1956

The Life Cycle of *Crassiphiala bulboglossa* (Trematoda: Strgeida): Development of the Metacercaria and Cyst, and Effect on the Fish Hosts

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The Journal of Parasitology, Vol. 42, No. 4, Section 1 (Aug., 1956), pp. 435-444

THE LIFE CYCLE OF *CRASSIPHIALA BULBOGLOSSA* (TREMATODA: STRIGEIDA). DEVELOPMENT OF THE METACERCARIA AND CYST, AND EFFECT ON THE FISH HOSTS

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INTRODUCTION

The adult of *Crassiphiala bulboglossa* was described by Van Haitsma (1925) from the kingfisher, *Ceryle alcycon*. It can be differentiated from *Uvulifer ambloplitis* (Hughes, 1927), apparently its closest relative in North America, by the presence of a large bulbous holdfast occupying most of a cup-shaped forebody, and the absence of a ventral sucker. In *U. ambloplitis* the holdfast is small, the forebody is not cupped, and a ventral sucker is present. These species can be readily identified with the keys given by Dubois (1953).

The metacercaria of *C. bulboglossa* was described as *Neascus bulboglossa* by Hughes (1928) from the perch, *Perca flavescens*. Van Haitsma proved it to be the metacercaria of *C. bulboglossa* by feeding experiments on kingfishers. It differs from all North American *"Neascus"* species by the absence of a ventral sucker (Hoffman, 1955a).

The life cycle of *U. ambloplitis* was determined by Krull (1934) and Hunter and Hunter (1934). Krull infected sunfish with the cercaria known as *Cercaria bessiae*, Cort and Brooks, 1928, from *Helisoma trivolvis* (Say), and found that the worm developed into *U. ambloplitis* (= *Neascus ambloplitis*). Hunter and Hunter studied the development in 2 species of snails, *Helisoma trivolvis* and *H. campanulatum*, and the resulting cercariae developed into *U. ambloplitis* in young small- and large-mouthed black bass, *Micropterus dolomieu* and *Huro salmoides*, rock bass, *Ambloplitis rupestris*, common sunfish, *Lepomis gibbosus*, and banded sunfish, *Enneacanthus obsessus*. Hunter and Hamilton (1941) observed the development of the cyst of *U. ambloplitis* in rock bass, common sunfish, and small-mouthed black bass, and found that the cyst of parasite origin appears in about 7 days and the host cyst becomes melanated during the third week. The nature of the cyst of *C. bulboglossa* in the skin of *Perca flavescens* was reported by Hunter and Hunter (1942).

Since *C. bulboglossa* and *U. ambloplitis* are closely related and have the same final host and species of *Helisoma* as molluscan hosts, as herein reported, one might expect that their developmental stages would be easily confused. The miracidium, sporocyst, and cercaria are very similar in the two species. Fortunately, fish and bird infections with *C. bulboglossa* predominated over infections with *U. ambloplitis* in the English Coulee, Grand Forks, North Dakota, and it was possible to complete the life cycle experimentally. This was briefly reported in abstract by Hoffman (1955b).

(Unless otherwise stated, the measurements given below are in microns)

Received for publication, February 14, 1956.

EGG AND MIRACIDIUM

Eggs of *C. bulboglossa* were obtained from feces and worms from 2 kingfishers taken in July and August, 1954. Both birds were heavily infected, 1 with at least 1,100 worms, so that many eggs were obtained. One worm in saline overnight expelled 68 apparently normal eggs. Ten eggs averaged 80 by 52, as compared with 90–104 by 56–70 for *U. ambloplitis* (Hunter and Hunter, 1934).

Eggs incubated in dechlorinated tap water (1 cc of 2% sodium thiosulfate per gallon) in standard Petri dishes at room temperature (23–25° C) yielded many miracidia. Some eggs still in the uterus of decomposing worms developed normally. The first miracidia hatched in 8–9 days.

The miracidia (Fig. 1) are very similar to those of *U. ambloplitis,* as described by Hunter and Hunter (1934).

