CULTURE of SOME COMMON FISH PARASITES for EXPERIMENTAL STUDIES

Heino Beckert

Zoology- Entomology Department Series
Fisheries No. 5

AGRICULTURAL EXPERIMENT STATION
AUBURN UNIVERSITY

E. V. Smith, Director May 1967 Auburn, Alabama

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Heino Beckert
Instructor

I. Introduction

Fish parasite cultures in the laboratory constitute an important aspect of research on control of these organisms. Basic information about the organism is a prerequisite to effective parasite control. Necessary information includes host range, life cycle, and special environmental conditions either advantageous or detrimental to the parasite, such as the organism's response to temperature, oxygen content, pH, and salinity of the water. To obtain information of this type the parasite must be available in sufficient numbers to permit study and experimentation.

A number of parasitic organisms have been cultured in the laboratory. Characteristically, internal parasites were cultured in vitro, usually on tissue cultures. Uzmann and Hayduk (1963) successfully cultured Hexamita salmonis, an intestinal flagellate of young trout and salmon. Williams, Hopkins, and Wyllie (1961) cultured the strigeid trematode Diplostomum phoxini in vitro. Jensen, Stirewalt, and Walters (1965) cultured cercariae of Schistosoma

*Supported by the Southeastern Cooperative Fish Parasite and Disease Project.

mansoni in vitro for as long as 112 days. Meerowitch (1965) raised Trichinella spiralis from larvae to immature adults in vitro. Hoffman (1958) reared metacercariae of Posthodiplostomum minimum to infertile adults. Hoffman and Putz (1964) maintained a population of Gyrodactylus macrochiri on 6 month old bluegills in running water in the laboratory. Hlond (1966) reported an in vitro culture of Ichthyophthirius multifiliis on a carp slime medium. However, personal communication with Mgr. Hlond, Instytut Zootechniki, Zator k. Oswiecimia, Poland, revealed that only the tomite state of I. multifiliis was reared and the parasite did not complete its life cycle in vitro. Tidd (1963) cultured Lernaea cyprinacea in the laboratory and infected goldfish as well as tadpoles and young adults of Rana pipiens with this parasite. Lahav and Sarig (1964) cultured L. cyprinacea in the laboratory, describing in detail the life cycle of this organism. Dr. Hoffman, Eastern Fish Disease Laboratory, Leetown, W. Va. describes in a personal communication a culture method for both Ichthyophthirius and L. cyprinacea. These parasites were maintained by dividing an aquarium with a screen and placing infected fish in one end and uninfected, non-immune fish in the other end. When the initially infected fish died they were replaced by more nonimmune hosts.

Most fish obtained from a natural habitat show some degree of parasitism. However, the fact that the incidence of parasitism is usually low in wild fish unless an epizootic is occurring, and the fact that wild fish are usually parasitized by more than one species of parasites make it difficult to obtain specific information about a single type of parasite. Therefore,it is necessary to obtain pure

cultures of specific parasites under laboratory conditions to eliminate variable environmental conditions that occur in a natural situation.

The following conditions should be met for culturing a fish parasite in the laboratory:

(1) An adequate supply of host fish is important. It is most desirable to use fish from the same population, that is individuals from the same parent stock, or at least fish spawned at the same time and in the same pond.

(2) Prospective host fish must be removed to an isolated environment as soon as possible to prevent natural infections by parasites and disease organisms. It is best to store young host fish in small ponds to facilitate their removal by seining. These storage ponds must be large enough to permit carrying out an adequate feeding program. Ponds with a surface area of 0.05 acre and an average depth of 2.5 feet have been used with good results at Auburn. Where facilities are available, host fish can be stored in troughs with running water. If an adequate flow of water can be maintained, a relatively large number of fish can be kept and fed in such a facility. The chief advantage of using troughs is that the fish can be observed and treated against parasites and diseases prior to experimental infection. This is not possible in ponds because close and regular observation of the fish population is not feasible. Where possible fish can be spawned in the laboratory and the fry reared to fingerling size in steel troughs with running water. This ensures parasite-free experimental fish that are not immune to parasites.

(3) It is essential that host fish used for maintaining a parasite culture be healthy and if at all possible free of disease and parasites. Periodic examinations of the fish should be made.

(4) The parasite culture is best maintained in a volume of water of manageable size. Forty-liter aquaria and 100-liter feeding troughs have been successfully used at Auburn.

(5) Since the life cycles of many parasites are influenced by temperature, it is desirable to maintain the parasite culture in an environment of relatively constant temperature.

(6) Dechlorinated water with a pH value of 7 or 8 is best suited for maintaining healthy host fish.

II. Laboratory Culture of *Ichthyophthirius multifiliis*

The organism. *Ichthyophthirius* is a one-celled, ciliated parasite of fishes that affects skin and gills, causing a condition known as white spot disease, or ichthyophthiriasis (Fig. 1 and 2). This organism is one of the most cosmopolitan and destructive parasites of fresh water fishes. Observations on the life cycle of *I. multifiliis* agreed with those given by Kudo (1960).

At maturity the parasite, lodged just under the host's epithelium, leaves the host and swims free in the water (Fig. 3). A short time after leaving the host, the parasite attaches itself to aquatic vegetation, stones, or other suitable substrate and encysts. The cytoplasm divides into as many as 1,000 small round daughter cells (Fig. 4 to 7). The daughter organisms or tomites (Fig. 8 and 9) break through the cyst wall, become elongate and swim actively about in search of a host. Once in contact with a host, the tomites bore into the skin where they parasitize the fish. Depending on the temperature the parasite reaches maturity in a

period of time ranging from a few days to several weeks.

Host fish. A number of host species for culturing Ichthyophthyrius have been used at Auburn. Carp, goldfish, bluegills, tilapia, fathead minnows, golden shiners, channel catfish and white catfish were used. Scaly fish were found to be unsatisfactory because in the course of a heavy infection with the parasite these fish secrete large amounts of mucus and lose scales; this interferes with the recovery of the mature parasites.

Exposure of host fish to the parasite. Ten channel catfish or white catfish fingerlings each were stocked into 40-liter (10 gallons) aquaria filled with dechlorinated tap water. Then, a single heavily infected catfish fingerling was killed and placed into each aquarium. The water temperature was kept between 70 and 80°F. Four or 5 days later, small white spots could be seen on the live fish. The resulting infection was usually heavy enough to maintain the culture and to allow experimentation with the parasite. It was not possible, however, to produce mild, uniform infections consistently. Only when host fish were exposed to large numbers of the parasite did uniform but heavy infections result. A minimum of 200 trophozoites per 10 fish in 40 liters of water should be used.

Recovery of mature parasites. The parasites should not be collected until they have almost reached maturity, that is until they are about to leave their host to encyst. When premature parasites are obtained by scraping them from the fish, the resulting number of daughter organisms is very small, yielding only a mild infection upon subsequent exposure to other fish. On the other hand, the parasites should be collected before any of them leave the fish since

Fig. 1. Channel catfish fingerling infected with I. multifiliis. The white spots covering the fish are trophozoites lodged just under the host's epithelium.

