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DYNAMICS OF GYRODACTYLUS POPULATIONS ON  
STICKLEBACKS (Gasterosteus aculeatus)

by

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## ABSTRACT

The relation between the monogenetic fluke Gyrodactylus alexanderi Mizelle and Kritsky, 1967, and its fish host Gasterosteus aculeatus was examined. Factors influencing the number of flukes on isolated fish at 15°C were measured, and the data combined to produce a computer model simulating a Gyrodactylus population. When tested in a multiple-host situation, the model predicted results that were similar to those observed experimentally.

Experimental populations of 20 flukes on individual fish increased for two weeks to a mean of 61 then decreased over two further weeks to a mean of 9. Fish that lost their fluke infestations were refractory to further infestation for about three weeks. A humoral immune response was not thought to be involved because of the relatively rapid rise and decay of the resistance, and because the course of the infestation on previously exposed fish did not differ from that on fish that had never been exposed. Handling of the fish was also shown not to affect the pattern.

Control of the numbers of Gyrodactylus appeared to be a mechanism by which fish shed the flukes on sheets of 'cuticle' formed by the epidermis. Hooklets of the flukes were attached to the cuticle and their purchase on the fish was lost when the cuticle was shed. Although all fish, whether infested or uninfested, shed cuticle every 1-2 days, that shed by the heavily infested fish was more opaque and more electron-dense, perhaps in response to the feeding activities of the flukes. The



change is suggested to be the basis for the increased 'shedding efficiency' of the fish which in turn was shown to be related to the number of flukes present on the fish five days earlier.

The mean life expectancy of flukes on fish was 16 days at 15°C. Flukes reproduced by giving birth to two daughters, one after 1.6 days and the other after 6.9 days. The rate of reproduction in fluke populations that were increasing was similar to that in decreasing populations on fish recovering from an infestation. The mean life expectancy of flukes away from fish was 1.8 days. It was estimated that of the flukes shed, less than half of the survivors reattached during any 24 hour period.

Other factors found capable of influencing population size were kept constant or minimised during the experiments and were not incorporated in the model. These included: predation on flukes by fish 15-30 mm in length; mortality of fish due to Gyrodactylus; stress on the fish; density of the fish population; and temperature.



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## INTRODUCTION

The original purpose of this work was to investigate quantitatively a parasite population to gain an insight into how it was maintained in the field. This has already been done for a number of parasites. Malaria was examined by Ross (1910) and other malaria workers, particularly MacDonald (1957). Schistosomiasis was also studied by MacDonald (1965) and by Hairston (1962). Experimental studies on populations of parasitoids and their hosts were carried out by Nicholson (1933), Nicholson and Bailey (1935), Thompson (1939), and De Bach and Smith (1941). Ractliffe et al. (1969), Michel (1970) and others have considered nematode populations in sheep. Crofton (1971) explored population changes in a theoretical host-parasite system. Quantitative work on populations of fish parasites has been mainly concerned with documenting variations in abundance, and then correlating these with changes in environmental conditions.

Population studies on most parasites are made complex by the series of distinct stages through which a parasite may pass during its life-time. Two or three hosts and one or two free-living stages are often essential to complete development, and the larvae may reproduce asexually in one environment while the adults reproduce sexually in another. In approaching the general problem a simple system was sought, with a single host in the life cycle and a simple pattern of reproduction. The live-bearing monogenetic trematode Gyrodactylus appeared to meet these requirements.



Gyrodactylus alexanderi was found on the body and fins of local sticklebacks (Gasterosteus aculeatus). The parasite required only a single host for the completion of its life cycle, though the flukes could transfer to other fish of the same species. Flukes produced a single offspring at each birth, and the daughter attached to the fish alongside the parent. Within a few days, the daughter itself was capable of reproducing.

To study the factors controlling the abundance of these flukes, isolated fish and flukes were maintained in the laboratory under different conditions. Later unattached flukes, and fluke populations on groups of fish were investigated.



## LITERATURE REVIEW

A large body of published information exists on the factors which were deemed important in regulating the numbers of Gyrodactylus. As will be seen in the following review, little of it is of a quantitative nature and many of the observations are contradictory, perhaps due to the fact that different species have been studied by various investigators, or because the same species have been studied under different conditions.

The review deals first with physical factors that have been associated with large or small Gyrodactylus populations, then with reproduction and mortality in individual Gyrodactylus. Following this there is an account of the effect of Gyrodactylus on its host, and the review is concluded by a consideration of the possible effects of the host on Gyrodactylus.



### Physical factors affecting Gyrodactylus population size

Physical factors such as low temperature have been linked to high infestations of Gyrodactylus. Field data indicated that G. elegans, G. rarus, and G. medius were more abundant during the cooler months of the year (Parker, 1965; Chappell, 1969; Sproston, 1946), and this has been supported by experiments on G. macrochiri and Gyrodactylus sp. (Hoffman and Putz, 1964; Malmberg, 1956a). Other field data indicated maximum fluke numbers of G. elegans and Gyrodactylus sp. occurred in the spring (Bauer, 1958; Meyer, 1970; Lewis and Lewis, 1963), summer (Wagener, 1909, G. elegans), or spring and late summer (Bykhovskaya-Pavlovskaya, 1962, G. elegans and G. medius). Srivastava and James (1967) found that G. medius was abundant in the spring and in the fall, and this was correlated with high mean tide levels and a temperature of 12°C. They concluded that temperature was the main factor controlling the abundance of this species of Gyrodactylus. In Russia, the gyrodactylids of carp (G. elegans and G. medius) are common in the south but rare in the north (Bauer, 1958). The validity of many of the species of Gyrodactylus is still in doubt, but it appears that, as Malmberg (1956a) has stated, different species of Gyrodactylus are adapted to different temperatures.

Other physical factors have been considered. Low infestations have been associated with low pH, oligotrophic conditions, and high humus content (Malmberg, 1956a and 1970).



### Gyrodactylus Reproduction and Mortality

The flukes are hermaphroditic, and though copulation has been described (Malmberg, 1956b), cross fertilisation is apparently not obligatory, at least for the first two births (Khalil, 1970). At birth a single daughter is produced which closely resembles the parent. The daughter already has a well developed embryo in utero , and within 1-2 days, gives birth to its own daughter. The uterus is then empty and it takes 5-7 days for another embryo to develop. Just prior to the second birth, the embryo can be seen to have another embryo in its uterus, and inside this embryo a third embryo can be identified. Thus the succeeding generations are arranged one inside the other like Chinese boxes.

Katheriner (1904) found four generations visible in one G. wagneri individual. Gyrodactylus species were thus described as producing succeeding generations in groups of four (Bychowsky, 1957). Braun (1966) however, found that in G. wagneri at least 12 generations were produced in succession.

On its fish host, G. eucaliae can live for at least a month at 4-6°C (Ikezaki and Hoffman, 1957). Bychowsky (1957) found the life span of Gyrodactylus spp. to be at least 12 days (no temperature cited). However, after removal from the fish the life span is much reduced.

Guberlet et al. (1927) found G. elegans died within 5 hours after leaving host. Tripathi (1957) found G. elegans indicus died within 8 hours at 29°C. Bychowsky (1957) reported that Gyrodactylidae died within 48 hours while Bykhovskaya-Pavlovskaya (1962) found that G. elegans and G. medius survived for a few days. Khalil (1964), working with a tropical species,



Macrogyrodactylus polypтери, found that at 30°C flukes survived as long as 10 days.

In a unique observation, Khalil also reported that specimens of Tilapia nilotica, 'when introduced into a tank with infested Polypterus, would pick up the worms from the surface of infested fish one by one and eat them and in a few hours there would be hardly any worms left on the Polypterus.' The only other suspected predators of gyrodactylids are the cleaner shrimps and cleaner fishes of coral reefs. However, direct observation and stomach analyses of cleaner fish have so far specifically recorded only crustacean parasites as being eaten (Youngbluth, 1968; Feder, 1966; McCutcheon and McCutcheon, 1964; Limbaugh, 1961; Randall, 1958).



### Effect of Gyrodactylus on its Host

Gyrodactylus infestations have apparently been the cause of a range of pathological conditions in the host. Infested Ictalurus melas were inactive and weak (Van Cleave, 1921); golden shiners produced excessive mucus (Lewis and Lewis, 1970); the gills of rainbow trout became shrivelled and functionless (Pratt, 1929), or the fish became covered with a bluish grey slime owing to an increased secretion of mucus (Davis, 1961). The skin of infested carp fry showed excessive mucus, loosened scales and some haemorrhages (Tripathi, 1957). In some areas the skin was found peeled off. Under pond fishery conditions, Bykhovskaya-Pavlovskaya (1962) found that infested carp were covered with a layer of mucus. The integument was destroyed and necrotic areas and small ulcers occasionally arose. In some cases, after a fin had been attacked, only the fin rays remained. Davis (1961) also reports that in heavy infestations rainbow trout fins became frayed and were worn down to stubs. Frayed fins were also evident in heavily infested plaice (MacKenzie, 1970).

Several authors have held Gyrodactylus responsible for fish deaths (Atkins, 1901; Embury, 1924; Guberlet et al., 1927; Nigrelli, 1940; Yin and Sproston, 1948; Davis, 1961; Tripathi, 1957; Bykhovskaya-Pavlovskaya, 1962; Lewis and Lewis, 1963 and 1970; Hoffman and Putz, 1964). Malmberg (1956a) attributed death in part to the toxic effect of all the secretions given off by the parasites. Yin and Sproston (1948) thought that excess mucus on the gills was the cause of death of infested goldfish. Bauer (1958) wrote that the copious effusion of mucus caused by Gyrodactylus



disturbed the respiratory function of the skin and ionic balance of the blood. Tinca tinca, infested on the gills with G. elegans, had a blood erythrocyte count 30-70% below average, and a haemoglobin content 20-27% below average (Kazdaev, 1954).

Malmberg (1956a) suggested that a small number of Gyrodactylus on a fish may be considered normal. Disease and death result only when large numbers of Gyrodactylus are present. Estimates of the number required to cause death have been made by several authors. Petrushevski and Shulman (1961) found sticklebacks dying with up to 1000 flukes per fish. Ten Polypterus, 20-25 cm in length, died when carrying 700-7000 Macrogyrodactylus polypteri per fish (Khalil, 1964). The carp fry observed by Tripathi (1957) died when carrying about 70 flukes each (G. elegans indicus). Bykhovskaya-Pavlovskaya (1962) found 80% mortality in immature and mature carp when there were on average 180 flukes per fish (G. elegans and G. medius). Goldfish 9-10 cm in length died when carrying 50-200 G. elegans indicus (Yin and Sproston, 1948). In other cases, large numbers of flukes have been present on fish without having any obvious effect. Bychowsky (1957) reported finding Pacific cod carrying thousands of gyrodactyloids each. Anthony (1969) kept an 8 cm goldfish that did not show symptoms of disease although it was carrying nearly 3000 G. elegans.

As dying fish are often infected with a number of potential pathogens, it is difficult to ascertain which organism is the prime cause of death. Some authors have suggested that the wounds caused by Gyrodactylus



permit the entry of bacteria (Sproston, 1946; Malmberg, 1956a) or fungi (Tripathi, 1957; Parker, 1965; Davis, 1961; Bykhovskaya-Pavlovskaya, 1962), or bacteria, fungi and Protozoa (Van Cleave, 1921; Noble, 1963). Noble et al (1963), Parker (1965) and Yin and Sproston (1948) found that fish infested with Gyrodactylus often had the ciliate Trichodina. Noble et al speculated that Gyrodactylus encouraged Trichodina by damaging epidermal cells. However, Anthony (1969) did not find any relationship between the two parasites on goldfish.



### Effect of Host on Gyrodactylus

The number of Gyrodactylus present on a fish may be influenced by the age or the size of the host. Younger fish appear to be more susceptible than older fish (Malmberg, 1964 and 1970; Hoffman and Putz, 1964; Bychowsky, 1957; Bauer, 1958), though the opposite has been found in sticklebacks (Polyanski and Shulman, 1956) and in rocklings (Strivastava and James, 1967). Fry of carp and stickleback were rarely infested (Bauer, 1958; Malmberg, 1956a).

Hoffman and Putz (1964) found that small blue-gills carried more G. macrochiri than larger fish in experimental infestations. Parker (1965) found that small golden shiners carried fewer G. elegans than larger fish in the field, but this relation disappeared when the fish were brought into the laboratory. Malmberg (1970) noted that regardless of age or species, fish less than 20 cm long were more frequently infested with Gyrodactylus spp. than larger fish. Nobel et al (1963) found no relationship between host size and frequency of infestation with G. elegans in Gillichthys mirabilis.

Unhealthy fish are apparently more susceptible to Gyrodactylus than normal fish (Guberlet et al., 1927; Malmberg, 1956a; Amlacher, 1970; Bykhovskaya-Pavlovskaya, 1962).

Crowding of fish may increase fluke numbers (Guberlet et al., 1927; Hoffman, 1967) as the parasites can spread by direct contact (Guberlet et al., 1927; Bychowsky, 1957; Malmberg, 1956a; Bauer, 1958), by fish contacting flukes on the bottom (Hoffman and Putz, 1964), or free in the



water (Parker, 1965).

Other investigations have suggested two mechanisms by which fish reject or inhibit monogenean parasites, though none have been described for Gyrodactylus. Wunder (1929), Paperna (1963), and Putz and Hoffman, (1964) found that gills infested with blood-feeding monogeneans of the genus Dactylogyrus developed an unusually thick epidermis. This resulted in the loss of the flukes, apparently because the flukes were cut off from their food supply. With subsequent healing, the gills again became a suitable habitat for Dactylogyrus and the cycle was repeated (Paperna, 1964). This alternation between susceptibility and resistance parallels the situation described in this thesis for Gyrodactylus on sticklebacks.

The second mechanism suggested involves humoral antibody. Paperna, in his 1964 paper mentioned above, described how carp eventually developed a resistance to D. vastator, and this, unlike the epidermal hyperplasia, was ineffective against two other Dactylogyrus species. Vladimirov (1971) associated loss of D. vastator with high levels of serum antibody. After observations on another monogenean, Epibdella melleni, in aquaria, Jahn and Kuhn (1932) suggested that a humoral immune mechanism might be involved. Nigrelli (1935) found that flukes of this species survived longer in mucus from susceptible fish than in mucus from 'immune' fish. In support of this idea, O'Rourke (1961) demonstrated that some blood antigens may be found in skin mucus, and Fletcher and Grant (1969) found immunoglobulins in the mucus of plaice.



In summary, conflicting data was revealed on most points, no doubt in part due to observations on different species of Gyrodactylus. However, factors were suggested which had to be considered in analysing the local Gyrodactylus-stickleback relationship.



## METHODS AND MATERIALS

The first experiments determined the course of the infestation under laboratory conditions. This was based on weekly observations on isolated infested fish. The reproduction and mortality rates of the flukes were then measured using single and multiple flukes. During the course of these experiments, a mechanism for the rejection of the flukes was discovered (Lester, 1972), and this led to examination of the rate of emigration of the flukes, their mortality rate when off fish, and their rate of reattachment. At various stages in this sequence, available data was incorporated into a model which simulated a fluke population. The results of the simulation suggested other experiments and these were later carried out. Finally, the model was used to predict quantitative relationships beyond the scope of experiments and observations.

Gasterosteus aculeatus leiurus, infested with Gyrodactylus alexanderi (Mizelle and Kritsky, 1967) were obtained from a pond in Queen Elizabeth Park, Vancouver, B.C. Fish and flukes were kept under a photoperiod of 12 hrs. darkness followed by 12 hrs. of light ( $350 \pm 150$  lux) at  $15^{\circ}\text{C}$  unless otherwise specified. Fish were maintained in 15 and 30 gallon aquaria. The method of maintaining the stock of flukes was similar to that used by Turnbull (1956) and followed the suggestions of Hoffman (1967). Four to five infested fish were kept in 1000 ml dechlorinated water, fed daily and the water changed weekly. Every week dead fish or those that had recovered from the infestation were replaced by fish that



had been free of flukes for at least four weeks.

Experiments on the course of the infestation were designed to eliminate as far as possible sources of variability recorded by other workers. Healthy fish, 25-35 mm in length and devoid of Gyrodactylus, were acclimatised to 15°C for at least four weeks. At the start of the experiment, they were experimentally infested with Gyrodactylus. The fluke-free fish were obtained either by treating infested fish with a 1:4000 formalin solution for 1 hour (Davis, 1961), or by raising fish from artificially fertilised eggs (Hagen, 1967).

Unattached flukes were obtained in two ways. Infested fish were placed in 15 ml dilute 2-phenoxyethanol (1:1500) for 30 seconds, after which flukes were blown from the fish surface by a jet of water, and the fish then removed. Flukes, brought to the centre of the dish by swirling were removed with as little anaesthetic as possible, and placed in 250 ml water. They generally showed normal activity within 5 seconds after transfer. They quickly fell to the bottom of the dish (rate of fall 2 cm/min) and were left for 10 minutes before being used in experiments. Alternatively, infested fish were killed and placed in 250 ml. water. Flukes left the fish within 1-2 days, depending on the rate of decomposition of the fish, and aggregated on the bottom of the bowl. The subsequent 'reaching' behaviour of flukes from the two sources was indistinguishable under 240 x magnification. Experimental infestations showed a similar course whichever source of flukes was used.

Fish to be infested were held in forceps by the relatively rigid pectoral girdle so that the fish was unharmed except for the scraping



off of some of the epidermis. The tail was then brought into contact with free flukes on the dish bottom. These experimentally infested fish were placed in individual bowls of 250 ml dechlorinated water. They were fed daily and the water changed weekly. Flukes on fish were counted under water using a wide field stereo microscope at 16 x magnification while the fish was held as before.