Four living miracidia, quieted with 0.4% chloretone, measured 94–118 by 23–30 depending upon their state of contraction or expansion. Details are as follows: cilia 10 long, those at anterior tip about 5. Two small lateral papillae 5 long, about 15 from anterior end of worm; often a globule exuding from tip of papilla; about 6 cilia on papilla, possibly tactile in function; these cilia do not beat in chloretone solution although other cilia do. "Penetration organ" elliptical, 20 by 12, its 2 ducts easily seen, much enlarged within the "organ," extend back to unicellular penetration glands which are more difficult to see. Pigmented eye spots, crescentic in dorsolateral view, 7–8 in greatest diameter. One pair of flame cells just posterior to eye spots, another near posterior end of worm. A globular body about 12 long by 16 wide slightly posterior to eye spots, cf. "brain" described by Hunter and Hunter (1934) in *U. ambloplitis.* Similar body near posterior end appears to contain 2 round, dense structures; may be the germ ball. Hunter and Hunter do not describe these round structures in germ ball of *U. ambloplitis.*

DEVELOPMENT IN THE SNAIL

Many embryonated eggs were placed in a gallon jar of dechlorinated tap water containing 35 laboratory-reared snails, *Helisoma anceps* Menke. The snails were fed commercial fox chow, calcium was supplied with blackboard chalk or pure calcium carbonate, and compressed air forced into the jar furnished aeration. Water temperatures ranged from 23–28° C, averaging 24° C.

At 30 days post infection the liver of 1 experimentally infected snail was a tangled mass of sporocysts, almost impossible to separate. One freed sporocyst measured 3 mm by 50–100 and contained germ balls and cercariae, some of which appeared fully developed. Cercariae appeared at 33 and 34 days post infection from 6 out of 27 exposed snails in individual containers, as compared to 42 days for *U. ambloplitis* (Hunter and Hunter, 1934). Many cercariae were shed by the 6 experimentally infected snails during the 7 days after they first appeared, but only 5 snails shed worms for 9 days, and only 1 of these yielded many; only 1 snail shed a few worms at 11 days. No cercariae were shed during 35 days of further observation. The liver of 1 snail autopsied at 17 days after cercariae first appeared was a mass of sporocysts but only 1 to 4 cercariae were in each sporocyst. The same was true of a snail examined at 26 days.

Of 200 *Helisoma trivolvis* collected from August 18 to November 18, 1954, from the English Coulee, 1 was infected with *C. bulboglossa* and yielded cercariae for 10 days; after 20 days' refrigeration it yielded cercariae for only 1 day upon returning to room temperature.

Of 200 *Helisoma anceps* collected October 17, 1954, from Turtle River, 21 began to yield cercariae 13 and 14 days after collection. They were refrigerated for 11 to 28 days but shed no more cercariae upon returning to room temperature.

Three more snails began to shed cercariae 25 days after collection and stopped after 5 days at room temperature. Four more began at 30 days after collection and shed worms for 2, 3, 6 and 16 days, respectively.

No accurate record was kept of the longevity of infected snails, but some died as soon as 15 days after cercariae first appeared and all were dead in 2½ months. Many uninfected snails survived similar conditions for longer lengths of time with less mortality. Although laboratory and natural conditions are not entirely comparable, it is likely that most infected snails die during the year, but that those infected late in the season probably survive the winter as the sporocysts develop slowly and then produce cercariae for a while the next spring.

THE CERCARIAE

The cercaria (Figs. 2, 3, 4) differs only slightly from that of *U. ambloplitis* (= *Cercaria bessiae*) as described by Cort and Brooks (1928) from *Helisoma lantum* from Michigan and redescribed by Miller (1936) from *H. trivolvis* from Louisiana. The cercariae used here were obtained from experimentally infected *H. anceps* and naturally infected *H. anceps* and *H. trivolvis*. All cercariae were proven to be *Crassiphiala bulboglossa* by recovering typical metacercariae from fish experimentally infected with the cercariae from the same snail. The writer believes that the metacercaria of this species is distinctive enough for species identification (see key in Hoffman, 1955a). The cercaria hangs in the water with the body downward and bent like a hook. Occasionally a rapid side to side motion of the tail drives the larva tail-first upwards in the water. Its behavior strikingly resembles that of the cercaria of *U. ambloplitis*. Maximum longevity was 25½ hours.