Fig. 2. Gill filaments of a channel catfish fingerling parasitized by I. multifiliis.

Fig. 3. Mature trophozoite of I. multifiliis showing the characteristic horse-shoe shaped macronucleus.

Fig. 4. Trophozoite of I. multifiliis after the first division. The two daughter cells are encased by a clear cyst wall.

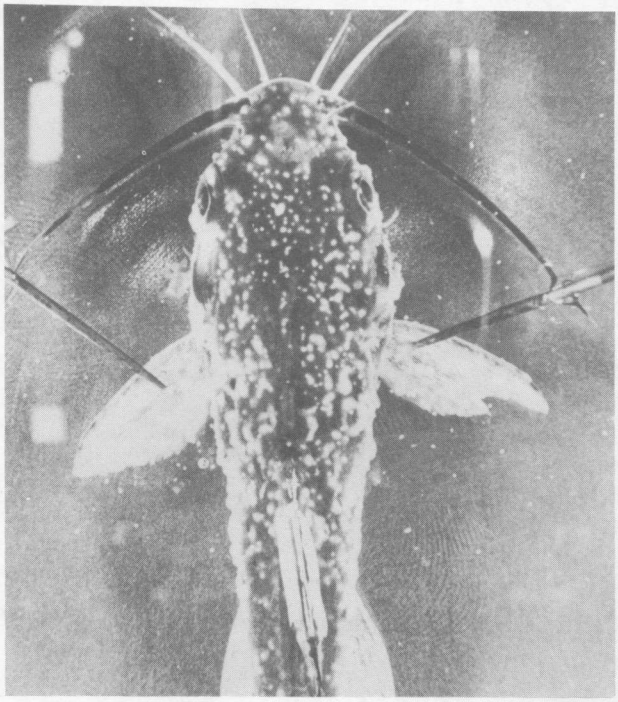

Fig. 1

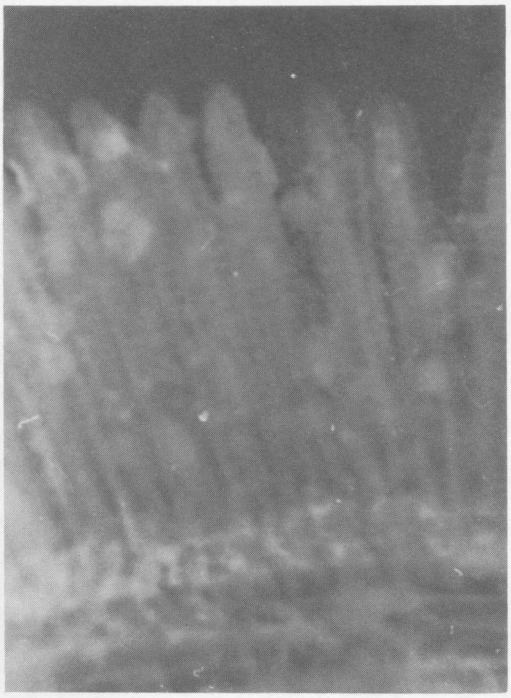

Fig. 2

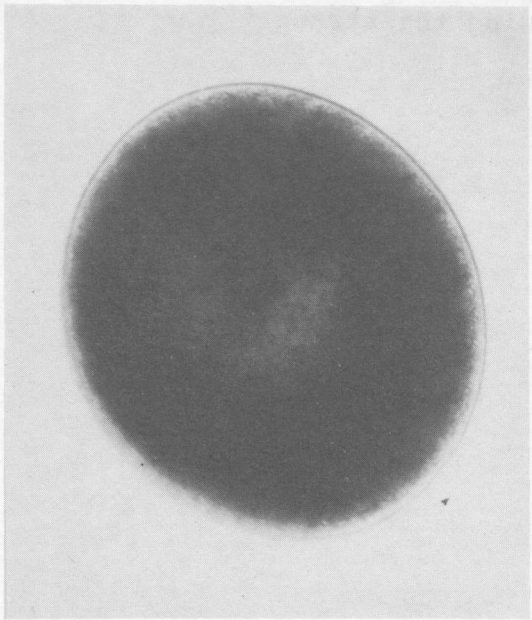

Fig. 3

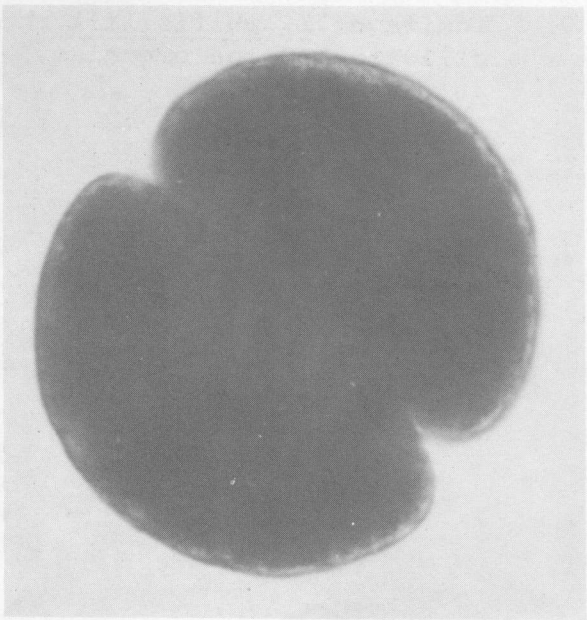

Fig. 4

Fig. 5. Trophozoite of I. multifiliis after the second division.

Fig. 6. Cyst of I. multifiliis.

Fig. 7. Cyst of I. multifiliis showing the cyst wall and containing almost mature tomites.

Fig. 8. Tomite of I. multifiliis just after leaving the cyst.

Fig. 9. Tomite of I. multifiliis showing the strong ciliation of the organism.