Two methods were used to estimate the reproductive rate of the flukes. In the first method, a single fluke was permitted to attach to an uninfested fish, 25-30 mm long. The fish was kept in 15 ml of water and examined daily when the water was changed. If the fluke had reproduced, either the parent or the daughter was removed. Parents could be distinguished from daughters because they had an empty uterus, whereas the uterus of the daughter contained a large well developed embryo. Initially, the flukes were removed with anaesthetic and placed on a separate fish. Later, to avoid any effect of the anaesthetic on the rate of reproduction, and also to permit easier manipulation, one of the flukes was pulled off with forceps and destroyed. It was found that the use of anaesthetic extended the average time before the first birth from 39 hours to 48 hours. The fish used in these experiments were not fed because of the small size of the containers, so for observations on fluke longevity, the flukes were anaesthetised and transferred to a different fish each week.

A total of 123 flukes were used in these experiments on fluke reproduction. Records of the offspring of individual flukes were kept on dendrograms on a time grid similar to those used by Bychowsky (1957, Fig. 133). Mean values for the times of reproduction in parents and



daughters were then calculated.

The second method involved mass staining of flukes by placing infested sticklebacks in a 1:10,000 solution of Neutral Red for one hour. Flukes retained the stain for at least 24 hours, often concentrating it in the gut, apparently using an excretory mechanism similar to that already reported for some Acoela and Tricladida (Hyman, 1951). Stained flukes could be readily distinguished from the recently born flukes which were colourless. The dye was not taken in with stained epidermal cells, as flukes transferred to stained fish remained unstained.

The mortality rate of flukes on fish was obtained from the dendrograms described above. The mortality rate of flukes off fish was measured by removing a total of 180 flukes from infested fish with anaesthetic, placing them in 250 ml bowls of water, and examining for live flukes every 24 hours.

The reattachment rate was measured by dropping anaesthetised flukes, in groups of 50, into clean water-filled containers of different sizes. After 30 minutes, by which time most of the flukes should have been on the bottom, an uninfested susceptible fish was added. Twenty-four hours later, the fish was removed and the number of adhering flukes recorded. A second fish was introduced into the tank to give an indication of the number of flukes that were still surviving in case a proportion of the flukes had never recovered from the anaesthetic.

After examining the infestation on isolated fish, experiments were designed to examine the effects of having several hosts present in the same chamber. The fish used were at different stages in the course of



the infestation so that as one fish was recovering, another was susceptible. This was done in the following way. A fish was infested with 100 flukes. After one week, an uninfested susceptible was added to the same chamber. After two weeks, a second uninfested fish was added, and after three weeks a third. After four weeks the original fish was replaced by an uninfested fish, and at the end of subsequent weeks the longest inhabitant of the chamber was replaced by a susceptible fish. Flukes on fish were counted weekly, under water as described above.

The flukes on infested fish clung to, and apparently fed on, the fish epidermal cells. The amount of damage the flukes caused therefore bore some relation to the regeneration time of the epidermis. Epidermal healing was found earlier to be very rapid, a 20 cell wide band of epidermis removed down to the dermis was replaced within 30 minutes (Lester, 1972). This was probably due to migrating cells so it was considered of interest to obtain an estimate of the 'turnover time' of the epidermis. A healthy uninfested fish, about 1 gm in weight, was injected with 0.001 mg colchicine (Henry et al., 1952). Six hours later, it was killed by pit/hing and fixed in Susa's fixative. After embedding and sectioning, cells were stained with alum haematoxylin and eosin, and the number of cells in metaphase were counted under 1250x magnification.

As infested fish occasionally died, the presence of organisms other than Gyrodactylus was suspected. To diagnose systemic bacterial infections, kidney tissue from 5 infested sticklebacks, two of which were close to death, was smeared on TSA plates and incubated at 20-22°C for four days (Bullock, 1971). Protozoans such as Trichodina and Costia



were found occasionally and were diagnosed in wet mounts. Fungus was also occasionally encountered on living fish, and in one case was specifically identified. This was done by culturing the fungus, taken from the nostril of a wild-caught fish two days after capture, on split boiled hemp seeds at 4°C and 20°C (Seymour, 1971).



## RESULTS

### Course of Laboratory Infestation

The typical pattern of a laboratory infestation at 15°C was established using 19 isolated fish, each initially infested with 20 flukes (Fig. 1, solid lines). The flukes increased in number over the first 1-2 weeks and then decreased over the next two weeks. Seven fish, isolated under similar conditions, died (Fig. 1, broken lines). The number of Gyrodactylus increased over the first 1-2 weeks and then continued to increase until there were 150-400 worms per fish and the fish died.

Because the typical infestation increased and decreased through four water changes, the organic debris and bacteria that accumulated between changes did not apparently contribute to the typical pattern.

If the fish was involved in controlling the population size of the fluke, it was possible that it took the fish 1-2 weeks to become adjusted to being handled and to living in a confined space. However, uninfested fish kept under these conditions of confinement and handling for two weeks prior to infection showed the same increase and decrease in fluke number as fish without the acclimation period (Fig. 2).

Fish that were osmotically stressed carried fluke populations that continued to increase in size until the fish died. Eight sticklebacks, caught and held in sea water, were placed directly into freshwater and infested with 20 or 30 flukes each. The course of the infestation is shown in Fig. 3. The osmotic stress on the fish apparently inhibited the



control of fluke numbers, and this further suggested that the host was normally involved in the control of the flukes. Incidentally, the flukes themselves could be stressed by salinity changes. Abrupt changes from sea water to freshwater or the reverse killed flukes within 30 minutes. Gradual changes of Cortland's saline for one day and 50% sea water for one day, enabled 18 of 133 marine flukes to live for at least 7 days, and 1 of 109 freshwater flukes to survive for up to 5 days.

Fish that had recovered from an infestation were refractory to a challenge infestation for several weeks (Fig. 4). The numbers on the horizontal axis refer to the time interval between their recovery and the administration of a challenge infestation of 20 flukes. Five of six fish reinfested after a one week interval had lost their worms by the end of the second week. When there was a two week interval, all six fish retained a small number of flukes (1-15) from the challenge dose. After three weeks, all 11 fish were carrying flukes and five were carrying 20 or more. Finally, after four or more weeks, all 19 fish were carrying 20 or more flukes.

The course of the infestation on fish in the multi-host situation was similar to that observed on isolated fish. On 24 occasions, a susceptible fish was introduced into a bowl containing fish carrying a total of 100 or more flukes. The mean numbers of flukes each week on the 24 fish were 0, 103, 47, and 14.

The foregoing account of the course of an experimental infestation demonstrated that although the flukes initially increased in numbers, they later declined on most fish, and the fish became temporarily refractory to further infestation. These changes were not caused by physical



changes in the environment as the temperature was constant and the water conditions were renewed at weekly intervals. It became necessary to decide whether the change in numbers was due to a change in the rate of reproduction, perhaps due to host influences, or to a changing mortality rate, or to some combination of these. The results of the experiments are presented next in the sequence: fluke reproduction and mortality; effect of the fluke on the fish; effect of the fish on the fluke; particularly the shedding mechanism; and finally a measure of fluke reattachment success. These factors and their relationships are summarised in the flow chart (Fig. 5). Those inside the black square were evaluated to produce a model of the system, those outside were kept as constant as possible during the quantitative experiments.



### Gyrodactylus Reproduction and Mortality

The reproduction of Gyrodactylus was examined in detail since a change in the reproductive rate could have been responsible for the decline in the fluke population on recovering fish.

Isolated flukes on fish reproduced twice during their life-time, the first time soon after birth. The upper line in Fig. 6 represents the life span, in days, of a fluke at 15°C. For 75 flukes, the average age at the time of the first birth was 1.61 days; for 31 flukes the time of the second birth was 6.90 days. The maximum life span recorded was 27 days though very few flukes reached this age, as indicated on the survivorship graph below (Fig. 6).

Neither birth apparently required cross-fertilisation in the parent. New-born flukes were sexually immature and lacked a cirrus. In some cases, parents were removed directly after giving birth, thus ensuring that they did not transfer sperm to the daughter, but the daughters still reproduced twice. There is the possibility that with cross-fertilization more flukes might have been born, so in three cases, a second fluke was added to the fish alongside the barren fluke, left for 24 hours and then removed. No further embryos were produced.

At 7°C, the ages at the two births were 5.2 and 18 days, and the maximum life span recorded was 71 days.

The rate of increase at 15°C was estimated in two ways. The fluke population on the 19 fish in Fig. 1 increased for the first seven days at an instantaneous rate of 0.12. Therefore the rate of reproduction of individual flukes had to be at least as great as this.



The second method calculated the reproductive rate from the details known about the reproduction of individual flukes. The age at the second birth was taken to be 7 days, and the age of the first birth was taken to be one quarter that of the second. Mortalities prior to the first and second births were incorporated (0.07 and 0.12 respectively, from data on Fig. 6), and all remaining flukes were assumed to die when they were 15.75 days old. Thus of 100 flukes born at the same instant, after one time period (1.75 days) 93 flukes would be still alive. These flukes would then reproduce giving a total of 186 flukes. After three more time periods, the 93 parents would have been reduced to 82, and these would then give rise to a further 82 daughters. This calculation was done for 16 time periods and from the total number alive at the end of each time period the instantaneous rate of increase was found to be 0.15.

This rate was further checked by applying the rule that the relative size of a particular age class in a population is constant with a constant rate of increase (Lotka, 1956). Using a calculation similar to that described in the previous paragraph, it was estimated that at any one time about 19% of the flukes were born within the previous 24 hrs. Experimentally, using the Neutral Red staining technique, of 347 flukes examined on 12 infested fish, 85 (24%) were colourless and thus had apparently been born within the previous 24 hours.

The decrease in fluke number evident in recovering fish may have been the result of a change in the fluke reproductive rate. However, by using the staining technique, it was found that flukes from decreasing



fluke populations continued to reproduce at the same rate as those from increasing populations. Forty-nine of 206 flukes (24%) from 6 decreasing populations were produced within the previous 24 hours, compared to 29 of 116 flukes (25%) from 5 increasing populations. Similarly, no difference was detected in the survival time of flukes removed from susceptible and recovering fish.

Flukes away from fish were subjected to a high natural mortality rate. Of 180 flukes removed from fish, the number surviving after successive 24 hr. periods was 154, 73, 18, 3 and 0.

Flukes were also subjected to predation by immature sticklebacks. Four unfed individually isolated fish, 24-26 mm long, which were recovering from an infestation, had a mean of 21 (range 10-25) pairs of Gyrodactylus anchors per intestine, compared to four similar fish, 34-36 mm long, isolated under the same conditions, that had a mean of 3 (range 0-9) pairs of anchors per intestine. Predation was not included as a factor in the model, and, to minimise its effect during the assessment of other factors, fish carrying experimental infestations were fed daily.

Incidentally, when only 1-2 flukes were present on a fish, flukes with large embryos, such as recently born individuals, remained in sheltered areas such as behind the bases of the spines and fins. Parents that had recently given birth, and hence had an empty uterus, were commonly found on the caudal or dorsal fin. In larger fluke populations this trend was obscured.



### Effect of Gyrodactylus on Its Host

Heavily infested fish frequently died. Over 1-2 weeks they became sluggish, emaciated and finally moribund. Fifty-four of 67 fish carrying over 150 flukes died, compared to only 33 of 174 fish carrying fewer flukes. Dying fish occasionally carried sessiline peritrichs such as Epistylis lwoffii but these are not believed to be pathogenic. Concomitant infections of G. alexanderi and Trichodina tenuidens were followed on one fish for four months without any relationship becoming evident. None of the kidney smears from infested sticklebacks produced bacterial colonies on agar, indicating that a systemic bacterial infection was not the cause of ill health. Saprolegnia parasitica was isolated from a local wild-caught stickleback but such fungus infections disappeared within a few weeks in the laboratory. None of the experimental fish which died showed any signs of a fungus infection when examined under 16x magnification prior to death. As no other organism was regularly associated with dying fish, it was concluded that Gyrodactylus caused the deaths.

To assess the possible damage inflicted by Gyrodactylus the rate of growth of the fish epidermis was measured. This was found by counting the number of cells in metaphase in sections of the colchicine-injected fish. Out of 14 fields taken from two sections, 51 epidermal cells were in metaphase out of a total of about 2,300 cells. So in 6 hours 2.2% had gone into mitosis, therefore in a day about 9% would divide, and all cells could be replaced every 11 days.



### Effect of the Host on Gyrodactylus

The course of the infestation and the following refractory period suggested that an immune response may have been involved. Five laboratory-raised fish, previously unexposed to Gyrodactylus, were infested with 20 flukes each. On successive weeks, the average number present was 43, 33, 6 and 0, thus showing a similar course of infection to that of previously infested fish. This suggested that a typical circulating antibody was not involved. The rapid time course of the resistance, (1-2 weeks to build up and 3-4 weeks to dissipate), also was unusual for known antibody responses in fish. However, if fish antibody was present in the epidermis it could have been ingested by the fluke during feeding. The gut of flukes occasionally contained host melanin granules and this may have indicated that the fluke ingested cellular material and sometimes penetrated as far as the dermis. Immunity is considered further in the discussion.

While doing these experiments, flukes were observed to leave fish riding on sheets of mucoid material. This material appeared to be synonymous with the 'cuticle' described by Whitear (1970) and was shed in patches of varying size from the surface of the epidermis every 1-2 days. As it was formed, the marginal hooklets of the fluke apparently became attached to it, rather than being directly attached to the epidermal cells, and thus when it was shed, the flukes were lifted off the fish. Cuticle from uninfested fish was less dense than that from infested



fish. A description of the cuticle and its role in removing flukes has been published (Lester, 1972) (See Appendix).

Shed cuticle plus flukes was removed daily from the bowls of three isolated infested fish. For the first few days of the infestation, no flukes were found away from the fish. As the infestation progressed, flukes were frequently encountered on the shed cuticle, particularly as the fish began to recover (Fig. 7). This technique was unsuitable for an evaluation of the changes in shedding efficiency because it was tedious and the flukes had to be removed to be counted. Shedding efficiency was therefore derived in the following way from data on the course of the infestation when fish were infested with 20, 60 or 100 flukes (Fig. 8). After one week, the fluke numbers had increased on all fish by factors of 2.2, 2.0 and 2.1 respectively. From this it was concluded that there was a delay of about a week before the shedding mechanism became effective.

After the first week, the rate of loss of flukes appeared to be highest on those fish carrying the most flukes (Fig. 8). This was confirmed by expressing the change in fluke number at the end of each week as a percentage of the number present at the start of the week (Fig. 9). Populations of over 100 decreased more rapidly than populations of 20-49. As the fluke loss was related to the shedding efficiency of the fish cuticle, it was concluded that the fish produced a response graded to the number of flukes present.

Flukes sometimes left fish that were not shedding cuticle. Fish carrying 300 or more flukes lay motionless on the bottom of the dish and



25-50% of the flukes left the fish and aggregated on the floor of the dish away from the dying stickleback.

#### Reattachment of Flukes to Fish

Flukes not attached to fish extended their bodies into the water 15-30 times a minute (6 observations) until the anterior end made contact with the same or another stickleback.

The rate at which the flukes reattached to fish was dependent on the proximity of the fish. Within 24 hours (at 15°C) in the absence of shed cuticle or debris, 23% of 200 unattached flukes had moved onto a stickleback in 67 litres of water, 37% (of 300 flukes) had attached to fish in 22 litres of water, 83% (of 150 flukes) in 1 litre of water, and 93% (of 200 flukes) in 0.25 litres of water.

Flukes would attach to other species of fish, and even to frogs (Rana pipiens), though they remained attached for a relatively short time. Of 20 flukes initially attached to a fish in one litre of water, none were left on a goldfish (Carassius auratus) or a pea-mouth chub (Mylocheilus caurinus) after one day, on a squawfish (Ptychocheilus oregonensis) after two days, or a gourami (Trichogaster trichoptera), kept at 20°C, after 5 days. However, flukes living in sea water or freshwater flourished equally well on both marine and freshwater races of sticklebacks (Gasterosteus aculeatus trachurus and G. a. leiurus).



### The Model

The change in the number of flukes on a fish from one day to the next was given by

$$X_{t+1} = (X_t - E_t) R + S_t$$

where

$$X_{t+1} = \text{no. of flukes on fish on day 't + 1'}$$

$$X_t = \text{no. on fish on day 't'}$$

$$E_t = \text{no. that left fish on day 't'}$$

$$R = \text{reproductive rate of the fluke}$$

$$S_t = \text{no. of flukes that reattached to fish.}$$

The number that left fish on day 't' was the product of the total number on the fish, and the shedding efficiency,  $C_t$ .

$$E_t = X_t C_t$$

A small error was introduced by not accounting for the reproduction of half  $E_t$  in the first equation.

Shedding efficiency,  $C_t$ , which affected fluke mortality, varied between 0.15 and 0.70. The lower limit was chosen because newly infested susceptible fish lost 0.1-0.2 of their flukes per day. The maximum value reflected the fact that fish shed cuticle on average every 1.5 days, therefore if the cuticle was operating with maximum efficiency in one day 0.75 of the flukes would be shed. 0.7 was used because during shedding it was possible for flukes to move from an area about to shed to an area which has already shed. Also different areas on the body may have differed in their ability to produce cuticle.



Between these two limits shedding efficiency  $C_t$  was calculated from an equation composed of 3 terms: the minimum value mentioned above (0.15), an increment proportional to the number of flukes present 5 days before, and a term which simulated the period during which fish were refractory to further infestation.

$$C_t = 0.15 + 0.1(1/1 + e^{-0.1(X_{t-5} - 50)}) + 0.95(C_{t-1} - 0.15)$$

The first item in the second term, 0.1, represented the maximum size of the increment per day. Its main function in the model was to round off the peak of the infestation as seen on a graph of population size against time. Values approaching 0.6 produced a sudden sharp drop in fluke number while values near 0.01 gave an extended peak even in heavily infected fish.

Enclosed within the first parentheses was a standard mathematical expression that produced a sigmoid curve when plotted against different values of  $X$ . The 0.1 controlled the slope at the mid-point of the curve. 0.01 would have produced almost a straight line, and would thus have indicated that the response was directly proportional to the number of flukes present. Values approaching 1.0 had the opposite effect and produced a 'square' sigmoid where the response would have been near its lower limit until a critical fluke number was reached and then would have rapidly increased to its upper limit.