The following measurements and observations were made on living specimens with the aid of the intra-vital stains neutral red and nile blue sulfate, and sometimes with 0.4% chloretone as an anesthetic: Body length of 23 cercariae, relaxed (or nearly so) 106–230, average 152; width 28–54, average 41. Tail stem, length 190–360, average 250; width, 24–42, average 31. Furcae, length 190–360, average 250; width, 24–42, average 31. Furcae, length 180–320, average 226; width from lateral view 30, from ventral or dorsal view, about 14. Body usually a little wider than tail stem, usually show 7–8 muscular annulations when extended. Anterior fourth of body with minute spines just visible under oil immersion. One pair posteriolateral flagellets about 5 long, on body. Penetration organ oval, 23–30 by 18–28. Three pairs penetration glands in posterior half of body, ducts easily seen in region of penetration organ. Gut short, rhabdocoel, difficult to see. Ventral sucker absent. Eye spots not seen. Nine flame cells on each side, one just posterior to penetration organ, second just posterior to first, 4 at irregular intervals near penetration glands, seventh at anterior level of excretory bladder, 2 in basal half of tail stem. Bladder at posterior end of body, constricted into 2 parts: anterior portion about 8 long by 15 wide, receives 2 collecting tubules; posterior bulbous part about 6 diameter, joins caudal excretory tubule. Heavily nucleated body about 12 in diameter just anterior to bladder is assumed to be genital primordium. Tail stem has about 25 annulations, approximately 10 long, regularly spaced; 10–15 flagellets each side, 15–25 long, closer together near ends of tail stem than at middle. Small globules sometimes seen at ends of flagellets; flagellet appears shorter as globule becomes larger, as seen by Cable (1955) in other cercariae. Two lateral rows of about 40 cells with conspicuous nuclei and a central "core" of about 75 prominently nucleated cells in tail stem. Each furca with 8 longitudinal rows of minute spines about 1 long on each side giving a striated appearance; each furca contains 2 rows of about 25 prominently nucleated cells.

The cercariae used by Krull (1934), Hunter and Hunter (1934), and Hunter and Hamilton (1941) in obtaining metacercariae of *Uvulifer ambloplitis* were identified as *Cercaria bessiae* but were not described. The cercaria of *Crassiphiala bulboglossa* differs only slightly from *Cercaria bessiae* as described by Cort and Brooks (1928) and Miller (1936), in the following respects: (1) Tail stem

longer and narrower, (2) Furcae longer and wider, (3) Seven flame cells on each side of body instead of 6, (4) Unpigmented eye spots, if present, could not be seen, (5) One pair of posterio-lateral flagellets on the cercaria of *C. bulboglossa* but not on *C. bessiae.*

(The writer wishes to acknowledge with gratitude the use of a mimeographed key to the furcocercous cercariae (1953) kindly supplied by Dr. Asa C. Chandler.)

DEVELOPMENT OF THE METACERCARIA

The metacercaria of *Crassiphiala bulboglossa* has been recorded from 11 species of 6 families of fishes (Host record verified by Allen McIntosh, Zoology Div., Bur. Animal Ind., Beltsville, Maryland): CYPRINIDAE—*Ericymba buccata, Leucosomus corporalis, Notemigonus crysoleucas, Notropis cornutus frontalis, Semotilus a. atromaculatus;* CYPRINODONTIDAE—*Fundulus diaphanus;* ESOCIDAE—*Esox lucius;* ETHEOSTOMIDAE—*Boleosoma nigrum olmstedi;* PERCIDAE—*Perca flavescens, Stizostedion vitreum;* UMBRIDAE—*Umbra limi.* It is probable that many other species of fish are susceptible.

Black spot *Neascus*-free individuals of 4 species of fish, fathead, *Pimephales p. promelas,* killifish, *Fundulus diaphanus menona,* brook stickleback, *Eucalia inconstans* and common black sucker, *Catostomus c. comersonnii* were exposed to variable numbers of cercariae in a small amount of dechlorinated tap water in gallon jars at 21 to 25° C. Of the 39 killifish exposed, 38 became infected; all of 22 fatheads became infected; 7 of the 8 sticklebacks became infected and the 1 sucker did not become infected, even though it was exposed to cercariae on 2 different occasions. During the development of the metacercariae in the fish, the temperature ranged from 21–25° C, averaging about 23° C. In 1 lot kept at 25° C continuosly, metacercariae became fully developed about 2 days sooner than in other lots.

The larvae readily penetrated the fatheads, killifish, and sticklebacks and could be recovered from the skin in large numbers after 4 days. The developing metacercariae were examined at nearly daily intervals but for convenience the observations will here be given for only those ages which show some morphological difference over preceding ones. The stages are given as they occur at 21–24° C, averaging 23° C. At 25–26° C all stages occur a day or so earlier.

Four days (Fig. 5)—Larvae found free in skin. No evidence of cysts either of host or parasite origin. Larvae not found earlier probably because of small size and numbers. Alive and relaxed, measured *ca.* 148 by 54; very active at that age, largest individual 180 by 50 extended, 120 by 110 contracted. Larva often ameboid, nearly round, occasionally moves to side by forming projection grossly like a pseudopod. Usually anterior part, containing penetration organ, extends slightly forward (Fig. 6). Phillips (1955) states that the metacercaria of *Troglotrema salmincola* was once described as an ameba. Fixed and stained specimens, like fresh ones, show only cercarial penetration organ; body stains very heavily.

