showed that infection of trout with *Discocotyle sagittata* was insignificant in the rivers draining into Windermere, but that it increased considerably when the host moved into the lake itself. Llewellyn (1962) and Anderson (1981) have shown the importance of host migrations in the epidemiology of marine monogeneans. All of these studies concern host-parasite interactions in which the host migrations occur at extended intervals. When they occur twice in a year, as in the case of *Fundulus heteroclitus* or *Gasterosteus aculeatus*, they could generate a significant degree of seasonal variation in monogenean abundance.

The population dynamics of ectoparasites may be influenced by the annual reproductive cycle of the host. During breeding, the skin surface may undergo structural changes, influencing the growth of ectoparasite populations (Pickering and Christie, 1980), and the changing levels of sex hormones immediately before breeding may also affect its capacity to mount a host response against ectoparasites (Pickering and Christie, *loc.cit.*), an effect well known in warm-blooded vertebrates (Michel, 1969). Other consequences of sexual reproduction, including territoriality, the formation of breeding shoals and parental care may also influence the epidemiology of the host-parasite interaction.

A third potential source of variation in parasite population size is interaction with other parasite species. Paperna (1964) showed that *Dactylogyrus vastator* usually replaced *D. extensus* when present in mixed infections on carp, and Wilson (1916) showed that the presence of *Ergasilus* on the gills of white crappie was inversely related to the presence of lamellibranch glochidia. Positive interactions between ectoparasites are also known: Paperna and Kohn (1964) observed such a relationship between the abundance of *Dactylogyrus* and *Trichodina* on carp, and Mackenzie (1970) noted a similar interaction between the abundance of ciliates on plaice gills. Negative interactions between parasites can generate seasonal variation in abundance because the impact of the interaction upon each parasite population can be minimised if their peaks of abundance are separated temporally (Whitfield, 1979). On the other hand, positive interactions favour the synchronous increase of parasite populations. Selective pressures generated by these interactions may modify direct environmental influences upon monogenean populations.

A considerable complexity probably surrounds the factors determining the seasonal abundance of fish ectoparasites. In order to determine the most important influences, it is necessary to consider the population dynamics.
of all the ectoparasites present on the host, in relation to each other, to environmental factors and to the annual cycle of the host. The interaction of *G. asterostei* with its host has been examined in Chs. 3 and 4, which has given an indication of the important factors affecting population size of this species in the laboratory. Using this study as a basis, the seasonal variation of gyrodactylid and other epibionts on sticklebacks in nature has been examined over a period of 27 months.

**MATERIALS AND METHODS**

(a) Sampling regime.

Fish were collected from two sites on the river Ver, Hertfordshire at Sopwell Mill (O.S. ref. TL154053) and Park Street (O.S. ref. TL148042). Each site was visited at 14 day intervals and samples of *Gasterosteus aculeatus* (Park St. and Sopwell) and *Pungitius pungitius* (Sopwell) collected by hand netting. Each sample of 15 fish was placed in river water and transferred immediately to the laboratory for examination. When fish were breeding, separate samples of fry and adults were collected. The number of dips with the net required to catch the total sample was recorded as an index of catch per unit effort.

The surface water temperature was measured (at approximately 11.00 a.m.) using a mercury bulb thermometer (accuracy ± 0.5°C) with the bulb shielded from direct sunlight. The river depth was measured using bridge supports as standard reference points.

(b) Examination in the laboratory.

Fish were kept in river water at a temperature close to that of the river when they were collected (5°C, 10°C, or 15°C) and usually examined within 24 hours (never more than 36 hours) of capture. They were killed by cutting the spinal cord and were placed in a petri dish containing dechlorinated tap water. The density of protozoan infections was estimated using a 3mm² quadrat frame, made from fuse wire, placed upon the dorsal, caudal and anal fins. The gills were then excised into a separate watch glass containing dechlorinated water. The exterior of the body was scanned, and all Gyrodactylus found counted and identified (Ch.2). When very abundant, a sub sample of 15 parasites was removed from each fish for identification, and the species composition estimated from this.
From the entire sample of 15 fish, 30 parasites were removed and aged according to the technique outlined in Ch.3. The gills were likewise examined, *Gyrodactylus* identified and counted and a sub sample removed for age determination.

After recording ectoparasite abundance the body length (tip of tail to snout) and sex (immature, breeding male, breeding female) were determined for each fish.

(c). Storage of samples.

In some cases it was impossible to examine fish immediately after capture. When this occurred the fish were killed in the normal way and protozoan densities assessed. The fish were then placed in individual vials and preserved in a small volume of 4% formaldehyde. At a later date, the *Gyrodactylus* present were counted, identified and aged in the normal manner, except that the fixative and sediment were also scanned. Comparison of samples preserved using this technique with those examined shortly after capture did not reveal significant differences in epibiont abundance.

**RESULTS**

(a) Description of sample sites.

The river Ver rises near Redbourn, Hertfordshire, from springs associated with the dip slope of the Chiltern hills (Fig. 39). It flows south through St. Albans, continuing to its confluence with the river Colne at Radlett, a distance of 18 km. Being spring fed, the river level does not show a close correlation with rainfall, and abstraction for domestic use in St. Albans has made the water level very erratic (Fig. 40). The river flows through arable farmland, and is enriched by agricultural waste along much of its length.

Site 1: Sopwell Mill.

At this point, South east of St. Albans, the river has been diverted through a water mill. It has been divided into two streams, one running under the mill (Fig. 39), the other passing over a weir and through a mill race around one side of the building. A very shallow, spring fed stream also joins the main river just below the mill race. Above the weir, the river is approximately 7m wide, and between 0.5 and 1.0m deep, with a substrate of very fine mud. It is slow flowing, and the presence of large trees on either bank prevent the development
Fig. 39. Map of sample sites from which sticklebacks were collected.
Fig. 40. Water level and rainfall in the P. Yer, Herts.
of submerged vegetation. Some *Phalaris* grows along the stream margins.

Below the weir, in the mill race, the stream is narrow (1.0m), shallow and fast flowing, with a substrate of large stones and gravel. *Ranunculus fluitans* grows abundantly in this section. In the small spring fed stream, opening into the mill race, the rate of flow is slight, and the substrate varies between soft mud, sand and gravel. At its confluence with the mill stream it is 5m wide, but near its source it narrows to less than 1m. It is 0.5m-1.0m deep containing an abundant vegetation of *Ranunculus fluitans*, *Petasitites hybridus*, *Ceratophyllum* Sp, *Apium nodosum* and *Rorippa nasturtium-aquaticum*. Fish were collected from the river above the weir, from the mill race and from the small spring fed stream.

**Site 2: Park Street**

The river occupies a single bed at this locality (Fig. 39), passing through gravel near the confluence with the Colne. It is 10m wide, 1m deep and relatively fast flowing, with a substrate which varies from fine mud to gravel. An old ford at this point creates a considerable area of very shallow water. Abundant submerged vegetation, principally *Ranunculus fluitans*, *Ceratophyllum* sp. and *Potamogeton compressus* has developed, and large beds of *Phalaris* line the river margins. Large, flooded gravel pits occupy the area to the north of the stream, and may connect with it during periods of flood.

(b) The ectoparasite community of sticklebacks from the river Ver.

Both *G. aculeatus* and *P. pungitius* were infected with a range of protozoan, monogenean and annelid ectoparasites and epibionts. In addition, diatoms were frequently observed adhering to the skin, and a variety of yeasts and filamentous algae could be seen attached to the epidermis using scanning electron microscopy. The commonest protozoans on the fish were petitrich ciliates, belonging to the genera *Trichodina*, *Apiozoa*, *Epistylis*, *Vorticella*, *Glossatella* and *Scyphidia*. These organisms were not identified to specific level, because considerable confusion surrounds their taxonomy (Corliss, 1961). Only *Trichodina* and *Apiozoa* were sufficiently abundant to allow estimation of density. Four species of *Gyrodactylus* were observed, *Gyrodactylus gasterostei* on the skin and *G. arculatus* on the gills of *Gasterosteus aculeatus*, and *G. pungitii* on the skin and *G. rarus* on the gills of *Pungitius pungitius*. *G. arculatus* was less site specific than the other species, and was at times found frequently on the skin, in addition to the gills of its host.
The leech *Piscicola geometrica* was frequently encountered on sticklebacks, and the oligochaete *Chaetogaster* was occasionally found on the skin surface. The jaw mechanism of this organism suggests that it may be able to feed on ectoparasites, and it is known that *C. limnicola* feeds on digenean larvae (Khalil, 1961). However, no evidence of predation on epibionts was obtained after an examination of the gut contents specimens collected, and it is thought that *Chaetogaster* epizoic upon fish normally feed on diatoms present on the skin.

(c) The annual cycle of the host.

The mean length of fish collected throughout the study are shown in Fig. 41. During the period September to May, only one size frequency class is present in the population, although in June, July and August, two size classes, corresponding to adult fish and newly hatched fry occur together. Fig. 42 indicates catch per unit effort data for both *P. pungitius* and *G. aculeatus* at Sopwell and Park Street. This varies greatly from sample to sample, due to weekly differences in fish behaviour and distribution. However, it shows the increasing effort required to catch adult fish in mid-summer, and ultimately these fish failed to appear in the samples. This suggests, in conjunction with the observations on length - frequency distribution of the fish, that both *G. aculeatus* and *P. pungitius* live for 13-15 months, attaining maturity and spawning in the summer after hatching. They then die, shortly after breeding.

The lengths of the fish collected in samples show considerable variation, which could be taken as evidence of the existence of two successive age classes in the population. However, this variation is due to the extended breeding season of the sticklebacks. Fish spawned in early June attain large size by September, whereas those hatched in early August have a short feeding period before the onset of winter, remaining small until recommencing growth in the following spring.

(d) Seasonal variation in *Gyrodactylus* abundance.

The abundance of *G. gasterosteii* shows a similar seasonal cycle of variation of both Sopwell and Park Street (Figs. 43 & 44). Populations on the fish are of moderate size in winter, with 100% prevalence and a mean intensity of 10-20 parasites per fish. During late winter and spring, the mean intensity of infection starts to increase, attaining a maximum of 80 parasites per fish in June and July. The level of infection may remain elevated until the disappearance of the fish in August (Park St. 1981) or it may decline before
Fig. 41. Mean length of fish collected throughout study.
Fig. 42. Catch per unit effort data for fish in R. Ver.
the death of the adult fish (Sopwell and Park St. 1980, Sopwell, 1981). The young fry become infected with *G. gasterostei* in mid-summer, and the parasite population on these rapidly increases to a maximum of 20 parasites per fish in late summer and autumn, when it may then become slightly smaller, or it may remain at this size throughout winter.

The seasonal abundance of *G. arcuatus* shows a similar trend, the population of this species remaining small (C.5-10 parasites per fish) throughout winter and increasing rapidly in size during spring and early summer (Fig.43 & 44). However, this species also shows a peak of abundance in late summer and autumn, when it is also most common on the skin of the host. *G. gasterostei* is relatively rare during this maximum of *G. arcuatus*.

*G. pungitius*, in contrast, does not show the same amplitude of population change observed in *G. arcuatus* and *G. gasterostei*. Throughout the sampling period, the mean intensity of infection remained within the range 5-30 parasites per fish, and a large increase in parasite populations in early summer was not observed. The infection of fry in late summer follows a similar pattern to that of *G. gasterostei* and *G. arcuatus* upon *Gasterosteus aculeatus*.

*G. rarus* was the least abundant species encountered in the study, rarely exceeding a mean intensity of infection of 5-10 parasites per fish. Populations of this species declined in prevalence and intensity in winter, increasing again in spring and early summer. The increase in prevalence and intensity of infestation of this species was slow relative to *G. pungitii*, suggesting that reproductive and transmission rates are slow in comparison with the skin parasite. No evidence for a decline in abundance before the death of the adult hosts was obtained for either *G. pungitii* or *G. rarus* (Fig. 43).

(e) Seasonal variation in protozoan infections.

The density of protozoan infections was estimated only in samples collected between May 1980 and December 1981.

On both *Gasterosteus aculeatus* and *Pungitus pungitius*, at Sopwell and Park St, *Trichodina* showed a similar seasonal pattern of abundance (Fig.43,44,45). Populations remained small throughout the winter, but increased in spring and early summer, attaining a peak density of 4-7 parasites per mm$^2$. The population then either declined, or remained large until the disappearance of the adult fish. After infection of the newly hatched fry, populations of
Fig. 43. Seasonal variation in ectoparasite abundance on Gasterosteus aculeatus at Park Street.
upon Gastropods annulatus at Solway

Fig. 44. Seasonal variation in abundance of ectoparasites

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Fig. 45. Seasonal variation in abundance of ectoparasites on Pungitius pungitius at Sopwell.
Trichodina increased rapidly to a zenith in June and July, before declining to their low winter level.

The seasonal variation in the dynamics of Apiosoma closely resembled that of Trichodina although this parasite failed to reach the same densities upon the fish. During the summer maximum of ectoparasite abundance, Apiosoma populations started to decline at an earlier stage of the infection than either Trichodina or Gyrodactylus.

(f) The infection of fry.

As sticklebacks breed throughout the summer period, giving rise to fish of a range of lengths, the relationship of infection prevalence with length was examined (Fig. 46) for each sampling date when fry were collected. This shows that the youngest fish, at a length of 11-13mm (the smallest free living stage) were seldom infected with ectoparasites, but that as they grew, the prevalence of infection increased.

(g) Sexual differences in infestation.

During the 1980 reproductive season (May, June and July), the epibiont burdens of adult fish were compared with respect to sex of hosts, which were divided into reproducing males (nuptial coloring present), reproducing females (containing maturing eggs) and non-reproductive fish composed of those still maturing and those which had recently spawned. No significant differences in levels of infestation were observed between these classes of fish (Table 26).

(h) Factors affecting parasite abundance.

In order to determine the factors influencing parasite abundance, the linear correlation coefficient between a range of parameters (mean weekly intensity of infestation of Gyrodactylus, Trichodina and Apiosoma, mean weekly air temperature, weekly water level and fish length) was determined for both host species.

It can be seen (Tables 27, 28, 29) that the abundance of Gyrodactylus gasterostei, G. arcuatus and Trichodina are closely correlated, particularly at Park Street.
Fig. 46. The infection of newly hatched fry with ectoparasites.
Table 26. Comparison of infection of male, female and immature sticklebacks with ectoparasites.

A) G. aculeatus, Park Street.

<table>
<thead>
<tr>
<th>Date</th>
<th>$\sigma^-$</th>
<th>$\sigma^+$</th>
<th>immature</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
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<td>42</td>
<td>5</td>
<td>90.5</td>
<td>13.8</td>
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<td>18</td>
<td>42.8</td>
<td>7</td>
</tr>
<tr>
<td>30/6/80</td>
<td>90</td>
<td>108</td>
<td>5</td>
<td>41</td>
</tr>
<tr>
<td>14/7/80</td>
<td>191</td>
<td>1</td>
<td>18</td>
<td>39</td>
</tr>
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</table>

B) G. aculeatus, Sopwell.

<table>
<thead>
<tr>
<th>Date</th>
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<th>$\sigma^+$</th>
<th>immature</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td>27/5/80</td>
<td>116.2</td>
<td>24</td>
<td>13</td>
<td>6.3</td>
</tr>
<tr>
<td>9/6/80</td>
<td>41.0</td>
<td>12</td>
<td>4</td>
<td>39</td>
</tr>
<tr>
<td>23/6/80</td>
<td>30</td>
<td>2</td>
<td>52.3</td>
<td>30.6</td>
</tr>
<tr>
<td>7/7/80</td>
<td>29</td>
<td>1</td>
<td>42.3</td>
<td>-</td>
</tr>
</tbody>
</table>

C) P. pungitius, Sopwell

<table>
<thead>
<tr>
<th>Date</th>
<th>$\sigma^-$</th>
<th>$\sigma^+$</th>
<th>immature</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>27/5/80</td>
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<td>1</td>
<td>6.5</td>
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<td>9/6/80</td>
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<td>4</td>
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<td>14.3</td>
<td>13</td>
<td>4</td>
<td>15.3</td>
</tr>
</tbody>
</table>

All samples tested using Quenouilles test for the difference of means (Elliot, 1977).

Legend

\[ \bar{x} \] arithmetic mean
\[ \sigma \] standard deviation
\[ n \] number of replicates
Table 27. Linear correlation coefficients between pairs of variables, G. aculeatus, Park Street.

<table>
<thead>
<tr>
<th></th>
<th>Air temperature</th>
<th>Water level</th>
<th>Gyrodactylus gasterostei</th>
<th>G. arcuatus</th>
<th>Trichodina</th>
<th>Apiosoma</th>
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</thead>
<tbody>
<tr>
<td>Air temp.</td>
<td>X</td>
<td>X</td>
<td>0.38</td>
<td>0.36</td>
<td>0.26</td>
<td>0.07</td>
<td>X</td>
</tr>
<tr>
<td>Water level</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>G. gasterostei</td>
<td></td>
<td>X</td>
<td>0.66</td>
<td>0.87</td>
<td>0.79</td>
<td>0.7</td>
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<tr>
<td>G. arcuatus</td>
<td></td>
<td>X</td>
<td>0.57</td>
<td>0.53</td>
<td>0.65</td>
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</tr>
<tr>
<td>Trichodina</td>
<td></td>
<td>X</td>
<td>0.53</td>
<td>0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apiosoma</td>
<td></td>
<td>X</td>
<td></td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body length</td>
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</tbody>
</table>
Table 28. Linear correlation coefficients between pairs of variables, *G. aculeatus*, Sopwell.

<table>
<thead>
<tr>
<th></th>
<th>Air Temperature level</th>
<th>Water level</th>
<th>Gyrodactylus gasterosteii</th>
<th>G. arcuatus</th>
<th>Trichodina</th>
<th>Apiosoma</th>
<th>Body length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Temp. X</td>
<td>X</td>
<td>X</td>
<td>0.15</td>
<td>0.15</td>
<td>0.02</td>
<td>-0.28</td>
<td>X</td>
</tr>
<tr>
<td>Water level X</td>
<td></td>
<td></td>
<td>0.06</td>
<td>0.08</td>
<td>0.25</td>
<td>0.42</td>
<td>X</td>
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<tr>
<td>Gyrodactylus gasterosteii X</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.72</td>
<td>0.09</td>
<td>0.58</td>
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<tr>
<td>G. arcuatus X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.11</td>
<td>0.53</td>
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<tr>
<td>Trichodina X</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.27</td>
<td>0.22</td>
</tr>
<tr>
<td>Apiosoma X</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>Body length X</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 29. Linear correlation coefficients between pairs of variables, *P. pungitius*, Sopwell.

<table>
<thead>
<tr>
<th></th>
<th>Air temperature</th>
<th>Gyrodactylus pungitii</th>
<th>G. rarus</th>
<th>Trichodina</th>
<th>Apiosoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air temp.</td>
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<td>0.12</td>
<td>0.31</td>
<td>-0.5</td>
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</tr>
<tr>
<td>Gyrodactylus pungitii</td>
<td>X</td>
<td>0.45</td>
<td>0.09</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Gyrodactylus rarum</td>
<td>X</td>
<td>0.49</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichodina</td>
<td></td>
<td>x</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The mean intensities of parasite infestations are in all cases correlated with increasing fish length. However, no relationship with air temperature or water level exists, suggesting that environmental influences upon parasite abundance are weak. The abundance of *Apisoma* shows a weak correlation with water level.

(i) Host reactions against ectoparasites in natural populations.

In an overdispersed distribution, the variance of the population is related to the mean in a manner which is approximately constant in a homogenous set of data (Southwood, 1966; Elliot, 1977; Boxshall, 1974). In the present work, large variations in the variance mean ratio in the laboratory are associated with the onset of the host reaction against *Gyrodactylus gasterosteii* (Ch.4). It was therefore considered that an examination of variance-mean ratio and population size could give an indication of the onset of a host reaction in nature (Fig. 47). It can be seen that such a reaction occurred in the *Gasterosteus aculeatus* population during June and July, 1980, but did not take place in 1981. A second, weaker reaction was also observed against the parasites of young (O+) fish in the late summer (August and September) in both 1980 and 1981. In the case of *Pungitius pungitius*, no host reaction against either *G. rarus* or *G. pungitii* was observed.