$X_{t-5}$  was the number of flukes on the fish five days previously and thus incorporated the delay in fish response described earlier in the



results. The '50' determined the mid-point of the curve, which is its point of inflexion.

During calculations, the first term remained constant and the size of the second term was significant only when there were 30 or more flukes present, so to simulate the refractory period the  $C_t$  value was maintained at a high level after all the flukes had been removed. This was accomplished by the third term which took the previous  $C_t$  value, minus the minimum value, and reduced it by 5% each day. Thus, in the absence of large numbers of flukes, the  $C_t$  value was reduced in about 3 weeks to a value that resulted in the total fluke deaths being less than the total births. The fluke population then started to rise and a relapse was simulated.

The total number reattaching to a fish ( $S_t$ ) was the sum of reattaching flukes that had been shed the same day, plus those from the previous two days. Flukes that had not attached within three days were assumed to die as, from the survival experiments, only 18 of 180 flukes survived for over three days.

$$S_t = O_t + P_t + Q_t$$

where  $O_t$  = those reattaching that were shed on the same day

$P_t$  = those reattaching that were shed on the previous day

$Q_t$  = those reattaching that were shed two days previously

In assessing  $O_t$ ,  $P_t$  and  $Q_t$ , an assumption had to be made. From the reattachment figures in the results, 93% of unattached flukes, in 250 ml. water, attached to a fish within 24 hours. If this had happened



in the experimental infestations, all the fish would have died with large numbers of flukes. It was concluded, therefore, that not all the flukes that left a fish on a particular day were able to reattach the same day. This meant that for some reason the reattachment experiments were inapplicable to the model, perhaps because they were carried out in clean containers. Experiments simulating the presence of shed cuticle using pieces of cellophane and dialysis tubing were unsatisfactory because the pieces were denser and stiffer than cuticle and did not effectively trap flukes. The possible role of shed cuticle and other debris in inhibiting fluke reattachment is considered in the discussion.

The proportion-reattaching used in the model was found by deduction, as the overall result was known (Fig. 8) and the other individual factors were known. Fig. 10 compares computer estimates of numbers of flukes present (crosses) with the observed values (lines). Here, one third of the flukes were assumed to reattach the same day ( $0_t$ ), while the other two thirds underwent some mortality. On the second day, a third of the survivors reattached ( $P_t$ ), and those remaining suffered further mortality. On the third day, again a third reattached ( $Q_t$ ) and the remainder were assumed to die. The use of proportions smaller than 0.33 predicted infestations that peaked earlier at a lower maximum value, and larger proportions had the opposite effect. However, the basic shape of the curves was unchanged for values ranging from 0.4 to 0.25.

When some variability was introduced into the model, as outlined below, the value used for the proportion reattaching had to result in



some simulated infestations ending with zero flukes whilst others showed a relapse. This was because, of 20 fish kept for 10 weeks, 14 lost all their flukes while they were recovering and six retained a small number of flukes and eventually showed a recurrence of infestation. Using reattachment values in the model of .4, .36, .33, .30, .28, and .25 in 50 simulated infestations flukes were still present after 10 weeks in 41, 26, 25, 13, 14, and 20 cases respectively. Incorporation of more sources of variability would increase the chances of populations going to zero. Therefore, it was concluded that a reattachment fraction of .33, which allowed a reasonably close fit on Fig.10 and also showed a proportion of relapse infestations, would be adequate for use in the model.

The numbers reattaching were calculated as follows:

$$O_t = E_t/3$$

$$P_t = 0.86 \times 2 O_{t-1}/3$$

$$Q_t = 0.47 \times 2 P_{t-1}/3$$

$O_t$  is one third of the flukes that left the fish on that day, as explained above.  $P_t$  and  $Q_t$  are one third of the flukes that are remaining after one, and after two days respectively. The 0.86 and 0.47 are the survival rates of flukes off fish as described earlier in the results.

To more closely simulate an actual fluke population the model was modified to incorporate some variability. This was done in one of two ways.

In the first method, variability was introduced by allowing  $E_t$  to fluctuate around the expected value. The amount of variability was



represented by a Poisson distribution. This distribution was applicable if all events were independent of each other, but as this may not have been so, due perhaps to behavioural patterns of the flukes, it may be an over estimate of the true variability.

The computer did not provide random numbers from a Poisson distribution, so cumulative Poisson distributions for means of 1 to 5 were fed in. A random number between 0 and 1, which was available, was compared to the cumulative distribution with the mean of  $E_t$ . The value of the segment in which the random number fell was taken as the new  $E_t$  value.

For expected  $E_t$  values greater than 5.5, the random numbers were taken from a normal distribution, since a normal distribution with the mean equal to the variance is similar to a Poisson distribution (where the mean and the variance are always equal) when the means are above 5. The normal distribution provided by the computer had a mean of 0 and a standard deviation of 1. To adjust this distribution, the random numbers were multiplied by the required standard deviation (i.e. the square root of the expected  $E_t$  value), thus giving a distribution with a variance of  $E_t$ . The product of the random number and the square root of  $E_t$  was subtracted from the expected  $E_t$  value to give the new  $E_t$  value.

In the second method, variability was introduced at the same point using a Monte Carlo technique. Instead of determining  $E_t$  from the product of  $X_t$  and  $C_t$ ,  $X_t$  random numbers between 0 and 1 were taken and compared to the value of  $C_t$ . The number of random numbers that were less



than the fraction  $C_t$  was taken to be  $E_t$ . The predicted course of 20 infestations using this method gave a result barely distinguishable from Fig. 12, a figure derived using the first method. The number of relapses predicted using the two methods was 20 out of 50 infestations for the Monte Carlo, and 19 out of 50 for the first method. All the results presented in the thesis used the first method of introducing variability.

The incorporation of variability permitted a better simulation of the recovery of fish. Using the deterministic model alone, a fish infested with 100 flukes partly recovered and eventually showed a relapse (Fig. 11). In reality, 14 of 20 fish lost all their flukes before a relapse occurred. By incorporating variability, of 20 simulated infestations, 13 declined to zero before a relapse occurred (Fig. 12).



### Multi-host Model

The question was posed as to the effect of the availability of susceptible hosts in the population at the time when the oscillation was being damped. The simulation of such a multi-host system started with one isolated fish infested with 100 flukes, and at one week intervals, an uninfested host was incorporated until a total of four fish were present. In the calculations, each of the fish was treated separately except that, of the total number of flukes reattaching at one time, an equal proportion of the flukes attached to each fish present. Variability was introduced using the first method described in the last section. The Fortran program for Fig. 16, which uses the multi-host model and variability, is included in the Appendix.

Without incorporating variability, the simulation of the multi-host system showed that as the flukes were shed from recovering fish they were picked up by uninfested fish, and these in turn shed flukes and recovered (Fig. 13). It also predicted a reduction in the total number of flukes present. In the experimental multi-host situation, the reduction did not occur because 22 of the 120 fish involved became sick and eventually died with large numbers of flukes. This tended to maintain high numbers of flukes in the bowls. However, the predicted movement of the fluke population from fish to fish as further susceptible fish were added was confirmed in the experimental observations. The mean numbers of flukes each week on introduced fish (0, 103, 47, and 14) showed a pattern of infestation very close to that predicted for the first introduced fish in Fig. 13.



Finally, as the simulation seemed close to the experimental situation, predictions were made, using the multi-host model over a longer time period than could be tested experimentally. Over a 10 month period, the numbers of flukes on all fish oscillated together between 10 and 40 flukes per fish (Fig. 14). However, by introducing some variability, the regular oscillations were lost. Fig. 15 shows the mean number of flukes present on four fish plotted at one week intervals, and the procedure repeated 20 times. The average numbers fluctuated between 5 and 50 flukes per fish, a result close to that found in the local pond. Forty fish caught when the water temperature was  $15^{\circ}\text{C}$  carried on average 22 (3-60) flukes per fish.

Although the oscillations were lost in a population of fish, individual fish continued to show fluctuations. All four fish in the multi-host model were assumed to have been carrying 27 flukes prior to, and at the start of calculations, thus largely removing the artificially-induced initial oscillation. The number of flukes on the fourth fish was then calculated over a two year period, and this was repeated five times (Fig. 16). Large fluctuations occurred at irregular intervals and in one of the five cases this led to the extinction of the flukes.



## DISCUSSION

### Course of Laboratory Infestation

The typical course of a laboratory infestation was similar to that reported by Khalil (1964). He found that gyrodactylid infestations on Polypterus increased over the first 3-4 weeks, then, if the fish survived, the number of worms decreased to close to zero over the next 4-5 weeks. He also found that if a few worms remained on the recovered fish, the fish were not susceptible to a second infestation. However, if they remained quite free of parasites for a 'short while', they could be reinfested when infested fish were introduced into the tank. Within one year, fish were reinfested four times. This loss of protection was similar to that observed in the sticklebacks.

A parallel situation has been reported for Dactylogyrus vastator (Paperna, 1964). Infestations evoked a cellular response that caused the worms to leave the fish. The fish remained refractory for a few weeks until the epidermis returned to its former state at which time the fish again became susceptible. This response was independent of a specific immunity which the fish developed later.

### Gyrodactylus reproduction

The reproduction pattern for G. alexanderi followed that found by Braun (1966) for G. wagneri. Daughters continued to reproduce uninterrupted for at least 20 generations, rather than reproducing in groups of



four as found by Bychowsky (1957). The time between successive generations was dependent upon temperature, and was 1.6 days at 15°C. This compares favorably with the 1 day found by Bychowsky (loc. cit.), 20 hours by Braun (1966), and 18 hours by Turnbull (1956).

From indirect evidence, Bychowsky concluded that a fluke reproduced 3 or 4 times during its life span of 12-15 days. In this work, the flukes reproduced only twice, although on average they lived for 15 days, long enough to reproduce three times. Turnbull (1956) also found that in some apparently healthy flukes, no embryos developed and the uterus remained empty. Khalil (1970) concluded from observations on the reproductive structures of Macrogyrodactylus polypteri that the worms could probably reproduce twice without being fertilised, Whether fertilisation would enable them to reproduce further was not known, however, Khalil did find fully mature specimens in which the female genital system had ceased to function. As the male system developed later than the female system, he suggested that these older worms were functional males in the population.

The interval between the two births was 5.31 days at 15°C compared to less than 2 days found by Turnbull at 25-27°C, and 4-5 days reported by Bychowsky (no temperature cited).

#### Effect of Gyrodactylus on Gasterosteus

A number of authors have stated that Gyrodactylus can kill fish although the mechanism producing death is obscure (see literature review). In this work, sticklebacks less than 35 mm in length invariably died if carrying over 200 flukes. No organisms other than Gyrodactylus were



regularly observed in dying fish. It seems that Gyrodactylus itself produces pathological changes which result in the death of the fish. Epidermal hyperplasia occurred in a few instances but typically there were no signs of injury either in live fish or in sections of infected fish. There was no excessive mucus production, and the fins were not frayed.

G. alexanderi apparently fed on epidermal cells (Lester, 1972). Host melanin granules were occasionally prominent in the fluke gut and as melanin is normally restricted to the pigment cells of the dermis, this suggested that the flukes had penetrated through the epidermis while feeding. However, macrophages carrying melanin granules have recently been found in the epidermis of plaice (Roberts et al., 1972). These apparently migrate through the epidermis and void the granules at the surface. How commonly this occurs, or whether it occurs at all in the stickleback, is not known.

If the flukes do feed on the epidermal cells, the rapid turnover rate of the epidermis (about 11 days at 15°C) would obscure fluke damage. However, a heavy infestation may result in so many damaged epidermal cells that normal epidermal functions, such as acting as an ionic barrier, are disrupted. Excessive leakage of ions from the fish into the water could result in the death of the fish. As osmotic stress inhibited the shedding response, the stress produced by 200 flukes may have had the same effect, thus ensuring a terminal infestation.



### Effect of the Host on Gyrodactylus

A humoral immune response may have been involved, though the periods of increase and decrease of resistance were short compared to the majority of antibody responses detailed in the literature. Antibody production in fish is temperature dependent. At 11°C trout took 3 months to reach their maximum titre, and high titres were maintained for at least 24 months (Krantz et al., 1963). At 14°C carp and goldfish showed a rise in antibody titre 15 days after injection whereas fish at 24°C showed an increase within 11 days (Cushing, 1942). Serum antibodies in carp at 18-20°C reached their highest levels around 30 days after infestation with Dactylogyrus vastator, a blood-feeding monogenean, and dropped to near normal levels after about 60 days (Vladimirov, 1971). At 24-29°C antibody was detected in catfish by the second week after inoculation, and reached a maximum titre by the end of the fourth week (McGlamery et al., 1971). In most cases, antibody remained at a high level for the next four weeks and was still present 15 weeks after the initial inoculation. Goldfish kept at 30°C, close to the lethal temperature, produced antibodies in 2-4 weeks (Uhr et al., 1962).

In the present work at 15°C, susceptible sticklebacks responded to flukes within two weeks and again became susceptible after a further four weeks, time periods which seem too short to correspond to the type of antibody production detected by the above authors. However, more rapid responses have been demonstrated. Trypanosome infections in perch and goldfish at 20°C were lost within a week (Barrow, 1955). Two to three weeks after recovery the fish serum lost its ability to lyse



trypanosomes. He concluded that the antibodies were rapidly dissipated. Whether this meant the fish again became susceptible was not demonstrated.

Hildemann (1958) grafted scales on to goldfish at 23°C and found the median survival time of the grafts to be 6-8 days. Homografts made as long as 26 days after the initial grafts survived only about 4 days, and this suggested to him that the rejection involved antibodies. Injections of whole blood from the donor fish produced high levels of haemagglutinins, but this conferred no protection, and conversely fish showed strong transplantation immunity at a time when only feeble concentrations of haemagglutinins were present. Hildemann concluded that cell-bound antibodies were responsible for graft breakdown. He did not determine how long the antibodies remained active in the fish.

If there had been some quick-acting immune mechanism involved, then the flukes could have come in contact with it when ingesting epidermal cells. The antibody could have weakened the flukes and caused them to loosen their hold on the fish. Such weakened flukes would be expected to show other physiological changes, for example a decrease in the reproductive rate or in the time of survival off fish, but observations on these points showed no appreciable change. Though the survival time of flukes shed on cuticle was not measured, shed flukes showed similar activity and behaviour to that of flukes that had been removed experimentally. Thus, there was no evidence that the flukes on recovering fish were physiologically different from those on susceptible fish.

In conclusion, it is not possible to say whether or not antibody is involved, although the evidence suggests that the humoral agglutinins



demonstrated by most workers are not involved.

Flukes left fish on a layer of mucoid material which has been referred to as 'cuticle' in this thesis. This layer has previously been referred to as 'slough' by the author (see Appendix). However, 'slough' generally refers to layers of cells or cell derivatives (Ling, 1972) whereas the layer was found to be non-cellular. It consisted of mucoid material, 0.5 to 1.0  $\mu$  thick, and was shed in sheets from the epidermis of infested fish every 1-2 days. It appeared to be synonymous with the 'cuticle' found on the surface of fish epidermis (Whitear, 1970) and was so designated in this thesis. Although Whitear examined the cuticle of eight teleost species including sticklebacks, she did not record whether cuticular shedding took place regularly. Cuticular shedding has been recorded in other teleosts by Gilchrist (1920) and Heldt (1927). As the cuticle was apparently secreted by the surface epidermal cells (Whitear, 1970), it was possible that this was the site of action of an antibody, perhaps a cell-bound antibody.

Though the cuticle became more dense on sticklebacks infected with Gyrodactylus, shedding was independent of any infestation. Its function may be to discourage epibionts such as Epistylis and Apiosoma. Occasionally fish became covered by these protozoans, and this may have been the result of not shedding, the 'mucus stasis' of Nigrelli et al (1955).

It may be possible to stimulate an efficient Gyrodactylus-shedding cuticle without using Gyrodactylus. For example, on healthy fish an abundance of epibionts may stimulate the formation of dense cuticle.



If this more general response could be demonstrated, it would argue against the involvement of antibodies.

Shed cuticle apparently inhibited fluke reattachment. Sheets of cuticle from an infested fish remained intact in the water for at least 24 hours before being destroyed by bacteria. When the sheets folded over, due to water currents, they tended to adhere. Flukes which were attached to this slough, particularly if inside a fold, were hindered from attaching to a fish. Though this effect was difficult to measure, it was thought to be responsible for the poor reattachment success rate (1/3) indicated by the model. Light debris on the bottom may also have hindered reattachment.

#### Limitations of Models

The approach used in this thesis evaluated a series of factors found to influence population size in Gyrodactylus, and combined these into models simulating population changes. While this provides a rational approach in investigating a system, predictions derived from the model that cannot be checked experimentally must be treated with caution. There are two sources of error. First, were the right factors included in the model, and second, if so were they given the right values?

In the single host situation, some factors were known to affect fluke population size, particularly those outside the black square in the flow chart (Fig. 5), though they were thought to have little effect in the experimental infestations. Besides those mentioned there may be others that were not evaluated.



Those factors that were included may have been given the wrong values. For example, the survival rate of flukes off fish was measured using flukes removed with anaesthetic rather than flukes shed on cuticle. Also, the experimental conditions, such as weekly handling of fish, may have produced results unusual under natural conditions.

Only one source of variability was included. In reality, all the factors would show variability, and in total this would enhance the chances of fluke populations going to extinction on a particular fish.

In the multi-host model perhaps the major omission was the absence of a system for incorporating deaths of fish, although in reality, on the death of a fish, all its Gyrodactylus are released into the pool of unattached flukes. Also high fluke numbers impair the ability of the fish to respond to the flukes, but this was also not included in the model. Such a feed-back system would cause some fluke populations by chance to increase to over 150 individuals, as apparently happens in reality, and these flukes would in turn be released into the pool of unattached flukes when the fish died.