Six days—Cyst of host origin just discernible in skin of fresh fish at 10× magnification. Metacercaria slightly larger, about 188 by 74, relaxed; otherwise no morphological change detected.

Seven days—Accumulation of black pigment around cysts evident in fatheads and stickleback, not in killifish. Pigmentation is first reported at 3–7 weeks for *U. amblopitis* (Krull, 1934; Hunter and Hunter, 1934). Congested or enlarged blood vessels noticeable about cysts especially in heavy infections. Bases of fins, opercula, ventral surface of head hyperemic in heavily infected fish and this condition usually persists almost until the metacercaria is fully developed. Host cyst 9–16 thick in histological cross section, composed of unflattened cells 7–9 in diameter. No connective tissue fibers visible.

Fourteen days—(Fig. 7)—Cysts heavily pigmented in fathead and stickleback, no pigment in killifish with one exception. Host cysts, exclusive of outer pigmented layer, 300–370 by 280–300, *ca.* 42 thick Strigeid characteristics first appear in metacercaria, hindbody a small conical posterior projection, forebody suggests leaf-like form of some strigeids; holdfast a bulbous projection *ca.* 66 in diameter. Relaxed worm *ca.* 245 by 150, contracted, 185 by 165. Oral sucker distinct, *ca.* 21–28 diameter. Pharynx subspherical, 18 wide. Reserve excretory system and cyst of parasite origin not evident. In stickleback, metacercariae do not develop this far; never develop strigeid constriction or cyst of parasite origin, but do develop rudimentary holdfast; remain alive 24–81 days, finally die and are resorbed; melanated host cyst remains intact long after death of parasite, at least 81 days post infection.

Sixteen days (Figs. 8, 9)—Metacercaria has typical *Neascus* shape, *ca.* 322 by 165, definite conical hindbody *ca.* 94 by 71. Ceca not evident in live specimens; present as very thin tubes in stained specimens. Body nuclei still very large, internal structure of worm difficult to see. Entire worm appears "vacuolated." Large bodies, 10 by 25 in holdfast and entire forebody, varying in shape from oval and elongate to nearly round but irregular; rhizoid extensions occur at ends of some large elongate ones. The writer believes these are cystogenous glands because they disappear after cyst of parasite origin is formed. In histological section there are numerous vacuoles which are probably the same. Holdfast still bulbous, 90 in diameter. Reserve excretory system not evident.

In section the host cyst appears as 2 layers, an inner cellular one about 6–10 thick composed of cuboidal cells in irregular rows 1 to 3 cells deep, and an outer connective tissue layer 4–36 thick, composed of many flattened cells and fibers. Melanophores, when present, lie in a loose layer in outer margin of cyst.

Seventeen and eighteen days—Metacercaria attains maximum size, 310–400 by 180–215. Hindbody 106–120 by 54–56. Bulbous holdfast about 94 long by 110 wide in ventral view. Forebody wide, not conspicuously "cupped" yet. Whole worm, including holdfast, looks swollen and thickened during at least part of period; large cells or vacuoles present in holdfast of live specimen.

Nineteen days (Fig. 10)—Worm somewhat flattened, including holdfast, forebody somewhat "cupped." Main branches of reserve excretory system as described for mature metacercaria by Hughes (1928) readily visible, contain rounded bodies, the largest 10 in diameter. Before formation of reserve excretory system, a quantity of granular material in forebody seems to deliniate future excretory tubules; it is absent after tubules are formed. Excretory bladder in hindbody has 2 main arms which join near excretory pore; each has a slight constriction about midway. Inner part of host cyst can be easily peeled from outer pigmented layer. Parasite lacks parasite cyst, can easily be forced out of host cyst.

Twenty days (Fig. 11)—Parasite cyst forming around some individuals; time of formation depends on temperature and number of metacercariae in fish, being delayed 4 or 5 days in a heavily parasitized fish. Newly secreted parasite cyst material gelatinous looking, very pliable, 9–23 thick. Metacercaria rounded, folded in typical *Neascus* manner as described by Hughes (1928). This time interval contrasts strikingly with 6–8 days for parasite cyst formation in *U. ambloplitis* (Hunter and Hamilton, 1941), but is comparable to 20–21 days in *Posthodiplostomum minimum centrarchi* (Hoffman, 1950). Host cyst no longer shows cuboidal inner cells, but entire cyst, 23–26 thick, composed of compressed connective tissue. Looser melanophore layer when present is on outer edge of cyst (see description given by Hunter and Hunter, 1942).