**DISCUSSION**

Populations of *Gyrodactylus gasterosteii* and *G. arcuatus* from the three spined stickleback show a distinct pattern of seasonal variation, attaining a peak of abundance in early summer, before being reduced to a very low level in late summer and autumn, a cycle similar to that described by Chappell (1969) for *Gyrodactylus rarus* (syn. of *G. gasterosteii* and *G. arcuatus*, Ch.2) from the same host. It is also similar to the seasonal variation observed in populations of *Fundulotrema* (*Gyrodactylus*) *prolongis*, *G. stephanus* and *Urocleidus angularis* from *Fundulus heteroclitus* by Barkman and James (1979), *G. macrochiri*, *Cleidodiscus robustus*, *Urodeidus acer* and *U. dispar* on *Lepomis macrochirus* and *Micropterus salmoides*, described by Rawson and Rogers (1972, 1973) and *Gyrodactylus medius*, observed on *Onos mustela* by Srivastrava and James (1967). At first sight, these observations on seasonal variation on *Gyrodactylus* from sticklebacks accord well with the hypothesis of Chubb (1977) that monogeneans have an optimum temperature above which population size is limited by high mortality, below which slow reproductive rate reduces growth.
Fig. 47. Variance/mean ratio as a means of identifying a host reaction against Gyrodactylus.
However, more detailed examination reveals that Chubb's (1977) hypothesis is inadequate to account for the observed seasonal variation in stickleback populations, which are greatly influenced by the annual cycle of abundance of the hosts. In the River Yer, catch per unit effort and length-frequency data indicate that both *G. aculeatus* and *P. pungitius* have an annual life cycle, hatching from spawn laid in summer and attaining maturity, spawning and dying in the following year. A similar annual life cycle has been reported for *G. aculeatus* in Monaco, Belgium and southern England (Bertin 1925; Heuts, 1947; Mann, 1971). In northern England, however, a life span of up to 3.5 years has been recorded (Jones and Hynes, 1950), and Pennycuick (1971) found *G. aculeatus* of a comparable age at an elevated, exposed site in South West England. Craig-Bennet (1971) correlated several maxima in the length-frequency distribution of a population of *G. aculeatus* with annual age classes, and concluded that the fish lives for several years in southern England. However, the extended reproductive period can generate sufficient heterogeneity in the length distribution to give this appearance of several age classes. It seems likely that the three spined stickleback has an annual life cycle throughout the southern part of its range, whereas in northern areas its life span is extended (Wootton, 1976). This is probably also the case with *Pungitius pungitius*, which has an annual life cycle in the river Ver, but which can live for up to 3 years in northern England (Jones and Hynes, 1950).

The annual life cycle of sticklebacks imposes the observed seasonal pattern of abundance upon the ectoparasite fauna. Being unable to survive away from the host for long periods of time, *Gyrodactylus* must be transmitted directly from the adult fish to the newly hatched fry in the short period after spawning and before the death of the parents. The fry can become infected after direct contact with an infected fish, or by direct contact with free living gyrodyactylids. The number of detached gyrodyactylids in the environment is dependent upon the proportion of individuals becoming accidentally detached from their hosts, and upon the number of hosts which have died, and which have been abandoned by their parasites (Ch. 3). The reattachment rate of detached, free living *Gyrodactylus* is very high, but these parasites are subject to considerable mortality (Ch. 3). The period when most individuals become detached (due to a host reaction, or to host death) is in June and July, coinciding with the period of highest water temperature (13-15°C), when parasites will survive for a maximum of 50 hours (Ch. 3) away from the host.
Survival will be further reduced by deoxygenation of the habitat over areas of fine mud (Ch.3). Moreover, the activity of the detached parasites declines towards the end of their life (Ch.3), when it is unlikely that they are capable of successful infection. The rate of reattachment may be affected by the nature of the sediment which detached parasites find themselves upon. Soft mud is probably less suitable for their movement than stones and vegetation, and infection by detached parasites is probably more important at Sopwell, with a well oxygenated, weedy river bed, than at Park Street, with its extensive areas of soft mud.

It can be concluded that although free living Gyrodactylus may be present in the environment in June and July, few may successfully infect young fish, because their survival period is curtailed by high water temperature and deoxygenation. The spatial distribution of adults and fry may also reduce the proportion of parasites reattaching to young hosts. The dispersive powers of gyrodactylids are poor when free living, and they are probably restricted to a small area close to the point where they originally became detached. Adult sticklebacks breed in water of 20-50 cm depth (Wootton, 1976), but the fry shoal in very shallow water, at the margins of the stream (personal observation). This partial segregation of adult sticklebacks and fry may reduce the transmission of detached Gyrodactylus to the fry. If the infection of fry by free living, detached parasites is insignificant, host-host contact, a more important route in the laboratory (Ch.4), may be important in nature. This is of particular interest in the sticklebacks, because of the intimate association which exists between the parent fish and the very young fry, which are brooded in a nest until they become free swimming at a length of 10-12 mm. This behaviour provides an ideal opportunity for parasite transmission, and Malmberg (1970) considered it an important source of infection of G. arcuatus on young fish. However, in the river Ver, transmission of Trichodina, Apiosoma and Gyrodactylus to the fry in the nest was unimportant as a source of infection (Fig. 46). The youngest fish had a very low prevalence of infestation, whereas this increased considerably amongst the larger, older, fry. This indicates that infection occurred after the fry had left the nest and had started shoaling together in the shallows. A few ectoparasites may successfully infect fry in the nest, accounting for the infections observed in the smallest fry. These parasites reproduce rapidly after the young fish have become free living, and their infections are rapidly transmitted to other fry (probably by host-host contact) in the large
shoals of small fish. Conditions for transmission are optimum in these shoals, the fish being densely crowded in very shallow water. The water temperature of these shallows may be slightly higher than in the main stream, allowing rapid multiplication of the parasite populations.

It is not clear why fry are not heavily infected before they leave the nest, when they are in close contact with large numbers of epibionts. However, the skin surface may be unsuitable for colonisation by epibionts until the fish is somewhat older. New born guppy fry (Lebistes reticulata can be infected by G. bullatarudis (Turnbull, 1956; Scott, personal communications), but in this case, the guppies are born at a more advanced stage of development than stickleback fry, and their skin may have attained its mature form. Infections of Gyrodactylus on fry of other hosts have rarely been reported.

Although Gyrodactylus infections must be transmitted between adults and fry in the very short midsummer period, the epidemiology of Trichodina and Apiosoma infections on sticklebacks may be more complex. The host specificity of these ciliates has not been determined experimentally, and as the taxonomic limits of the numerous described species are poorly known (Corliss, 1961), it is possible that species can infect several hosts and that sticklebacks first become infected from a reservoir host present throughout the summer period. The existence of such a reservoir would reduce the importance of host death as a means of regulating epibiont abundance, and would allow infection of fry to take place over a much longer period. The seasonal variation in abundance of Trichodina is very similar to that of Gyrodactylus, which suggests that such a reservoir host is unlikely to be important.

In the case of Epistyliis, Glossatella, Y. verticella and Scyphidia, a range of substrates, including inorganic debris, invertebrates and vertebrates can be colonised by the zooids (Kudo, 1966). These sessile peritrichs occurred irregularly on sticklebacks, and may have colonised these unusual habitats only during periods of abundance on other more favourable substrates.

The epidemiology of ectoparasite infections upon sticklebacks is unusual because of the extreme brevity of the period when vertical transmission between generations can take place. With a few exceptions, teleosts are long lived, with pulsed reproduction occurring annually in temperate climates.
Their populations contain several age classes and infection of young fish from older individuals can take place over an extended period. In many cases, spatial separation of feeding and breeding grounds may prevent infection of young fish until they mature and join the adult population. The best example of this is that of the eel, *Anguilla anguilla*, infected with *Gyrodactylus anguillae*, which Malmberg (1970) found on migrating elvers and Ogawa and Egusa (1978) found in freshwater eel farms. This suggests that it is a parasite of the maturing, freshwater phase of the eel life cycle, and that mature fish and leptoccephalus larvae, which are marine, are uninfected. Fish migrations do not need to be as spectacular as those of the eel to significantly affect the epidemiology of *Gyrodactylus* infections. Srivastrava and James (1967) noted an increase in transmission of *G. medius* amongst adult *Onos mustela* when these fish migrated inshore to spawn. Similarly, the habit of many cyprinids of forming shoals of fish of a similar age may reduce the rate at which these hosts become infected with *Gyrodactylus*.

Although, in the case of sticklebacks, the annual cycle of the host is the most important factor governing the seasonal abundance of the ectoparasite populations, other studies (Srivastrava and James, 1967; Rawson and Rogers 1973; Barkman and James, 1979) have emphasised the importance of seasonal variation in temperature. In England, the mean weekly air temperature has an annual range of -5°C to +30°C (Manley, 1952). However, the variable climate generates considerable variation throughout the year, and a wide range of temperatures can be experienced in any month (Manley, loc. cit.). A number of authors (Edington, 1966; Smith and Lavis, 1975; Boon and Shires, 1976; Crisp and Howson, 1982) have shown that the water temperature of small streams is closely related to air temperature, although it shows less variation (Hynes, 1961), rarely falling below 0°C or exceeding 15°C. In addition, the larger the stream under consideration, the smaller is the range in variation experienced (Smith and Lavis, 1975). Other factors, including stream topography, discharge rate (Smith and Lavis, loc. cit.) and tree cover (Gray and Edington, 1969) modify stream temperature slightly but, in general, the range in variation is small and subject to considerable short term fluctuations. The reproductive rate of *Gyrodactylus* is strongly dependent on temperature (Lester and Adams, 1974a; Harris, 1980a; Ch. 4) and, with its short generation time, this parasite might be expected to 'track' short term temperature fluctuations closely. In fact, no correlation can be observed between parasite population size and weekly water temperature (Table 28, 29). An increase in the size of populations of *Gyrodactylus* and *Trichodina* during spring and summer accompanies the gradual increase in the average temperatures during this period, but is due to the gradual increase in parasite populations throughout the life of the host.
The increase in parasite burdens shows no correlation with short term temperature fluctuations, continuing to increase during short periods of declining water temperature. The lack of a significant relationship between temperature and ectoparasite burdens is probably due to the simultaneous effect of temperature on both mortality and fecundity.

Another environmental factor which could influence the size of parasite populations is the river current velocity. In the river Ver no relationship exists between rainfall and river water level, partly because the river is spring fed and partly because of abstraction in the Luton and St. Albans areas. With the exception of *Apiosoma*, which shows a slight positive correlation, ectoparasite abundance is not related to water level (Tables 28, 29, 30).

*Apiosoma* is an ectocommensal probably feeding upon organic particles and bacteria in suspension (Lom, 1973a; Sleigh, 1973). In periods of spate, the density of food particles in suspension is increased (Hynes, 1961), possibly allowing an increase in the abundance of this organism.

In laboratory studies, a host reaction has been observed to limit the abundance of *Gyrodactylus* on sticklebacks (Lester, 1972; Lester and Adams, 1974a, 6; Harris, 1980a; Ch.4). In nature, considerable difficulty is experienced when separating declines in epibiont abundance caused by a host reaction from those caused by stochastic variations in death rate, and by sampling error. Variations in the growth rate of parasite populations on different hosts cause individuals to follow separate shedding cycles, and the synchronous rejection of parasites by a large proportion of the host population is probably rare (Ch.4). Such events would take place only when the overall rate of parasite population growth is sufficiently rapid to mark differences in population growth rates on individual hosts. The variance-mean ratio of *Gyrodactylus gasterosteai* populations increases considerably when the hosts reject the parasites in laboratory infections. (Ch.4). This is due to the marked heterogeneity of hosts at this time, some, which have already rejected their parasites, being uninfected, while others, which have yet to react, carry heavy epibiont burdens. An increase in variance-mean ratio, combined with a decline in absolute abundance can be used to identify host reactions in nature. As shown in Fig. 47, these criteria indicate that a host reaction occurred at both Park Street and Sopwell in May and June, 1980. During the remainder of the year, the variance-mean ratio is
Boxshall (1974) has shown that in Lepeophthirus, the index of dispersion increases with the mean density of parasites upon the host. This relationship could generate the large variance-mean ratios observed in May and June, when absolute densities of the ectoparasites were also high. However, in the case of G. gasterosteii, although the variance-mean ratio varies considerably from week to week, it is not closely related to parasite density. It can therefore be concluded that the increase in ratio in May and June 1980 was due to a real increase in the overdispersion of the parasite population, and is not a spurious increase correlated with parasite density. Such an increase is probably due to the occurrence of a host shedding reaction.

The variance-mean ratio of the ectoparasite populations did not increase in the same way in 1981, despite the large parasite populations present on the fish at this time, and the hosts were still heavily infected at the time of their disappearance in mid summer. In August 1980, a decline in the infection on fry was observed, which was also correlated with an increase in the variance-mean ratio. This may represent the occurrence of a weak host reaction in the fry.

The population dynamics of Gyrodactylus rarus and G. pungitii on P. pungitius do not show the effect of a host reaction. The population of G. pungitii on this host remains within narrow limits of abundance in comparison with G. gasterosteii or G. arcuatus from Gasterosteus aculeatus. It is not known whether P. pungitius reacts against Gyrodactylus in the same manner as that described in G. aculeatus by Lester (1972). It is possible that a different mechanism may limit the abundance of Gyrodactylus species on hosts other than the three spined stickleback, including G. bullatarudis from the guppy (Scott, personal communication), G. eucaliace on the brook stickleback (Ikezaki and Hoffman, 1957) and G. unicopula on plaice (Mackenzie, 1970). Alternatively, the difference in parasite population dynamics on P. pungitius may be due to the behaviour of this host. In particular, the ten-spined stickleback shows a preference for fast flowing water and thick weed beds in comparison with G. aculeatus (Wootton, 1976; personal observation), features which may have a significant effect upon the population dynamics of Gyrodactylus pungitii and G. rarus.

On Gasterosteus aculeatus, a close relationship was observed between the size of Gyrodactylus gasterosteii, G. arcuatus and Trichodina populations.
The correlation between these populations suggests that they are subject to the same controlling factors, of which the most important are the high mortality during transmission to fry in late summer and the action of synchronous host reactions in reducing population size. A similar close relationship has been observed between the abundance of epibionts in several previous studies. Noble, King and Jacobs (1963) observed such a relationship between Gyrodactylus and Trichodina on the gills of Gillichthys mirabilis, and Paperna and Kohn (1964) found a close correlation between the abundance of Trichodina and Dactylogyrus on the gills of carp. Mackenzie (1970), on the other hand, found no relationship between the abundance of Gyrodactylus unicopula and Trichodina borealis on plaice, although the latter was closely correlated with another peritrich, Scyphidia adunconucleatta. In this case, the absence of a relationship may have been an artifact, as Mackenzie (loc. cit.) only considered the prevalence of these ciliates and of Gyrodactylus unicopula.

The relationship between Gyrodactylus, Trichodina and Apiosoma is well established empirically, although nothing is known of the biological basis of the interaction. Unlike Gyrodactylus, which feeds upon host epidermal cells (Kearn, 1963a, 1976; Ch.3), the sessile peritrichs are ectocommensal (Lom, 1973a; Fernando and Hanek, 1976), feeding on suspended particles which are swept over the surface of the fish. The trophic relationships of Trichodina are obscured by the fact that, although structurally adapted for ectocommensalism (Sleigh, 1973), they are sometimes pathogenic to fish (Bauer, Musselius and Strelkov, 1973). The differences in feeding mechanisms and source of food of these epibionts makes it unlikely that their trophic relationships would form a basis for their interaction. However, Gyrodactylus, Apiosoma and Trichodina all utilise the same site of attachment, the skin surface of the host, and it is probable that the presence of one genus attached to the skin will affect the probability of success of another which attempts to colonise a fish. Gyrodactylus, Trichodina and Apiosoma populations on sticklebacks may increase until one exceeds a threshold density, triggering a host response which results in a decline in abundance of all three epibionts. Alternatively, the presence of one epibiont upon the skin may increase the probability of successful colonisation by another, until both are adversely affected by a host reaction. A basis for such a positive interaction has already been demonstrated by Lom (1973) in the case of Epistyliis, which colonises teleosts by growing upon an Apiosoma zooid which has previously attached directly to the fish skin. It is to be expected that both Trichodina and Apiosoma will be affected by changes in the microtopography
of the skin induced by other components of the skin fauna. The attachment of *Apisoma* involves secretion of a pad of mucus onto the skin surface (Lom, 1973). *Trichodina* utilises an adhesive ring which 'pinches' the edges of a group of epithelial cells (Lom, 1973). The strength of these attachment mechanisms may be adversely affected by an increase in host mucus thickness, which is probably influenced by the presence of *Gyrodactylus* (Ch.3). Excessive mucus production is associated with both *Apisoma* and *Trichodina* infections (Bauer, Musselius and Strelkov, 1973), and it is possible that these epibionts can stimulate a host reaction in the same way as *Gyrodactylus*.

A negative interaction probably occurred between *Gyrodactylus gasterostei* and *G. arcuatus* on the three spined stickleback. *G. arcuatus* is frequently recorded from the skin of its host (Bychowsky and Polyansky, 1953; Malmberg, 1970), but in fish from the river Ver it was found most frequently on the gills. During autumn, when the infection of *G. gasterostei* was small, *G. arcuatus* was often found upon the skin and fins, in addition to the oral cavity and gills. This suggests that *G. gasterostei* interacts with *G. arcuatus*, restricting it to the gill chamber. When water temperature is high enough to permit rapid reproduction, but while *G. gasterostei* is rare, *G. arcuatus* can spread from the gills onto the skin. The basis for such an interaction is not known, but as both species can be affected by a host reaction (Fig. 47), it is not likely to be simply related to the differences in attachment strategy in these two species (Ch.3). Interactions between *G. pungitii* and *G. rarus* on *Pungitius pungitius* have not been observed, probably because these species exhibit strict site specificity, and are unlikely to meet.

During spring and early summer, the infestation of *Gasterosteus aculeatus* with *Gyrodactylus gasterostei* increased beyond the level (approximately 20 parasites per fish) which stimulated a host reaction in the laboratory (Ch.4). The rapid increase in infection at this time was not closely correlated with temperature, and may have been due to an increase in the carrying capacity of the host, rather than to an increase in the growth rate of the parasite population. This increase in the level at which the hosts react to epibiont infection may be associated with the onset of reproduction in the host. Pickering and Christie (1980) have shown that sexual dimorphism in the skin structure of *Salmo trutta* can influence ectoparasite burdens, and although a similar separation between male and female sticklebacks was not observed in the present work (Table 26), it is possible that a difference exists between sexually active and immature fish. Leatherland and Lam (1969) have shown that
prolactin, which is associated with migration and the reproductive cycle in sticklebacks (Lam and Hoar, 1967) can affect the structure of the skin of _G. aculeatus_, and can therefore possibly influence the size of ectoparasite populations.

A second factor which may influence the size of ectoparasite populations in nature is stress upon the host. In _Gasterosteus aculeatus_, breeding causes considerable stress, because of the high cost of reproduction in this species (Wootton, 1976). Stress alters the structure of fish skin (Pickering and Macey, 1977; Pickering, Pottinger, and Christie, 1982) and has been implicated as a factor determining the overall size attained by ectoparasite populations. Many authors (Malmberg, 1970; Rawson and Rogers, 1974; Johnsen, 1978) have suggested that stressed fish are more susceptible to _Gyrodactylus_ than healthy fish. On the other hand, Pascoe and Mattey (1977) observed that _Gyrodactylus_ disappeared from stressed sticklebacks, although their controls were inadequate to rule out the involvement of other factors. Overall then, it is probable that stress in summer may partially account for the increase of parasite populations on sticklebacks at this time.

_Gyrodactylus pungitii_ and _G. carus_ on _Pungitius pungitius_ do not show such a distinct increase in the size of their populations in spring as do those of _G. aculeatus_ and _G. gasterosteis_. This may be related to the observation (Wootton, 1976) that reproduction stresses _P. pungitius_ less than _G. gasterosteis_ because interactions between males are less intense.