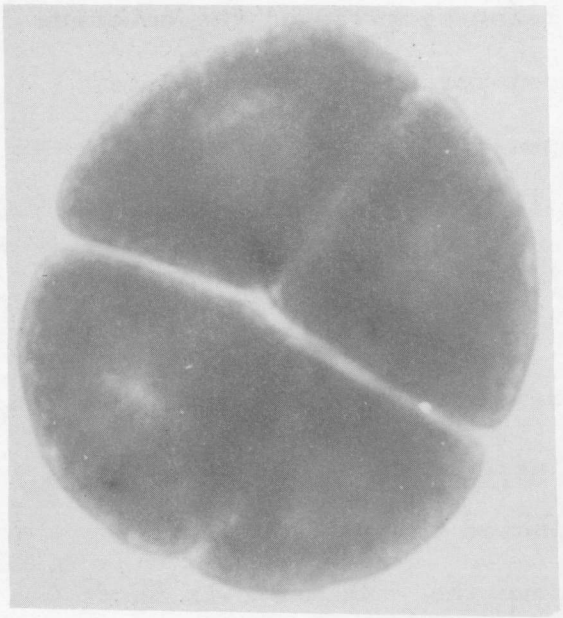

Fig. 5

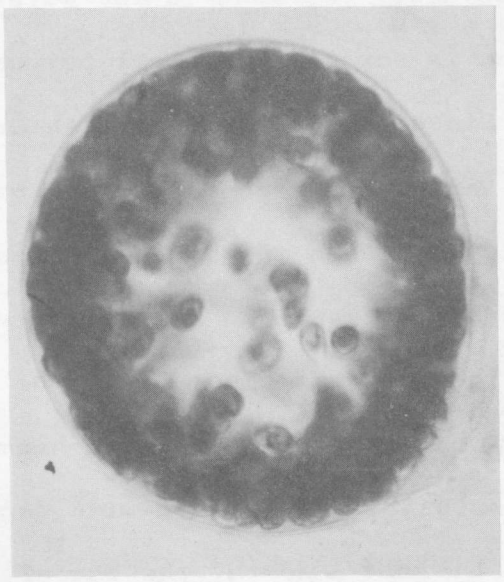

Fig. 7

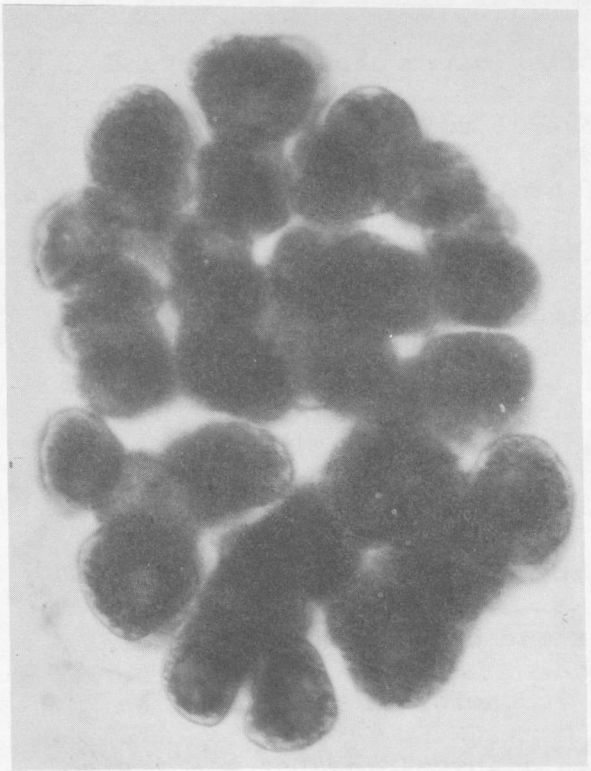

Fig. 6

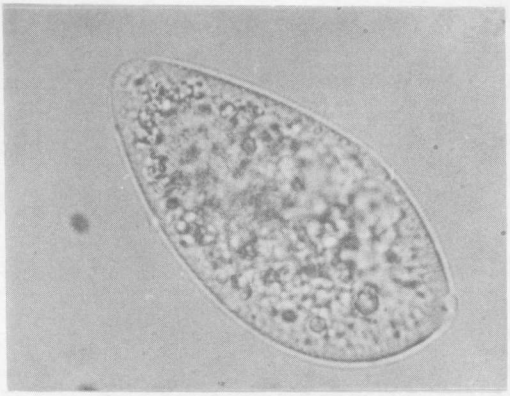

Fig. 8

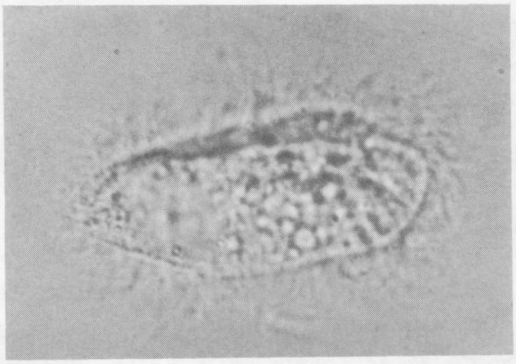

Fig. 9

any parasite swimming in the water or being encysted on the aquarium bottom is for all practical purposes lost for use in the culture.

Since all of the host fish are exposed to the parasite at the same time and since most of the parasites are in the same stage of development when introduced to the host fish, most of the parasites are equally far along in their development when collected.

The most efficient method of recovering and collecting mature Ichthyophthirius is pithing a heavily infected host fish and placing it in a petri dish filled with dechlorinated tap water. Shortly after the host's death, the parasites leave the fish and swim about in the water.

To avoid a low oxygen situation by leaving the dead fish in the container with the parasites that already have left the host, it is desirable to remove the pithed fish after about 1 hour and place it into another dish. After several consecutive changes about 1 hour apart, a large number of healthy and normal trophozoites of the parasite can be collected in the dishes.

Counting of trophozoites. In culturing Ichthyophthirius in the laboratory, it is often desirable to count a specific number of parasites for further use in infecting other fish. The counting of trophozoites is greatly facilitated by placing the dish containing the parasites on a dark background. Mature trophozoites are usually 0.5 to 1.0 mm. in diameter and of a whitish or gray color. Against a dark background the trophozoites can easily be counted with the unaided eye. However, a large-diameter magnifying glass and strong illumination are helpful and less fatiguing. The best time for counting trophozoites is immediately after the parasites

have begun to settle to the bottom of the dish. At that time the parasites adhere to the glass instead of actively swimming through the water and are easily collected.

If only a small number of trophozoites is required, individual parasites can be sucked up with a finely drawn-out pipette and placed in a staining dish. The parasites are then poured into another aquarium to infect a new host population.

Where larger numbers of trophozoites are needed, it is desirable to draw with a grease pencil eight wedge-shaped sections on the bottom of the dish containing the pithed fish. If, for instance, 300 trophozoites are needed, this number can be counted and the sections containing the desired parasites marked with a grease pencil. Since the parasites are adhering to the glass, they can be washed off into an aquarium with the aid of a wash bottle. This washing off must be done gently to prevent injury to the parasites.

Care should be taken in avoiding great temperature differences between the water in dishes containing the parasites and the water in aquaria containing the fish to be infected.

III. Laboratory Culture of *Cleidodiscus pricei*

The organism. *Cleidodiscus pricei* (Fig. 10) is a common gill parasite of channel catfish. It is a monogenetic trematode, namely this gill fluke does not have an intermediate host and exhibits no polyembryony like digenetic trematodes such as the liver fluke. After copulation the adult lays adhesive, three-lobed eggs (Fig. 11 and 12) that cling to the mucus on the gills or drop off the fish into the water.