Twenty-one to twenty-two days (Figs. 12, 13, 14)—At *ca.* 23°C all cysts of parasite origin formed, congealed, and contracted to normal mature thickness of 1–2. Metacercaria appears as described by Hughes (1928), but ellipsoidal parasite cysts in writer's material measured 189–260 in length, 147–190 in lateral width (average 241 by 164), *ca.* 108 in dorsoventral depth; Hughes' specimens averaged 200 by 160. Host cyst, excluding outer pigmented layer, diameter totals *ca.* 600. Most cysts were just beneath epithelium of fish, either above or below a scale, but several were found on gill arches and under epithelium of gullet in heavily infected fish. Many nuclei appear in mature cysts, but fewer than in younger cysts. Dense inner layer 22–49 thick, loose outer layer 0–50 thick in oldest specimens studied, 60 days.

Mature metacercariae can be removed from cysts by digestion with either pepsin (Hoffman, 1955a) or trypsin (Hughes, 1928).

Fischthal (1949) found that black grubs (*Neascus spp.*) and yellow grubs (*Clinostomum marginatum*) survived "wintering over" in fish. The author has attempted to keep *Pimiphales p. promelas* infected with *Crassiphiala bulboglossa* and *U. ambloplitis* in the laboratory for long periods to study longevity of *C. bulbo-*

glossa. The fish usually do not survive much over a year, but in one which survived 37 months, all of the worms examined of both species were still alive. Hoffman (1950) found that another strigeid, *Posthodiplostomum minimum centrarchi* survived at least 11 months in naturally infected fish in laboratory aquaria.

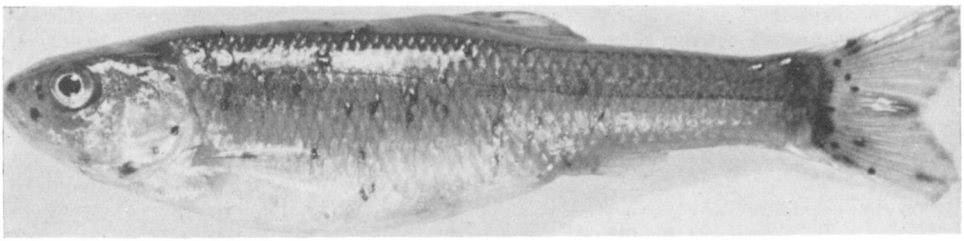


FIG. 14. Fathead, *Pimephales p. promelas,* with fully developed cysts of *Crassiphiala bulboglossa.*

HOST DIFFERENCES

It has already been stated that the stickleback becomes infected but the parasite does not attain full development and probably does not become infective in that fish. It lives up to 81 days in this apparently abnormal host and eventually dies; the writer is not aware of experimental demonstration of this phenomenon in other fish trematodes, and it may be unusual.

On several occasions killifish taken from the same experimental jar as fatheads contained metacercariae which were one to several days younger in appearance than those of the fatheads. One heavily infected fathead 2½ inches long contained about 600 cysts in which the parasites did not secrete the parasite cyst until 5 days after those of similar but less heavily infected fish.

The pigmentation of the cyst in the fathead was similar to that occuring in other fish, except that there were many cysts with accumulated xanthophores instead of melanophores about them. The majority of these yellow cysts occurred in the less heavily pigmented areas of the fish, i. e., the ventral and ventro-lateral areas. Similar yellow pigmentation has been reported by Chapman and Hunter (1954) for cysts of *Cryptocotyle lingua* in the cunner, *Tautoglabrus adspersus.*

Pigmented cysts were observed in but 1 of the 38 killifish studied. The accumulation of pigment cells was not great but was definite around 2 cysts on the caudal fin. Normally, the pigment cells are close to the fin rays in the killifish but on this occasion some of them appeared to have migrated down the ray to the cyst and a row of pigment cells could be seen along the ray on either side of the cyst. The mechanisms of the pigmentation of parasite cysts is not known. Hunter and Hamilton, (1941), theorized that the cells of the fish contain the enzyme necessary for pigment production and that the parasite, in some way, provides chromogen or vice versa. Chapman and Hunter (1954) applied the dopa test to the developing cysts at 7–33 days (the time during which pigmentation occurs) and elucidated no evidence of dopa oxidase. They also saw no evidence of melanophore migration into the area of the cyst. Therefore, the mechanism still remains to be determined.