During early summer, the epibiont burdens of _G. aculeatus_ increase above the levels at which parasite induced host mortality was observed in the laboratory (Ch. 4). In laboratory infections experiments to examine the level at which mortality occurred, fish were maintained at low density and fed _ad libitum_. In nature however, stress induced by reproduction and high epibiont burdens may render hosts significantly more susceptible to parasite induced mortality. It seems likely therefore, that during summer the survival of a large proportion of the adult _G. aculeatus_ population is adversely affected by their epibiont burdens. Although parasite induced host mortality has a theoretically important role in the regulation of both hosta and parasite populations (Crofton, 1971a; Anderson and May, 1978, 1979a, 6; May and Anderson, 1979), in general the effect of metazoan parasites upon their hosts is considered small. It is normally thought that vertebrate populations may be regulated by resource shortage (Caughley, 1977), predator-prey (Ricklefs, 1973) or parasitoid-host interactions (Varley, Gradwell and Hassell, 1979) the effect of bacterial, viral
and protozoan 'microparasites' (Anderson and May, 1978, 1979a,b) or by social factors (Wynne-Edwards, 1962). However, the ability of Gyrodactylus to induce mortality amongst hosts in the laboratory (Lester and Adams, 1974a; Ch.4) suggests that this parasite may have a role in host population regulation in nature. Newly hatched stickleback fry are preyed upon by a range of invertebrates and vertebrates (Wootton, 1976), but adult fish are attacked by very few predators, because of their complement of spines. In the River Ver, the only organisms observed capable of feeding on adults were kingfishers (Alcedo althis), coots (Fulica atra), heron (Ardea cinerea) and perch (Perca fluviatilis). It is probable that pike (Esox lucius) also occurred in the river. The density of these predators was low, and in the presence of an abundant alternative food source (minnows, Phoxinus phoxinus), their effect upon stickleback population was probably small. During June and July, when adult sticklebacks were disappearing from the population, large numbers of dead and dying fish were observed, suggesting that predation was not an important cause of death. Stickleback mortality may have been due to reproductive stress or to heavy ectoparasite burdens, or it is possible that stress prevents the fish regulating its ectoparasite populations by a host reaction, allowing them to increase to lethal proportions. Both parasite induced host mortality and the host reaction (Ch.3) can control the abundance of ectoparasites (Ch.4). In the river Ver, both factors probably operate in a complementary manner. In 1980, populations of G. gasterosteoi were reduced on adult fish by a host reaction. However, the number of flukes surviving for transmission to fry was further reduced by host death. In 1981, a host reaction was not observed in adult fish (possibly because the cool summer delayed its onset), and host death was the primary regulating factor upon the parasite populations. It is interesting to note that adults of Pungitius pungitius also disappear in summer, although their ectoparasite burdens are not as high as those of G. aculeatus, and although they are not stressed so severely during reproduction.

The population dynamics of stickleback's epibionts are determined by the annual cycle of the host fish, the most important factor regulating their abundance being the high mortality associated with transmission from adults to fry in mid-summer. As parasites are free to reinfect another host after they have become free living, the host response and host death do not automatically regulate the parasite population. However, unless hosts are crowded at very high density, the proportion of detached parasites successfully reinfecting
another host is so small that both the host reaction and host death are effective regulatory mechanisms. Host death occurs in mid summer, when the importance of restraints on ectoparasite population growth may have been reduced by reproductive stress. A host reaction may also occur at this time, further limiting the ectoparasite population. A host reaction may also act to limit parasite population growth at other times of year, when water temperature is high (for example in late summer and autumn). Throughout winter, when low temperature limits reproduction, the parasite population grows slowly, remaining small.
Ch. 6. THE BIOLOGY OF *GYRDIÖTLUS GALLIENI*.
INTRODUCTION

Although *Gyrodactylus* is a very large genus containing several hundred species, the other twelve genera of the Gyrodactylidae are relatively rare, and only *Macrogyrodactylus* has been studied in any depth (Malmberg, 1956a; Khalil, 1964, 1970; Amirthalingam, 1965; Saoud and Mageed, 1969). Three genera parasitic upon hosts other than fish (*Isancistrum* on cephalopod molluscs, *Metagyrodactylus*, a hyperparasite of *Argulus*, and *Gyrdicotylus*, on the African clawed toad) are particularly poorly studied. An investigation into the biology of these genera would provide an interesting insight into the adaptive significance of viviparity in the infection of these unusual hosts.

*Gyrdicotylus gallieni* has never been readily available for scientific study. It was described by Vercammen-Grandjean (1960) from five specimens, of which only two, poorly preserved individuals remain. Thurston (1970) obtained abundant material (70 parasites from one toad) but, again, only two were preserved. Thus, until the present study, only four specimens of this parasite have been available for comparison with other gyrodactylids. Tinsley (personal communication) has collected *Gyrdicotylus* from four taxa of *Xenopus*, but never in sufficient quantities to allow a redescriptions, or a study of its biology.

During the course of the present work, *Gyrdicotylus* was obtained from *Xenopus laevis* imported from South Africa. A sufficient number was collected to provide ample material for a redescriptions of the morphology of *G. gallieni*, and to allow the establishment of a laboratory colony, from which biology could be studied.

The morphology of *G. gallieni*

Vercammen-Grandjean (1960) gave a very brief account of the morphology of *G. gallieni*, indicating a number of interesting features. He worked largely from living material, and his preserved type specimens do not allow the re-examination of internal anatomy. Prior to the description of *G. gallieni*, Bychowsky (1957) had suggested a relationship between the gyrodactylids and the polystomatids. Vercammen-Grandjean (1960) implied that several characters of *Gyrdicotylus gallieni* including its suckorial attachment mechanism, hamulus morphology and penis structure, might provide further evidence for such a link between the gyrodactylids and the polystomatids. Bychowsky's (1957) hypothesis has passed out of favour in recent years (Llewellyn, 1981a).
although Lambert (1979, 1980a,b) has suggested that the gyrodactylids arose by neoteny from a form ancestral to the polyopisthocotyleans. Vercammen-Grandjean (1960) provided a detailed account of the excretory system of G. gallieni, which Malmberg (1974) considered to be primitive, despite the parasite's sophisticated attachment mechanism. Thus, on the one hand Gyrdicotylus has been regarded as evidence of a link between the gyrodactylids and the polyzootamids (Vercammen-Grandjean, 1960), and on the other hand as a primitive gyrodactylid with a secondarily derived sophisticated attachment mechanism (Malmberg, 1970). The interest generated by these two views of the relationships of Gyrdicotylus with the remainder of the Gyrodactylidae made it desirable to re-examine its morphology, and to test the accuracy of the original description.

The ecology of G. gallieni

Vercammen-Grandjean (1960) described the site of infection of G. gallieni as the host intestine and stomach, a very unusual site of infection for monogeneans, which are normally ectoparasitic. Only one other monogenean, the dactylogyrid Entergyro cichlidarum, has been recorded from this site of infection (Paperna 1963). Amongst the gyrodactylids, only Gyrodactylus cryptarum has been recorded from an enclosed site of infection, the acoustico-lateralis canals of its host (Malmberg, 1970). Thurston (1970) briefly recorded the site of infection of Gyrdicotylus gallieni as the oral cavity of the host, but no further information was given concerning the ecology of the parasite. This confusion affects the significance of Vercammen-Grandjean's (1960) observation for, if G. gallieni infects the intestine of its host it would require adaptations to resist host digestive enzymes unnecessary if it is found in the oral cavity.

The internal site of infection (whether mouth or intestine) suggests that the route of transmission must be more complex than that of other Gyrodactylids. The enclosure of the site prevents fortuitous transmission during periods of host contact, and a migration is necessary between the initial site of colonisation and the final habitat occupied by the parasites. Southwood (1977) has suggested that the dispersal of free living organisms between habitats can be correlated with population dynamics within habitats. Thus, if dispersal between habitats allows an organism to reproduce more successfully than if it remains in one habitat, it will develop a colonisation strategy (r-strategy of Macarthur and Wilson, 1967) with rapid reproductive rate, short persistence in a habitat and good powers of dispersal. If, however, the organism will be more successful by remaining in a single habitat patch, because of the difficulties involved in colonising other habitats, then it will develop a persistence
strategy (K-strategy of Macarthur and Wilson, 1967), characterized by a slow reproductive rate and poor powers of dispersal. In the case of parasitic organisms, the transmission rate is often constrained by host behaviour and ecology (Crofton, 1971a). Gyrdicotylus gallieni must have a different route of transmission to that of Gyrodactylus gasterosteii (Ch.4), and yet it possesses similar structural adaptations for transmission (Ch.3). This suggests that the population dynamics of Gyrdicotylus gallieni would be correlated with the transmission of these parasites, in a way which might be predicted from Southwood's (1977) analysis. The transmission route and population dynamics of Gyrdicotylus gallieni have been studied in comparison with Gyrodactylus gasterosteii (Chs. 3,4) in order to test the applicability of Southwood's (loc. cit.) theory to parasitic organisms.

The distribution, taxonomy and host specificity of Gyrdicotylus gallieni

The taxonomy of Gyrodactylus has been studied in some depth, and it has been shown that the genus contains a very large number of highly specific species (Malmberg, 1970; Ch.2). Macrogyrodactylus is represented by several species, which are thought to be host specific (Paperna, 1979), but in other genera of the family the limits of species variability and host specificity are unknown. Gyrdicotylus is suited for a study of host specificity and morphometric variation because it is apparently restricted to infecting Xenopus, for which the inter-relationships between the component species are well known (Tinsley, 1981a,b). Tinsley (personal communication) has collected Gyrdicotylus from a range of Xenopus species and sub species, providing material for a comparison of its morphometric variation on different hosts. In addition, the establishment of a laboratory colony has allowed an experimental study of the host specificity of G. gallieni from South African Xenopus laevis.

MATERIALS AND METHODS

Xenopus laevis laevis were obtained through a commercial importer from Cape Flats, near Cape Town, South Africa. They were kept individually in 1l of dechlorinated water, until sacrifices for examination. Prior to dissection the toads were anaesthetised by chilling in iced water until immobile (Whitear and Mittal,1979) and killed by a sharp blow to the head. The jaw and gut were removed and placed in dechlorinated tap water (unlike other internal parasites, Gyrdicotylus was found to become more active in dechlorinated water than in 0.6% saline, surviving for 24-36 hours in this medium). The linings of the buccal cavity and the gut were scanned using a fibre-optic light source (Schott KH150B), and any parasites located were individually removed on a piece of host tissue to a watch glass containing dechlorinated water.
Parasites were flattened and fixed in formol-saline and stained in alum carmine or fast red Salt B for morphological examination. To facilitate study of the opisthaptor sclerites, specimens were fixed and mounted in ammonium picrate-glycerin (Malmberg, 1970). The excretory system was observed in living parasites using positive phase contrast microscopy (Leitz-Dialux).

For scanning electron microscopy, the entire buccal mucosa was fixed, immediately after dissection, in 1% osmium tetroxide in 0.2M cacodylate buffer (pH 7.4). The tissue was post-fixed in 4% glutaraldehyde for 1 hour, followed by washing in buffer and distilled water (1 hour each), dehydration in acetone and critical point drying (Polaron 3000). The specimens were mounted on aluminium stubs, coated with gold (Polaron 5000 sputter coater) and examined at 7.5 KV accelerating voltage in a Cambridge 600 scanning electron microscope.

Toads from the Westfield College and London Zoo study centre colonies, both of which were known to be free of Gyrdicotylus, were used to maintain experimental infections of the parasite. The uninfected toads were kept in 150ml crystallizing dishes, which during the infection process, were held on the stage of a binocular microscope. A single living parasite, freshly dissected from a wild caught host, was placed against the skin of the experimental host until it attached to it, and its progress followed using epi-illumination until it disappeared from view. After infection, the toad was kept in the 150ml dish for up to 3 months at 25°C in a 12 hr. L:D photoperiod. Toads were not fed after infection, and the water was changed every 3-4 days, the sediment being scanned for detached parasites. In order to test the possibility of parasites entering the host through ingestion of infected prey or sheets of sloughed epidermis (Tinsley and Whitear, 1980), the toads were fed upon strips of meat, to which individual flukes were attached. Subsequent maintenance was as described above.

Gyrdicotylus from freshly imported hosts had a red tinge to the body, which disappeared if the hosts were maintained in the laboratory for a few weeks. In order to identify this pigment, a living parasite was mounted on a slide in a drop of water, and flattened under a cover-slip. It was examined using a microspectroscope (Leitz).

To study the morphometric variation of Gyrdicotylus gallieni from different hosts, specimens were examined from the following host taxa: Xenopus laevis collected from Cape Flats and Transvaal, South Africa, X. l. victorianus collected from numerous

1. A solution of 4% formaldehyde in 0.6% saline.
collected from sites, Uganda), X.1. petersi (Zambia), X.borealis (Kenya), X.withei (Uganda) and X.clivii (Ethiopia). In addition, the type material of Gyrdicotylus, collected by Vercammen-Grandjean (1960), and specimens collected by Thurston (1970) were also examined.

Because fixation and flattening cause an unpredictable distortion of the soft tissues of gyroactyldids, only the opisthaptor sclerites were used for comparative measurement. The dimensions of the opisthaptor sclerites used were:

- **Hamulus**
  - Total length
  - Shaft length
  - Dorsal root length
  - Ventral root length
  - Point length

- **Marginal hook**
  - Total length
  - Shaft length
  - Sickle length
  - Sickle distal width
  - Sickle proximal width

The limits of these dimensions are shown in Fig. 48.

The host specificity of *Gyrdicotylus gallieni* from *Xenopus laevis laevis* was examined experimentally using *X. withei*, *X.vestitus*, *X.muelleri* and *X.laevis victorianus*, in comparison with control infections in *X.laevis laevis*. The toads used were originally collected from natural populations, but had been maintained, in the Westfield College colony, for many years, and were known to be free of natural infestations of *Gyrdicotylus*. They were exposed to infection according to the technique outlined above, and maintained for one week or one month under standard conditions.

**RESULTS**

**Observations on the morphology of G. gallieni**

(1) **Body form.**

The parasite has a fusiform body, with a length in living specimens of 1.0-1.2mm. The anterior has two indistinct cephalic lobes, with spike sensillae at the tips. The opisthaptor, separated from the body by a stout peduncle is cupped (Plate 16). The central hamuli are closely associated, forming a girder which supports the roof of the haptor. The body is transparent, often with a pink tinge.
Fig. 48. Limits to dimensions used in morphometric analysis of Gyrdicotylus.
Plate 16. Gyrdicotylus gallieni, attached to the oral mucosa of Xenopus.

The opisthaptor (o) of this parasite is pressed closely onto the host epithelium (e).

A depression (h) is present on the dorsal surface of the haptor, indicating the line of the hamuli.
(2) The alimentary canal.
The pharynx is made up of a thin walled anterior chamber and a glandular and muscular posterior region, bearing a ring of short pharyngeal processes. The gut is divided immediately behind the pharynx into two crura which extend the length of the body. These are lined by an unpigmented epithelium, and the gut contents are opaque and white.

The pharynx is surrounded by large glands, which empty through ducts in the cephalic lobes (Fig. 49).

(3) The opisthaptor
The opisthaptor lacks the finger-like processes bearing the marginal hooks which are present in other gyrodactylids. Instead, tegument has grown between and beyond the marginal hooks, giving a cupped appearance (Fig. 50). The haptor is partially divided by a longitudinal strip of tegument enclosing the hamuli (Plate 17). The walls of the haptor are reinforced by radial muscle fibres, and each half forms a partially independent sucker.

The hamuli are 78 (72-88) μm long, strongly curved, with both dorsal and ventral roots (Fig. 51). The shafts are 59 (53-67) μm long, the dorsal roots are 13 (9-17) μm long and the ventral roots are 36 (29-67) μm long. The points are 26 (9-17) μm long and the ventral roots are 36 (29-46) μm long. The points are 26 (21-28) μm long, with a constriction a short distance behind the tip. Both ventral and dorsal bars are absent, and the hamuli are firmly bound together with fibrous tissue, visible in whole mounts when examined using phase contrast.

The sixteen marginal hooks are 16 (15-17) μm long, articulated, with large sickles. A sickle filament loop (Malmberg, 1970) is present (Fig. 51).

(4) The female reproductive system
The ovary consists of a few oocytes on the posterior wall of the thin walled, transparent seminal receptacle. This lies at the rear of the large uterus, which occupies most of the centre of the body. It may contain a large, nearly mature embryo, containing in turn a developing F₂ generation daughter. It opens to the exterior through a mid ventral birth pore.

(5) The male reproductive system
The crescent shaped testis partially surrounds the seminal receptacle. It contains globular cells in new born individuals, which later become irregularly
Fig. 49. The morphology of Gyrdicotylus gallieni (whole mount).

- ph - pharynx;
- p - penis;
- ov - mature oocyte in seminal receptacle;
- cg - cephalic glands;
- t - testis;
- em - embryo mass;
- s - sucker;
- ha - hamulus;
- mh - marginal hook;
- g - gut.
Fig. 50. Lateral view of Gyridicotylus haptor, attached to host.
Plate 17. Ventral view of haptor of Gyridicotylus gallieni.

Broken tip of hamulus can be seen in Plate 18, projecting from the host epithelium.
Fig. 51. The opisthaptor solerites of Gymnocotylus gallieni from Xenopus laevis laevis.
rounded, with large interstices which become filled with living sperm. A thin walled, transparent vas deferens (visible only in living specimens) extends anteriorly from the testis along one side of the body to the sperm-filled seminal vesicle, immediately behind the pharynx. The penis, anterior to the seminal vesicle, is globular (20 μm diameter), with a flattened external face bearing a corona of 18-21 large, identical grooved hooks, each of which is 5-6 μm long.

(6) The excretory system.

The complete excretory system is shown in Fig. 52. It consists of two separate looped longitudinal canals, each running the length of the body. Four flame cells are placed along each main canal, five drain into the anterior loop of each, six into the posterior. A short collecting duct empties into small contractile bladders, which communicate with the exterior through pores in the pharyngeal region.

(7) Embryology and post-embryonic development.

From a consideration of a large number of developmental stages seen in living and preserved Gyridicotylus, the following sequence of embryonic development was determined. A single oocyte grows to a large size within the seminal receptacle, and enters the uterus shortly after the birth of a daughter. The cell divides until it has formed a small mass of cells, with a single large cell at the centre, which ultimately gives rise to the F₂ generation embryo. The marginal hook sickles and hamulus rudiments differentiate from the cell mass in the manner described by Braun (1966) for Gyrodactylus. The shafts are next to develop and finally the roots appear. The pharynx, gut and female reproductive system do not differentiate until the parasite is ready to be born. When the F₁ embryo is fully developed, the hamulus shafts of the F₂ embryo are visible.

At birth the female reproductive system of the newborn fluke is fully formed, with a large embryo in utero and an oocyte in the seminal receptacle. The testis has attained its maximum size, but the cells are tightly packed and inactive, and do not commence sperm production until after the birth of the first daughter, when the penis, seminal vesicle and vas deferens also develop. The distinctive characters of this developmental sequence allow parasites to be divided into newborn, pre-1st birth and post-1st birth developmental stages, in the same way as in Gyrodactylus gasteroste'i (Ch.5).
Fig. 52. The excretory system of Gyrdicotylus gallieni.
The biology of *Gyrdicotylus gallieni*

(1) Body pigment

The body of *Gyrdicotylus gallieni* is coloured pink by a pigment which is concentrated around the uterus and ovary. The pigment showed two peaks of absorption, when oxygenated and after reduction with sodium dithionate, characteristic of a haemoglobin. However, insufficient pigment was present in a single worm to allow precise estimation of the absorption wavelengths. When pyridine was added the characteristic pink coloration of pyridine haemochromogen developed, but diffused away before an estimate of the absorption wavelength could be obtained. The coloration of *G. gallieni* became less apparent after toads had been maintained in captivity for several weeks. Maintenance of toads in unchanged water did not influence the disappearance of the pigment.

(2) The route of invasion of the parasite.

When placed on the dorsal surface of a toad, parasites wandered across the body until they encountered the external nares. The cephalic lobes were inserted into the nostrils, and after a few seconds the parasites disappeared into the internal chamber. Subsequent dissection of toads infected in this way revealed parasites in the oral cavity. Tracks followed by a number of individual parasites are shown in Fig. 53.

Although topographic features of the toad skin (sensory plaques, eyes) could be used in locating the nostrils, the parasites do not appear to orientate on the surface of the host. It is not thought that sensory cues are used in the initial phases of infection.

During entry the parasites probably respond to sensory cues provided by the nostril. In particular, they possess a marked rheotactic response, lashing violently when stimulated by a jet of water. When this occurred on the edge of the external nare, the movements of the parasite continued until the cephalic lobes were lodged deeply within the nostril. As the toad regularly exhales a current of water through the nostril this response may be important in stimulating the entry of the parasite into the host.

Although it is unlikely that *Gyrdicotylus* could enter the oral cavity through the mouth when wandering around the skin surface the mouth opens infrequently, and has a horny ridge which would be difficult for the parasite to negotiate, it is possible that some could enter through the mouth when
Fig. 53. Routes taken by Gyrlicotylus gallieni when infecting Xenopus.
the toad eats its sloughed skin (Tinsley and Whitear, 1981). The relative success of infection from the skin surface in comparison with entry upon food items is shown in Table 30. The success of invasion on food is significantly less than that through the nostril (P < 0.05, \( \chi^2 \) test).

(3) The attachment of Glyricotylus gallieni.