Both models remain hypotheses because of the calculated reattachment success which was not directly demonstrated. Experimental verification could perhaps be achieved by taking shed cuticle to which flukes were attached and placing it in a bowl with a susceptible fish. Both models are also restricted to a specific temperature (15°C), and to a specific density of fish (1 fish to 250 ml water).



### General Discussion and Implications of Model

Using a model derived from theoretical principles, Crofton (1971) found that continuous oscillations in numbers of parasites and in numbers of hosts were produced when an immune response was incorporated into the system. In the Gyrodactylus simulation, regular oscillations were also produced by the deterministic model (Fig. 14). These were still present in the stochastic model although different groups of fish became out of phase with one another within a few weeks (Figs. 15 and 16). This suggested that in large non-homogeneous populations of sticklebacks individual fish may show fluctuations in fluke number, but the total population of flukes would remain relatively constant.

The relationship between the fish and the flukes is probably temperature dependent. At 7°C, fluke reproduction was one third the rate at 15°C. Data was not obtained on the time required for the fish response at 7°C, but immune responses in most fish are not detectable at this temperature. Therefore, one might expect that populations of Gyrodactylus would be large at winter temperatures. In the summer, the fish may 'over-respond' to the flukes, and hence the fluke population may become small. Preliminary studies indicated that infestations on fish at 15°C became heavier during the next week after the temperature had been raised to 25°C. Presumably, the fish was stressed for a longer period than the fluke. Therefore, daily temperature fluctuations, particularly in the spring and fall, may enhance the chances of an epizootic due to Gyrodactylus.



With more experimental data on factors such as temperature and fish density, interactions in the system could be investigated, perhaps using the 'response surfaces' idea of Holling and Ewing (1971). Ultimately, the critical factors and the stability of the system could be detected by systems analysis.

Knowledge of the course of the infestation and its time period may be of value in reducing fish losses in cultural conditions since precautions can be taken when the infestation is reaching its peak. For example, fish might be protected by providing a flow-through water supply or continuous filtering of the water for the second and third weeks (at 15°C). This is preferable to the traditional method of subjecting the fish to dilute formalin (1:4000) which causes extensive gill damage (Smith and Piper, 1972). If the fish are liable to be exposed to further influxes of Gyrodactylus, at least in the system examined, it is better that each fish carries a few flukes permanently to provide premunition.

The typical course of the infestation may also provide a tool for examining different races of Gyrodactylus and/or sticklebacks. There are several types of Gasterosteus in the Pacific Northwest, and they may constitute sibling species (Hagen, 1967). Thus it is possible that there are several races of G. alexanderi. Different races of host may show different degrees of susceptibility to flukes, and similarly fluke races may be separable by their virulence or their salinity tolerance. Morphological similarities in the host races may indicate similar fluke races, and similarities in the fluke races may indicate relationships between races of hosts.



If the model as described incorporates the basic relationship between the fish and the parasite, the following line of reasoning leads to an unexpected conclusion. If the fish do not respond to the flukes, all infested fish and parasites die. If fish respond, infested fish and parasites survive. This appears to be an example of the idea that a host response which results in acquired resistance can increase rather than decrease the number of parasites. This has already been suggested on theoretical grounds by several authors (Crofton, 1971; Sprent, 1963; Dineen, 1963). It is possible that the immune mechanism in vertebrates, which is involved in resistance to numerous parasites and diseases, may not have evolved as a protection against disease (Sprent, 1969). Several authors, including Good (1971), have suggested that it evolved in order to identify aberrant cells in organs as animals became more complex, and only incidentally came to react against foreign proteins. In the case of the stickleback, cuticular shedding may have been evolved for purposes other than removing gyrodactylids.

If this line of argument is continued, again from the parasite point of view, it would appear that it is beneficial to the parasite to produce a host response. This conflicts with the traditional view that a parasite should be as unobtrusive as possible, but if the model approaches the true situation, it is because the fluke stimulates the fish to respond that the fluke is able to maintain 10-20% of its lethal number on each fish.

I would like to conclude by suggesting that one of the more important results of this thesis was the demonstration of the use of component



analysis and computer modeling in investigating a parasitological problem. The approach has much to offer parasitology, particularly ecological parasitology. By considering the system as a whole and then examining each factor in turn, it provides a rational framework in which to work. Being quantitative rather than qualitative, its precision forces hitherto unrecognized factors to one's attention. Finally, when the complete system has been described, the importance of individual factors becomes clear. For example, the significance of the extensively-investigated responses of free-living stages, and the recent work on changes in host behaviour with infection, can only be fully assessed after an analysis of the whole of the life cycle of the parasite. By further application of the 'whole system' approach, we may better understand how parasites maintain their populations in the wild.



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Figure 1. Course of experimental infestation on isolated fish

Of 26 fish each initially infested with 20 flukes, 19 fish recovered (solid line) and 7 died (dotted line). The mean numbers of flukes present at the end of each week are indicated together with one standard error either side of the mean.



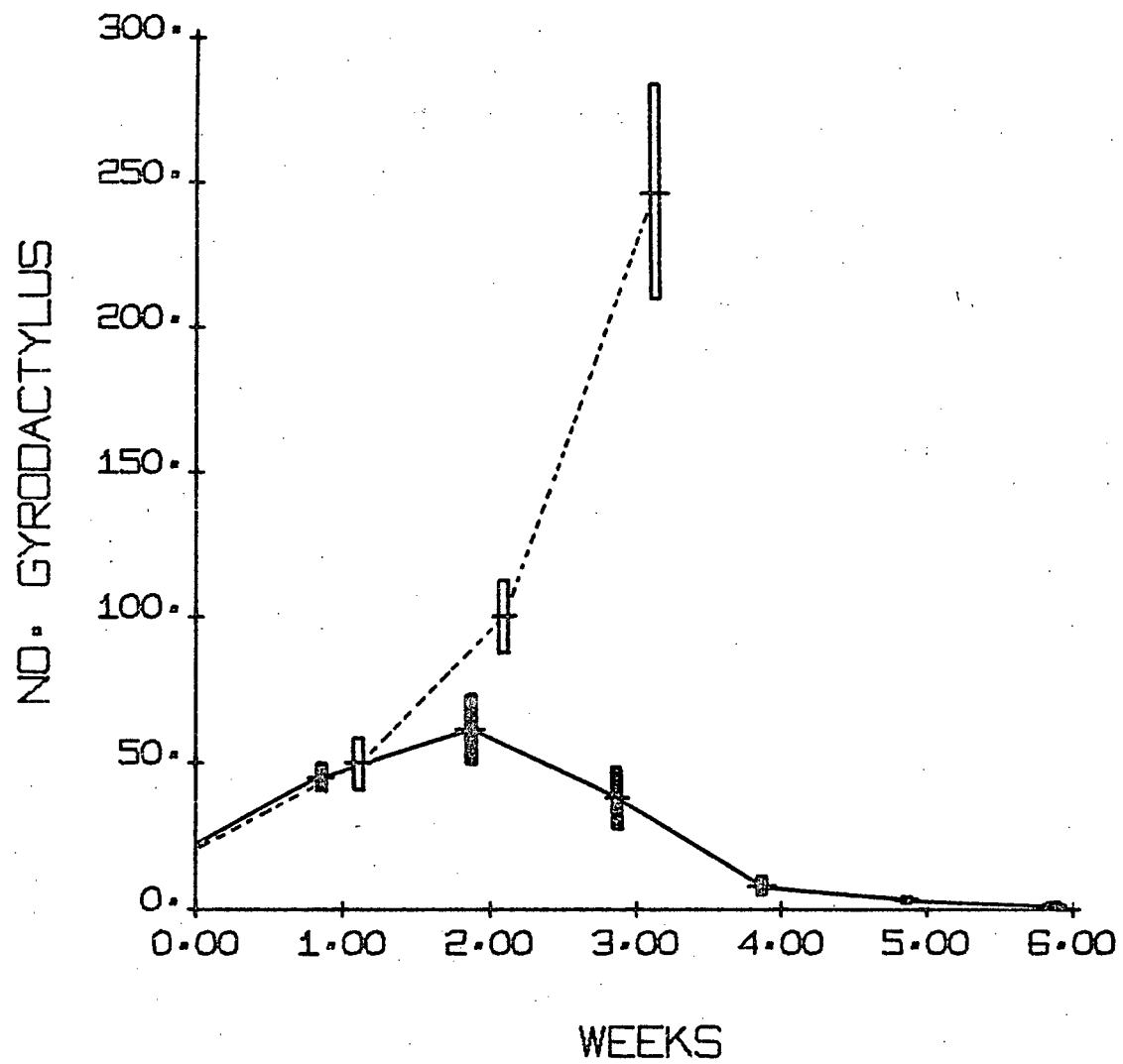




Figure 2. Effect of Handling on Course of Infestation

Solid line indicates course of infestation of 8 fish subjected to experimental handling and confinement for two weeks prior to infestation. Dotted line indicates the course of the infestation of the 19 fish shown in Fig. 1.



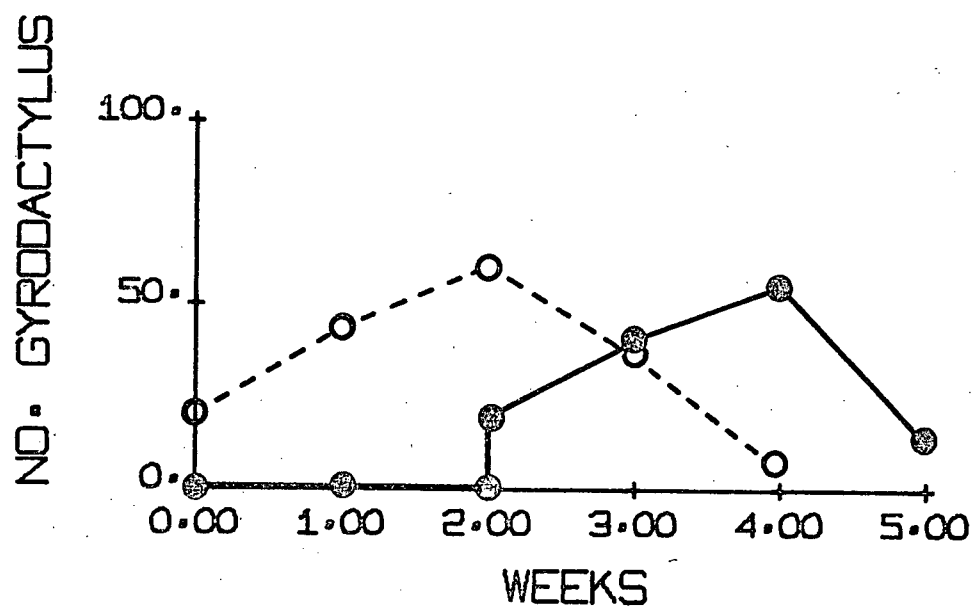




Figure 3. Effect of osmotic Stress on Infestation

Eight sea water adapted fish were infested with freshwater flukes and held in freshwater. Circles indicate observations, open circles indicate death within the next week.



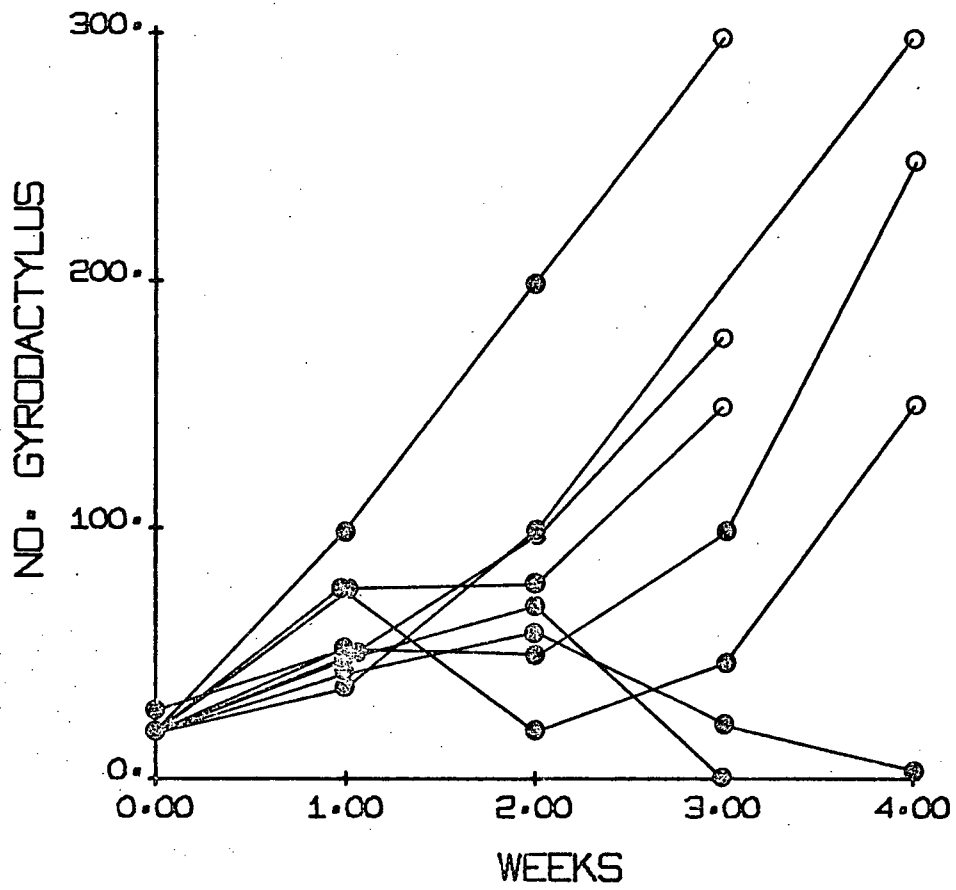




Figure 4. Refractory Period of Recovered Fish

The horizontal axis represents the time interval between recovery from infestation (taken from when they carried 10 or fewer flukes) and being given a challenge infestation of 20 flukes. Means and range of data from 6, 6, 11, and 19 fish.



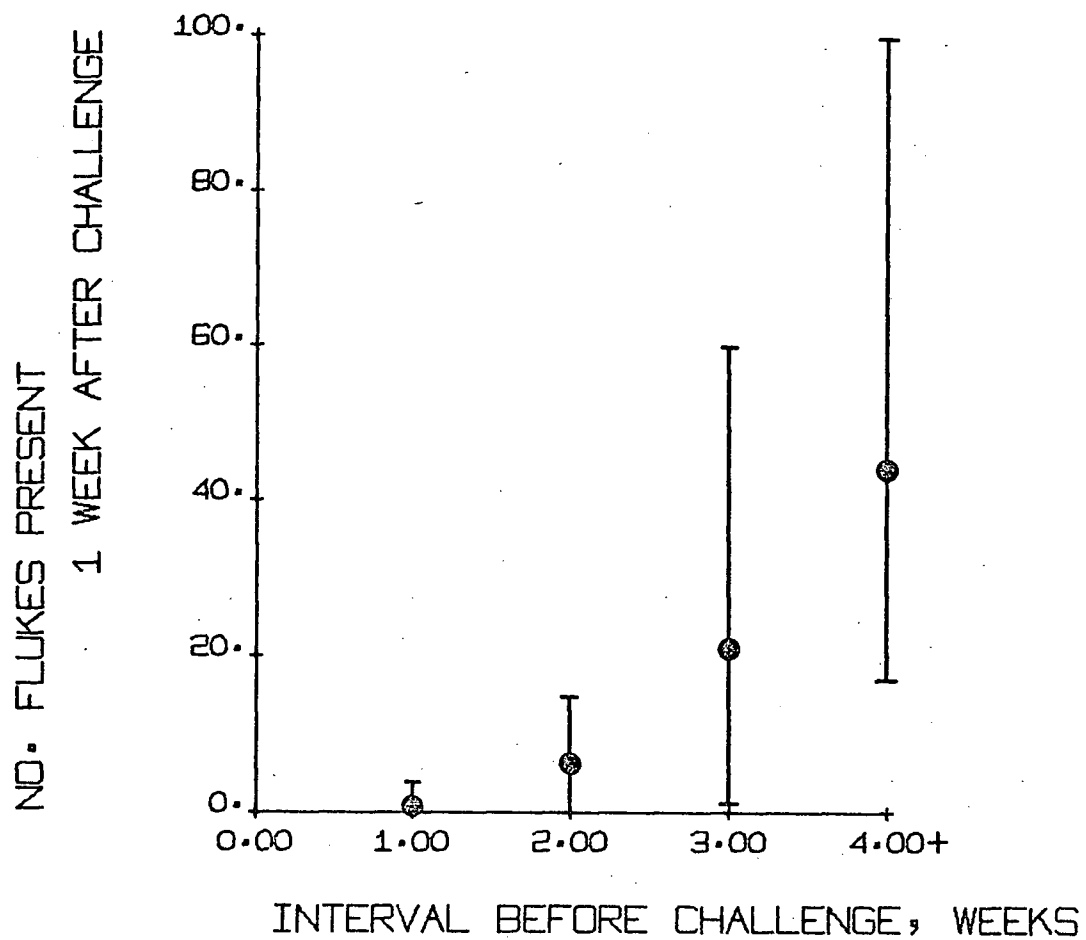




Figure 5. Factors Found to Influence the Size of Gyrodactylus Populations

The flow chart indicates their relationships. The factors inside the black square were evaluated and used to produce a model which simulated a laboratory fluke population.