Fig. 10. Adult of C. pricei attached to a gill filament of a channel catfish. The posterior end of the worm is armed with a set of hooks with which the parasite fastends itself to the host's gill epithelium. The anterior end of the worm has four eye-spots.

Fig. 11. Adult C. pricei with fully formed egg just before it was laid.

Fig. 12. Egg of C. pricei with its characteristic spine. When the larva is ready to emerge the operculum at one of the corners breaks open.

Fig. 13. Larva of C. pricei. The four eye-spots and the hooks can be distinguished.

Fig. 14. Larva of C. pricei showing a tuft of cilia at the posterior end and a lateral band of cilia around the midsection. The band of cilia at the anterior end is not visible.

Fig. 15. Gill filaments of a channel catfish infected with C. pricei.

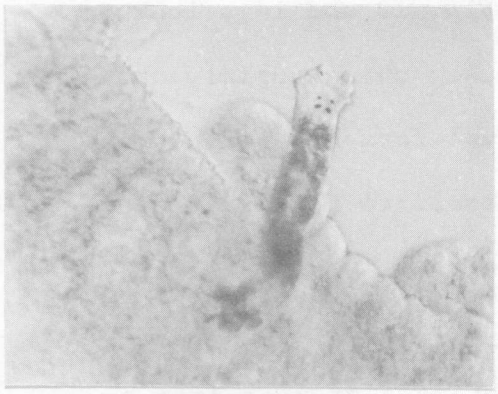

Fig. 10

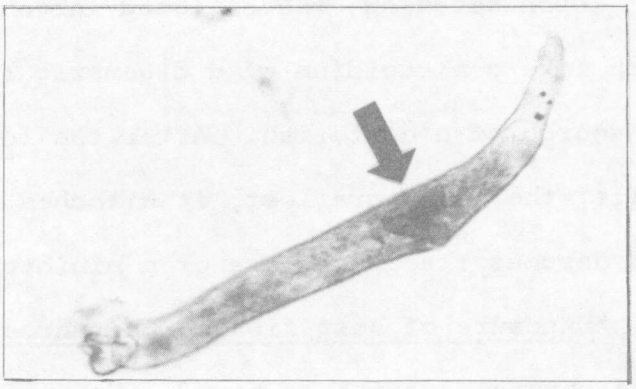

Fig. 11

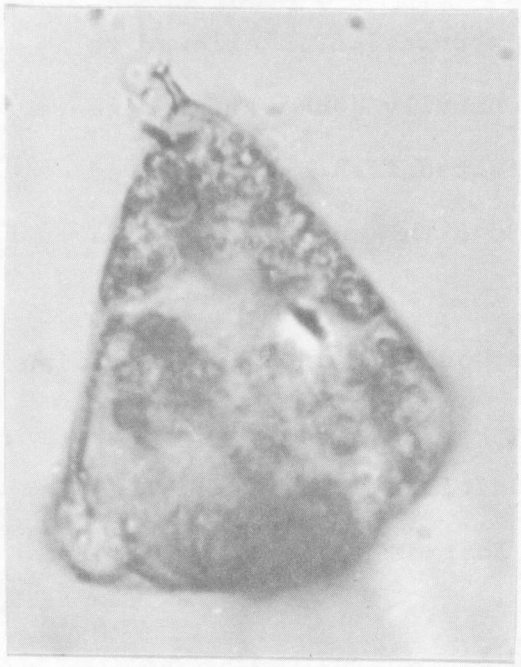

Fig. 12

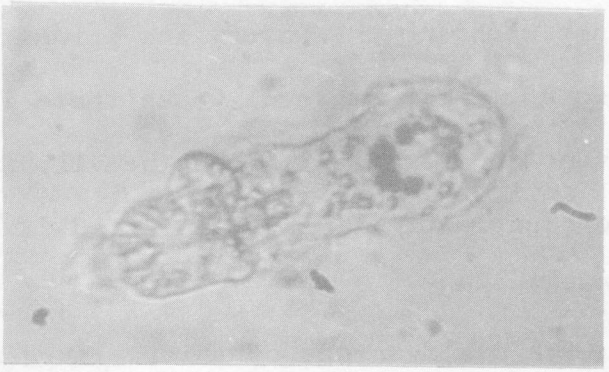

Fig. 13

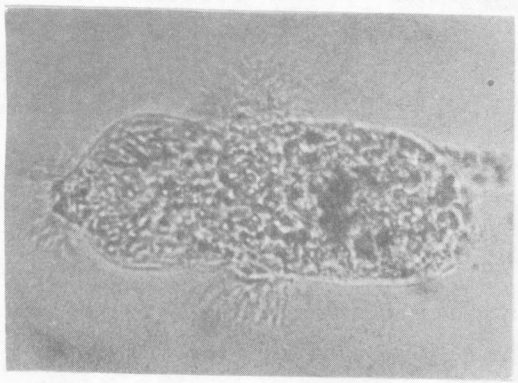

Fig. 14

Fig. 15

Upon hatching, the ciliated larva (Fig. 13 and 14), looking much like a miracidium of a digenetic fluke, swims rapidly about in search of a host fish. After the larval form has reached the gills, the cilia are lost; it attaches itself to a gill filament and assumes the appearance of a miniature adult worm (Fig. 15).

Exposure of host fish to the parasite. Rarely can a gill fluke-free channel catfish be found. Use can be made of this fact by placing 10 or 15 channel catfish fingerlings in a 40-liter aquarium. By not changing the water, the parasite population is allowed to build up. After several weeks there is usually a heavy infection present on most fish. These heavily infected fish can be placed into aquaria with lightly infected fish and the culture may thus be maintained.

The disadvantage of this culture method is that, while the fish are being held in aquaria, feeding is not possible. Consequently, all fish are in a very poor physical condition. Weaker individuals may die and the parasite culture may be lost as the host population density decreases.

When channel catfish are being held in aquaria, even without feeding, a certain amount of debris accumulates on the bottom. If the fish have a gill fluke infection, many of the eggs can be found in that debris. This material can be syphoned into a container, preferably a urine sedimentation glass, to concentrate the debris. The debris should be checked microscopically for gill fluke eggs, and introduced into aquaria containing channel catfish to maintain the culture.

At Auburn, debris containing fluke eggs was collected and

aliquots of the material introduced into several aquaria. The culture was maintained in this manner for several months.

When gills infected with adult worms are removed from a channel catfish, placed in a petri dish and covered with water, the parasites are in a stress situation. It was observed that within 30 minutes most adult worms had laid one egg each. The eggs can easily be removed from the gill tissue with a fine pipette. Most of the eggs collected in this manner yielded infective larvae. By this method a relatively large number of eggs can be obtained for experimentally infecting new host populations.

Use of feeding troughs with running water. Since the disadvantage of culturing gill flukes in aquaria is the resulting poor condition of the fish, an experimental system was used at Auburn that allowed light feeding of the fish while being exposed to the parasite.