When attached to the host, the haptor is closely appressed to the host oral epithelium (Plate 10). As can be seen from the imprint left in the epithelium (Plate 18), the haptor is pressed firmly to the host around its entire circumference. The hamuli support the roof of the haptor (Plate 16; Fig. 50). When the specimen of Glyricotylus was removed for examination of the ventral surface of the haptor, the tips of the hamuli snapped, and remained embedded in the wound (Plates 17 & 18). This suggests that they penetrate the host epithelium to some depth. The tegument around the marginal hooks forms a marginal flap to the haptor, and is folded inwards in life. Host epithelium in contact with this flap is deformed, and the cells are flattened (Plate 18), suggesting that the flap is pressed firmly against the host. It may function as a valve, preventing ingress of water under the edge of the haptor when attached. Within each half of the haptor (Plate 18), the epithelium is drawn up into a plug, indicating that this parasite uses suction when attached.

The oral epithelium of Xenopus consists of an epidermis of 6-8 cell layers, on a fibrous dermis. The epidermal cells are living, but a thin layer of Keratin is present on the outer layer. A row of goblet mucus cells is present in the epidermis, approximately two cell layers below the surface (Fig. 54).

(4) The growth and age structure of Glyricotylus populations.

The growth of both laboratory and natural Glyricotylus gallieni populations is shown in Fig. 55. Laboratory infections were derived from a single parasite, and as hosts were maintained in isolation, population growth due to transmission from other toads was precluded. Detached parasites were commonly found in the sediment 40-60 days after infection, and some infected toads were found to have lost their parasites after this period.

The age structure of Glyricotylus populations (Table 31) is biased in favour of older (post - 1st. birth) parasites, and very few newborn individuals were found. The age distribution of detached parasites was similar to that of attached flukes.

The host specificity and taxonomy of the genus Glyricotylus

(1) Morphometric variation.

The specimens of Glyricotylus examined fell into two distinct types. All specimens found on Xenopus laevis laevis (South Africa), X. l. victorianus (Uganda) X. l. petersi (Zambia) and X. borealis (Kenya) were similar in size.
Table 30. A comparison of the success of two routes of infection of Xenopus by Gyridicotylus.

<table>
<thead>
<tr>
<th>Parasites placed on skin</th>
<th>Parasites fed to toad with strip of meat.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seen to enter nostril</td>
<td>Not seen to enter nostril</td>
</tr>
<tr>
<td>No. trials</td>
<td>23</td>
</tr>
<tr>
<td>No. of successful</td>
<td>10</td>
</tr>
<tr>
<td>infections</td>
<td>5</td>
</tr>
<tr>
<td>% success of infections</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
</tr>
</tbody>
</table>

Success rate of parasites fed with food significantly different \((P<0.005)\) from those placed on surface of skin \((\chi^2\text{ test.})\).
Plate 18. The wound inflicted by Gyrdicotylus gallieni upon the mouth of the toad.

v-imprint of marginal valve.
s-epithelium sucked up into suckers.
h-tips of hamuli remaining in wound.
Fig. 54. The structure of the oral epithelium of Xenopus.

ep- epidermis; d-dermis; g-layer of goblet mucus cells; k-keratinised tips of epithelial cells.
EXPERIMENTAL INFECTION

NATURAL POPULATION (imported 10/4/81)

DETACHED PARASITES

No. of Parasites

PERIOD OF INFECTION (DAYS)

DEATHS

As above (imported 20/10/80)

Detached parasites

36 32 28 24 20 16 12 8 4 0

PERIOD OF INFECTION (DAYS)

No. of Parasites

DEATHS

As above (imported 20/10/80)

Detached parasites

36 32 28 24 20 16 12 8 4 0

PIE. 55. The growth of G. duodenalis populations in the mouth

of isolated Xerophilus larvae.

PIE. 55. The growth of G. duodenalis populations in the mouth

of isolated Xerophilus larvae.
Table 31. The age structure of Gyrdicotylus populations.

A) From the mouth of toads.

<table>
<thead>
<tr>
<th></th>
<th>percentage</th>
<th>percentage</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>new-born</td>
<td>4.2</td>
<td>16.9</td>
<td>78.8</td>
</tr>
</tbody>
</table>

Number of flukes examined: - 71.

B) Detached parasites

<table>
<thead>
<tr>
<th></th>
<th>percentage</th>
<th>percentage</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>new-born</td>
<td>4.5</td>
<td>11.3</td>
<td>83.3</td>
</tr>
</tbody>
</table>

Number of flukes examined: - 44.
Some variation exists between these species, but no the variation in species from one host taxon (Xenopus laevis victorianus) is also considerable, it is thought that Gyrdicotylus from all of these hosts are referrable to one species, Gyrdicotylus gallieni Vercammen-Grandjean, 1960. Five specimens of Gyrdicotylus from Xenopus wittei have opisthaptor dimensions which fall outside the normal range of G. gallieni sensu stricto. These specimens have hamuli 58-61 μm long, and overall are considerably smaller than G. gallieni. It is proposed to erect a new species, Gyrdicotylus parvus n.sp. to accommodate these specimens. A single individual, collected from Xenopus clivii in Ethiopia was intermediate in size between G. gallieni and G. parvus. Additional material from this host is necessary to determine the status of this Gyrdicotylus form. A comparison of the morphometrics of Gyrdicotylus from each host taxon is presented in Table 32 and Figs. 51 and 56.

**Gyrdicotylus parvus n.sp.**

**HOST:** Xenopus wittei Tinsley, Kobel and Fischberg, 1979

**SITE OF INFECTION:** Oral cavity.

**TYPE LOCALITY:** S.W. Kigezi, Uganda.


**DIAGNOSIS:** Small species, body length of fixed contracted specimens 0.39 (0.25 to 0.4) mm, maximum width 0.11 (0.08 to 0.16) mm. Opisthaptor cupped, with two hamuli joined down centre by fibrous tissue, and 16 articulated marginal hooks. Opisthaptor modified into two suckers, ventral and dorsal bars absent. Hamuli 56 (56 to 59) μm long, shafts 49 (48 to 53) μm long, with two roots, dorsal 8.5 (8 to 11) μm long, ventral 15 (14 to 17) μm long. Points 22.4 (21 to 24) μm long, with constriction behind tip. Marginal hooks 14 (12 to 17) μm long, sickles 5 (4 to 5) μm long, with sickle filament loop. Penis spherical, present in only one paratype, 14 μm diameter, armed with complete ring of large spines.

The host specificity of Gyrdicotylus gallieni

Gyrdicotylus gallieni taken from Cape Flats Xenopus laevis laevis was used in all host specificity experiments. Four criteria were used to measure the success of this parasite in infecting other species of Xenopus. These were the proportion of parasites successfully entering the nostril after being placed upon the skin of the toad, the proportion of infections which became successfully

* Dimensions refer to holotype. Parentheses give range of dimensions of 5 paratypes.
Fig. 56. Opisthaptor sclerites of Gyrdicotylus gallieni from Xenopus borealis.
Table 2. Dimensions of opisthaptor sclerites of Gymnicycles from species and sub-species of Xenopus.

<table>
<thead>
<tr>
<th>Species of Xenopus</th>
<th>Locality</th>
<th>Total Shaft</th>
<th>Inner root</th>
<th>Outer root</th>
<th>Point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>length</td>
<td>length</td>
<td>length</td>
<td>length</td>
</tr>
<tr>
<td></td>
<td></td>
<td>x α</td>
<td>x α</td>
<td>x α</td>
<td>x α</td>
</tr>
<tr>
<td>laevis</td>
<td>Cape flats</td>
<td>78.4 4 59</td>
<td>13.2 2.4</td>
<td>35.9 4.6</td>
<td>25.9 2.3</td>
</tr>
<tr>
<td>laevis</td>
<td>South Africa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>laevis</td>
<td>Transvaal</td>
<td>78.4 3 63</td>
<td>11.2 0</td>
<td>28.0 2.3</td>
<td>24.2 0.7</td>
</tr>
<tr>
<td>laevis</td>
<td>South Africa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>petersi</td>
<td>Zambia</td>
<td>82.2 3 66</td>
<td>14.7 0.8</td>
<td>35.2 2.6</td>
<td>26.8 2.4</td>
</tr>
<tr>
<td>laevis</td>
<td>Kajansi</td>
<td>91.5 2 71</td>
<td>9.7 3.8</td>
<td>39.3 5.5</td>
<td>29.0 0.8</td>
</tr>
<tr>
<td>victorianus</td>
<td>Uganda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>laevis</td>
<td>Kajansi</td>
<td>79.8 4 62</td>
<td>9.1 1</td>
<td>24.5 1</td>
<td>23.1 1</td>
</tr>
<tr>
<td>victorianus</td>
<td>Uganda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>laevis</td>
<td>Kivu</td>
<td>- - 74</td>
<td>8.4 -</td>
<td>- - 24.5</td>
<td>-</td>
</tr>
<tr>
<td>victorianus</td>
<td>Rwanda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>laevis</td>
<td>L. Bulera</td>
<td>77.5 3 63</td>
<td>12.6 1.5</td>
<td>30.3 0.8</td>
<td>28 0</td>
</tr>
<tr>
<td>victorianus</td>
<td>Uganda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>borealis</td>
<td>Kenya</td>
<td>79.1 3 59</td>
<td>11.2 0</td>
<td>28.7 1.0</td>
<td>21.7 0.99</td>
</tr>
<tr>
<td>wittei</td>
<td>Kigesi</td>
<td>59.5 1 49</td>
<td>6.7 1.3</td>
<td>17.5 0.8</td>
<td>22.1 1.8</td>
</tr>
<tr>
<td></td>
<td>Uganda</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>clivii</td>
<td>Ethiopia</td>
<td>64.4 - 45</td>
<td>9.8 -</td>
<td>15.4 -</td>
<td>23.8 -</td>
</tr>
</tbody>
</table>

1:- Specimens collected by Thurston (1970)
<table>
<thead>
<tr>
<th>Total Shaft length $\bar{x}$</th>
<th>Sickle length $\bar{x}$</th>
<th>Sickle distal width $\bar{x}$</th>
<th>Sickle proximal width $\bar{x}$</th>
<th>No. of specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x}$</td>
<td>$\sigma$</td>
<td>$\bar{x}$</td>
<td>$\sigma$</td>
<td></td>
</tr>
<tr>
<td>16.1 11.6 1</td>
<td>5.5 0.4</td>
<td>5.1 0.7</td>
<td>5.1 0.7</td>
<td>20</td>
</tr>
<tr>
<td>16.8 11.9 1</td>
<td>5.6 0</td>
<td>4.9 0.8</td>
<td>4.9 0.8</td>
<td>4</td>
</tr>
<tr>
<td>17.0 11.6 1</td>
<td>5.1 0</td>
<td>4.9 0.8</td>
<td>4.9 0.8</td>
<td>6</td>
</tr>
<tr>
<td>18.5 12.0 1</td>
<td>5.8 1</td>
<td>6.1 0.4</td>
<td>5.8 0.3</td>
<td>2</td>
</tr>
<tr>
<td>16.1 11.2 1</td>
<td>4.9 1</td>
<td>4.2 0</td>
<td>4.2 0</td>
<td>3</td>
</tr>
<tr>
<td>18.2 11.9 1</td>
<td>5.3 0.5</td>
<td>5.3 0.5</td>
<td>5.3 0.5</td>
<td>2</td>
</tr>
<tr>
<td>16.1 11.7 1</td>
<td>5.6 0</td>
<td>4.7 0.4</td>
<td>4.7 0.4</td>
<td>3</td>
</tr>
<tr>
<td>16.3 11.0 1</td>
<td>5.6 0</td>
<td>5.6 0</td>
<td>5.6 0</td>
<td>4</td>
</tr>
<tr>
<td>14 8.8 0.7</td>
<td>4.9 0.8</td>
<td>3.3 0.8</td>
<td>3.3 0.8</td>
<td>5</td>
</tr>
<tr>
<td>15.4 9.3</td>
<td>5.6 4.3</td>
<td>4.2</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

All dimensions in $\mu$m.
established in the toad (parasites present in mouth after 1 week), the maximum persistence of the parasites within the host, and their ability to breed successfully.

The proportion of parasites entering the nostril of *X. laevis* did not differ significantly from that of control infections on *X. laevis*. A smaller proportion entered the nostril of *X. wittei, X. vestitus* and *X. muelleri*, but there was no evidence of a specific behavioural response to an unnatural host, as was observed in *Gyrodactylus gasterosteii* (Ch.2).

The establishment success of infections of *G. gallieni* was less in all experimental hosts than in the control *X. laevis*, possibly indicating a physiological adaptation to the normal host. However, in all hosts some parasites were able to become established, persisting for at least 1 month in *X. wittei*, 2 weeks in *X. victorianus* and 1 week in *X. muelleri* and *X. vestitus* (these refer to minimum periods of persistence, as determined by dissection). Reproduction of *G. gallieni* was also observed in *X. victorianus* and *X. vestitus*. These observations (summarised in Table 3) indicate that although some physiological specificity may exist, *G. gallieni* not surviving as successfully is novel hosts as in *X. laevis*, the strict behavioural specificity observed in *Gyrodactylus gasterosteii*, which prevents infection of novel hosts (Ch.2) does not occur in *G. gallieni*.

DISCUSSION

The morphology of *Gyrdicotylus* is very similar to that of *Gyrodactylus*, differing in the form of the attachment apparatus, penis and excretory system. The anatomy of the female reproductive system (the most conspicuous organ system) is identical to that described by Braun (1966) for *Gyrodactylus wageneri*. The very small ovary and the development of several embryos within each other indicate that viviparity involves the same processes (Katheriner, 1894; Braun, 1966) as in *Gyrodactylus*. As these complex adaptations are unlikely to have arisen more than once in the evolution of the Monogenea, they provide the clearest evidence of a relationship between *Gyrdicotylus* and the other gyroactylid genera.

The form of the penis and of the excretory system of *Gyrdicotylus*, however, do not indicate a close relationship with other gyroactylids. The penis of *Gyrodactylus* bears a single large grooved hook, surrounded by a ring of small spines, (Braun, 1966). This structure has also been observed in most other
Table 33. The host specificity of Gyrodactylus gallieni from Xenopus laevis laevis.

<table>
<thead>
<tr>
<th>Host:</th>
<th>X. l. laevis</th>
<th>X. l. victorianus</th>
<th>X. l. victorianus</th>
<th>X. muelleri</th>
<th>X. vestitus</th>
<th>X. wittei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original locality</td>
<td>Cape Flats</td>
<td>Kajansi, Uganda</td>
<td>L. Bulera, Ghana</td>
<td>Kigezi, Uganda</td>
<td>Kigezi, Uganda</td>
<td></td>
</tr>
</tbody>
</table>

| Proportion entering nostril | 19/31 | 7/10 | 10/13 | 3/10 | 6/10 | 7/20 |
| Percentage | 61 | 70 | 76 | 30 | 60 | 35 |

| Proportion infections succeeding | 16/28 | 2/10 | 4/10 | 1/10 | 2/10 | 3/20 |
| Percentage | 57 | 40 | 20 | 10 | 20 | 15 |

| Maximum persistence in host | 3 months | 7 days | 14 days | 5 days | 6 days | 1 month |
| Maximum intensity of infection | 16 | 2 | 6 | 1 | 2 | 1 |

Breeding: Yes Yes Yes No Yes No
Malmberg (1970) considered that the excretory system of primitive gyroactylids was more complex than that of advanced forms. This assumption is supported by the description of Öogyrodactylus farlowellae, which has a more complex excretory system than that of any of the viviparous genera (Harris, 1982c; Ch.7). Malmberg (1974) considered Gyrdicotylus to be primitive, for although it has a sophisticated attachment mechanism, the excretory system is relatively complex (Vercammen-Grandjean, 1960). Malmberg's (loc.cit.) analysis can be extended by considering the fate of individual flame cells. During the evolution of the gyroactylid genera, it is probable that flame cells have been lost and gained throughout the excretory system. However, it is unlikely that they would be lost, and subsequently reappear in an identical position in the system. Hence, variations in the excretory system of different genera will indicate the divergence of the gyroactylid genera from each other during their evolution and can form the basis for a tentative phylogeny of the group. Fig. 57 and Table 34 present a comparison of the system in those genera of the Gyrodactyloidea for which it has been adequately described.

The numbering system used by Malmberg (1970) to describe the excretory system has been extended to accommodate the complexities of the Öogyrodactylus system. This analysis shows that although Öogyrodactylus is the most primitive genus described it is not directly ancestral to the gyroactylids as it lacks excretory bladders and two flame cells of the anterior loop (II. f. 1,3). These elements of the system were probably lost after the divergence of the gyroactylids and the öogyrodactylids from a common ancestor. Only three viviparous genera have been examined in sufficient detail to allow this analysis. As shown by Malmberg (1970), all sub-genera of Gyrodactylus are closely related, and can be placed upon a direct sequence of progressive simplification of the excretory system, although sub-genus Gyrodactylus
Fig. 57. Relationships of the gyrodactylid genera as suggested by the morphology of the excretory system.

1). Oogyrodactylus
2). Gyrdicotylus
3). Macrogyrodactylus
4). sub genus Gyrodactylus
5). Other Gyrodactylus sub-genera
Table 34. A comparison of the excretory system in the genera of the Gyrodactyloidea.

<table>
<thead>
<tr>
<th>Flame cells</th>
<th>Oögyrodactylus</th>
<th>Macrogyrodactylus</th>
<th>Gyrdicotylus</th>
<th>Gyrodactylus</th>
</tr>
</thead>
<tbody>
<tr>
<td>of anterior canal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l.lf.1</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>l.lf.2</td>
<td>X</td>
<td>-</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>l.lf.3</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

| Flame cells | of posterior canal |                |                   |              |              |
|-------------|--------------------|-----------------|-------------------|--------------|
| II.lf.1     | X                  | X               | X                 | X            |
| II.lf.2     | X                  | X               | X                 | X            |
| II.lf.3     | X                  | X               | X                 | X            |
| II.lf.4     | X                  | X               | X                 | X            |
| II.lf.5     | -                  | -               | -                 | -            |

<table>
<thead>
<tr>
<th>Anterior flame cells</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I.f.1a</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>I.f.1b</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>I.f.2</td>
<td>X</td>
<td>-</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>I.f.3</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>I.f.4</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>I.f.5</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Posterior flame cells</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>II.f.1a</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>II.f.1b</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>II.f.1c</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II.f.1d</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II.f.2</td>
<td>X</td>
<td>X</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>II.f.3a</td>
<td>X</td>
<td>X</td>
<td>-</td>
<td>X</td>
</tr>
<tr>
<td>II.f.3b</td>
<td>X</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>
Table 34. Comparison of excretory systems... (Cont'd).

<table>
<thead>
<tr>
<th></th>
<th>Öögyrodactylus</th>
<th>Macrogyrodactylus</th>
<th>Gyricotylus</th>
<th>Gyrodactylus</th>
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</thead>
<tbody>
<tr>
<td>II.f.3c</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II.f.4a</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>II.f.4b</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II.f.5a</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>II.f.5b</td>
<td>X</td>
<td>X</td>
<td>X&lt;sup&gt;3&lt;/sup&gt;</td>
<td>-&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>II.f.5c</td>
<td>X</td>
<td>X</td>
<td>-&lt;sup&gt;3&lt;/sup&gt;</td>
<td>X&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>II.f.5d</td>
<td>X</td>
<td>X&lt;sup&gt;4&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**Excretory bladders**

- Present  
- Absent

**Notes**

1) One cell (either II.f.4 or 5) missing.

2) Homologies of II.f.2-5 obscure in Öögyrodactylus as all branch from same point.

3) Homologies of flame cells in haptor difficult to determine.

4) Bladders of Macrogyrodactylus of an unusual pattern.