EFFECT OF THE PARASITE ON THE FISH

Krull (1934) reported that the cercariae of *Uvulifer ambloplitis* produce a spectacular nervous response in fish, and heavily infected fish die in 2–4 days. Hunter

and Hunter (1938) observed that heavy infections caused significant weight loss. The writer exposed 2 adult fatheads to as large a concentration of cercariae as could be obtained. At no time, however, did these fish demonstrate severe nervous symptoms. At 7 days the fish were "peppered" with developing black-spot cysts, and there was extensive congestion of blood vessels at the bases of the fins and ventral and posterior portions of the opercula. Histological examination demonstrated severe congestion but no hemorrhage. Vascular enlargement also occurred around the cysts themselves but was much more evident in the locations just mentioned. One fish which seemed most affected died at 10 days post infection and was literally covered with developing cysts. The other one, although in distress, was more lethargic than nervous. The scales were raised over the entire body giving the fish a very rough appearance. At 18 days it was not feeding, but at 21 days it was taking food and seemed livelier. A part of a fin containing cysts was removed each day to study the development of the metacercariae. At 25 days, 4–6 days later than in less heavily infected fish, the parasite cyst was formed and the fish was autopsied. Approximately 600 cysts were present.

On another occasion each of 6 killifish about 2½ inches long were exposed to 400, 800, 1600, and 2600 cercariae, respectively. The fish exposed to 400 cercariae developed a small degree of hyperemia at the bases of the fins, but appeared normal in other respects and was preserved at 27 days when the cysts of parasite origin were being formed. The fish exposed to 800 cercariae demonstrated much vascular congestion by 15 days, was not feeding at 22 days and went into a sort of frenzy when disturbed. The fish exposed to 1600 cercariae had developed much vascular congestion at 15 days, demonstrated the same frenzy as the preceding fish, and died at 19 days. The fish exposed to 2600 cercariae showed much congestion as early as 9 days, and at 15 days there were spotty hemorrhages over the entire body. At 16 days the fish died and was estimated to have 400 cysts. They were so numerous that in some instances 2 or 3 parasites were enclosed in a single host cyst.

These observations on the effect of the parasite on the fish are rather cursory, but they do show that the type of damage done must be considerably different from that reported by Krull (1934) for *U. ambloplitis*. Under natural conditions it is doubtful that a fish would be exposed to as concentrated a dosage of cercariae as was used experimentally, and except under very unusual circumstances, this parasite probably would not contribute to the mortality of the fish.

IMMUNITY

Krull (1934) reported that older sunfish previously infected with a few *Uvulifer ambloplitis* were refractory to further infection. Ferguson (1943) and Hoffman (1950) were able to reinfect previously infected cyprinid fish with *Posthodiplostomum minimum*. The writer has been able to reinfect previously infected fish with *Diplostomulm baeri eucaliae*. In the present study, 4 fatheads, previously infected with *Crassiphiala bulboglossa,* and 6 uninfected fatheads as controls, were exposed to the cercariae of *C. bulboglossa;* the 6 control fish became infected and the other 4 were autopsied at 16 days or less and examined for superimposed *C. bulboglossa* metacercariae; the superimposed metacercariae could easily be differentiated from those of previous infection because they lacked the cysts of parasite origin which had already been formed in the older natural infec-

tions. In all cases the fish did become successfully reinfected with numerous parasites and no evidence of immunity was observed.

SUMMARY

1. The life-cycle of *Crassiphiala bulboglossa* was completed by experimentally infecting the snail, *Helisoma anceps,* with miracidia hatched from ova obtained from adult *C. bulboglossa* from the kingfisher, *Ceryle alcyon,* and subsequently infecting fish, *Pimephales p. promelas* and *Fundulus diaphanus menona,* with the resulting cercariae.

2. The miracidium and cercaria are described.

3. The development of the metacercaria and its cysts are described.

4. Large numbers of cercariae killed fish but not until 10–15 days post infection. No evidence of immunity was observed.

ACKNOWLEDGMENTS

The writer wishes to thank the following students for technical assistance at one time or another during the project: Miss Kathleen Piggott, Messrs. James B. Hundley, James B. Hoyme, and Richard Olafson. Dr. J. P. E. Morrison, Smithsonian Institute, Washington, D. C., kindly identified the snail species.

REFERENCES

CABLE, R. M. 1955 Personal Communication.