At first, a 100-liter stainless steel feeding trough was stocked with 50 channel catfish fingerlings. A very small amount of water was passed through the trough and the fish were fed approximately 1 per cent of their body weight each day. The fish were examined for gill flukes twice weekly for 4 weeks. No significant increase in the gill fluke population was found. It was assumed that most of the parasite eggs and larvae were washed from the trough.

To determine if better results in culturing gill flukes could be obtained by impeding the flow of water through feeding troughs, the following procedure was used: One feeding trough was used as control; the water entered from the faucet and went

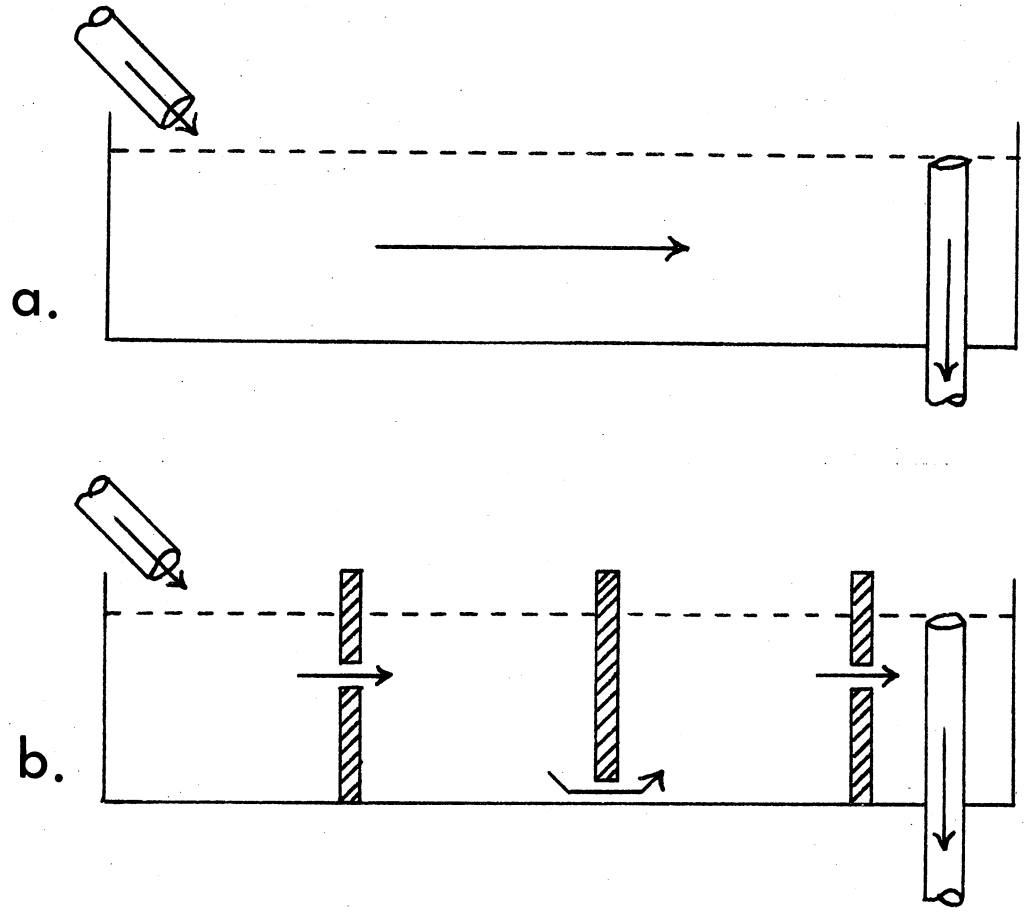

Fig. 16. a. Regular feeding trough, b. feeding trough with baffles.

out through a standpipe. In the other trough a series of Styrofoam baffles was used to impede the water flow and prevent loss of eggs and larvae by the running water (Fig. 16). Each trough was stocked with 50 channel catfish fingerlings that were fed a maintenance diet of 1 per cent of their body weight each day. The water temperature was 55°F. Prior to stocking, 10 fish from the experimental population were checked for infection with C. pricei. Periodic counts of gill flukes were made from five fish from each trough and the number of parasites found was recorded. All gill

fluke counts were made from the outer right gill (Fig. 17 and 18).

On the average, the fish harbored 6.3 gill flukes per outer gill prior to stocking. The parasite population on the fish stocked into the feeding trough without baffles decreased during the 65-day experimental period. It can be assumed that the unimpeded water flow carried away a sizeable portion of parasite eggs and larvae. On the other hand, the parasite population increased on the fish held in the feeding trough with baffles. It appears that with a decrease in water flow Cleidodiscus pricei increases in number on the host. An experimental set-up similar to the one described would be well suited for culturing C. pricei in the laboratory in large numbers.

Collecting gill flukes. The best method to collect and count gill flukes for quantitative determinations is to remove one or several gill arches, rinse them gently with a wash bottle and place them into a vial half filled with water. The vial should then be placed in a freezer to remain for 24 hours. Freezing relaxes the flukes and causes them to loosen their hold on the gill filaments. After being frozen for 24 hours, the vial content is thawed and shaken briskly, using an agitator with vortex action. This shaking removes most of the mucus clinging to the flukes and makes the parasites clearly visible (Mizelle, 1936). The contents of the vial is then poured into a small dish, preferably one with a grid bottom and placed on a dark background. Under a low power dissecting microscope, the gill flukes can readily be seen and counted.