Based on the accounts of Malmberg (1956a, 1970), Vercammen-Grandjean, 1960 and upon original data.
line of evolution, having lost excretory bladders after its divergence from the main stock. Both of the other genera in which the excretory system has been studied, *Gyrdicotylus* and *Macrogyrodactylus*, show significant differences from *Gyrodactylus*. *Gyrdicotylus* lacks a branch of the posterior excretory system, with the associated flame cells II f. 2 and 3, which is present in *Macrogyrodactylus* and *Gyrodactylus*. From this it may be assumed that *Gyrdicotylus* split from the main gyrodactylid stock, subsequently losing this branch of the excretory system. *Macrogyrodactylus* must also have diverged from the gyrodactylid stock of evolution, losing flame cells 1.If. 1,2,3 and 4 from the anterior loop of the main excretory canal. The excretory bladders of this genus are also unusual, lacking a separate collecting duct (Malmberg, 1956a). They may represent a stage in the disappearance of these organs, or they may be structures which have evolved de novo in this genus. Within the viviparous genera, or at least three lines of evolution can be discerned, forming the *Macrogyrodactylus*, *Gyrdicotylus* and *Gyrodactylus* stocks. The gyrodactylid stock must have been distinct by the time of the first radiations of the primitive sub genera of *Gyrodactylus* which being restricted to infection of teleost fish, probably occurred at the time of the radiation of this host group, during the Jurassic and Cretaceous (Nelson, 1969). The separation of the Gyrodactylidae into the gyrdicatyldes, gyrodactylids and macrogyrodactylids must therefore have occurred at some time before this. As the penis structure of *Macrogyrodactylus* is more similar to that of the gyrodactylines than to that of the gyrdicatyldes, it is probable that this genus is more closely related to the former group. These relationships are summarised in Fig. 58. Vercammen-Grandjean (1960) separated *Gyrdicotylus* into a separate sub-family, the *Gyrdicotylineae*, on the basis of the structure of the attachment apparatus, a subdivision supported by the evidence presented in the present work concerning the structure of the penis and the excretory system.

At present, *Gyrdicotylus* has been recorded only from pipids, a phylogenetically ancient group of anurans. Fossils of this group extend back to the early cretaceous, and the origins of the group are thought to lie in the late Jurassic period, 120 million years B.P. (Tinsley, 1981a). The parasites of *Xenopus* are, in general, not closely related to others of their respective groups, being 'primitive' and yet at the same time highly specialised to this host (Tinsley, loc. cit.). Although *Gyrdicotylus* is a primitive gyrodactylid, with specialised adaptations (attachment mechanism, route of invasion) for infecting *Xenopus*, it cannot represent a line of gyrodactylid evolution which radiated onto early tetrapods and evolved in parallel with them, now being
Fig. 58. Probable interrelationships of the Gyrodactyloidea.
restricted to a relict host group. The Amphibia probably arose in the Devonian, 300m years B.P. and 180m years before the appearance of the Pipidae (Tinsley 1961). The subsequent evolution of the amphibians is complicated (Young, 1973) by phases of terrestrial and aquatic evolution, but the pipids, although ancient relative to the modern Lissamphibia, arose comparatively recently in the phylogeny of the Amphibia. If, as suggested above, the gyrodactylids had separated from the gyroactylid stock before the radiation of Gyrodactylus onto bony fish in the late Jurassic and early Cretaceous (Malmberg, 1970), then they probably existed prior to the appearance of Xenopus, fossils of which date from the Paleocene, 70m years B.P. (Estes, 1975). The occurrence of Gyrodactylus in Xenopus is probably therefore an ecological rather than a phylogenetic phenomenon. The pipids may have become infected by a gyrodactylid from fish sharing a similar habitat during the late Jurassic or early Cretaceous. Xenopus are found in most freshwater habitats in tropical Africa, of which the most characteristic are stagnant water bodies, inhabited, when large enough, by a range of fish with accessory air breathing organs (Clarias, Protopterus, Polypterus and anabantids). A similar association of fish and amphibia in swampy deoxygenated water must have existed throughout much of the period since the appearance of the tetrapods. The extant hosts most likely to harbour gyrodactylines are probably primitive swamp dwelling fish (Polypterus, Calamoichthys and lung fish) rather than other amphibians. In this respect, it is interesting to note that an undescribed species of Gyrodactylus, collected from Polypterus endlichteri by Shotter and Medaiyedu (1977), closely resembles Gyrodactylus gallieni. This species lacks suckers, but the hamuli which have two roots, are closely associated with a very small ventral bar. The marginal hooks of this species have disproportionately small shafts, similar to those of Gyrodactylus. It would be of great interest to examine the excretory system and penis of this species, to determine its affinities within the Gyrodactylidae.

If the trend suggested by the analysis of the excretory system is reliable, then Macrogyrodactylus is also a very primitive genus, and is also liable to be restricted to primitive hosts. Although Macrogyrodactylus polypteri, the only species of the genus which has been studied in depth (Malmberg, 1956; Khalil, 1964, 1970), is found on Polypterus, other species of the genus occur on more advanced fish groups (Paperna, 1979); M. clarii and M. congoense on Clarias, and M. anabantii and M. ctenopomii upon Ctenopoma. However, although these hosts are unrelated, they share the same habitat with Polypterus and
and other primitive swamp dwelling fish, from which they may have originally become infected with Macrogyrodactylus. Ecological transfer of this type has been suggested as the mechanism by which many Gyrodactylus species in temperate Eurasia attained their present host distribution (Ch.2). One species, Macrogyrodactylus latesii infects a host which inhabits open water (Lates niloticus). However, as only one specimen has been described, some doubt as to the accuracy of this record must exist (Paperna, 1979).

Bychowsky (1957) linked the polystomatids and the gyrodactylids because both groups possessed 16 marginal hooks. Vercammen-Grandjean (1960) inferred that Gyrdicotylus supported this relationship, being found in the most primitive and aquatic representatives of the modern Amphibia and having a number of characters (penis morphology, hamulus shape, suckers) in common with the Polystomatidae and Sphyranuridae. However, the attachment apparatus is derived, paralleling but not homologous with that of the polystomatids. The double roots of the hamuli are an adaptation for this suctorial attachment, the dorsal roots and shafts forming a girder supporting the roof of the suckers and peduncle, while the ventral roots reinforce the anterior wall of the haptor (Fig. 50). Hamuli with twin roots are of general occurrence within the Monogenea, except in those gyrodactylids (Ch.3) in which the dorsal root has been suppressed. They do not, therefore provide a link between the gyrodactylids and the polystomatids. Because they are adaptive, they do not indicate a relationship between Gyrdicotylus and those other gyrodactylids (Gyrodactyloides, Archigyrodyactylus or Metagyrodactylus) which also have double rooted hamuli. The resemblance of the penis of Gyrdicotylus to that of the polystomatids is also coincidental, as the morphology of the individual sclerites is different. In Gyrdicotylus these are curved, and have a deep central groove, whereas in the polystomatids they form simple spines.

Microspectroscopic tests have indicated that Gyrdicotylus may contain haemoglobin, concentrated around the developing embryos and ova. Haemoglobin has been recorded from one other monogenean, Oculotrema hippopotami, in which it is probably a respiratory pigment (Thurston, 1963). Its significance in Gyrdicotylus is unclear, but it may have a respiratory function, enabling the parasite to obtain sufficient oxygen when the toad remains submerged for long periods. The pigment fades in parasites from hosts in captivity, without apparently affecting their survival. It is possible that in the laboratory
Toads spend less time fully submerged than in nature and that the oral mucosa becomes less extensively deoxygenated, reducing the need for a respiratory pigment in *Gyrdicotylus*.

During the present work, *Gyrdicotylus* has occurred upon the oral mucosa, and has never been found in the gut beyond the longitudinal oesophageal rugae. This site of infection was recorded by Thurston (1970), although Vercaumen-Grandjean (1960) originally described the parasite from the stomach and intestine. Tinsley, Kobel and Fischberg (1979) also recorded *Gyrdicotylus* from the mouth of *Xenopus wittei*. In view of the considerable body of data suggesting that the mouth is the normal site of infection, it is thought that the original description is inaccurate. Only one other monogenean *Enterogyrus cichlidarum*, has been recorded from the host intestine (Paperna, 1963), and this parasite has a curiously thickened tegument, which is thought to offer protection against digestive enzymes. In the absence of such an adaptation, it is unlikely that *Gyrdicotylus* could survive in the intestinal environment. The highly aquatic *Xenopus* leaves the water occasionally, and the oral cavity is one of the few sites which could be tolerated by a monogenean when the host is on land (Tinsley and Whitear, 1980). The outer keratinised layer of the epidermis is moulted regularly, and would be unsuitable for colonisation by ectoparasites (Tinsley and Whitear, 1980). Although the mouth is also lined by a thin layer of keratin (Fig. 54), this is probably moulted haphazardly, and can probably be disrupted mechanically by the parasites during feeding. The oral epithelium contains a continuous layer of goblet mucus cells, similar to that observed by Fishelson (1972) in the skin of the teleost *Lepadichthys lineatus*. The significance of this layer for the attachment and feeding of *Gyrdicotylus gallieni* is not clear.

The principal route of entry of *Gyrdicotylus* into the toad is probably through the nostril. Some infections succeed when parasites are fed to toads on strips of meat (Table 30). The success rate of infections is significantly lower by this route, and its importance in nature is not clear. *Xenopus* moultis and eats its thin, keratinous outer cuticle every few days (Tinsley and Whitear, 1980). It is possible that *Gyrdicotylus* attached to the cuticle at this time would be ingested, enabling them to invade the mouth of the host. Cannibalism may also provide a route of infection for *Gyrdicotylus*. The nostrils offer a more favourable route of infection invasion than the mouth, for although partially sealed by a flap of skin, they are frequently fully dilated when the host is submerged, offering maximum opportunity for a parasite to enter. By contrast, the mouth is closed for most of the time, restricting the periods when it can be entered by a parasite attached to the skin of the host.
The passage of Gyrodicotylus into the host is associated with high mortality. The success rate of infections when parasites spend some time searching on the skin is not significantly different from that when the parasites rapidly locate and enter the nostril, suggesting that high mortality is associated with the period immediately after the parasites have entered the mouth (the nostril is a simple tube, unlikely to interfere with the movements of parasites). The cause of this failure of infections to become established after the parasites have successfully entered the nostril is not known.

This route of infection is very unusual amongst monogeneans. Tinsley (in press) has recently shown that it is also used by the oncomiracidia of Pseudodiplorchis and Neodiplorchis when they invade spadefoot toads. Similar strategies are also seen in other monogeneans from enclosed sites, including the larvae of Merizocotyle, which enters the nasal fossae of elasmobranchs, and the adult of Gyrodactylus cryptarum, which inhabits the acoustic-lateralis canals of its host (Kear, 1968b; Malmberg, 1970).

The most important problem for gyrodactylids inhabiting enclosed sites of infection is not the route of entry, but the route of exit used. In oviparous monogeneans, including Merizocotyle, Calicocotyle, Dictyocotyle and the polystomatids, eggs are released and allowed to pass passively from the relevant orifice. In gyrodactylids no specialised transmission stage is present in the life cycle. Skin parasites rely on fortuitous host-host contacts or accidental dislodgement and subsequent reattachment for transmission (Ch. 4). Gyrodicotylus, however, from an enclosed site, cannot take advantage of fortuitous host contacts, and if dislodged would probably reattach to the oral mucosa. If unable to reattach, the parasites would probably be carried down the oesophagus into the stomach and killed. Parasites have not been observed leaving the host mouth, but it is possible that some may escape when the toad feeds, or alternatively it is possible that Gyrodicotylus actively migrates from the host oral cavity through either the mouth or the nostrils.

The growth of Gyrodicotylus gallieni populations within the mouth of the toad is very slow (Fig. 55). Although Thurston (1970) recorded 70 G. gallieni from the mouth of one toad, such high intensities of infection have not been recorded during the present work, in which a maximum of only 20 parasites was recorded from the mouth of a toad in the three month experimental period. This contrasts with Gyrodactylus alexanderi, and G. gasterostei, in which population growth is rapid and final population size is potentially very large (Lester and Adams, 1974a; Ch. 4). The slow growth rate of G. gallieni populations may be
due to high mortality of established parasites, or to a low fecundity. It is impossible to measure these parameters directly, because of the enclosed site of infection. However, a consideration of the parasite population age structure indicates that the majority of parasites recovered possessed a penis, indicating that they had given birth on at least one previous occasion. Very few newborn flukes were encountered in the populations within each host. This suggests that parasites are long lived, yet reproduce infrequently, contrasting strongly with the strategy of Gyrodactylus gasterosteii, in which the parasite populations contain many newborn flukes, few of which survive beyond the first birth. The fecundity of G. gallieni is low, as is the mortality amongst established flukes.

After 1-3 months of infection, there was an increase in the number of Gyrodicotylus found detached in the water containing the toads. (Fig. 55). This phenomenon was observed in both laboratory and natural infections, and has also been reported (Tinsley, personal communication; Jackson, personal communication) during routine screening of toads for Protopolystra infections. A number of toads, which were known to have been infected, were found to have lost their infection when subsequently examined. This decline in parasite infections may have been due to poor culture conditions, particularly the starvation of the hosts for three months. However, the toads were apparently healthy throughout the experimental period, and detached parasites were active and able to infect another host. Alternatively, a host reaction of the type observed in sticklebacks (Chs. 3, 4) may be responsible, although if parasites were simply dislodged, they might be expected to reattach to other parts of the oral mucosa, or be swept down the oesophagus. It is possible that a chemical reaction against the parasites takes place, leading to a migration out of the mouth as some aspect of their environment is rendered unfavourable.

The decline in infestation may represent a migration from the host facilitating transmission, although it is surprising that such a large proportion of parasites should leave the host at one time.

The low fecundity of Gyrodicotylus is probably related to its route of transmission. Unlike Gyrodactylus, it faces considerable difficulties when infecting new hosts, first gaining access to the toad mouth after a "difficult" migration and subsequently leaving it again in order to effect transmission to other toads. It has no opportunity for fortuitous transmission when other hosts are near, which would reduce the risk associated with movement from one toad to another. It has therefore adapted for maximum persistence in individual hosts, with a very firm attachment mechanism, which reduces the risk of accidental dislodgement to a minimum. One factor influencing colonisation strategy in free living organisms (Macarthur and Wilson, 1967; Southwood, 1977) is the persistence of suitable patches of environment.
In the case of gyrodactyliids, the life span of the host (the environment patch) is long relative to that of the parasites. However, the suitability of the host can be reduced if it mounts a host response against its parasites. The low fecundity of *Gyrodicotylus* may be an adaptation reducing the harm done to the host by preventing the build up of large populations within the toad mouth. This may delay the onset of a host reaction, maximising the period for which the host remains suitable for infection.

The strategy adopted by *Gyrodicotylus gallieni* inevitably results in populations of this parasite containing a large proportion of older flukes with functional male reproductive systems, which may be important in determining the role of cross fertilisation in the genus. Copulation may take place more frequently than in populations of *Gyrodactylus gasterosteii*, in which few individuals survive to develop a functional male reproductive system (Ch.4). Insufficient is known of the mechanisms of gyrodactyliid reproduction to predict the extent to which sexual reproduction is relinquished in *Gyrodactylus* or *Gyrodicotylus*. The slow reproduction of *Gyrodicotylus gallieni* will reduce the genetic heterogeneity generated by the rapid reproductive rate of other parasites (Maynard Smith, 1978; Price 1979), including *Gyrodactylus*, although this will be counteracted by the more frequent occurrence of cross-fertilisation.

*Gyrodicotylus gallieni* was first recorded by Vercammen-Grandjean (1960) from Xenopus levis victorianus. It was subsequently recorded from Xenopus sp. by Thurston (1970), a host later identified as *X.1. victorianus* by Tinsley (1973). Tinsley, Kobel and Fischberg (1979) also recorded *G. gallieni* from Xenopus wittei. During the present study, specimens have been examined from Xenopus laevis, X.1. victorianus, X.1. petersi, X borealis, X. wittei and X. clivii, subsequentially extending the host range of the genus. These records are from a wide area of sub-saharan East and Central Africa (Uganda, Kenya, Ruanda, Zambia and Ethiopia) and South Africa. No specimens have been obtained from West Africa.

The specimens examined fall into two taxonomic groups, referrable to *G. gallieni* Vercammen-Grandjean, 1960 and *G. parvus* n.sp. Although the morphometric variability of *G. gallieni* from *X. laevis* sub species and *X. borealis* is greater than that of *Gyrodactylus* species, all specimens from these hosts fall within the same range of variation.

Specimens collected by Thurston (1970) from Kajansi in Uganda, are slightly larger than Vercammen-Grandjean's type material, and other specimens examined
during the present study. However, further specimens from Kajansi, collected by Tinsley, are also smaller than those collected by Thurston, and it is considered that this variation is normal for \textit{G. gallieni}. It may be environmentally induced, as has been observed in \textit{Gyrodactylus} by Kulemina (1977).

\textit{Gyrdicotyly}l\textit{us parvus} n.sp, from \textit{X. wittei} was originally described as \textit{G. gallieni} by Tinsley, Kobel and Fischberg (1979). However, these specimens are considerably smaller than \textit{G. gallieni} collected from any of its other hosts.

Other parasites of \textit{Xenopus} (including \textit{Protopolystoma xenopodia}, \textit{Cephalochlamys namaquensis} and \textit{Chitwoodchabaudia} spp) are distributed amongst the host species in a way which reflects host chromosome complement (Tinsley, 1981b). However, insufficient is known of the distribution of \textit{Gyrdicotyly}lus to apply this analysis rigourously to this genus. \textit{G. gallieni} has been recorded only from host species with 36 chromosomes, whereas \textit{X. wittei}, the host of \textit{Gyrdicotyly}lus \textit{parvus} n.sp has 72 chromosomes. If the specimen recorded from \textit{Xenopus clivii} is also found to belong to \textit{Gyrdicotyly}lus \textit{parvus}, then this species would occur on both 36 and 72 chromosome \textit{Xenopus} taxa. Despite extensive examination, Tinsley (personal communication) has never recorded \textit{Gyrdicotyly}lus from \textit{Xenopus tropicalis}, which has only 20 chromosomes. However, this parasite has also never been recorded from several other \textit{Xenopus} taxa, and much further information on the host-parasite distribution, of \textit{Gyrdicotyly}lus is required, before its pattern of speciation relative to that of the host can be assessed.

In comparison to \textit{Gyrodactylus} (see Malmberg, 1970; Ch.2), \textit{Gyrdicotyly}lus shows a relatively large range of morphometric variation. This may reflect the probable importance of cross fertilisation in this genus, and the degree of genetic heterogeneity thus generated. No consistent correlation of morphology with environment was observed, despite the range of habitats (including sites in equatorial, savannah and mediterranean climate zones) from which toads were available.

The widespread occurrence of \textit{G. gallieni} in sub-species of \textit{X. laevis} and in \textit{X. borealis} is supported by observations upon the host specificity of \textit{G. gallieni} from \textit{Xenopus laevis laevis} from South Africa. The success of infections of these parasites in \textit{X.1. victorianus} was similar to that in their natural host (Table 34). However, fewer infections succeeded in becoming established in other species of host (\textit{X. muelleri}, \textit{X. vestitus} and \textit{X. wittei}), and population growth and persistence in these hosts was reduced.
The parasites were able to breed in *X. vestitus* and *X. laevis victorianus*. These observations indicate a different pattern of host specificity to that of *Gyrodactylus gasterostei* (Ch.2), in that *Gyrodactylus gallieni* can infect most of the species of *Xenopus* which were tested experimentally. Although its success in novel hosts is reduced. Each sub-species of *Xenopus laevis* may be infected by its own strain of *Gyrodactylus gallieni*, morphologically identical to each other but physiologically adapted to their own particular host taxon. There is no evidence to suggest that *G. gallieni* possesses adaptations allowing it to discriminate between host species in the manner shown for *Gyrodactylus gasterostei* (Ch.2). This difference in specificity between *Gyrodactylus gasterostei* and *Gyrodactylus gallieni*, is of considerable interest. Although strict host specificity is often considered evidence of a phylogenetically ancient relationship (Llewellyn, 1982), in this case the association of *Gyrodactylus gallieni* with *Xenopus* may be more ancient than that of *Gyrodactylus gasterostei* with the three spined stickleback. Alternatively, the difference may be due to the closer relationships between the species of *Xenopus* used to demonstrate host specificity than those between the hosts used to test the specificity of *Gyrodactylus gasterostei*. However, the karyotypes of *Pungitius pungitius* and *Gasterosteus aculeatus* are similar (Muramoto, Igaishi, Ito and Makino, 1969; Chen and Riesman, 1970), showing less variation than those of *Xenopus* species (Tymowska and Fischberg, 1973). The difference in specificity may therefore be due to ecological factors. In temperate freshwater, several species of fish may be present, often shoaling together (Glaser, 1974). Each host may be infected by several *Gyrodactylus* species, suggesting that competition between species may be intense (Ch.2). Parasites usually show a close adaptation to their normal host, and it is therefore unlikely that a species of *Gyrodactylus* would be able to successfully colonise a novel host in the face of competition from species already adapted to that host. As transmission rates in such habitats are potentially very high (Ch.4), it may be advantageous for a parasite to possess adaptations preventing its movement onto a foreign host. This strategy would place considerable selective pressure upon the development of sensory mechanisms capable of discriminating normal hosts when first contacted (Ch.2). In contrast, *Xenopus* often occupies habitats in which there is less opportunity for competition between *Gyrodactylus*. The internal site of infection reduces the need for sensory adaptations for identifying potential hosts, and in relation to the overall range of *Xenopus* species, areas of sympatry are rare (Tinsley, 1981b).
The biology of *Gyrdicotylus gallieni* reflects its adaptation to a novel host, in this case the anuran *Xenopus*. The difference in the structure of the skin, which has a regularly moulted keratinous layer, and the occasional movements of the host onto land (Tinsley and Whitear, 1980) has necessitated the infection of an internal site, the oral cavity. Infection of this site is achieved by migration through the nostril, and presents the parasite with considerable problems during transmission. Being unable to make use of fortuitous host-host contacts, in the manner of *Gyrodactylus* (Ch.4), individuals of *Gyrdicotylus* face a high risk of mortality during transmission. This has led to the adoption of a strategy of persistence within an individual host, with a very firm attachment mechanism and a low fecundity, which reduces the risk of the parasite population stimulating a host reaction. As a consequence of low fecundity and mortality, the age structure is biased in favour of older flukes, with a mature male reproductive system, which may increase the genetic heterogeneity of *Gyrdicotylus*. This heterogeneity, in combination with the reduction in the probability of contact with gyrodactylids from other host species, has resulted in *Gyrdicotylus gallieni* being less host specific than *Gyrodactylus gasterostei*, and to the genus *Gyrdicotylus* having speciated less than *Gyrodactylus*. 
Ch. 7. THE MORPHOLOGY AND LIFE CYCLE OF COGYRODACTYLIUS FARLOWELLAE n. gen.et sp.