CHANDLER, A. C. 1953 Key to the furcocercous cercariae. Mimeographed.

CHAPMAN, J. A. AND HUNTER, G. W. III 1954 Studies on host-parasite reactions VII. The pigmentation cells surrounding the metacercarial cysts of *Cryptocotyle lingua* in the cunner, *Tautoglabrus adspersus* (Walbaum). Trans. Am. Micr. Soc. **73:** 28–36.

CORT, W. W. AND BROOKS, S. T. 1928 Studies on the holostome cercariae from Douglas Lake, Michigan. Trans. Am. Micr. Soc. **47:** 179–221.

DUBOIS, G. 1953 Systématique des Strigeida, Complément de la Monographie. Mém. Soc. Neufchateloise des Sci. Nat. Tome **8,** 141 pp.

FERGUSON, M. S. 1943 Experimental studies on the fish hosts of *Posthodiplostomum minimum* (Trematoda: Strigeida). J. Parasitol. **29:** 350–353.

FISCHTHAL, J. H. 1949 The over-wintering of black grubs and yellow grubs in fish. J. Parasitol. **35:** 191–192.

HOFFMAN, G. L. 1950 Studies on the development and biology of two trematodes, *Bunodera eucaliae* and *Posthodiplostomum minimum.* Ph. D. Thesis. State University of Iowa: 70 pp.

———1955a *Neascus nolfi* n. sp. (Trematoda: Strigeida) from cyprinid minnows with notes on the artificial digest recovery of helminths. Amer. Midl. Natur. **53:** 198–204.

———1955b Studies on the life-cycle and development of *Crassiphiala bulboglossa* (Trematoda: Strigeida) J. Parasitol. **41** (Suppl.): 22.

HUGHES, R. C. 1928 Studies on the trematode family Strigeidae (Holostomidae) No. X. *Neascus bulboglossa* (Van Haitsma). J. Parasitol. **15:** 52–57.

HUNTER, G. W. III AND HAMILTON, J. M. 1941 Studies on host-parasite reactions to larval parasites IV. The cyst of *Uvulifer ambloplitis* (Hughes) Trans. Am. Micr. Soc. **60:** 498–507.

——— AND HUNTER, WANDA S. 1934 Further studies on fish and bird parasites. Suppl. 24th Ann. Rep. N. Y. S. Conserv. Dept., No. IX, Rep. Biol. Surv. Mohawk-Hudson Watershed: 267–283

——— AND ———1938 Studies on host reactions to larval parasites. I. The effect on weight. J. Parasitol. **24:** 447–481.

——— AND ———1942 Studies on host-parasite reactions V. The integumentary type of strigeid cyst. Trans. Am. Micr. Soc. **61:** 134–140.

KRULL, W. H. 1934 *Cercaria bessiae* Cort and Brooks, 1928, an injurious parasite of fish. Copeia, 1934 (2): 69–73.

MILLER, E. L. 1936 Studies on North American cercariae. Ill. Biol. Monogr. **14** (2): 125 pp.

PHILLIPS, C. B. 1955 There's always something new under the "parasitological" sun. (The unique story of helminth-borne salmon poisoning disease). J. Parasitol. **41:** 125–148.

VAN HAITSMA, J. P. 1925 *Crassiphiala bulboglossa*, nov. gen., nov. spec., a holostomatid trematode from the belted kingfisher, *Ceryle alcyon* Linn. Trans. Am. Micro. Soc. **44:** 121–131.

PLATE I

Explanation of Figures

FIG. 1. Composite drawing of the miracidium.

FIG. 2. Composite drawing of the cercaria.

FIG. 3. Furca as seen from the lateral view.

FIG. 4. Normal resting position of the cercaria.

FIG. 5. Four-day old metacercaria in normal extended position.

FIG. 6. A. Four-day old metacercaria in contracted position. B and C. Sketches showing ameboid movement of the metacercaria.

FIG. 7. Fourteen-day old metacercaria.

FIG. 8. Sixteen-day old metacercaria freed from host cyst.

FIG. 9. Same as Fig. 8, but from lateral view.

FIG. 10. Nineteen-day old metacercaria showing the newly formed main tubules of the reserve excretory system.

FIG. 11. Twenty-day old metacercaria showing the relatively thick newly secreted cyst of parasite origin.