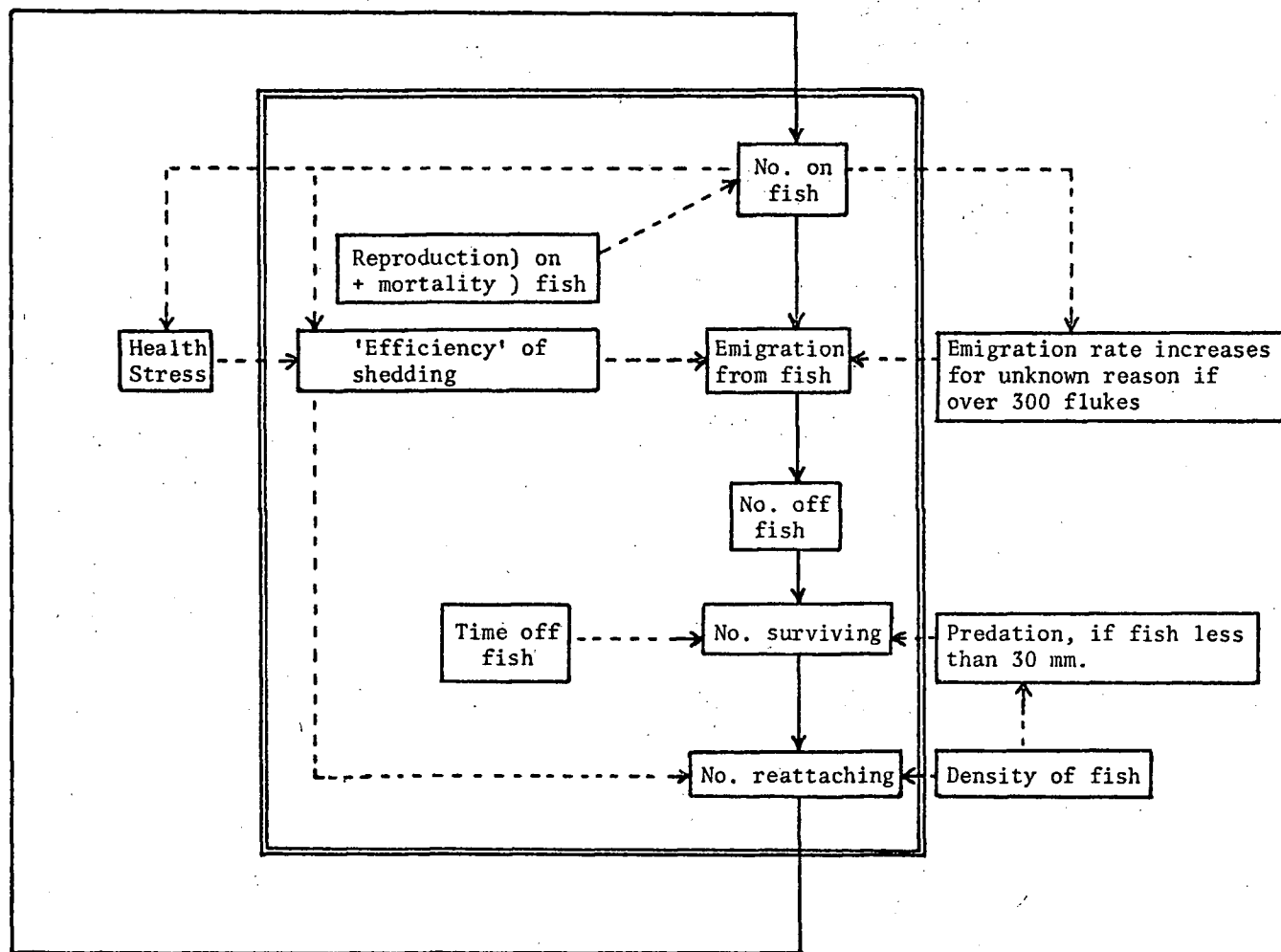




Figure 6. Fluke Birth and Death Statistics

Upper line: life span of fluke  
marked off in days. Graph below:  
survivorship graph for the fluke.  
All data derived from observations  
on individual flukes.



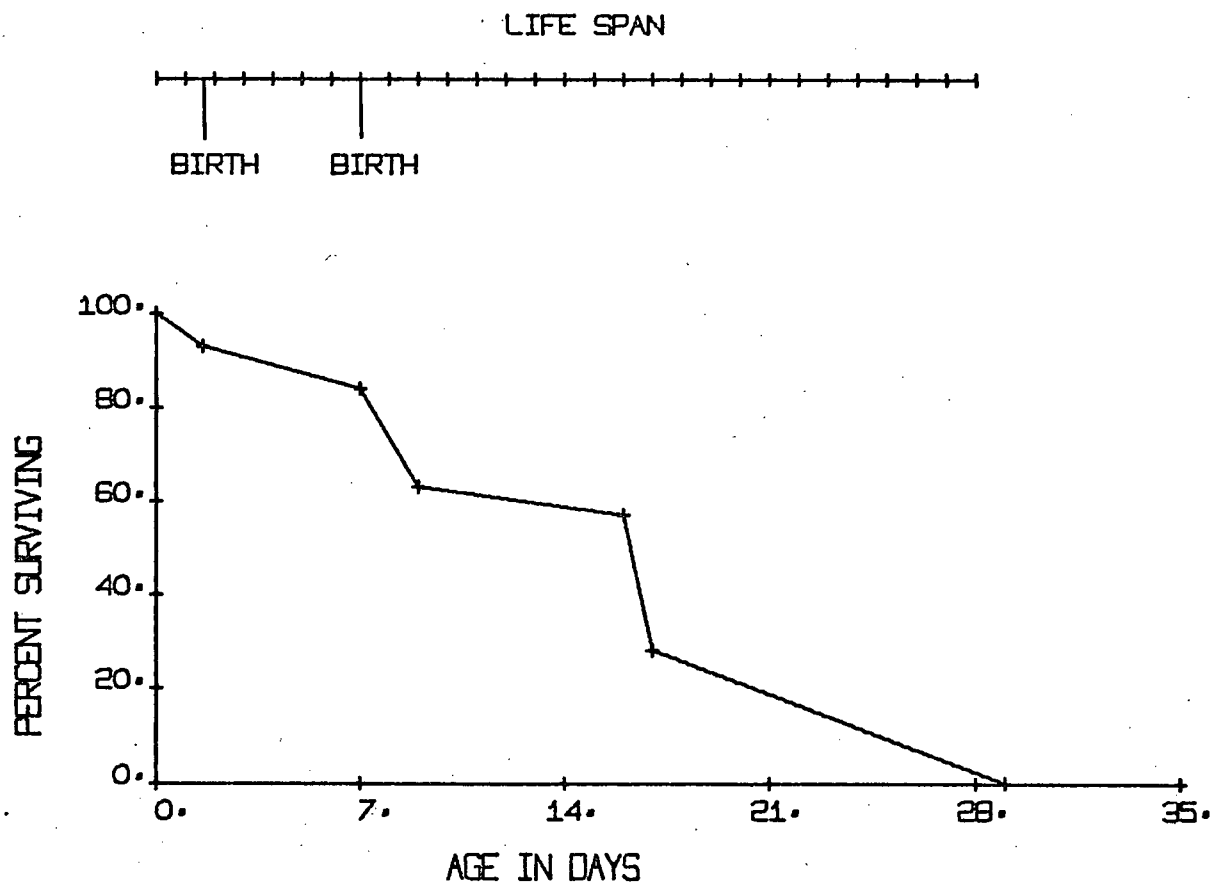




Figure 7. Pattern of Shedding of Flukes on Cuticle

Solid circles indicates numbers of flukes  
on three isolated fish, recorded weekly.

Open circles indicates daily records of  
the number of flukes on shed cuticle re-  
moved from the container.



NO. GYRODACTYLUS

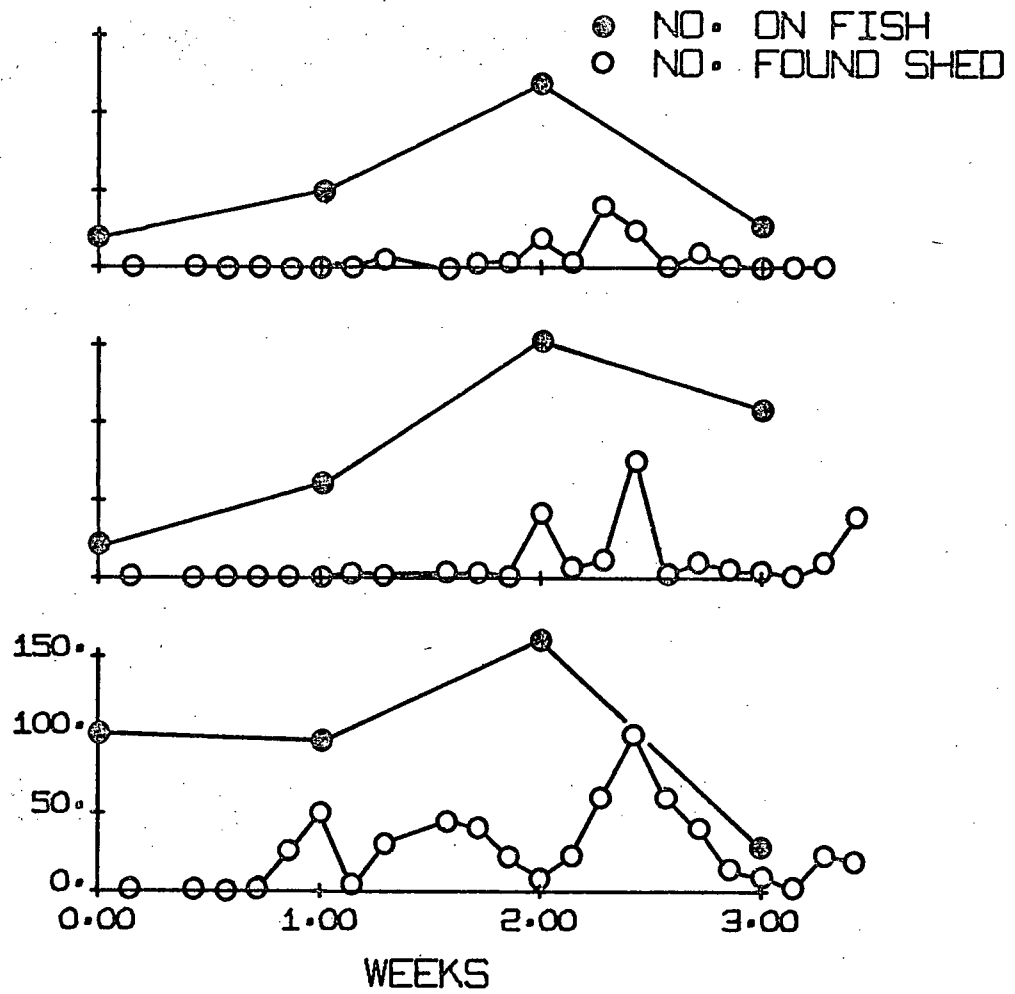




Figure 8. Courses of Infestation Resulting from 100,  
60 and 20 Flukes per Fish

Circle and dot: mean numbers of flukes present on 10 fish infested with 100 flukes each.

Solid circle: mean numbers present on 6 fish infested with 60 flukes each.

Open circle: mean numbers of flukes on 19 fish infested with 20 flukes each.

6 of the 10 fish infested with 100 flukes died during the second week.



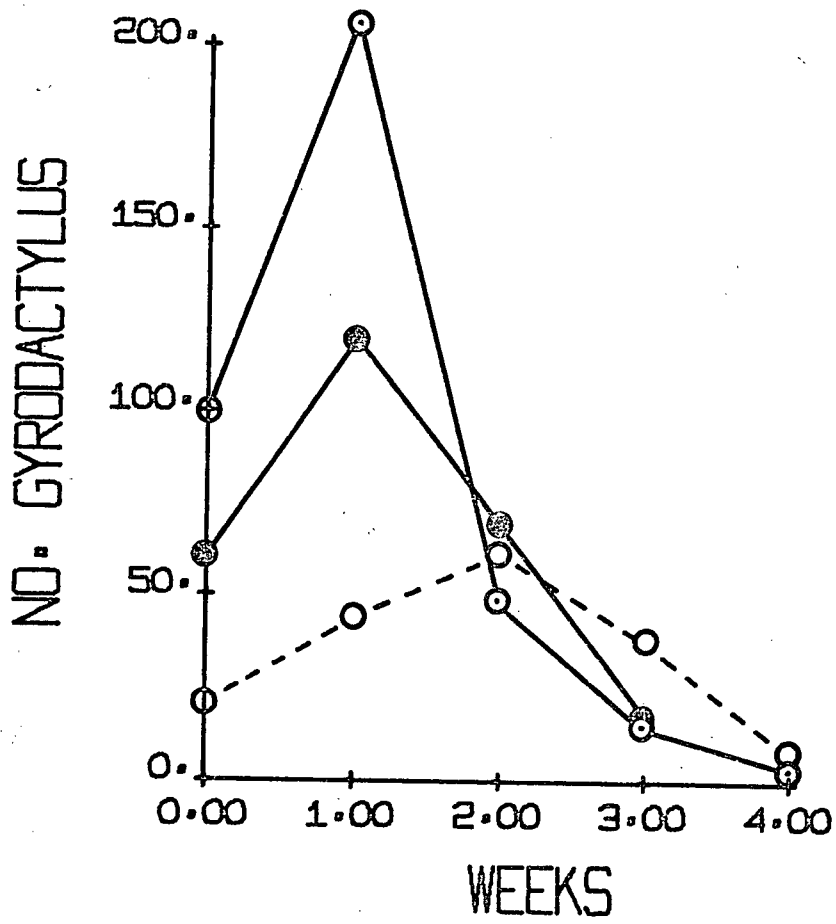




Figure 9. Change in Fluke Number on Recovering Isolated Fish

Horizontal axis indicates the number present at the start of each week, and the vertical axis the percent change by the end of the week. The means from left to right were derived from 37, 28, 25, and 17 fish respectively with initial infestations within the ranges indicated.



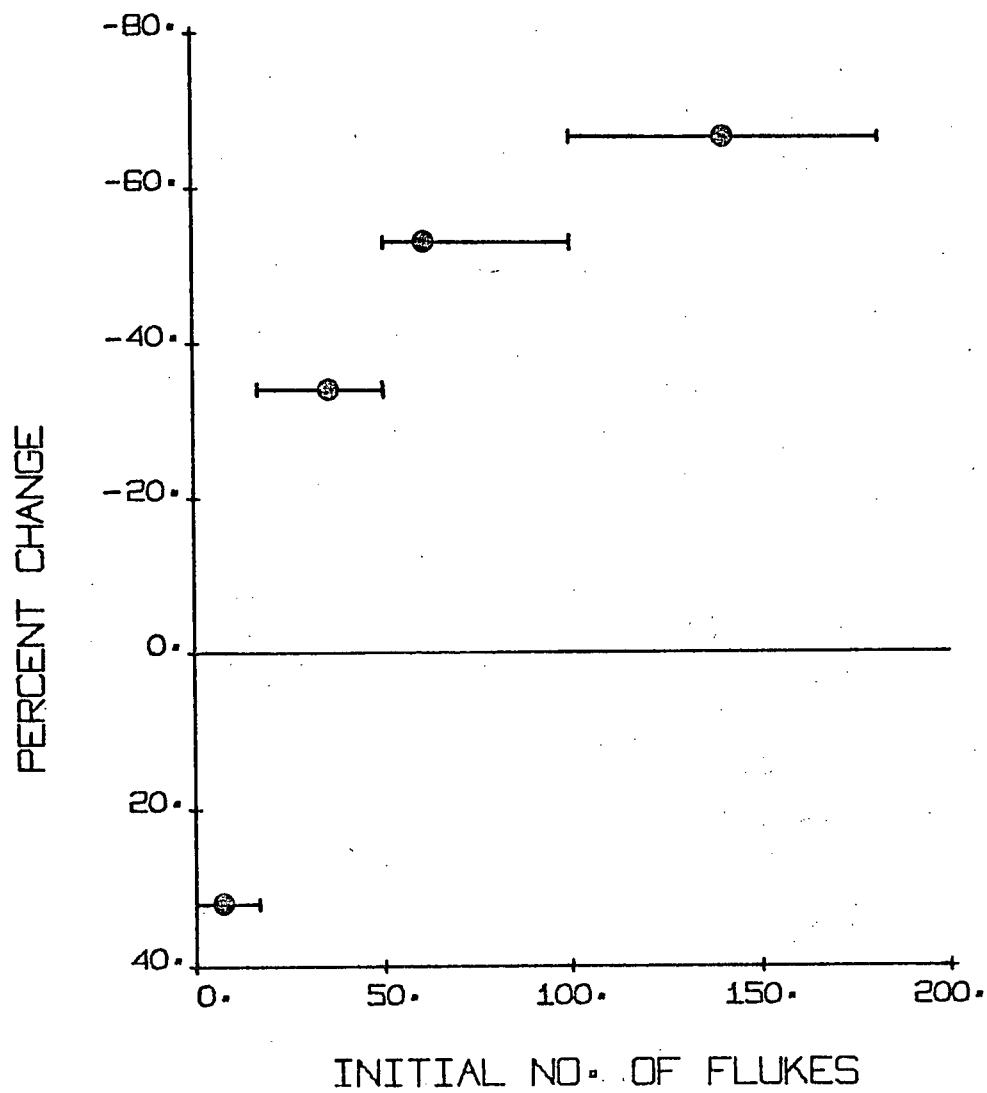




Figure 10. Course of Experimental Infestation Compared  
to Course of Simulated Infestation  
(Deterministic Model).

Straight lines join means of fish infested with 20, 60 or 100 flukes (as in Fig. 8). Crosses indicate daily estimates of the number of flukes present, calculated using the single host model.