This chapter forms the basis of an account submitted for publication in 'Parasitology'.
INTRODUCTION

The Gyrodactylidae, the only viviparous monogenean family, is a group of thirteen genera and several hundred species, which all have a relatively uniform morphology. The most distinctive feature of the group is the retention of embryos until fully grown. Several generations of offspring develop within each other sequentially, so that a parasite may contain a large daughter which already has an embryo in utero, containing in turn a developing third generation. The reproductive mechanism has attracted considerable attention, and provides the clearest separation between gyrodactylids and oviparous monogeneans.

Many characters of taxonomic importance (including the reproductive system and the larva) have been modified in the viviparous genera, making their relationships with the oviparous monogenean families the subject of considerable debate. Gyrodactylids have been related to both monopisthocotyleans (by Johnston and Tiegs, 1922) and to polyopisthocotyleans (Bychowsky, 1957), and more recently it has been suggested that the family represents an isolated group within the Monogenea (Baer and Euzet, 1961; Lambert, 1980a, b; Llewellyn, 1981a).

In addition to the obscure phylogenetic relationships of the gyrodactylids, the ecological and evolutionary significance of viviparity is unknown. The Monogenea are characteristically oviparous, producing eggs which are released into the environment where they hatch to give a free swimming oncomiracidium. This is the infective stage in the life cycle, which attaches to a new host and grows to maturity. Apart from the gyrodactylids, which are all viviparous, some other genera show varying degrees of development of eggs in utero. This occurs in a number of polystomatids, as a response to selective pressures related to the semi-terrestrial nature of the hosts (Combes, 1981). Callorhynchcola multiformis, a chaetocerosid parasite of holocephalans, retain eggs until the larvae are ready to hatch; however, this phenomenon is seen in only a few scattered genera of fish monogeneans. The adaptive significance of larval development in utero in the monogeneans of fish is unclear, but it may be associated with reducing reproductive losses (Llewellyn, 1981b).
During the course of work on gyrodactyloid ecology, I obtained two specimens of a South American loricarid catfish, Farlowella amazonum (Gunther, 1864), which were infected with large numbers of an oviparous monogenean, closely related to the gyrodactyloids. The parasite, which is described in this paper, provides important new evidence on the origin of viviparity, in addition to clarifying the relationships of the gyrodactyloids within the Monogenea.

MATERIALS AND METHODS

Two catfish (Farlowella amazonum, Loricaridae), imported from Peru by Aquatic Nurseries Ltd., Hampton, Middlesex, were obtained shortly after importation and maintained in 4 litres dechlorinated, softened tapwater (pH 7.2) at 25-27°C. The fish fed on the abundant algal growth in the aquarium.

Observations were made on the parasites attached to the fins and skin of the living, unrestrained hosts. Eggs and larvae were collected by pipette and larger parasites were removed from the host using insect pins. Specimens were flattened, fixed in 10% formol-saline and stained with alum carmine or haematoxylin and eosin. The egg shell tanning system was explored using catechol or fast red salt B after the methods of Johri and Smyth (1956).

The excretory system was studied using positive phase contrast illumination (Leitz Dialux) on living parasites. Argyrophilic structures were silver stained by one hour's incubation in 1% silver nitrate solution in the dark at 2°C followed by rinsing in distilled water and exposure to sunlight for 10 minutes. To allow three dimensional
reconstruction of the reproductive system, unflattened formalin-fixed specimens were embedded in Epon 812 epoxy resin after dehydration in acetone, and 2μm sections were cut on a Huxley LKB II ultramicrotome and stained with iron haematoxylin (12 h iron aluminium sulphate mordant, 6 h aqueous haematoxylin).

On the death of one fish, the caudal peduncle was fixed in 1% osmium tetroxide (1 h) postfixed in 2% glutaraldehyde (1 h), rinsed in 0.2M cacodylate buffer (pH 7.2) and distilled water (1 h each) and dehydrated in acetone, critical point dried (Polaron 3000) and sputter coated with gold (Polaron E5000). The specimen was examined in a Cambridge 600 scanning electron microscope.

OBSERVATIONS

Morphology of Oogyrudactylus gen. et sp. nov.

(1) Body form

The parasites were present in large numbers on the skin of the catfish, and in life had a transparent cylindrical body up to 1.25 mm long, through which the opaque, off-white vitellaria and gut contents could be seen. The opisthaptor is clearly demarcated from the body by a slender penduncle. Two distinct cephalic lobes are present on the anterior of the body, which bear single cilium sensillae and "spike" sensillae of fused cilia, identical to those described by Lyons (1969) from Gyrodactylus sp. Post-pharyngeal glands empty through ducts at the tips of the cephalic lobes, and may produce an adhesive secretion used in attaching the anterior of the body during locomotion.

(2) Opisthaptor

This haptor is divided into sixteen finger-like lobes, each containing a marginal hook. These are dorsal to a pair of large hamuli, associated with ventral and dorsal bars. The marginal hooks are small, with inconspicuous sickles. The distal ends of the shafts are flexible, allowing the sickle to rotate about the end of the shaft. Malmberg (1970) described this form of marginal hook as articulated, a term which will be used throughout this paper.

1. Measurements of holotype and of paratype series included with description, p. 269.
A long shank ligament (as defined by Mizelle and Kritsky, 1967) extends from the base of the marginal hook shaft into the centre of the opisthaptor. The marginal hooks are fully developed in the newly hatched larva, and do not grow subsequently.

The fully developed hamuli are very long, with well developed stout points and slightly curved shafts. Two roots are present on each hamulus, the outer being much longer than the inner, which is connected to the dorsal bar. The ventral bar lacks both membrane and processes, and is not articulated with the hamuli.

When viewed laterally, the hamuli and bars are contained in a ventral lobe of tegument, which is partly separated from the dorsal lobe containing the marginal hooks (Pl 19). The dorsal lobe of the haptor is also deeply divided into sixteen papillae bearing the marginal hooks. This gives the hamuli and marginal hooks the capacity for a considerable amount of independent movement.

(3) Gut and Pharynx

The pharynx opens through a ventral subterminal mouth (Pl 20). It has two chambers, a posterior muscular and glandular part, with a rim of tentacular processes, and an anterior, thin walled chamber through which the posterior is extended during feeding. This structure is also seen in many gyrodactylids, and it probably functions in the same way. The processes are placed in contact with the host tissue and bring about lysis of epithelial cells, which are then sucked into the gut by muscular action. The gut divides immediately behind the pharynx into two unbranched crura which extend the length of the body into the peduncle. The gut cells are colourless, and the gut contents are normally white, homogenous and opaque. Pigmented cells have not been observed in the gut wall. The parasite normally feeds on host epidermis, but on one occasion, when the host was very heavily infected, parasites were seen with host erythrocytes and granules of black dermal pigment in the gut lumen. This indicates that the parasites are able to erode the epidermis and to feed on the dermis, although the rate of regeneration of the epidermis is probably normally sufficient to replace cells damaged by feeding parasites, reducing damage to the dermis.

(4) Female reproductive system

The mature female reproductive system is shown in Fig. 59. The single germarium shows a range of stages of oocyte maturation. At the anterior edge of the
Plate 20. Comparison of the ventral surface of *Oogyrodactylus farlowellae* using SEM and silver nitrate staining.

B Silver nitrate stain

s-sensillae; m-mouth.

A Scanning Electron Micrograph.

Note group of six sensilla clustered anterior to mouth.
Fig. 59. The holotype of Oogryroductylus farlowellae, whole mount.

p-penis; e-egg; ot-ootype; sr-seminal receptacle; bg-basophilic glands; ov-ovary; v-vitellaria; ha-hamulus; db-dorsal bar; vb-ventral bar.
germarium is a spherical, transparent seminal receptacle. This contains oocytes (up to three) and a mass of sperm in a wispy matrix. The seminal receptacle opens into the ootype, which is separated from the uterus by a slight anterior constriction. The ootype is lined by single celled eosinophilic cells and the ducts of much larger basophilic glands, which fill the body between ovary and pharynx, also open into it. The uterus has a thin wall, and is capable of considerable distension when an egg is present. The egg is retained in the uterus before laying, and a posterior peg on the egg projects into the ootype, where a sticky droplet is secreted. Only one egg is retained in the female duct at a time.

Fifty to seventy irregularly rounded vitelline follicles occur in four post-ovarian longitudinal rows, associated in pairs with the gut crura. Two vitelline ducts run forward between the follicles, fusing in the region of the ovary. The single common vitelline duct then runs into the ootype. The vitelline follicles react positively with both catechol and Fast Red Salt B, indicating the presence of both phenolase and of the phenol required for egg shell tanning.

The egg shell is oval, attenuated anteriorly and more rounded posteriorly.

The shell is golden yellow when fully developed. The egg is retained in utero until embryonation is comparatively advanced, though the precise stage at which it is released has not been established. In eggs containing active larvae, an operculum can be distinguished, one third of the length of the shell from the anterior tip.

(5) Male reproductive system

The large single testis is median and post-ovarian. It is surrounded by a thin wall, and contains groups of cells separated by channels containing inactive sperm. The vas deferens leaves the centre of the testis and runs anteriorly along the side of the body. It opens into the centre of a large, elongate seminal vesicle, containing active sperm. From the seminal vesicle, a short duct leads into the basal bulb of the penis (Fig. 60). The penis is elongate with a small basal bulb at right angles to its base. The walls of the penis and bulb are muscular. The penis is extrusible (Pl 21) and the tip bears a thin ring of sclerotised tissue (hooks of the type seen in Gyrodactylus and associated genera are absent). Two glands, at tip and base, are associated with the penis.
Fig. 60. Paratype of Oogyrodactylus farlowellae with functional male reproductive system and immature female system.

sp- spike sensilla; ph-pharynx; ph.g-pharyngeal glands; p-penis; pb-penis bulb; ut-uterus; g-gut; sr-seminal receptacle; sv-seminal vesicle; ov-ovary; t-testis; v-vitellaria.
Plate 21. SEM of Oogyrodactylus farlowellae showing extended penis.

p - penis; u - uterine pore.
(6) The excretory system

The excretory system is made up of two looped canals running longitudinally down each side of the body. A short collecting duct runs from each main canal to the surface, opening to the exterior through a small pore in the pharyngeal region. The two halves of the system anastomose in the cephalic and peduncular regions. Each longitudinal canal has 5 flame cells placed along its length on each side and another 18 flame cells communicate with the main canals via secondary ducts. 13 of these cells drain into the posterior loop of the main canal, and 5 enter the anterior. The complete system is shown in Fig. 61C.

(7) Argyrophilic structures

The distribution of silver staining structures corresponds closely with the distribution of single cilia sense organs, derived from SEM. A comparison of the same area of the body shown by both techniques is given in Pl 20a and Pl 20b. The sensillae are arranged in four transverse bands with additional clusters around the cephalic lobes, mouth, genital aperture, and opisthaptor. The concentration is greatest on the dorsal surface (Figs. 61A and B).

(8) The newly hatched larva

Hatching of larvae was not observed, but the smallest free-living larvae removed from the aquarium were the same size as the largest active larvae seen in unhatched eggs, and were therefore considered to be newly hatched.

These larvae were unciliated, with well developed opisthaptor, anterior attachment glands and sense organs. The body is cylindrical and the cephalic lobes are prominent. The cup-shaped haptor is indistinctly separated from the body, and is armed with sixteen fully developed marginal hooks. The hooks line the rim of the opisthaptor and have long shank ligaments which come together in the peduncular region (Fig. 62). The hamuli are represented by thin sclerotised spines in the peduncle, on either side of the marginal hook shank ligaments.

The gut and pharynx of the larva have the same form as those of the adult, although the large basophilic glands posterior to the pharynx are undeveloped. The reproductive system is primordial. Single cilia sense organs are present over the entire body surface, but their distribution has not been mapped. Multiciliate spike sensillae are present on the tips of the cephalic lobes.
Fig. 61. Excretory system and single cilium sensilla of *O. farlowellae*.

A- Dorsal surface; B- Ventral surface.
C- Excretory system.
Fig. 62. Newly hatched larva of Oogyrodactylus farlowellae.

co - cerebral organ; ph - pharynx; g - gut;
sl - shank ligament; hr - hook rudiment; mh - marginal hook.
A refractile, lenticular structure is present between the cephalic lobes, anterior to the pharynx. This appears pale green in living specimens and appears to have a laminate structure. It persists throughout the life of the parasite, but is most conspicuous in the larva. Its position and appearance suggest that it may be homologous with the 'cerebral organ' of Macrogyrodactylus polypteri recorded by Khalil (1971). The function of this organ is obscure, but its proximity to the cephalic lobes (known to possess a concentration of sense organs), and its prominence in the free living newly hatched larva suggest that it may be sensory, of some importance in host location.

(9) Life Cycle and post-larval development

Eggs start to embryonate in utero, and are then released from the genital pore. The exact stage of development at which this occurs is not known. The eggs adhere to the substrate by the sticky droplet and may be capable of adhesion to the skin of the host, although examination with SEM failed to provide evidence of this. Empty egg shells were first observed in the sediment from the bottom of the aquarium 5 days after the introduction of the hosts. Prior to this, eggs containing fully developed larvae had been found, and these were removed from the aquarium and cultured for up to 9 days away from the host without hatching, although the larva could be seen moving within the egg. Chemical factors may therefore be implicated in the hatching of these larvae, as has been demonstrated for Entobdella soleae and Acanthocotyle lobianchi by Kearns and MacDonald (1976). After the hosts had been in the aquarium for 6 days, newly hatched larvae were observed on their skin. The post-larval development of the parasites takes up to 8 days, and the life cycle (egg to adult) is completed in 11 to 13 days at 27°C. The life span of the parasites is not known.

Newly hatched larvae have undeveloped hamuli and attach themselves to the host by the marginal hooks. These are held at a steep angle to the surface of the host (Pl 22). The hamuli start to develop in the peduncular region, and then elongate and grow through a semicircle to form the points (Fig. 63). The shafts elongate and the hamuli migrate posteriorly until the shafts lie ventral to the marginal hooks. The haptor increases in size, and becomes deeply divided to form the papillae within which the marginal hooks lie. This takes place without growth of the marginal hooks and causes the haptor to flatten until the adult form is reached. When the hamulus shafts have developed, and the posterior migration has taken place, the hamulus roots and dorsal and ventral bars develop.
Plate 22. Young larva of Oogyrodactylus farlowellae.
Fig. 63. The post-larval development of the haptor of O. farlowellae.

A- Dorsal aspect of haptor; B-Lateral aspect of haptor;
hr- hamulus rudiment; mh-marginal hook.
The reproductive system starts to mature after the larva has infected the host. The male system develops first, as in all other oviparous monogeneans. The testis, seminal vesicle and penis develop rapidly, and are functional about 7 days after hatching, when the parasite is 0.67 (0.47-0.89) mm long. At this stage, the only parts of the female system which have developed are the uterus and seminal receptacle, which is thin walled, globular and fully developed. The ootype and its associated glands are undifferentiated at this stage, and the wall of the female duct is muscular throughout its length. The ovary is made up of a small patch of undifferentiated cells on the posterior wall of the seminal receptacle, and the vitellaria, vitelline ducts and ootype glands are undeveloped.

This stage of development of the reproductive system (shown in Fig. 60) is accompanied by a phase of intense copulatory behaviour. During copulation, the extended penis of one individual is lodged deeply within the uterus of the partner. When flukes are copulating, they face each other and elongate the anterior third of the body, which is entwined with the head of the partner. The pharyngeal regions of the body, bearing the genital apertures are brought together. The apertures are provided with large numbers of sensory cilia, which are probably responsible for co-ordinating correct alignment. Copulation lasts for up to 30 secs, then the partners disengage. The parasites are promiscuous and after separating from one partner, copulation may immediately take place with another. Sperm was found in a wispy matrix in the seminal receptacle of all mature parasites. On the hosts examined, parasite density was high enough to ensure that cross-fertilisation was widespread. It is not known whether self-fertilisation is important in nature, but the positioning of the genital apertures suggest that it is possible.

After the male reproductive system has been functional for some time, the female system develops further and egg laying begins. The parasite also grows to its full size. The ovary increases in diameter, and oocytes start to mature. The vitellaria grow rapidly, the ducts develop, and finally vitelline droplets appear in the cells. The ootype differentiates from the posterior of the uterus, and the glands discharging into it develop. The uterus becomes less muscular, and more capable of distension to accommodate the egg. The testis becomes obscured by the vitellaria and ovary, and it is not visible in fully developed parasites. It is possible that it recrudesces, ceasing production of sperm. Copulation is seen less frequently in egg-laying flukes.
Fig. 64. Copulation in Oogyradactylus farlowellae.
The pharyngeal glands also develop in the mature parasites, but apart from growth, the gut and pharynx do not show any other changes. Development of the excretory and sensory systems was not followed. The spike sensillae and the cerebral organ do not change in size, and become progressively less conspicuous as the parasite grows.

The attachment of O. farlowellae to the host
The loricarid catfish lack scales, but are armoured with sub-dermal bony plates. The hamuli of the parasite can be extended from the tegument for a considerable length, and can be actively embedded in the host skin (Pl 23). The marginal hooks seem relatively unimportant in attachment, due to their small size and shallow penetration of the skin. Their function may be to prevent the hamuli turning about their longitudinal axis, which would cause them to tear free of the host's skin. The dorsal bar may also have a similar function, preventing the hamuli from splaying. The ventral bar is pressed onto the surface of the fish (Pl 24), and may act as a pressure pad, holding the anterior of the haptor firmly against the host. The attachment of the parasites causes a severe wound in the skin of the host. The hamuli produce deep pits which probably take some time to heal (Pl 25).

The ecology of the host, Farlowella amazonum
The loricarids are exclusively South American catfish, characterised by their heavily armoured bodies and scaleless skin. They show adaptations for living on the bottom of rivers, feeding on the algal mat present on stones. The diet of Farlowella is not precisely known, for although Placobates, with a gut 25X the body length has shown to be an algal feeder, Loricaria, with a gut only 2.5X body length, is thought to be carnivorous (Alexander, 1966). Farlowella has a short gut (1.7X body length, personal observation), so this genus also may take animal food. Even if Loricaria and Farlowella are carnivorous, they are not active predators, and they probably feed on small invertebrates encountered in the Aufwuchs community (Hora, 1930).

The morphology of the fish suggests that they are poor swimmers, as the body is elongate and flattened, with very small fins. The swim bladder is reduced (Alexander, 1966), and the body is encased in heavy bony scutes which reduce the lateral movement of the tail. In the aquarium, these fish move very little, remaining attached to the sides of the tank by the suckorial
Plate 23. Ogyrodactylus farlowellae, attached to skin of host.

mh - marginal hooks; h - hamuli embedded in skin.
Plate 24. Ventral surface of haptor of *O. farlowellae*.

hp - point of hamulus; mh - marginal hook; vb - ventral bar.
Plate 25. Skin lesion on Farlowella amazonum, caused by haptor of Oogyrodactylus farlowellae.

h-wound caused by hamulus; mh-wound caused by marginal hook.
mouth, swimming only when provoked. Hora (1930) and Alexander (1966) considered these to be adaptations for torrent dwelling, maintaining the fish in one position on the stony stream bed.