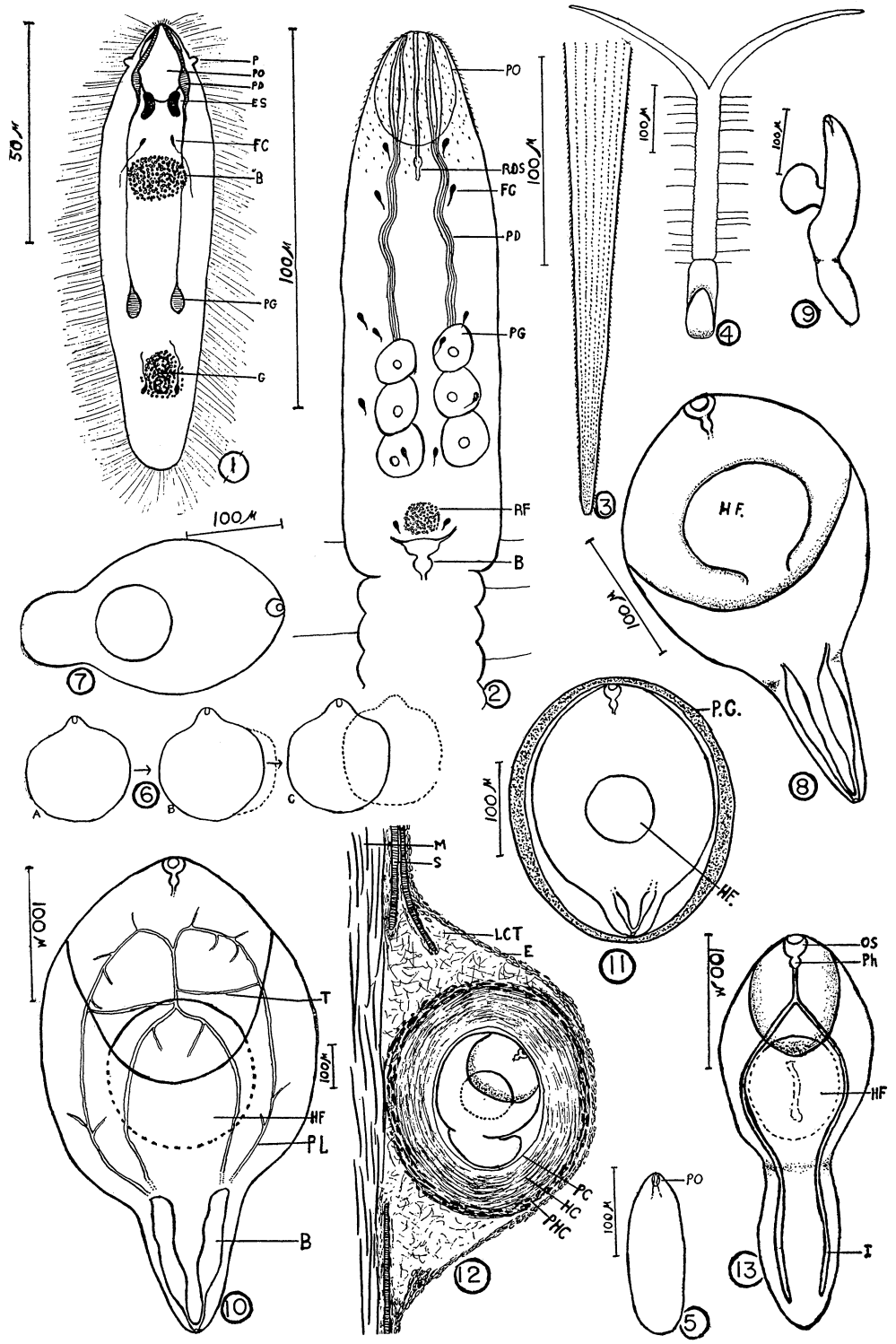
FIG. 12. Freehand drawing showing the appearance of a fully developed cyst as seen in cross section.

FIG. 13. Mature metacercaria freed from its cyst; excretory system omitted.

Abbreviations

B—Bladder, excretory
"B"—Brain
E—Epithelium
ES—Eye-spot
FC—Flame cell
G—Germ ball
HC—Host cyst
HF—Hold-fast
I—Intestine
LCT—Loose connective tissue
M—Muscle
OS—Oral Sucker
P—Papilla
PC—Parasite cyst
PD—Penetration duct
PG—Penetration gland
Ph—Pharynx
PHC—Pigmented host cyst
PL—Primary lateral excretory vessel
PO—Penetration organ
RDS—Rudimentary digestive system
RF—Reproductive fundament
S—Scale
T—Transverse commisural excretory vessel

PLATE I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