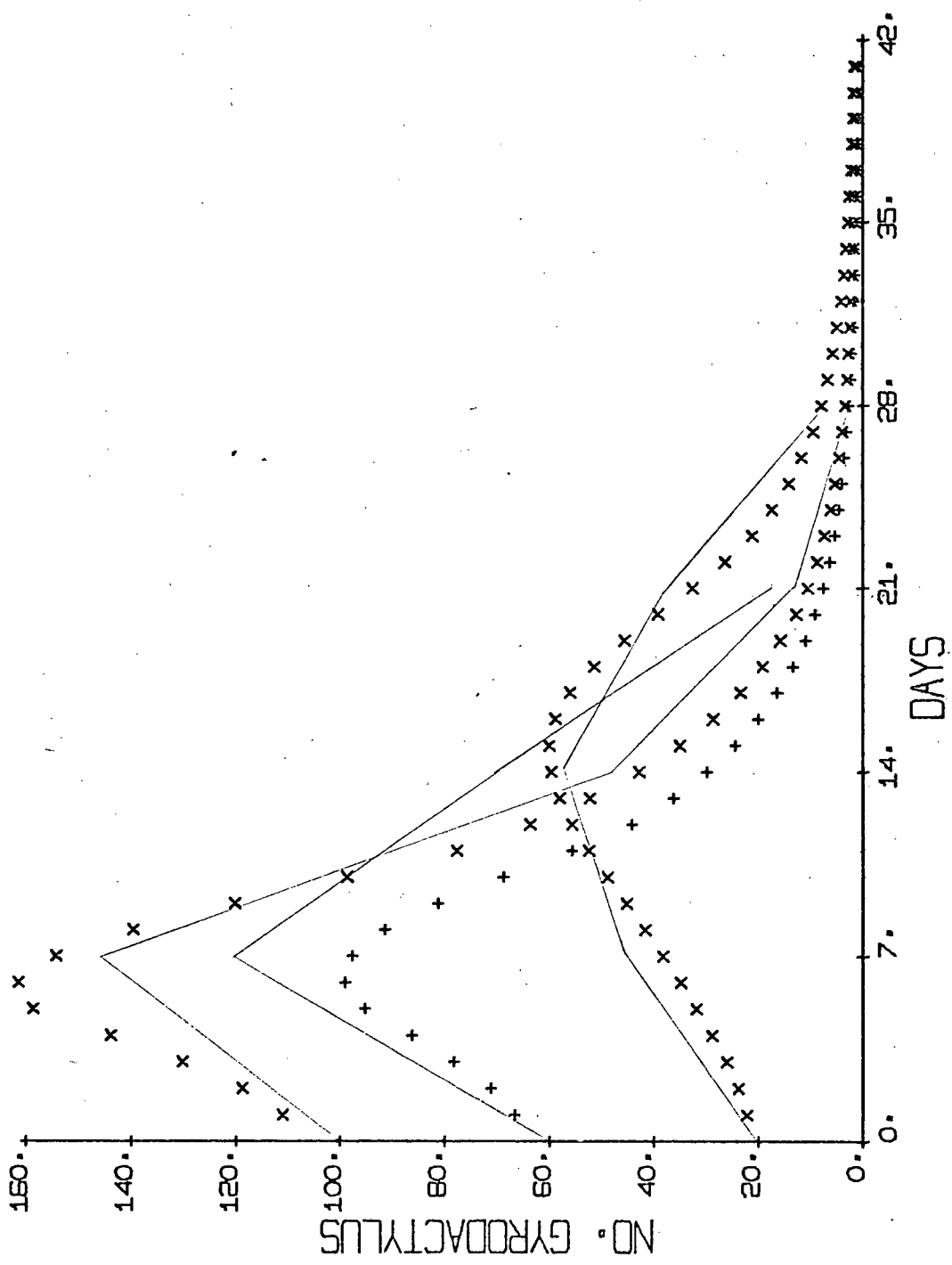




Figure 11. Calculated and Observed Relapses  
(Deterministic Model)

Solid line: computer estimates of  
number of flukes present on one  
isolated fish over 140 days. Dot-  
ted line: example of a relapse  
observed in the laboratory.



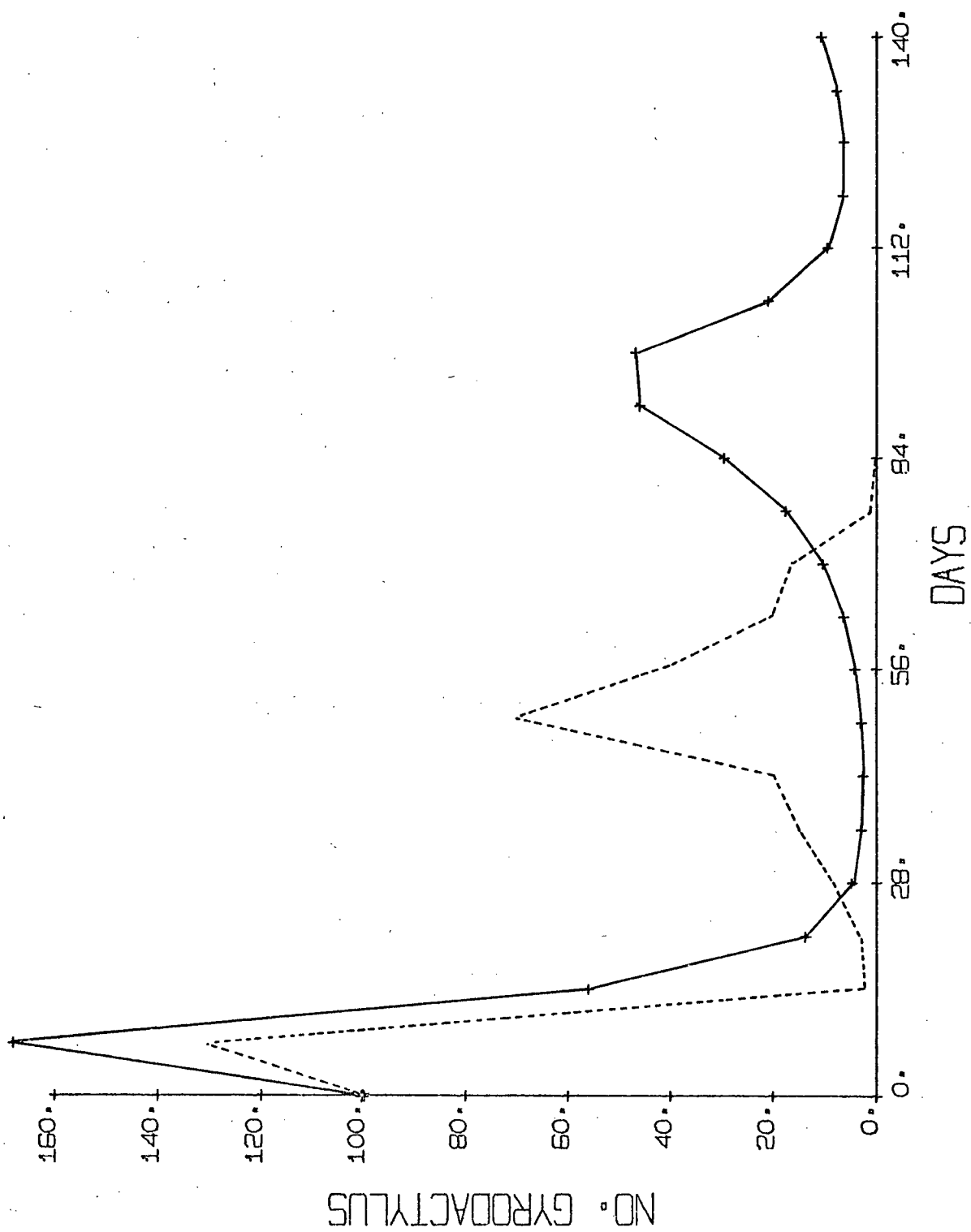




Figure 12. Simulated Course of Infestation on 20 Isolated Fish (Stochastic Model)

Each line represents the number of flukes on one fish (vertical axis) calculated over 140 days (horizontal axis). All start with 20 flukes, and calculations incorporate the random variability.



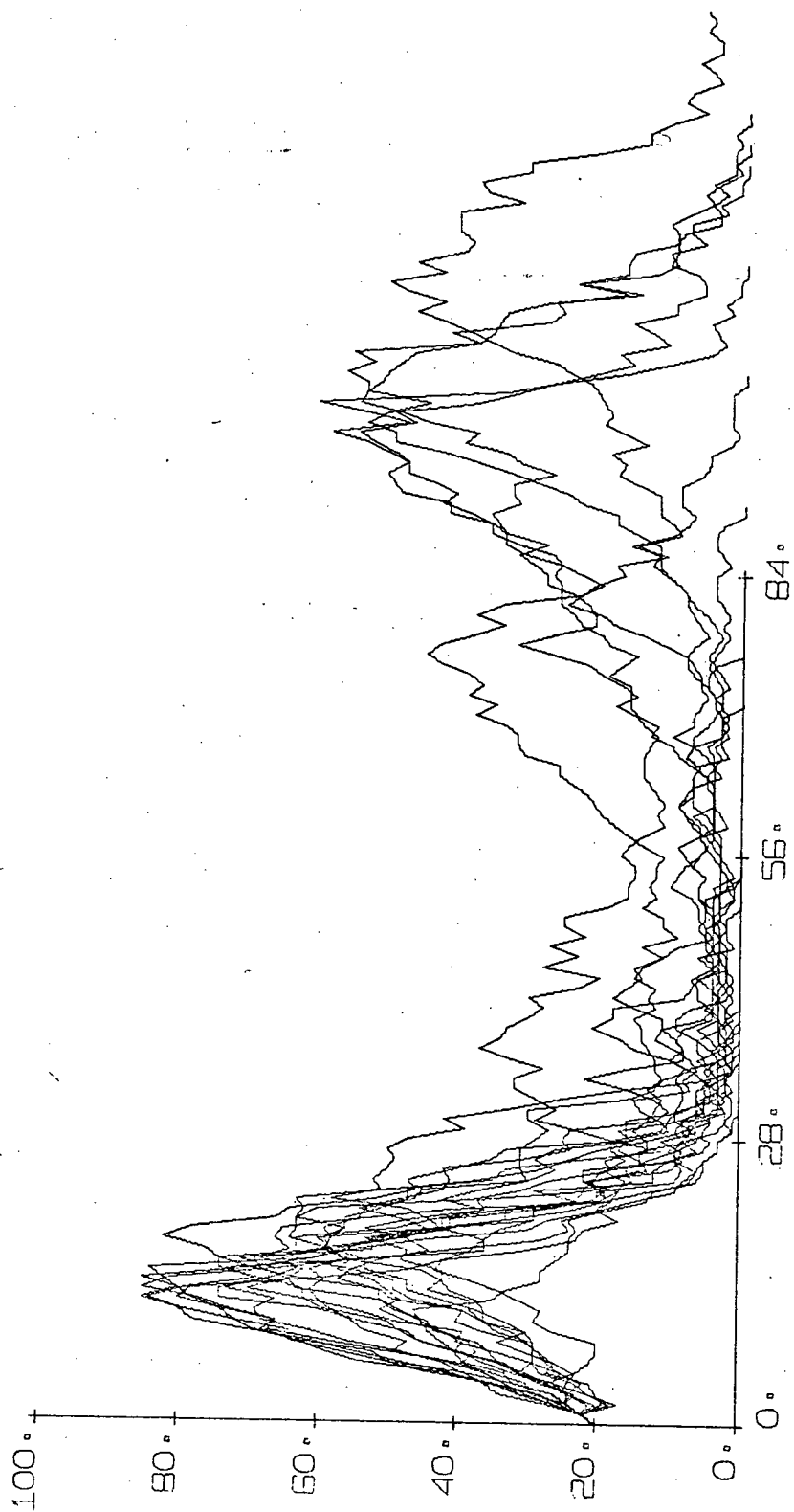




Figure 13. Simulated Infestation in Multi-Host Situation  
(Deterministic Model)

The symbols represent estimates of the numbers of flukes present on four fish that are assumed to be in the same chamber. The first fish (x) was assumed to have an infestation of 100 flukes, and the other three fish, considered to be initially uninfested, were introduced at weekly intervals.



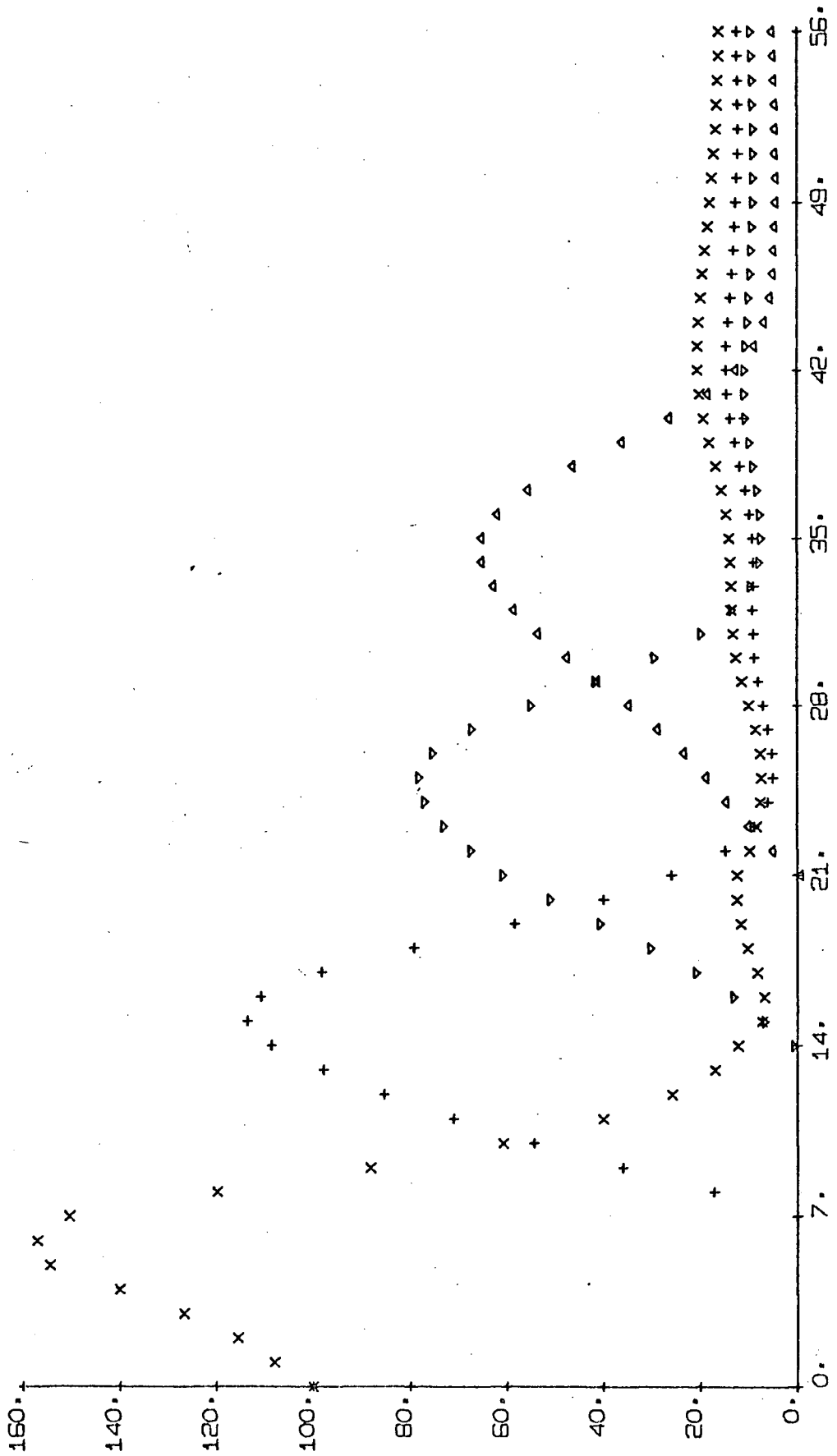




Figure 14. Multi-Host Simulation over 280 Days  
(Deterministic Model)

The symbols represent the numbers of flukes present on the four fish shown in Fig. 13. Points here are plotted weekly, and calculations made for a 280 day period.



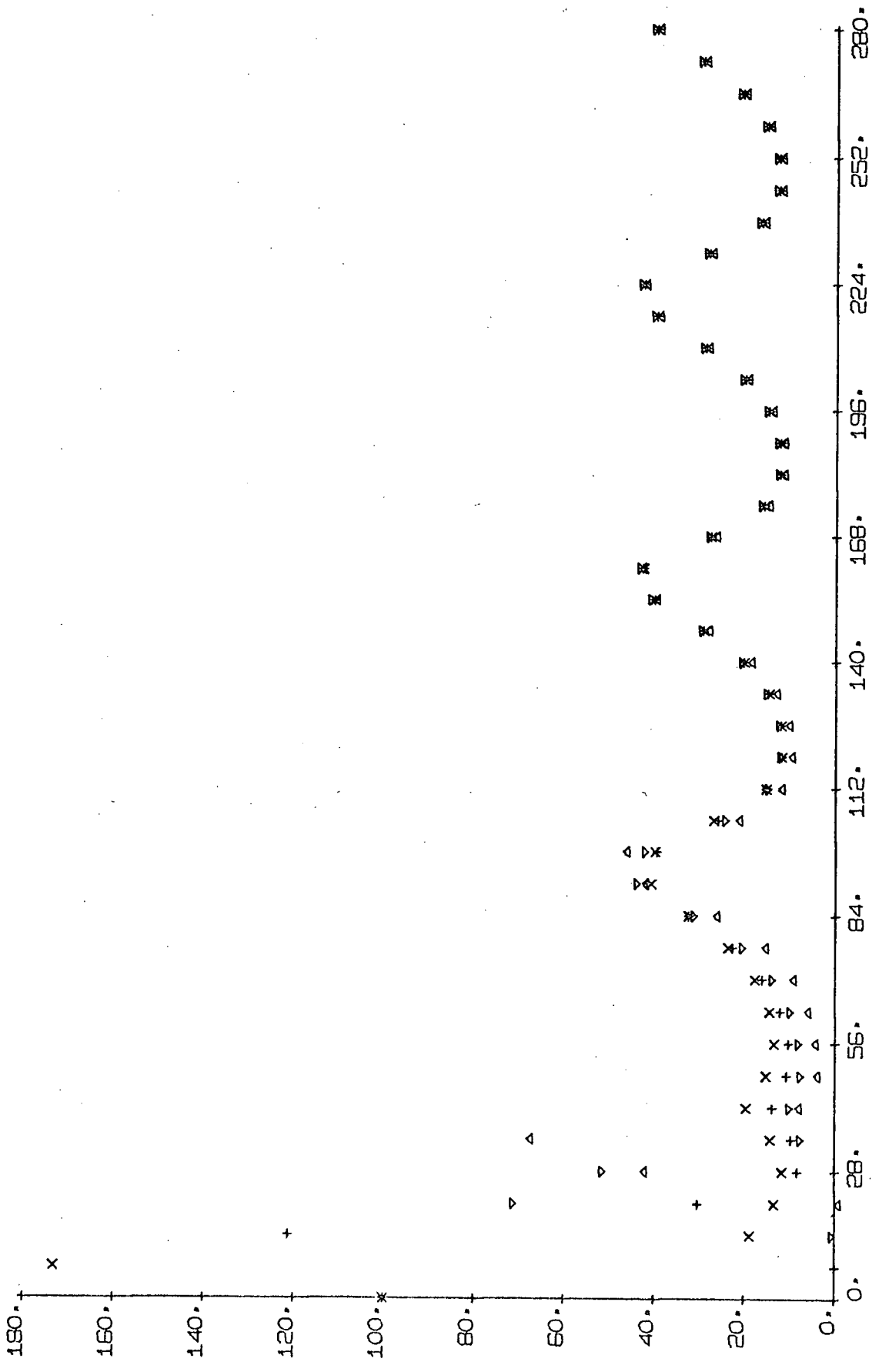




Figure 15. Simulated Infestation in Multi-Host Situation  
(Stochastic Model)

The calculations, which incorporate variability, were done 20 times over a 360 day period. Each of the 20 lines represents the mean number of flukes on four fish.