Few observations have been made on the ecology of Farlowella in nature. Some loricarids are undoubtedly torrent dwellers, as has been recorded for the related Argas marmorata in Colombia (Johnson, 1912).

Hora (1930) stated that the loricarids are torrent dwellers in hill streams, but this is unlikely in view of the wide distribution of the family in South America (Isbrucker, 1978). It is probable that Farlowella often occurs in seasonal streams, where adaptations for torrent dwelling are necessary during the wet season, but where in the dry season they are restricted to stagnant pools as described by Lowe-McConnell (1975). Little can be said of the environment of F. amazonum, from which Oogyrodactylus farlowellae was obtained, although the work of Patrick (1964) suggests that it is not as markedly seasonal as that studied by Lowe-McConnell (1975).

DESCRIPTION

Oogyrodactylidae fam. nov.

Diagnosis: Monogenea (Nonopisthocotylea) with sixteen articulated marginal hooks, all placed at opisthaptor margin. Single pair of ventral hamuli, articulated with a dorsal bar and closely associated with a ventral bar. Cephalic lobes well developed, bearing spike sensillae.

Reproductive system protandrous, oviparous, with separate male and female genital apertures, vaginae absent. Single ovary, single post-ovarian testis and four longitudinal rows of post-ovarian vitellaria. Male copulatory organ a tubular, weakly sclerotised penis. Larva an unciliated oncomiracidium with well developed cephalic lobes.

Type genus: Oogyrodactylus gen. nov. Also contains Phanerothecium Kritsky and Thatcher, 1977.
Oogyrodactylus **gen. nov.**

**Diagnosis:** With characters of the family. Hamuli with well developed roots, ventral bar lacking membrane. Penis muscular and extrusible, with small ring of sclerotisation at tip. Penis bulb present at base, penis sac absent. Seminal vesicle elongate, lying along long axis of body, vas deferens entering centrally.

**Type species** Oogyrodactylus farlowellae *sp. nov.*

Oogyrodactylus farlowellae *sp. nov.*

**Host:** *Farlowella amazonum* (Gunther, 1864). The taxonomy of the genus *Farlowella* is very confused, and is at present undergoing revision (Isbrucker, personal communication). The identification of *F. amazonum* is therefore provisional, and the specimens are deposited in the Institute of Taxonomic Zoology, Amsterdam, pending critical examination.

**Habitat:** Skin and fins.

**Locality:** Not precisely known, but from Amazon headwaters in region bordering north east Peru, south east Colombia and western Brazil (0-5°S, 70-75°W).

**Diagnosis:** With characters of genus and family. Body length 1.46 (0.9-1.2) mm extended, 0.5-0.7 mm contracted. Maximum width 0.74 (0.15-0.34) mm.

Marginal hooks 35 (29-36) μm long, sickles 4.2 (4.0-6.0) μm, shafts 32 (28-36) μm long, with conspicuous shank ligaments. Length of hamuli 106 (99-104) μm, shafts curved, 67 (58-70) μm long, dorsal root 7 (6-10) μm ventral root 39 (36-50) μm long. Dorsal bar 24 (21-28) μm, slightly curved, connected to dorsal roots of hamuli. Length of ventral bar 31 (29-35) μm, width 10 (7-12) μm at centre, widening at ends, without membrane or processes.

Pharynx with long processes, 67 (54-73) μm long, anterior chamber 67 (48-70) μm wide, posterior chamber 92 (70-100) μm wide. Gut dividing immediately behind pharynx into two unbranched crura. Single thin walled vas deferens, saccate seminal vesicle, 70-160 μm long, 25-40 μm wide. Penis muscular and elongate, 67 (57-80) μm long, with penis bulb at base 28 (25-40) μm long.

Ovary spherical, 112 (100-150) μm diameter, in centre of body. Seminal receptacle globular, 49 (35-56) μm diameter, lying between ovary and ootype.

* Dimensions refer to holotype. Range of paratypes expressed in parentheses, based on 5-9 specimens. Dimensions not visible in holotype expressed only as range of paratypes.
Uterus thin walled when mature, containing single egg. Ootype lined by unicellular glands and surrounded by large basophilic glands. Up to 70 vitelline follicles present, each $74(42-98) \mu m$ diameter, bordering the gut crura in four longitudinal rows. Eggs oval, $120(96-136) \mu m$ long, $60(44-74) \mu m$ broad, with an attenuated anterior and rounded posterior. Small posterior peg, bearing sticky droplet.

Larva unciliated, $140-200 \mu m$ long, with sixteen fully developed marginal hooks and two hamulus rudiments.

**Material** Holotype (BM(NH) 1982.3.30.1) and paratypes (BM(NH) 1982.3.30.2-9) deposited in British Museum (Natural History). Other paratypes in United States National Museum Helminthological Collection (USNM No.77095) and in author's collection.

**DISCUSSION**

*Ogyrodyactylus farlowellae* most closely resembles *Phanerothecium caballeroi*, described by Kritsky and Thatcher (1977) from the Colombian catfish *Cephalosilurus tungara*. *P. caballeroi* was reported to be viviparous, but re-examination of the type material suggests that this is unlikely. No eggs or embryos are present in any of the type specimens, possibly because they are all too young to possess a mature female system. However, the holotype and one paratype show developing vitelline follicles (some containing vitelline droplets) in the posterior of the body, and one specimen also contains vitelline ducts. These structures are absent in the viviparous gyrodactylids. Kritsky and Thatcher (1977) described a saccate uterus in *Phanerothecium*, but this organ appears to be glandular, lacking a limiting membrane, and is probably an aggregation of large basophilic glands surrounding the ootype. The ovary is large and globular, similar to that of *Ogyrodyactylus*, but unlike that of the viviparous gyrodactylids, in which it is restricted to a small group of cells on the posterior wall of the seminal receptacle (Braun, 1966). The penis of both *Ogyrodyactylus* and *Phanerothecium* is tubular, unlike that of gyrodactylids in which it is globular, armed with short spines.

The opisthaptor sclerites of both genera are also very similar. The hamuli have two roots, whereas in most gyrodactylids, the inner root is absent.
The ventral bar is identical, having a peculiar hour-glass shape, lacking both processes and membrane. In both genera, the marginal hooks are inconspicuous, and have very small sickles.

The male genitalia of Phanerothecium and Oögyrodactylus are sufficiently different to justify their generic separation. The penis of Oögyrodactylus is unarmed, except for a small ring of sclerotisation at the tip, and has a muscular basal bulb. In Phanerothecium however, the penis is sclerotised throughout its length, bearing a complex coiled part of the vas deferens within a cirrus sac. The seminal vesicle is a larger structure than in Oögyrodactylus, and is irregularly saccate instead of elongate.

Oögyrodactylus and Phanerothecium are closely related, and it is proposed to place them both in the same family, the Oögyrodactylidae fam. nov., distinguished from the Gyrodactylidae because its representatives are oviparous, rather than viviparous. The two families share a number of features in common. They both have sixteen marginal hooks with articulated sickles placed around the edge of the opisthaptor. The only other monogenean family with articulated marginal hooks is the Acanthocotylidae, but in this group two of the sixteen hooks are placed at the centre of the haptor. A second character connecting the gyroactylids with the oögyrodactylids which reinforces their separation from other monogeneans, is the form of the hamuli and bars. All gyroactylids, except those in which sclerites have been secondarily lost or gained, have a single pair of ventral hamuli, closely associated with a ventral and dorsal bar. The dorsal bar is connected with the hamuli, and the ventral bar is often articulated with them. In the Dactylogyridae, the bars are not closely associated with the hamuli (except in the Tetraonchidae and Ancyrocephalidae), and are never connected with them. As pointed out by Lambert (1980a), the pattern of development of the bars in this group suggests an independent origin from the hamuli. In Oögyrodactylus and the viviparous gyroactylidae, however, the simultaneous development of the hamulus roots and the dorsal bar (Ergens, 1965; personal observations) and the physical connection between them suggests that they all develop from the same group of onchoblasts.

The anterior of the body is similar in both the oögyrodactylids and the gyroactylids, but somewhat different from all other monogenean families. The cephalic lobes bear spike sensillae in association with large numbers of individual sensory cilia. Although other types of compound uniciliate
receptors have been recorded from monogeneans (Fournier, 1981), the structure and position of these spike sensillae is unique to these two families. The cephalic lobes have a sensory role in addition to an adhesive function. They may be used in host location by the larva of Oögyrodactylus and by adult gyrodactylids. In all other monogenean families, the cephalic lobes are indistinct, absent from the larva (Lambert, 1980b), and lacking spike sensillae. In these groups the primary role is to provide an adhesive prohaptor, carrying the ducts of very large adhesive glands. Both the gyrodactylids and the oögyrodactylids lack the one or two pairs of pigmented dorsal eyes present in most other monogeneans.

Although considerable superficial differences exist between the female reproductive systems of the two families (due to the adaptations for viviparity shown by the Gyrodactylidae), a number of similarities can be seen. Both families have separate male and female genital apertures, whereas in most monogeneans the two systems open into a common orifice. The seminal receptacle is a dilatation of the oviduct, lying immediately adjacent to the ovary. A similar arrangement is seen in a few other monogeneans, but in most families the receptacle is a secondary sac lying to one side of the oviduct and communicating with it by a short duct.

Both the excretory system and the pattern of single cilia sensillae have been used to elucidate the relationships of the Gyrodactylidae (Malmberg, 1970; Lambert, 1979, 1980a,b). The excretory system of Oögyrodactylus farlowellae is intermediate between the longitudinal system of the gyrodactylids (Malmberg, 1970) and the circular system of the oviparous monogenean larvae studied by Llewellyn (1963). A true comparison is not possible, however, because Malmberg and Malmberg (1970) showed that in Dactylogyrus the circular system becomes longitudinal as the parasite matures. Similarly, Lambert (1980b) worked only on the sensilla patterns of larval parasites; as no observations have been made on the changes taking place in this system as the parasite matures, it is not possible to relate the sensilla patterns of the larvae of oviparous monogeneans to the system of the adult gyrodactylid.

The undeveloped hamuli of the Oögyrodactylus larva indicate that it is an unciliated oncomiracidium, not a post-oncomiracidium. This larva differs from that of other monogeneans since at hatching it bears spike sensillae on the tips of well developed cephalic lobes.
The resemblances between the Oogyrodactylidae and the Gyrodactylidae suggest that the two families should be placed in the same supra-familial group. The gyroactylids have been related to several different groups in phylogenetic classifications of the Monogenea. Johnston and Tiegs (1922) created the order Gyrodactyloidea, encompassing the gyroactylids, calceostomatids, dactylogyrids and protogyrodactylids. Bychowsky (1957) separated the gyroactylids from this group and included them with the polystomatids in the order Gyrodactyloidea on the basis of similarities in the number and configuration of the marginal hooks. Llewellyn (1963) pointed out that this was an artificial grouping, because the polystomatids possess a genito-intestinal canal and are clearly members of the Polyopisthocotylea as originally defined by Ohdner (1912). They are generally sanguinivorous, and possess 'haematin' cells in the gut. The gyroactylids however, are monopisthocotyleans, lacking a genito-intestinal canal and haematin cells, and feeding principally on mucus and epithelial cells. The most recent classifications have regarded the gyroactylids as an isolated group within the Monogenea. This was first suggested by Baer and Euzet (1961) who retained the gyroactylids within the monopisthocotylean Gyrodactyloidea, while separating all the other families originally placed in this order into other groups. Lambert (1977, 1980b) suggested that the gyroactylids arose from a polyopisthocotylean ancestor by neoteny, and this origin was thought to be responsible for the absence of tertiary attachment organs, genito-intestinal canal and haematophagy. The discovery of Oogyrodactylus farlowellae, an oviparous monopisthocotylean which is closely related to the gyroactylids, seriously weakens the hypotheses of Bychowsky (1957) and Lambert (1977, 1980b). It confirms the opinion of Llewellyn (1981a) that the gyroactylids are an isolated group of monopisthocotyleans. It is proposed to place the Gyrodactylidae and Oogyrodactylidae within the monopisthocotylean order Gyrodactyloidea Johnston and Tiegs, 1922, as amended by Baer and Euzet (1961). It is at present impossible to relate the Gyrodactyloidea to any other monogenean group, although the presence of a number of primitive characters (articulated marginal hooks, separate genital openings, seminal receptacle formed from oviduct) suggests that they separated from the main monogenean stem at a very early stage in the evolution of the group.

The origin of viviparity within the Gyrodactyloidea

Previously, the origin of viviparity in the Gyrodactylidae has been obscure because of the absence of related oviparous forms. However, the description
of *O. farlowellae* makes it possible to suggest a route by which the phenomenon arose in the family.

During the maturation of *O. farlowellae*, the male reproductive system becomes functional at a time when the only parts of the female system which have developed are the uterus and seminal receptacle (Fig. 60). At this point the reproductive system closely resembles that of the mature *Gyrodactylus* (Fig. 55), in which the vitellaria, vitelline ducts and ootype are undeveloped, and the ovary is restricted to a few cells on the posterior wall of the seminal receptacle (Braun, 1966). The only differences between the female systems of the two genera at this stage of development are that in *Gyrodactylus* the uterus is expanded to accommodate the developing embryo, and the seminal receptacle has the additional function of retaining the developing oocyte (Braun, 1966). At some point during the evolution of the Gyrodactylidae, it is probable that precocious oocyte maturation has occurred, resulting in oocytes entering the uterus before the male system is mature, because the gyrodactylids are progynous, giving birth to one daughter before the male system becomes functional (Turnbull, 1956; Khalil, 1970; Lester and Adams, 1974). All other families of the Monogenea are protandrous, the male system developing before the female.

The development of eggs *in utero*, despite its selective advantage in reducing larval wastage (Llewellyn, 1981b), is a rare strategy within the Monogenea. Apart from the gyrodactylids, which are exclusively viviparous, and the polystomatids, in which egg retention *in utero* is an adaptation maximising transmission during the short period when the host enters water, it is restricted to only a few isolated genera. One disadvantage of this strategy is that the development time of eggs is long relative to their rate of production, a bottleneck effect which has been overcome in *Acanthocotyle greeni* by retaining a cluster of eggs outside the uterus (Macdonald and Llewellyn, 1980), or, as in *Callorhynchicola multitesticulata*, by expansion of the uterus (Manter, 1955).

Retention of eggs reduces larval wastage, but it also reduces the dispersal of eggs. If the hosts are randomly distributed in the environment, the probability of reinfection is maximised if they too are randomly distributed. Therefore, because eggs are released along the track of one host moving through its environment, it is advantageous for them to be dispersed by water currents and turbulence. Active dispersal by the larvae is insignificant,
Fig. 65. A comparison of the reproductive system of the mature Gyrodactylus and the immature Oögyrodactylus.

A- Immature Oögyrodactylus
B- Gyrodactylus.

p- penis; sv- seminal vesicle; u- uterus;
vd- vas deferens; sr- seminal receptacle;
o- ovary; t- testis.
because of their short free-swimming life (Kearn, 1971). However, when hosts are highly aggregated in their environment (through shoaling or restriction of suitable microhabitats), the probability of reinfection can be maximised by concentrating the infective larvae in areas of high host density. This can be achieved by retaining eggs in utero and by having unciliated crawling larvae, two adaptations which reduce the passive dispersal of the infective stages.

A close correlation is seen between the occurrence of egg retention and the possession of an unciliated larva in the Monogenea. In Callorhynchicola multitesticulata, Acanthocotyle greeni and Ongyrodactylus farlowellae, both adaptations are present (Kearn, 1967; Llewellyn, 1963, 1981b). In Dionchus, an unciliated larva is associated with the presence of filaments on the egg shells which cause the eggs to become firmly entangled in the gills of the host (Ktari, 1977), an adaptation equivalent to egg retention in its effect on reducing egg dispersal. The hyperparasite Udonella also has an unciliated larva, as do species of Acanthocotyle, which do not retain developing eggs. All of these parasites have hosts which are highly aggregated in their environment. Callorhynchicola, parasitic on holocephalans, and Acanthocotyle, parasitic on skates and rays, are slow moving, sedentary and demersal. Ongyrodactylus infects a host which, living in torrents, is likely to be sedentary and aggregated into parts of the river which are suitable for it. It also probably spends much time in contact with the river bed. Udonella is parasitic on Caligus, a copepod parasite found in the gill chamber of teleost fish. The distribution of Caligus in its environment (the fish population is liable to be over-dispersed, and the copepods remain in intimate contact with the gill substrate of the host, over which the Udonella larvae crawl. Dionchus is a parasite of Echeneis, the remora, which in turn is attached to the skin of larger fish, a comparable case to that of Udonella on Caligus.

In addition to the association of larval retention and unciliated larvae with parasitism of overdispersed sedentary hosts, many monogeneans with these adaptations live in an environment in which a strong unidirectional water current is present. In the majority of monogeneans, released eggs fall away from the host and settle onto the substrate. They are not exposed to constant water currents, although they may be stirred by turbulence. The eggs and larvae of Ongyrodactylus, however, are constantly exposed to the torrent in which their host lives. The eggs and larvae of Dionchus and
Uodonella are also washed by a constant water current, because the adult flukes are hyperparasitic on fast swimming fish. The parasites all show a number of adaptations, including egg retention, unciliated larvae and attachment of eggs to a solid substrate (by a sticky droplet in Oogyrodactylus, or by an egg-shell filament in Dionchus) which prevent the eggs and larvae from being swept from the habitat. Similar adaptations are seen in a range of stream dwelling organisms, including coelenterates, molluscs and crustaceans.

Retention of eggs to an advanced stage of development, as seen in *O. farlowellae*, reduces the reproductive rate of the parasite because the short uterus limits the rate at which mature eggs can be produced. In fully viviparous parasites, the disadvantage would probably become more important. *Gyrodactylus*, for example, rarely gives birth to more than four daughters in its life span (Turnbull, 1956; Bychowsky, 1957; Lester and Adams, 1974). Precocious oocyte maturation overcomes this disadvantage of viviparity, and has been extended in the gyrotrictylids so that several generations of oocytes develop within each other, inside the uterus of the parent. In addition, when the F₂ generation has started to develop within the uterus of the F₁ daughter, a second oocyte develops to the point where it is ready to mature immediately after the first F₂ daughter has been born. These adaptations reduce the generation time of the gyrotrictylids to the point where it is the shortest in the Monogenea (Turnbull, 1956; Bychowsky, 1957; Lester and Adams, 1974). Associated with suppression of the larval phase in gyrotrictylids, transmission is achieved by the movement of adult parasites between hosts. An important factor in the evolution of the group has been the retention of larval sensory adaptations in the adult.

*Oogyrodactylus farlowellae* is adapted to a host with a highly specialised ecology. The oogyrodactylids have not been recorded from the comparatively well studied areas of Eurasia, North America and Africa, and in contrast to the abundant gyrotrictylids, they do not appear to be an important part of the ectoparasite fauna of any fish family. However, *O. farlowellae* provides an understanding of the evolutionary pathway followed by the viviparous gyrotrictylids, which have become one of the most important and successful monogenean groups.
Ch. 8. CONCLUSIONS
The viviparous gyroactylids have a host range and ecology paralleling that of the oviparous Monogenea. The discovery of *Oggyrodactylus farlowellae* has shown that they are derived from a monopisthocotylean stock, but has not revealed any close relationship with any other oviparous family. Indeed, the Oggyrodactylidae and the Gyrodactylidae show considerable differences from other monopisthocotylean monogeneans. The most significant of these differences is the possession, in *O. farlowellae* and the gyroactylids of 'spike' sensillae on the cephalic lobes. These organs, found only in these groups are retained throughout the life cycle, and were thought by Lyons (1969) to be chemosensory, and in the present work they have been implicated in host location and identification (Ch. 2). In other oviparous monogeneans, this function is fulfilled by a group of six dorsal sensillae, which are lost after a host is contacted (Lambert, 1980a, b). The absence of sense organs which could be utilised for host selection in adult monogeneans has been a major obstacle in the development of a theory to account for the origin of viviparity in the group. Llewellyn (1981b) suggested that viviparity arose from monogeneans with an oncomiracidium larva by a process of retention of larvae to a progressively more mature stage, reducing the wastage of eggs and larvae. However, in postulating an origin from a form with an oncomiracidium larva, it is necessary to suggest intermediate forms in which the adults develop the adaptations necessary for transmission, before the total suppression of the larval phase. In *Oggyrodactylus farlowellae*, which has a small, infective larva, these sensory adaptations are present throughout the life cycle in a form which is otherwise similar to other oviparous monogeneans. This genus may have the capacity for adult transmission without showing extreme egg retention, and suggests an alternative route for the evolution of viviparity in the Monogenea.