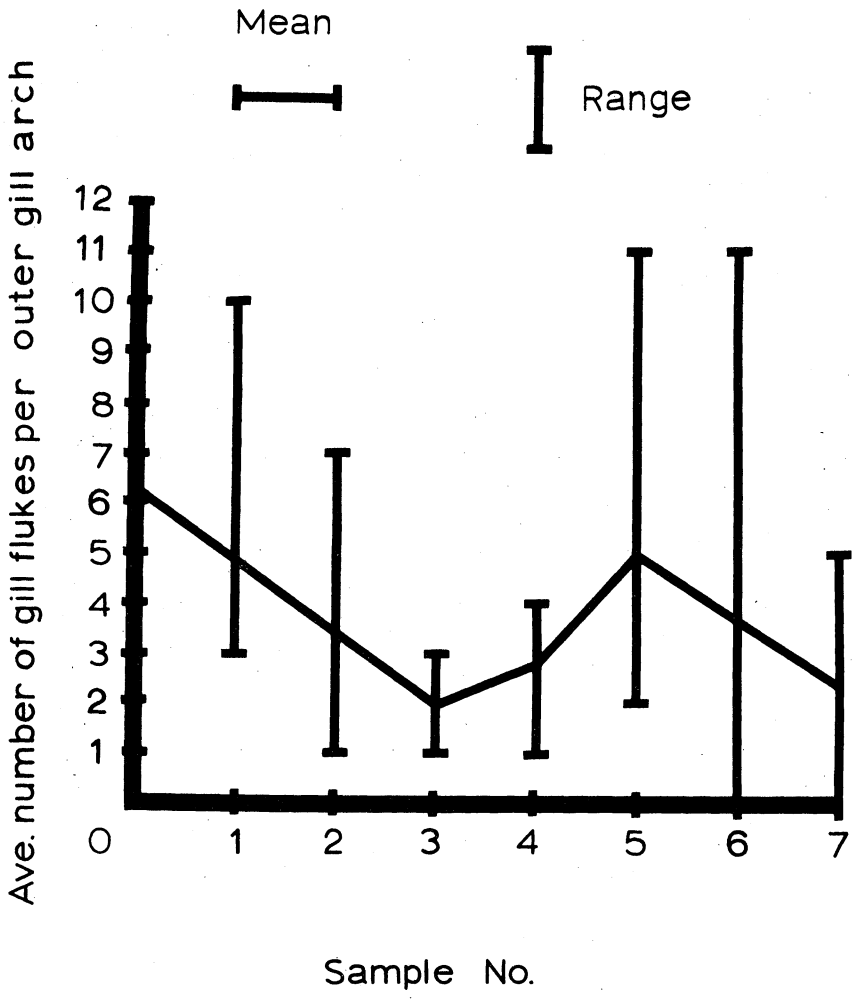

Fig. 17. Average number of Cleidodiscus pricei on the gills of channel catfish fingerlings held in a 100-liter feeding trough without baffles for 65 days. Samples were taken at approximately 9-day intervals.

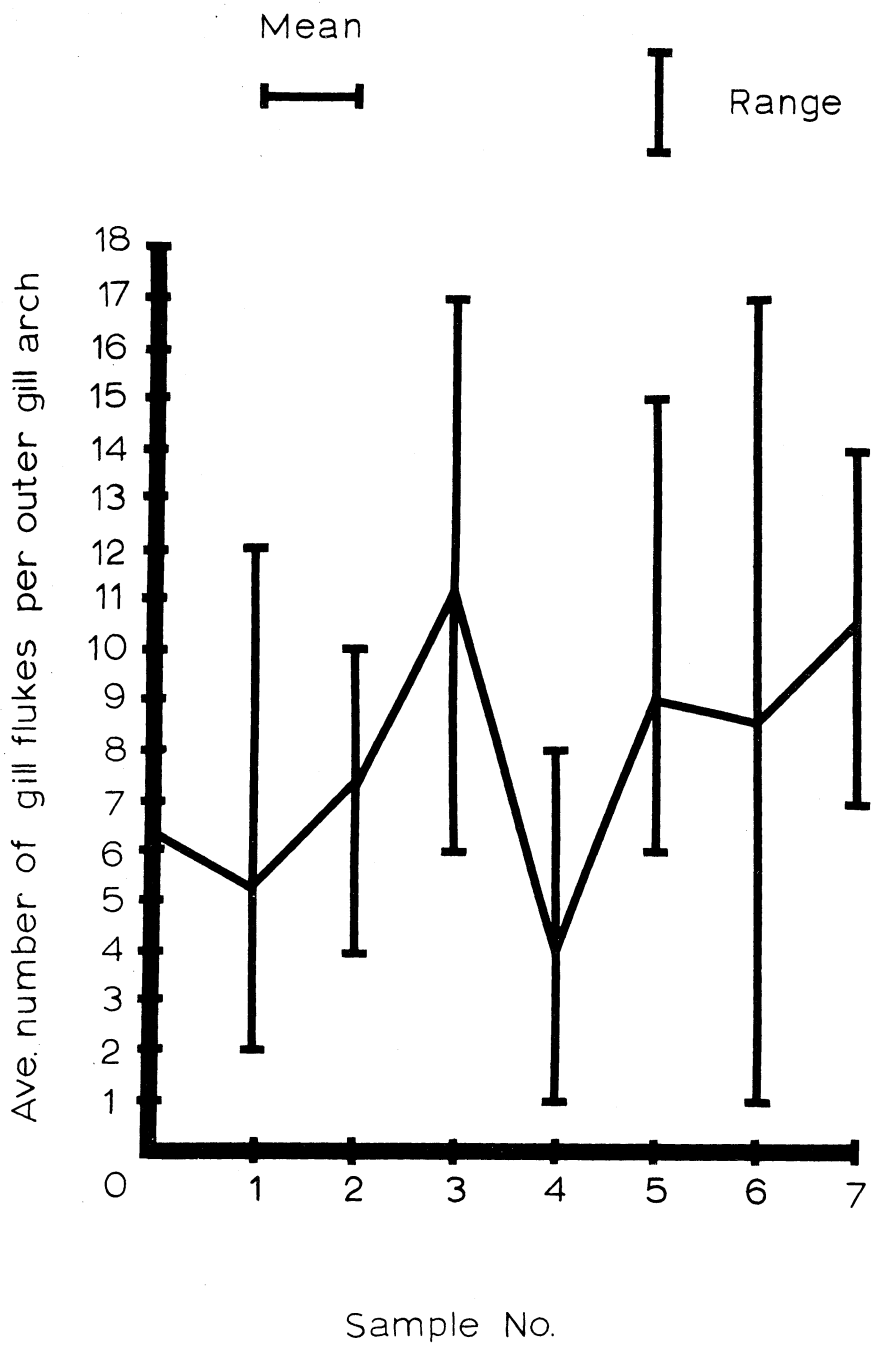

Fig. 18. Average number of Cleidodiscus pricei on the gills of channel catfish fingerlings held in a 100-liter feeding trough with baffles for 65 days. Samples were taken at approximately 9-day intervals.

IV. Laboratory Culture of Lernaea cyprinacea

The organism. Lernaea cyprinacea is a parasitic copepod infecting primarily cyprinid and catastomid hosts, but may parasitize many other freshwater fishes.

This parasite is commonly known as anchor worm because its anterior end if modified into an anchor-like structure (Fig. 19 a) that enables the parasite to lodge itself firmly under the skin of its host.

Studies of the life cycle of this parasite (First Annual Report, Cooperative Fish Parasite and Disease Project, July 1, 1964 to June 30, 1965; Agricultural Experiment Station, Auburn University) show that mature eggs develop into parasitic forms after having undergone as much as seven molts, going through the naupliar and metanaupliar stages (Fig. 19 b and c). The first parasitic form, namely the first copepodid stage (Fig. 19 d) infects fish. Five more molts occur. Mating takes place during the last copepodid stage. The male does not develop further. The female burrows into the skin where it becomes embedded. The characteristic anchors keep the parasite affixed to its host. Under good conditions one female will produce several pairs of egg sacs. A temperature of approximately 80°F is optimal for this parasite.

Host fish. Since the anchor worm parasitizes readily all cyprinids, goldfish or carp lend themselves readily for use as host fish. These species are hardy and can be kept in aquaria for a considerable period of time when fed lightly.