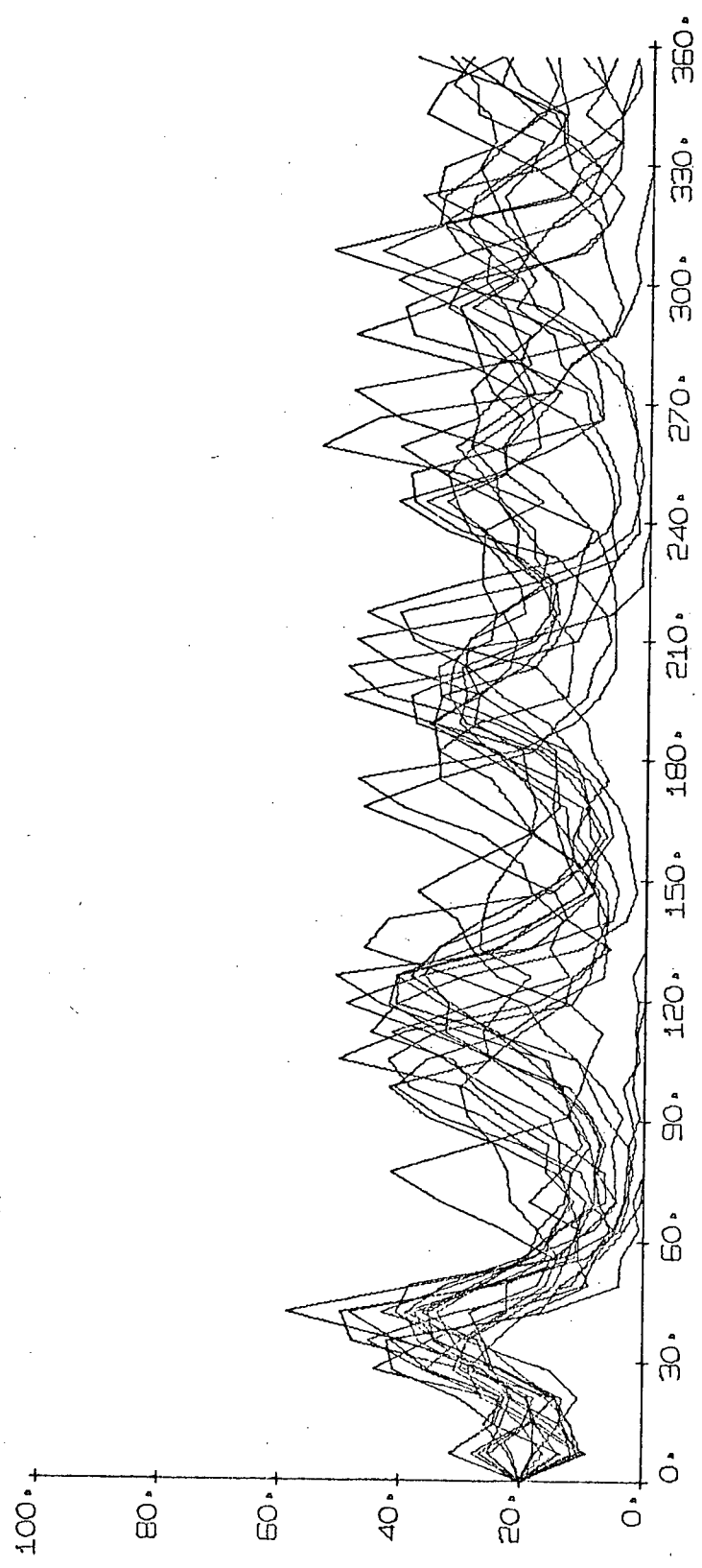
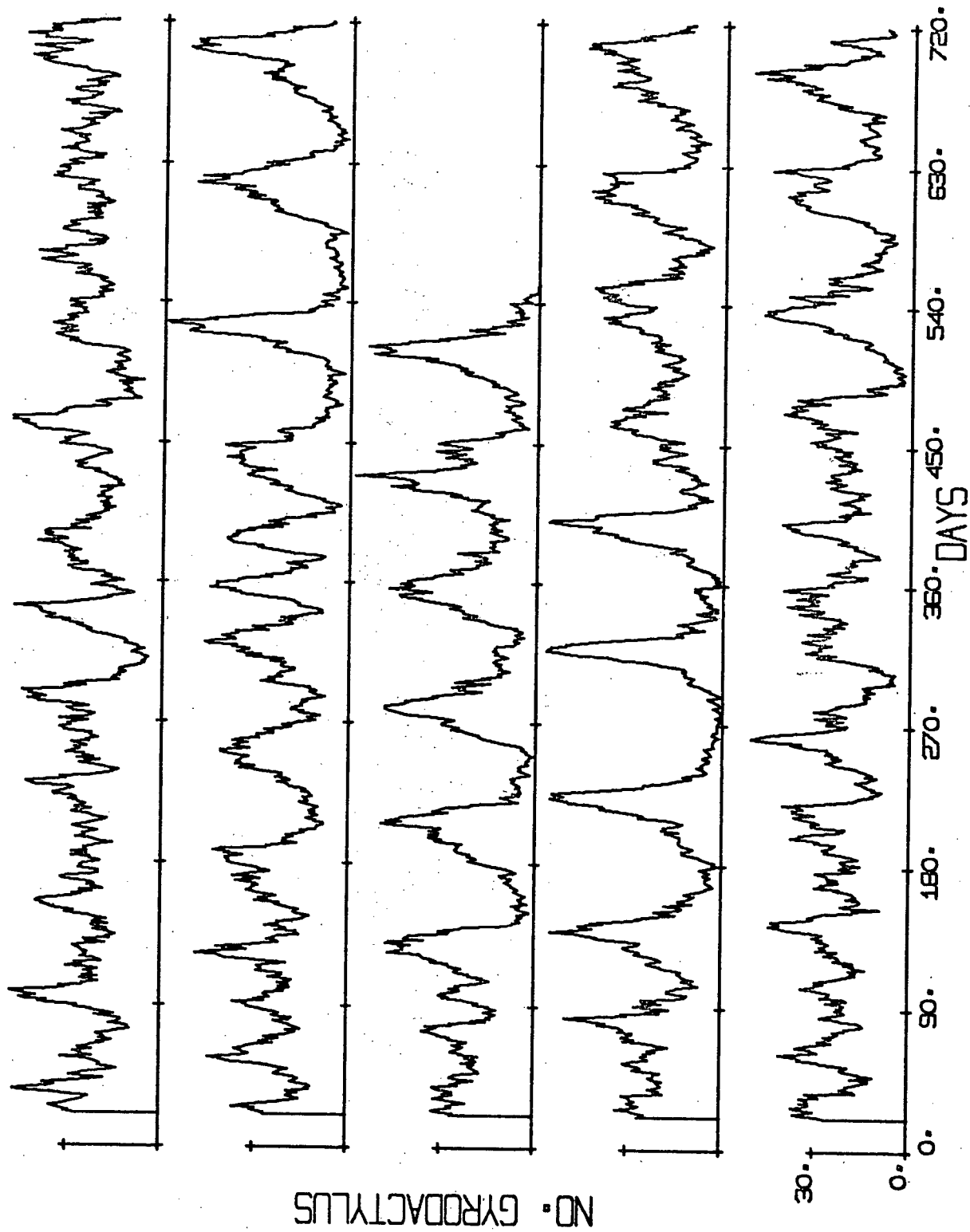




Figure 16. Numbers of Flukes on Individual Fish in the Multi-Host Model Situation (Stochastic Model)

Estimates of the number of flukes on one of the four fish in the multi-host situation, calculated over 720 days and repeated five times.







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UNIVERSITY OF BRITISH COLUMBIA BIOLOGY DATA CENTRE ON MONDAY DEC. 18, 1972 USER JOB