Both Bychowsky (1957) and Llewellyn (1963, 1965) considered that the Monogenea evolved from a ciliated 'protomonogenean' similar to the modern rhabdocoels, which developed a parasitic relationship with early fish. The life cycle of rhabdocoels lacks a distinct larval phase (Hyman, 1951), and the juvenile resembles a miniature adult. Ectocommensal groups, for example the temnocephalids, have a life cycle in which both adults and juveniles are capable of transmission (Jennings, 1971), and which may be analogous to the life cycle of *Oggyrodactylus farlowellae*. It is therefore possible that the life cycle of *O. farlowellae* resembles that of the ancestral 'protomonogenean' more closely than any other extant monogenean. After the invasion of vertebrate hosts by the creeping larvae and adults of the protomonogeneans, the group may have diverged into two stocks, depending on the ecology of the hosts concerned. On slow moving, sedentary hosts,
the frequency of host contact may have resulted in adult transmission being more successful than larval transmission. This would have led to the suppression of the larval phase and the development of viviparity. On the other hand, on more active pelagic fish, selection may have favoured the evolution of a specialised infective stage, the actively swimming oncomiracidium, and the suppression of adult transmission, preventing established flukes dispersing away from the host. This hypothesis implies that the separation between viviparous forms, transmitted as adults, and oviparous forms, transmitted by larvae, is a very ancient phenomenon within the Monogenea, and suggests that the gyroactylids and the oviparous monogeneans have coexisted throughout much of the group's history.

As discussed elsewhere (Ch.7), the retention of eggs in utero and the suppression of the larval phase reduces the fecundity of the parasites. A comparison of the immature *Oögyrodactylus* with *Gyrodactylus* suggests that in the latter group, viviparity is associated with precocious oocyte maturation (Ch.7). This precocity, in conjunction with embryo clustering, forms the second major difference between the gyroactylids and the oviparous groups, as it greatly reduces the generation time of the parasites, giving them the fastest reproductive rate in the Monogenea (Ch.4). The fully viviparous gyroactylids have attained considerable success, radiating onto a wide range of solitary and fast moving fish, utilising host behaviour to facilitate transmission (Bychowsky, 1951). The group probably radiated in parallel with the expansion of the host groups, as suggested by Llewellyn (1982) to account for the phyletic host range of the Monogenea. By the Jurassic and Cretaceous epochs, when the teleost families expanded considerably, at least three gyroactylid stocks, the gyroactylines, the macrogyrodactylines and the gyrdicotylines had already diversified, of which the gyroactylines colonised the teleosts and radiated in parallel with them (Ch. 6). Some evidence suggests that the gyroactylids were originally parasites of freshwater fish. The most primitive form, *Oögyrodactylus farlowellae* is a skin parasite, and the gyroactyloids have articulated marginal hook sickles, which may be an adaptation for skin parasitism. The articulation allows the sickle to be turned through a considerable angle when gripping a flat surface, and is an adaptation also seen in the skin parasitic acanthocephalids. The rigid marginal hooks of other monogeneans are more suitable for gaffing gill tissue, and when used for attaching to skin, when the larva migrates to the gills after infection, the sickles are brought into the same angle as those of gyroactylids by flexure of the shafts (Cone and Burt, 1981). The gyroactylids are the only monogenean skin parasites found i
... where they may have evolved free from competition with the marine acanthocotylids, capsalids, monocotylids, microbothrids and enoplocotylids.

The ability of adult gyrodactylids to move freely between hosts, and the survival of these parasites away from their host (Ch.4), highlights an important difference in the ecology of Gyrodactylus from that of oviparous monogeneans. Amongst oviparous genera, detachment is equivalent to death, and strong selective pressures will act to maximise the persistence of the parasite upon the host. In viviparous forms, detached parasites are capable of reattachment, and selection for persistence upon a host is balanced by the probability of survival and reattachment of detached individuals. The attachment of skin parasitic genera shows a trend towards reducing damage done to the host, which is also paralleled in other groups of skin parasitic monogeneans (Ch.3). Amongst gill parasitic gyrodactylids, the strength of attachment is increased, regardless of the damage inflicted upon the host. This trend is also seen in the oviparous dactylogyrids, tetraonchids and ancyrocephalids, which actively gaff host tissue with the hamuli (Kearn, 1968a). This difference in attachment strategy may be due to a difference in the response of gills and skin to parasitic infection. Although Lester (1972) demonstrated a host response against the skin parasite G. alexanderi, similar to that described in the present work against G. gasterostei, the effect of responses against gill parasites is not clear. Paperna (1964) reported such a response, but this may only take place against larger dactylogyrids. Firmer attachment may therefore be possible if the gills are less likely to react against parasitic infection than the skin. Alternatively, the firm attachment of gill parasites may be associated with the inherent difficulties of transmission in this habitat. The skin parasite Gyrodactylus gasterostei is freely transmitted by both host-host contact and by reattachment of detached flukes (Chs. 4 & 5). This species is able to make use of fortuitous host contacts, and may possess behavioural adaptations increasing the speed with which it can react to such contacts (Ch.3). On the other hand, Gyrodactylus gallieni, from the mouth of Xenopus, has a difficult transmission route, involving migration through the host's nostril, and has a poor rate of establishment once it has entered the mouth (Ch.6). It is probably unable to make use of fortuitous host-host contacts for transmission.

The demographic strategies of these parasites are correlated with their route of transmission and strength of attachment. Skin parasites from fish, for example Gyrodactylus gasterostei, have a good chance of successful reattachment...
to another host if they become detached, which is correlated with a weak attachment mechanism, high accidental rate of dislodgement and a high rate of reproduction (Chs. 3 & 4). Moreover, the host reaction by the stickleback renders the fish unsuitable for parasites after a relatively short period of infestation (Ch.4). In these conditions of a short period of suitability of the host and a good chance of transmission to other hosts, a colonisation strategy, with high reproductive and potential dispersed rates, is the most suitable life history strategy which can be adopted (Southwood, 1977). If, however, the host remains susceptible to infection for a longer period, and transmission is arduous, then a persistence strategy, with firm attachment and low fecundity is more appropriate (Southwood, loc. cit.) The population dynamics of a gill parasite, which might show an intermediate strategy, have not been studied experimentally, but observations upon G. rarus in nature suggest that they are transmitted slowly, and have a slow reproductive rate, (Ch.5).

The survival of Gyrodactylus away from the host, and its potential ability to reattach to the host is of some significance for the regulation of its populations. At low host densities dislodgement is equivalent to death, as so few parasites survive to reattach to another host. Then, host death and a host reaction can limit the parasite population in the manner demonstrated by Anderson (1976). However, at high host densities, the proportion of parasites surviving dislodgement is such that both host reaction and host death are ineffective at regulating parasite abundance (Ch.4), and an epizootic may occur. This accounts for the pathogenicity of Gyrodactylus species to cultured fish. During the present study, the regulation of ectoparasite populations in a natural host population was examined. The host chosen, the three spined stickleback, is annual in the streams examined, and parasite population regulation was principally achieved by a period of high mortality in mid summer, when transmission from adults to fry takes place (Ch.5). However, the regulation of Gyrodactylus populations on this host cannot be considered typical, as most fish species live for longer than one year.

The ecological strategy of the gyroactylids probably has a strong influence upon their reproductive biology. Because these organisms are progynous (Ch. 4 & 6), their mortality pattern determines the proportion of functional male individuals present in a population. Skin parasites, with a high mortality rate and a population age structure biased in favour of younger
flukes (Ch.4) have few males in a natural population, reducing the extent to which cross fertilisation can occur. The population of Gyridicotylus gallieni, however, contains numerous older flukes which have a functional male reproductive system (Ch.6), and cross fertilisation between these parasites may take place freely. The reproductive mechanism of gyrodactylids is not fully understood (Katheriner, 1904; Braun, 1966), but the observation that isolated individuals may continue to give birth for several generations without the agency of cross-or self-fertilisation (Ch.3) indicates that normal sexual reproduction may not be important. The restriction of genetic flow in Gyrodactylus populations may have led to the extensive speciation seen in this genus. On the other hand, genetic flow may be more important in Gyridicotylus, reducing the degree of speciation seen. Gyridicotylus gallieni is considerably less host specific and more adaptable to different hosts than Gyrodactylus (Chs. 2 & 6).

The hypothesis advanced above concerning the speciation of Gyrodactylus does not account for the existence of several species on individual hosts (Ch.2). It is probable that speciation of Gyrodactylus has occurred in several distinct phases, different species groups radiating onto phylogenetically or ecologically related host groups at different times. The successive radiations of species groups onto new hosts has increased the complexity of the Gyrodactylus fauna of natural hosts, creating the pattern of Gyrodactylus communities seen on modern host groups. These radiations increase the possibility of interactions between different species, further influencing the pattern of speciation in the genus.

The hypothetical evolutionary sequence outlined above suggests that the gyrodactylids become distinct at a very early stage in monogenean evolution, and that they are not closely related to any other group within the class. Their biology differs considerably (due to viviparity and adult transmission) from that of other monogeneans, and yet they have achieved a modern day importance equal to that of the oviparous forms. Further analysis of the evolution and ecology of the group will require two approaches. In the first place, studies based on comparative anatomy (Bychowsky, 1957; Llewellyn, 1963, 1965, 1981a; Lambert, 1979, 1980a,b) provide insights into the relationships of the monogenean groups. However, it is also necessary to consider experimentally the selective pressures determining the evolution of the ecological strategies seen in these groups. In this respect analysis of Southwood (1977), relating reproductive strategy and dispersal mechanism in free living organisms will be valuable in determining the
significance of the ecological strategies and transmission syndromes of parasites, including monogeneans. A combination of such studies will provide an integrated account of the processes determining the evolution of the gyrodactylids, and may allow the development of a host-ectoparasite model of general application to a range of parasite taxa.
Appendix: Publications based upon the content of this thesis.


* The unrefereed manuscript of this paper forms the basis of Ch. 7. of this thesis.
Journal of Zoology 148, 88-152.

Bulletin of the Fishery Research Station of Ceylon 17, 237-239.

Journal of the Marine Biological Station of the United Kingdom 61, 833-842.


Parasitology 94, 3-33.


Nature 280, 455-461.


Meredith Corporation, New York.

Iowa State Journal of Research 24, 77-81.


Journal of Fish Biology 20, 39-51.


In: Parasites of Freshwater fish and the Biological basis for their Control.
I. P. S. T., Jerusalem.

I. P. S. T., Jerusalem.

Parasitology 47, 40-45

Beachamp, P. de (1912). Isancistrum loliginis n. g. n. sp. Trematode parasite de Calmar et l'existence de Solenocotyle chiaji Dies.


Archiv fur Protistenkunde XXI, 1.

Bychowskaya-Pavlovskaya, I. E. and others (1964). Key to the parasites of freshwater fish of the U. S. S. R.
I. P. S. T., Jerusalem.

Trudy Borodinskoi biologicheskoi stantsii 6, 51-55.


Trudy zoologicheskogo instituta Akademiya nauk SSSR (Leningrad) 13, 91-125.

John Wiley, Chichester.

Chappell, L. H. (1969). The parasites of the three spined stickleback (Gasterosteus aculeatus) from a Yorkshire pond. I. Seasonal variation of a parasite fauna.
Journal of Fish Biology 1, 137-152.

Cytogenetics 2, 321-332.

Chubb, J. C. (1964). A preliminary comparison of the parasite fauna of the fish of Llyn Padarn, Caernarvonshire, an oligotrophic lake, and Lyn Tegid (Bala lake), a late oligotrophic or early mesotrophic lake.


Oikos 15, 265-273.

Einzsporn, T. (1965). Nutrition of *Ergasilus sieboldi* Nordman II.
The uptake of food and the food material.

Freshwater Biological Association Scientific Publication 25
Ambleside.

Edward Arnold, London.

Ergens, R. (1962). Weitere neue Arten der Gattung Gyrodactylus
Nordmann, 1832 (Monogenoidea) fur die Fauna der
Tschechoslowakei.
Zoologisk Listy 11, 327-331.

Ergens, R. (1965). Die morphogenese der chitinoiden teile
des Haptors bei *Gyrodactylus tincae* (Malmberg, 1956) Malmberg
1964 (Monogenoidea) und ihre morphologisch-metrische
Variabilitat.
Zeitschrift fur Parasitenkunde 26, 173-184.

Ergens, R. (1930). On the problem of three species of the genus
*Gyrodactylus*, members of the *G. wageneri* group. (*Gyrodactylidae: Monogenea*).
Helminthologia 17, 257-267.

Ergens, R. and Bychowsky, B. E. (1967). Revision of the species
*Gyrodactylus nemacheili* Bychowsky, 1936 (Monogenoidea).
Folia parasitologica, Praha 14, 225-238.


Evans, D. A. (1953). Experimental evidence concerning contagious
distributions in ecology.
Biometrika 40, 136-211.


Ikezaki, F. M. and Hoffman, G. (1957). *Gyrodactylus eucaliae* n. sp. (Trematoda: Monogenea) from the brook stickleback *Eucalia inconstans*.


*Parasitology* 46, 106-116.


*Advances in Parasitology* 19, 1-63.


*Arbeit zoologisch und zootomisch Institut Wurzburg* X 128-164.


*Journal of the Marine Biological Association of the United Kingdom* 42, 93-104.

Kearn, G. C. (1963). Feeding in some monogenean skin parasites: *Entobdella soleae* on *Solea solea* and *Acanthocotyle* sp. on *Raja clavata*.

*Journal of the marine Biological Association of the United Kingdom* 42, 749-766.

Kearn, G. C. (1964). The attachment of the monogenean *Entobdella soleae* to the skin of the common sole.

*Parasitology* 54, 327-335.


*Parasitology* 55, 473-480.

Kearn, G. C. (1967). Experiments on host finding and host specificity in the monogenean skin parasite *Entobdella soleae*.

*Parasitology* 57, 585-605.
Parasitology 58, 149-163.

Parasitology 58, 921-928.

Kearn, G. C. (1970). The production, transfer and assimilation of spermatophores by Entobdella soleae, a monogenean skin parasite of the common sole.
Parasitology 60, 301-311.

Kearn, G. C. (1971). The physiology and behaviour of the monogenean skin parasite Entobdella soleae in relation to its host (Solea solea).

Parasitology 68, 173-188.


International Journal of Parasitology 2, 545-552.

Parasitology 82, 57-59.


Kritsky, D. C. and Thatcher, V. E. (1977). Phanerothecium gen. nov. and Fundulotrema gen. nov. Two new genera of viviparous Monogenea (Gyrodactyloidea) with a description of P. caballeroi sp. nov. and a key to the sub-families and genera of the family. In: Exerta Parasitologia en memoria del doctor Eduardo Caballero y Caballero. Institute de Biologia, Mexico.


The host specificity, microecology, adhesive attitudes and comparative morphology of some trematode gill parasites.

*Journal of the Marine Biological Association of the United Kingdom* 25, 113-127.


*Journal of the Marine Biological Association of the United Kingdom* 42, 587-600.


*Advances in Parasitology* 1, 287-326.


*In: Third symposium of the British Society for Parasitology* Blackwell, Oxford.


Llewellyn, J. (1979). The related biologies of the monogenean parasite *Isancistrum* and its cephalopod host *Alloteuthis subulata*.

*Haliotis* 8, 97-98.


*Parasitology* 82, 165-167.


*Parasitology* 82, 64-66.


Journal of the Marine Biological Association of the United Kingdom 60, 73-80.

Folia Parasitologica, Praha 20, 105-112.

Lom, J. (1973b). The adhesive disc of Trichodinella epizootica—ultrastructure and damage to the tissue.

Longmans, London.

Parasitology 32, 159-173.

Parasitology 59, 625-636.

Journal of Parasitology 56, 1110-1117.

Suppl. 1. to the Zoological Journal of the Linnean Society 51, 19-
Lyons, K. M. (1973). The epidermis and sense organs of the Monogenea and some related groups. 


*Journal of Fish Biology* **2**, 23-34.


Freshwater Biology Association Scientific Publication No.27. Ambleside.


*Skrifter utgivna av Sodra Sveriges Fiskeriforening Arsskr.* 19-76.

In: *Parasitic worms and aquatic conditions.* (ed. R. Ergens and B. Rysavy).
Czechoslovak Academy of Science, Prague.


Translated by Z. Kabata, Oliver and Boyd, Edinburgh.

of host parasite population interactions. II. Destabilising 
processes. 


Mead-Briggs, A. R. (1964). Some experiences concerning the interchange 
of rabbit fleas, Spilopsyllus cuniculi (Dale) between living 
rabbit hosts. 

Mellanby, K. (1944). The development of symptoms, parasitic infection 
and immunity in human scabies. 
Parasitology 35, 197-206.

infections of grazing animals. 
Advances in Parasitology 7, 211-282.

digenean Transversotrema patialense (Soparkar, 1924) on the 
zebra fish Brachydanio rerio (Hamilton-Buchanan). 
Journal of Fish Diseases 1, 443-447.

Mittal, A. J. and Whitear, M. (1979). Keratinisation of fish skin with 
special reference to the catfish Bagarius bagarius. 

trematodes XXXIII. New species of Gyrodactylus and a key to 
the North American species. 
Transactions of the American Microscopical Society 86, 390-401.

trematodes XXX. Five new species of Gyrodactylus from the 
Pacific Tomcod Microgadus proximus (Girard). 


Journal of Parasitology, 21, 438.


Nigrelli, R. F. (1937). Further studies on the susceptibility and acquired immunity of marine fishes to Epibdella melleni, a monogenetic trematode.
Zoologica, New York 22, 185-197.

Nigrelli, R. F. and Breder, C. M. (1934). The susceptibility and immunity of certain marine fishes to Epibdella melleni, a monogenetic trematode.
Journal of Parasitology, 20, 259-269.


Ecology 44, 295-305.

Nordmann, A. von (1832). Mikrographische Beitrage zur Naturgeschichte der Wirbellosen Thiere.
Berlin.

Zoologischer Anzeiger 39, 337-351.

Fish Pathology 15, 95-100.

Paling, J. E. (1965). The population dynamics of the monogenean gill parasite Discocotyle sagitatta Leuckart on Windermere trout Salmo trutta L.
*Parasitology* **52**, 667-694.


Annales de la Musee royal del'Afrique centrale, No 226. Tervuren.


Parker, J. D. (1965). Seasonal occurrence, transmission and host specificity of the monogenean trematode *Gyrodactylus elegans*, from the golden shiner *Notemigonus chrysoleucas*.


Patrick, R. (1964). A discussion of the results of the Catherwood expedition to the Peruvian headwaters of the Amazon.


*Proceedings of the Helminthological Society of Washington* 29, 159-162.

*Journal of Wildlife Diseases* 2, 174-177.

Nelson, London.

*Journal of Fish Biology* 4, 87-98.

*Tulane Studies in Zoology* 16, 22-25.


Academy of the Rumanian Peoples Republic.


*Molecular and Biochemical Parasitology Suppl. 1982*, 435.

*Parasitology* (in press).


Smart, J. (1942). *Lice*. British Museum (Natural History), London.


Transactions of the Zoological Society of London 25, 153-600.

Srivastava, L. P. and James, B. L. (1967). The morphology and occurrence of Gyrodactylus medius Katheriner, 1894 (Monogenoidea) from Onos mustela.
Journal of Natural History 1, 481-489.

Angewandte Parasitologie 14, 1-10.

Tagliani, G. (1912). Enoplocotyle minima nov. gen., nov. sp., trematode monogenetico parasita sulla cute di Muraena hellena L. Richerche anatomiche e sistematiche.
Archiv fur Zoologia di Napoli 5, 281-318.

Journal of Zoology 154, 475-480.

Revue de Zoologie et Botanie d'Afrique 82, 349-369.


Monitore Zoologico Italiano n. s Suppl. XV. 133-150.

Monitore Zoologico Italiano (N.S.) Suppl. XV, 367-385.

Parasitology (in press).

Proceedings of the Royal Society of Edinburgh 70B, 127-129


Canadian Journal of Zoology 34, 583-594.

Chromosoma (Berlin) 44, 335-342.


Musee Royale de l'Afrique Centrale, Tervuren.

Irish Naturalists Journal 10, 189-190.

Archiv fur Anatomisch und Physiologisch Wissenschaft 768-797.


Warne, London.

Journal of Zoology 160, 437


Edward Arnold, London.

Acta Parasiticoligica Polonica 22, 149-163.

Springer-Verlag, Berlin.

Williams, C. B. (1944). Some applications of the logarithmic series and the index of diversity to ecological problems.
Journal of Ecology 32, 1-44.


Parasitology 66, 473-485.

Parasitology 54, 155-172.