Collection of parasite eggs. As the adult female develops, the worm's posterior portion can be seen protruding from the host's

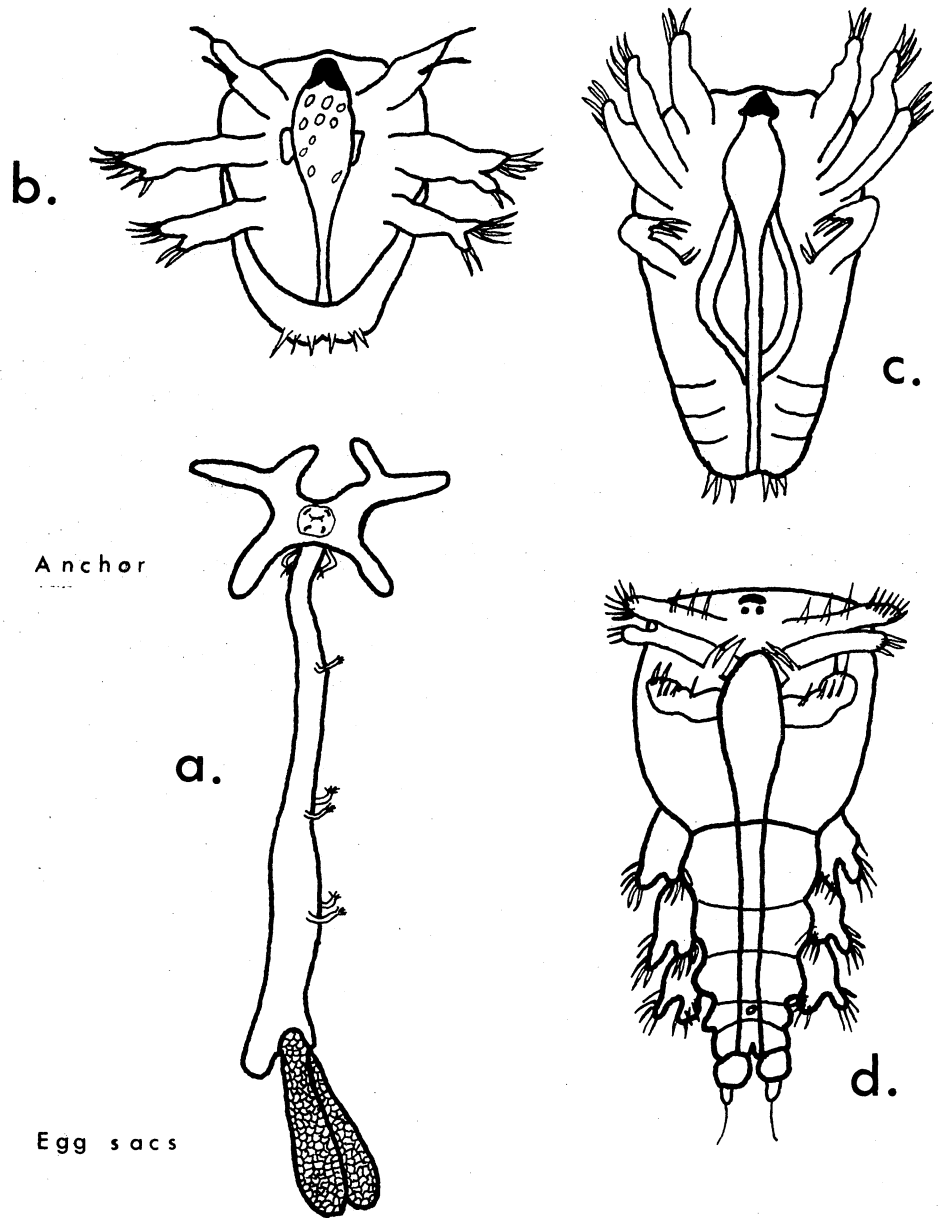

Fig. 19. Stages in the life cycle of Lernaea cyprinacea. a. adult, b. nauplius, c. metanauplis, d. copepodid stage. (After Putz and Bowen, 1964).

Fig. 20. Adult anchor worm protruding from the abdomen of a goldfish. (Photograph by Dr. H. S. Swingle)

Fig. 21. Almost mature egg sacs of an anchor worm. (Photograph by Dr. H. S. Swingle)

Fig. 22. Naupliar stage of L. cyprinacea. (Photograph by Dr. R. Allison)

Fig. 23. Shed skin of one of the naupliar stages. (Photograph by Dr. R. Allison)