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C-ERRS...STNO.C..... FORTRAN SOURCE STATEMENTS ..... IDENTIF

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      DIMENSION X(10,4),SL(10,4),ON(10,4),SUMON(10),SURV(10,4),
1 SURV2(10,4),ON2(10,4),ON3(10,4),POIS(15,5),EXIT(10,4)
      CALL RANDI(50.)
      READ(2,1)POIS(1,1),POIS(2,1),POIS(3,1),POIS(4,1),POIS(5,1),
1 POIS(6,1),POIS(7,1)
      FORMAT (15F4.3)
      READ(2,1)POIS(1,2),POIS(2,2),POIS(3,2),POIS(4,2),POIS(5,2),
1 POIS(6,2),POIS(7,2),POIS(8,2),POIS(9,2),POIS(10,2)
      READ(2,1)POIS(1,3),POIS(2,3),POIS(3,3),POIS(4,3),POIS(5,3),
1 POIS(6,3),POIS(7,3),POIS(8,3),POIS(9,3),POIS(10,3),POIS(11,3)
      READ(2,1)POIS(1,4),POIS(2,4),POIS(3,4),POIS(4,4),POIS(5,4),
1 POIS(6,4),POIS(7,4),POIS(8,4),POIS(9,4),POIS(10,4),
1 POIS(11,4),POIS(12,4),POIS(13,4),POIS(14,4)
      READ(2,1)POIS(1,5),POIS(2,5),POIS(3,5),POIS(4,5),POIS(5,5),
1 POIS(6,5),POIS(7,5),POIS(8,5),POIS(9,5),POIS(10,5),POIS(11,5),
1 POIS(12,5),POIS(13,5),POIS(14,5),POIS(15,5)
      N = 1
      CALL P1130
      CALL FPL0T (0, 2., 7.75)
100 CALL SCALF (0.0167, 0.0333, 0., 0.)
      CALL FGRID (0, 0., 0., 90., 8)
      CALL FGRID (1, 0., 0., 30., 1)
101 CALL FPL0T (-2, 21., 0.)
      I = 2
      J = 1
      SUMON(I-1) = 0.
      X(I-1,J) = 27.
      EXIT(I-1,J) = 2.
      DO 24 II = 1,720
      J = 1
      I = MOD(II,7) + 1
      IIM1 = II - 1
      IM1 = MOD(IIM1,7) + 1
      IIM5 = II - 5
      IM5 = MOD(IIM5,7) + 1
      M = II
2 IF (J.EQ.1.OR.M.GT.1) GO TO 3
      SL(I,J) = 0.3
      EXIT(IM1,J) = 0.
```



# **ATTACHMENT OF *GYRODACTYLUS* TO *GASTEROSTEUS* AND HOST RESPONSE**

**R. J. G. Lester**

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## ATTACHMENT OF *GYRODACTYLUS* TO *GASTEROSTEUS* AND HOST RESPONSE

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**ABSTRACT:** *Gyrodactylus alexanderi* (Mizelle and Kritsky, 1967) initially uses its marginal hooklets to cling to the surface of *Gasterosteus aculeatus leiurus*. Tension on muscles associated with the hooklets then causes the anchors to sink into the epidermis, compressing the epidermal cells rather than piercing them. When strong shearing forces are exerted on the fluke, e.g., by fish movement, the anchors gaff the epidermis and prevent the haptor from being torn free. When shearing forces are absent, the anchors do not have this effect and the haptor is held close to the fish by the marginal hooklets alone. Healthy sticklebacks carrying 100 or more flukes shed a layer of mucoid material about 1  $\mu$  thick every 1 to 2 days. When this is shed, flukes are removed with it, apparently because the hooklets are attached to this slough instead of the surface of the epidermis.

The monogenetic trematode genus *Gyrodactylus*, found on many marine and freshwater fish, has a mode of attachment that differs from those previously described (Llewellyn, 1956a, 1957, 1958, 1960; Kearns, 1964, 1965, 1966, 1967; Palling, 1966). Terminology used here for sclerites of *Gyrodactylus* is that proposed by Mizelle and Kritsky (1967).

Several investigations have suggested mechanisms by which the fish rejects or inhibits monogenean parasites. Wunder (1929), Paperna (1963), and Putz and Hoffman (1964) described proliferation of gill epidermis during *Dactylogyrus* infestation and this resulted in the loss of the parasites. Jahn and Kuhn (1932) suggested humoral immunity to *Epibdella melleni* after observations on aquarium-held fish. Nigrelli (1935) found that *E. melleni* survived longer in mucus from susceptible fish than in mucus from "immune" fish. O'Rourke (1961) demonstrated that some blood antigens may be found in skin mucus.

This study was initiated when it was observed that *Gyrodactylus* on sticklebacks began to decrease in numbers after about 2 weeks, coincident with the fish sloughing off layers of "mucus" to which the worms were attached.

### MATERIALS AND METHODS

*Gasterosteus aculeatus leiurus*, infected with *Gyrodactylus alexanderi* (Mizelle and Kritsky, 1967), were obtained from a pond in Queen Elizabeth Park, Vancouver, B. C. Uninfected fish were obtained either by treating infected fish with a 1:4,000 formalin solution for 1 hr (Davis, 1961),

or by raising fish from artificially fertilized eggs (Hagen, 1967). Stocks of fish and flukes were maintained in aquaria under a 12:12 photoperiod at 15 C. Experimental fish were isolated in 250 ml dechlorinated water under the same conditions of light and temperature. The fish were fed every day and the water in the bowls was changed once a week. Living flukes were observed in wet mounts using an inverted microscope.

Infected and uninfected fish were killed by pithing, fixed in Bouin's fluid, sectioned in paraffin wax, and stained with hematoxylin and eosin. Slough material was collected from live fish within a few hours of its production and examined under a phase contrast microscope or dried on slides, fixed in picric-formalin, and stained using Hale's Colloidal Iron, DNFB, Chèvremont-Frédéric (Chayen et al., 1969), PAS (Pearse, 1968), or a combination of Colloidal Iron/PAS (Mowry, 1963).

Material was prepared for electron microscopy by fixation in 6% glutaraldehyde (Sabatini et al., 1963) in phosphate buffer M/15, pH 6.98, with 4% sucrose added. Postfixation in 1% osmium in phosphate buffer followed and material was rapidly dehydrated in a graded series of ethanols and finally propylene oxide. Infiltration with Epon 812 was carried out over 8 hr and material was then embedded in flat aluminum dishes. Thin sections were cut on an LKB Ultramicrotome and stained with uranyl acetate (saturated solution in 70% EtOH) and lead citrate (Reynolds, 1963). They were viewed using a Hitachi HU-11 A electron microscope.

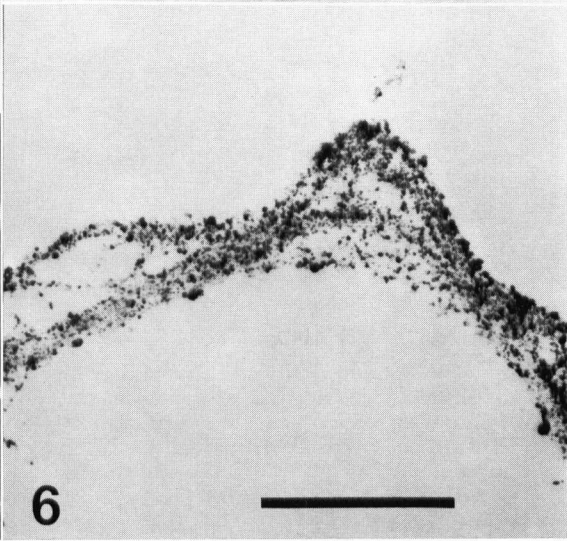
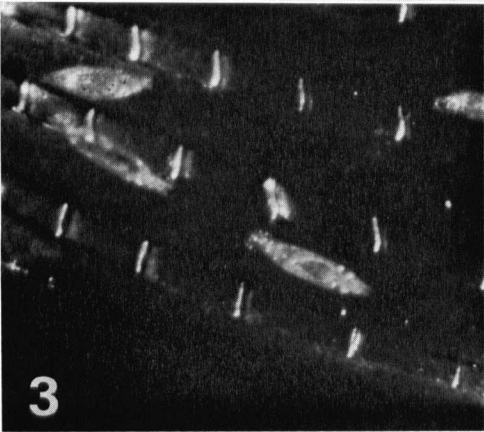
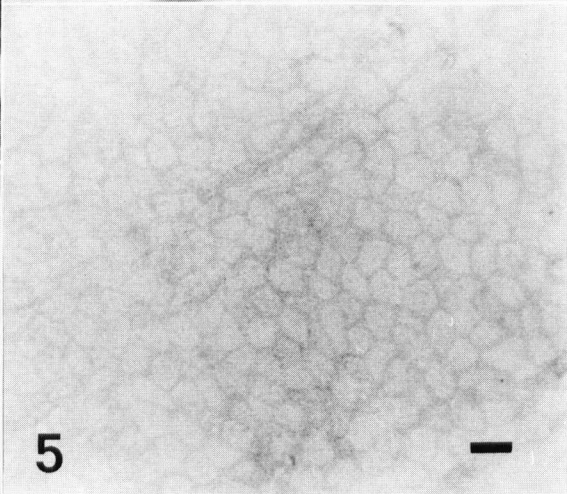
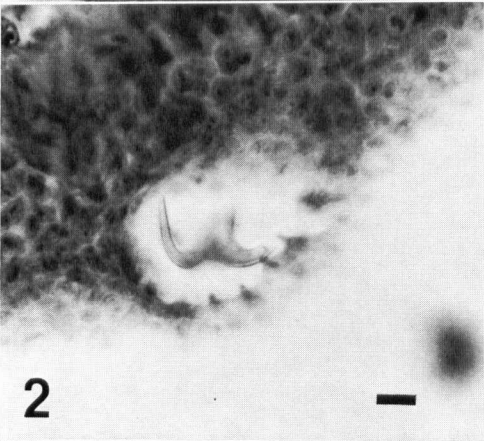
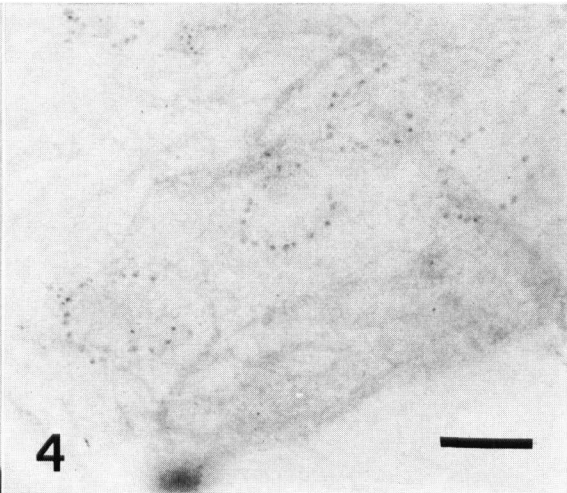
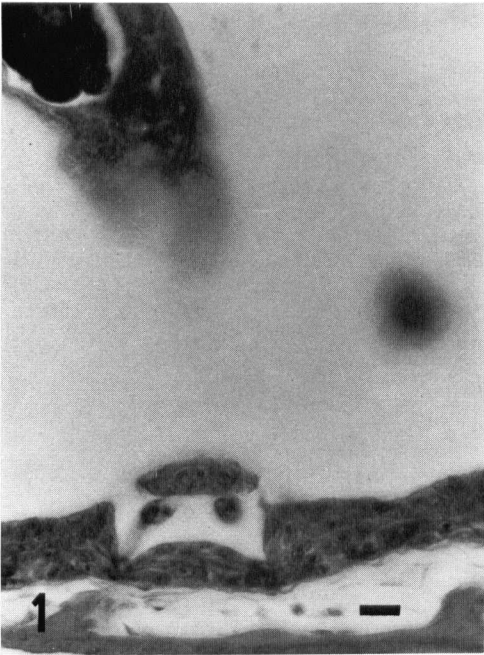
Fresh slough was also shaken at 37 C for 5 hr in a phosphate buffer (pH 7) containing 0.5% trypsin and 0.2% EDTA (ethylenediaminetetraacetic acid).

### RESULTS

In sections of fish epidermis, the points of the anchors depressed but did not penetrate the epithelial layer (Fig. 1). Tension on the marginal hooklets, attached to the surface

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membrane of the epidermis; distorted the shape of some of the epidermal cells, pulling them into fine points at the point of attachment (Fig. 2). The cumulative effect of 16 hooklets under tension appeared to provide the force necessary to push the anchors into the epidermis (Fig. 7A).

Observations on living flukes on active fish showed that the body of the fluke lay downstream of its haptor (Fig. 3). In this position the anchor points were directed toward the tail.

On glass, the flukes were unable to attach their haptors unless the head organs had previously produced a sticky secretion to which the hooklets could adhere. The head organs attached first, using their secretion, then the haptor was placed on the glass so that it partly overlapped the head organs. The first and second most anterior pairs of hooklets clung to the secretion, the head organs freed themselves, and the animal stood erect. As the fluke swayed, it pivoted on the anterior hooklets of the haptor.

The skin of healthy fish, experimentally infected with 100 or more flukes, sloughed off a layer of mucoid material 0.5 to 1  $\mu$  thick every 1 or 2 days, often with flukes attached to it. The flukes did not appear to be harmed in any way. After an hour they readily attached to another, or the same fish, and subsequently reproduced as normal.

Staining of the slough gave a negative reaction for sulphhydryl groups (Chèvrement-Frédéric), a weakly positive one for proteins (DNFB), strongly positive for acid mucopolysaccharides (Colloidal Iron) and for general mucosubstances (PAS). Colloidal Iron also stained knobs on the surface of the slough. These knobs were in groups of 16 and apparently marked the site of fluke attachment (Fig. 4). Slough stained with PAS, or the PAS/Colloidal Iron combination, showed a reticular network with spaces 5 to 6  $\mu$  across

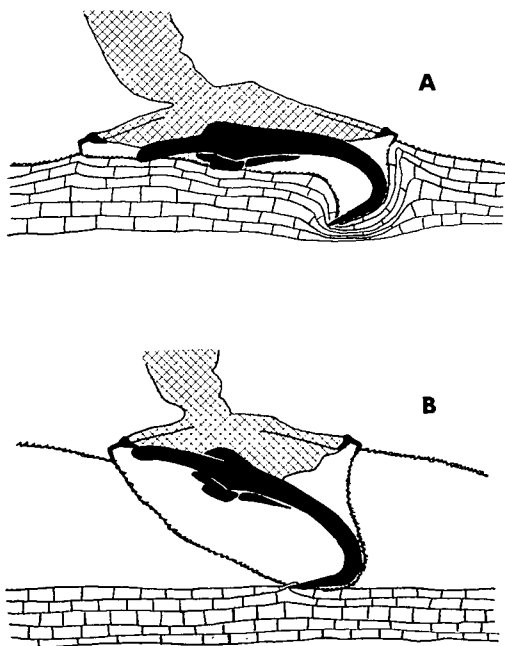


FIGURE 7. Diagrams showing position of haptor in relation to epidermis. A. Normal situation. B. During shedding of slough.

(Fig. 5), the same dimensions as the superficial cells of the epidermis. Both the "footprints" and the reticular pattern were clearly seen using phase contrast microscopy. Cells and bacteria were also seen at intervals adhering to the slough. To determine whether or not the slough was a thin cellular layer, sections were examined under the electron microscope (Fig. 6). No membranes or other cellular components were recognized. Slough was incubated with trypsin and EDTA for 5 hr and though this removed all bacteria and cellular debris, the slough and its reticular network remained intact. It was concluded that the layer was a secretion.

Laboratory-raised fish, unexposed to *Gyrodactylus*, produced slough. It was also regularly produced by infected field-collected fish that

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FIGURES 1-6. Attachment of *Gyrodactylus alexanderi* to surface of *Gasterosteus aculeatus*. 1. Section across haptor attached to fish epidermis. Bar denotes 10  $\mu$ . 2. Tangential section of fish showing epidermis and site of haptor attachment. Scale as in Fig. 1. 3. Live *Gyrodactylus* on tail. Anterior of fish to the left. 4. Marks on slough indicating site of previous haptor attachment. Bar denotes 100  $\mu$ . 5. Slough stained to show reticular network. Scale as in Fig. 1. 6. Electron micrograph of section of slough from an infected fish. Bar denotes 1  $\mu$ .



had had their flukes removed. This slough was more difficult to see under a dissecting microscope than that produced by an infected fish. It tended to break up more easily and was rarely found in large sheets. The reticular pattern could still be seen with phase contrast microscopy, and under the electron microscope, sections had a similar appearance to those from infected fish (Fig. 6) except that there was less electron-dense material.

Sixteen field-collected fish that had had their flukes removed for 4 weeks were each experimentally infected with 20 flukes. The average numbers of flukes present at the end of successive weeks were 45, 68, 45, and 9. Five laboratory-raised fish, previously unexposed to *Gyrodactylus*, were also each infected with 20 flukes. They lost flukes at least as rapidly as those fish that had been infected 4 weeks prior to the experiment. Their average numbers were 43, 33, 6, and 0. Fish that were reinfected within a few days of their recovery lost all flukes within a week, whereas fish kept for 4 weeks before reinfection repeated the increase/decrease cycle. It appeared that fish were protected for a few days after their recovery but that the protection wore off within a month.

### DISCUSSION

The haptoral attachment of some monogeneans involves suction (Kearn, 1964), or an adhesive haptoral secretion (Kearn, 1965). From its performance on glass, *Gyrodactylus* does not use either of these methods.

On fish, the anchors of *Gyrodactylus* are pushed into the epidermis by tension on the hooklets which are attached to the delicate surface membrane of the fish. The typical posture of this genus (Braun, 1966, and Fig. 3), and of other monogeneans (Llewellyn, 1956b), is for the body of the fluke to lie downstream of its haptor. In this position, the anchors hold the haptor against the fish when there is a strong shearing force on the fluke, even though the hooklets may be torn free. In Figure 7A, if the fish moves suddenly to the right the anchors will passively gaff the epidermis. The anchors are prevented from tearing out of the fluke by ligaments that run from the anchor roots, fuse at the junction of haptor and body, then spread out and connect with

elastic elements in the parenchyma (Braun, 1966).

Though the hooklets left knobs on the slough marking their site of attachment, anchors left no trace. This supports the idea that the anchors are essentially passive structures. In *G. wagneri*, the tips of the anchors can be moved toward each other or farther apart, but no other movements are possible independent of the haptor (Braun, 1966).

The force required for the anchor to actually penetrate the host tissues apparently comes from external forces on the parasite, such as those due to the water current or to overcome inertia. These forces appear to be insufficient to deeply embed the anchors, probably because of the small (0.5 to 1.0 mm) and very elastic body of the fluke. The anchors can be withdrawn to allow the fluke to move about on the surface of the fish. In some larger monogeneans, there are hooks that are embedded in the same way, but these hooks are more or less permanently fixed in the host tissue and the flukes rarely change their position (Llewellyn, 1956a, 1957). In other large monogeneans, the force that embeds the hooks comes from the fluke itself, as in *Tetraonchus monenteron* which has mobile hooks that actively gaff host tissue. These flukes are able, like *Gyrodactylus*, to disengage their hooks and move about on the surface of the host (Kearn, 1966).

The marginal hooklets of most monogeneans play an important part in attachment only in the larval stage. In the adult, this function is largely taken over by anchors or "hamuli" which develop as the larva matures (Llewellyn, 1963). Though the hooklets may persist in some species, their role is relatively minor (Llewellyn, 1960; Kearn, 1964, 1966). In *Gyrodactylus*, the hooklets perform a major function in the attachment of the adult. On a motionless fish they appear to be the only mechanism of attachment. The anatomy of the hooklets and their associated structures has been described by Braun (1966), and he proposed a mechanism to account for the typical clawing motions of the hooklets.

On *Gasterosteus*, the hooklets of *Gyrodactylus* apparently become attached to the developing slough. When the slough is shed, the



hooklets lose their purchase on the fish, the anchors rise to the surface of the epidermis (Fig. 7B), and a quick movement by the fish leaves the slough and flukes behind in the water. The slough appears to be a mucoid secretion rather than a cellular layer. It remained intact when incubated for 5 hr in a medium 10 times stronger than that designed to separate HeLa cells in 15 min (White, 1963). It differs from typical fish mucus in that it is insoluble in water and is shed simultaneously from much of the surface of the fish. The number of mucous cells is not increased in recovering fish, and it is possible that the slough comes from the superficial epidermal cells, each cell producing a thin layer over its surface. Histochemically the absence of sulphhydryl groups in the slough indicates an absence of keratin. This agrees with the findings of Burgess (1956).

Though the slough is not produced as a reaction to *Gyrodactylus*, its increase in density is associated with the flukes. The stimulus may come from the hooklets and anchors which distort the epidermal cells. Alternatively, flukes may elicit the response during feeding. When this occurs, the pharynx is firmly attached to the surface of the epidermis for 20 to 30 sec and presumably epidermal cells or parts of them are ingested. Kearn (1963) showed that *Entobdella soleae* fed on the epidermal cells of sole, and found evidence for the secretion of proteolytic enzymes. In the sections of infected fish, no consistent signs of feeding sites were found. This is to be expected as the flukes fed less than once per hour (at 15 C), and a 20-cell-wide band of epidermis, experimentally removed down to the dermis, was regenerated within half an hour. Under phase contrast, slough showed patches of debris near "footprints" and these may indicate feeding sites.

A humoral immune response is not thought to be involved for three reasons. First, fish infected 4 weeks prior to the experiment lost flukes at a similar rate to previously unexposed fish. Second, the response is very rapid. Fluke numbers were controlled within 2 weeks whereas Krantz et al. (1963) found that an immune response in brown trout took 3 months to reach its maximum titer at 11 C. Third,

intramuscular injections of whole fluke antigen conferred no protection. It is more likely, therefore, that a local tissue response is involved.

#### ACKNOWLEDGMENTS

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      X(IM1,J) = 22.
      SUMON(IM1) = 0.1
      GO TO 4
3     IF (M.GE.5) GO TO 103
      SL(I,J) = 0.3
      GO TO 4
103   SL(I,J) = 0.15 + 0.1 * (1. / (1. + EXP(-0.1 * (X(IM5,J) - 50.))))
      1 + (SL(IM1,J) - .15) * .95
      IF (SL(I,J).GT..7) SL(I,J) = .7
4     X(I,J) = (X(IM1,J) - EXIT(IM1,J)) * 1.2 + SUMON(IM1)
      IF (X(I,J).GT.0) GO TO 41
      EXIT(I,J) = 0.
      GO TO 42
41    EX = X(I,J) * SL(I,J)
      IF (EX .GE. 5.5) GO TO 22
      EX5 = EX + .5
      K = EX5
      R = RANDV(0)
      DO 33 L = 1,15
      IF (R.LE.POIS(L,K)) GO TO 66
33    CONTINUE
66    P = L-1
      A = P-EX
      GO TO 80
22    R = RANDN(0)
      A = R * SQRT(EX)
80    EXIT(I,J) = EX + A
      IF (EXIT(I,J).GT.X(I,J)) EXIT(I,J) = X(I,J)
42    ON(I,J) = X(I,J) * SL(I,J)/3.
      SURV(I,J) = (EXIT(I,J)-ON(I,J)) * 0.86
      IF (M-2) 5,6,7
5     ON2(I,J) = 0.
      ON3(I,J) = 0.
      GO TO 8
6     ON2(I,J) = SURV(IM1,J)/3.
      SURV2(I,J) = (SURV(IM1,J)-ON2(I,J)) * 0.47
      ON3(I,J) = 0.
      GO TO 8
7     ON2(I,J) = SURV(IM1,J)/3.
      SURV2(I,J) = (SURV(IM1,J)-ON2(I,J)) * 0.47
      ON3(I,J) = SURV2(IM1,J)/3.
      GO TO 8
8     GO TO (9,10,11,15),J
9     IF (II.LT.7) GO TO 12
      J = 2
      M = II- 6
      GO TO 2
10    IF (II.LT.14) GO TO 13
      J = 3
      M = II - 13
      GO TO 2
11    IF (II. LT. 21) GO TO 14
      J = 4
      M = II- 20
      GO TO 2

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12  SUMON(I)=ON(I,1)+ON2(I,1)+ON3(I,1)
    GO TO 16
13  SUMON(I)=(ON(I,1)+ON(I,2)+ON2(I,1)+ON2(I,2)+ON3(I,1)+ON3(I,2))
    1/2.
    GO TO 16
14  SUMON(I)=(ON(I,1)+ON(I,2)+ON(I,3)+ON2(I,1)+ON2(I,2)+ON2(I,3)+
    1 ON3(I,1)+ON3(I,2)+ON3(I,3))/3.
    GO TO 16
15  SUMON(I)=(ON(I,1)+ON(I,2)+ON(I,3)+ON(I,4)+ON2(I,1)+ON2(I,2)
    1+ON2(I,3)+ON2(I,4)+ON3(I,1)+ON3(I,2)+ON3(I,3)+ON3(I,4))/4.
    GO TO 16
16  IF (J.EQ.4) GO TO 17
    GO TO 24
17  Q = II
    IF (X(I,J).GT.60) GO TO 24
    CALL FPLT (0, Q, X(I,J))
    IF (X(I,1).LE.0.1 .AND. X(IM1,1).LE.0.1) GO TO 31
24  CONTINUE
31  WRITE (3,32) II,J,X(I,J),EXIT(I,J),SUMON(I)
32  FORMAT (1X, 2I15, 3F12.2)
    IF (N.GE.5) GO TO 20
    CALL FPLT (1, 0., -60.)
    N = N+1
    IF (N-5) 18,19,20
18  GO TO 100
19  CALL GGS (0.,720.,0.,30.,90.,30.,12.,1.,0.15)
    GO TO 101
20  CALL FCHAR (379.,-17., .2, .3, 0.)
    WRITE (7,21)
21  FORMAT ('DAYS')
    CALL FCHAR (-30.,100., .2, .3, 1.57)
    WRITE (7,23)
23  FORMAT ('NO. GYRODACTYLUS')
    CALL EXIT
    END

```

## FEATURES SUPPORTED

ONE WORD INTEGERS  
STANDARD PRECISION  
LOAD & GO  
IOCS-  
PLOTTER  
1132 PRINTER  
CARD

## CORE REQUIREMENTS FOR -

COMMON- 0, VARIABLES AND TEMPORARIES- 854, CONSTANTS AND PROGRAM- 1428

## COMPILATION

// XEQ

\*\* COMMENCE PROGRAM EXECUTION \*\*

JOB TERMINATED BY OPERATOR