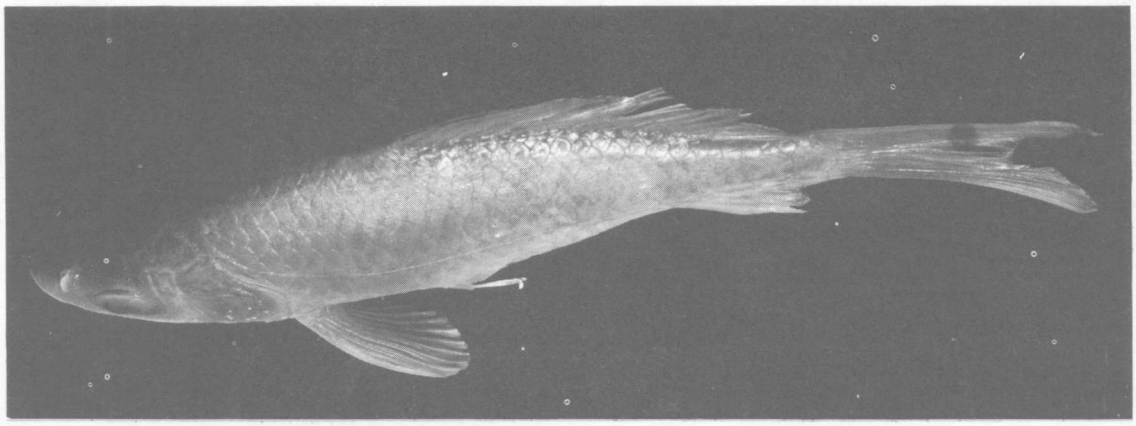

Fig. 20

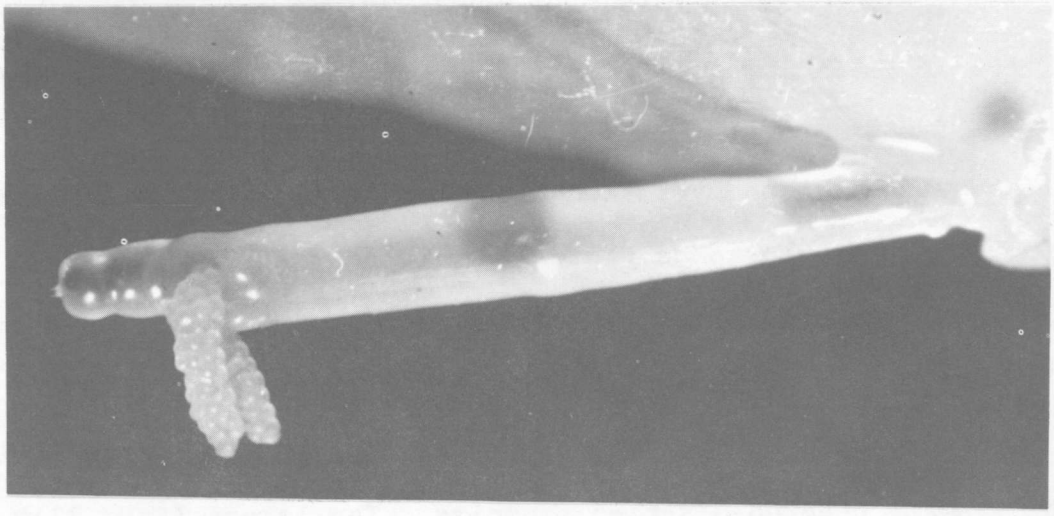

Fig. 21

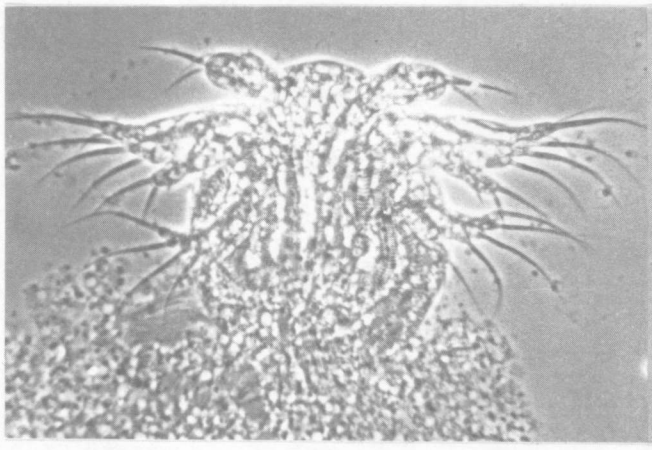

Fig. 22

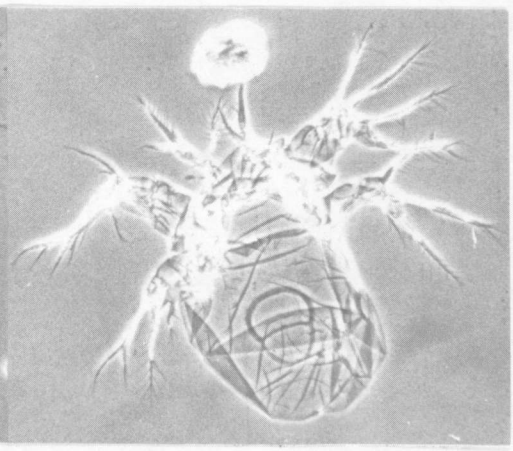

Fig. 23

skin (Fig. 20). A pair of egg sacs (Fig. 21) is formed at the posterior end. At first these egg sacs are greenish, with maturity they become whitish. At that point they should be removed from the adult. This is best done by removing the host fish from the aquarium, holding it under a strong light. Preferably the fish should be removed from the aquarium with a wire basket rather than with a net. This will reduce the chance of injury to the worms. The fish should be held firmly in a paper towel. The egg sacs can then be pinched off with a pair of sharp forceps. Care should be taken that the adult worm is not injured or accidentally dislodged in the process.

Incubation of the eggs. Once the egg sacs are recovered from the parasite they should be placed in a small dish filled with dechlorinated water. Water temperature should be 80°F. The dish should then be placed in an incubator set at 80°F.

Most eggs will hatch within 24 hours and the still free-living organisms will undergo a series of molts. The larvae (Fig. 22) should be checked frequently. Shed skins (Fig. 23) are evidence that molting is taking place. At 80°F approximately 72 hours will elapse before the first copepodid stage is reached. This stage is characterized by an elongation of the abdomen, which differentiates it from the last metanaupliar stage.

Exposure of host fish to the parasite. It is best to introduce only the first copepodid stage to the host fish since earlier, non-parasitic stages may be eaten by the fish, thus reducing the chance for infection. Water containing the infective larvae should

be poured gently into the aquaria containing previously unexposed fish. The water in the aquaria should be of the same temperature as that in which the larvae were kept.

At Auburn it was not possible to obtain uniform, light infections in the laboratory. However, fairly satisfactory results were obtained by exposing 3, 6-inch goldfish in a 40-liter aquarium to the first copepodid stages obtained by incubating one egg sac. If a too light infection results, this method of infecting fish could be varied by increasing the infection rate.

The fish in the aquaria should be fed lightly. Starving fish will feed on the protruding anchor worms of other fish.

Symptoms of anchor worm infection. Two or 3 days after introduction of the parasitic stages, the fish usually show lack of mobility, folding of fins while staying close to the bottom of the aquaria.

Later small bloody lesions appear on the base of fins and on the sides of the body. The fish may show jerking movements and rub themselves on the bottom or sides or the aquarium. Approximately 10 days after exposure to the first copepodid stage, small anchor worms can be seen on the fish.

SUMMARY

Ichthyophthirius multifiliis was cultured in the laboratory by the following method: Ten non-immune channel catfish or white catfish fingerlings each were stocked into 40-liter aquaria filled with dechlorinated tap water. Then, either a recently killed, heavily infected catfish fingerling or at least 200 trophozoites of the parasite were introduced into each aquarium. After the new host fish became visibly infected, the parasites were recovered by killing the host fish, placing one fish into a petri dish filled with water. The trophozoites left the host almost immediately after its death and swam in the water. They were collected with a fine pipette at that time or later after they began to adhere to bottom of the dish.

Cleidodiscus pricei was cultured in the laboratory by crowding 15 channel catfish fingerlings, which usually have a mild, natural infection, into one aquarium. The parasite population then gradually built up. Gill fluke eggs can be collected either by syphoning them off the bottoms of aquaria containing infected fish or by removing gills harboring the worm and collecting the eggs as they are being laid. To avoid starving the host fish in aquaria, 100-liter feeding troughs equipped with baffles to impede the water flow were stocked with 50 channel catfish fingerlings, having a natural, light parasite infestation. By feeding the fish approximately 1 percent of their body weight each day, the hosts remained healthy and the parasite population was allowed to build up. The baffle system prevented, to a large extent, the loss of parasite eggs and larvae.

Lernaea cyprinacea was cultured in the laboratory on cyprinid fishes. Mature egg sacs were removed from the adult parasite and incubated at 80°F. for approximately 72 hours. At that time infective larvae had developed. The first copepodid stage was then introduced to non-immune host fish. Three 6-inch cyprinids in a 40-liter aquarium were exposed to the first copepodid larvae obtained by incubating one egg sac. The water temperature was between 75 and 80°F.

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