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HOST-PARASITE STUDIES OF TRICHOHYRA INFESTING CUTTHROAT TROUT (SALMO CLARKI) AND LONGNOSE SUCKERS (CATOSTOMUS CATOSTOMUS) FROM YELLOWSTONE LAKE, WYOMING

R. A. Heckmann1 and T. Carroll2

ABSTRACT.—Trichophrya sp. (Protozoa) on the gills of cutthroat trout (Salmo clarki) and longnose suckers (Catostomus catostomus) was studied using light and electron microscopy and tracer techniques. All cutthroat trout, 14 cm in total length and above, from Yellowstone Lake, Yellowstone National Park, Wyoming, were infested with the suctorian. No trichophryans were found on fry or fingerling cutthroat trout. Sixty percent of the examined longnose suckers from the same location were infested. Light microscopy disclosed extensive pathology of gill epithelium in longnose suckers infested with Trichophrya that was not observed for infested cutthroat trout. Electron micrographs show damage to immediate host gill cells by both parasites, depicted by a reduction and lack of mitochondria. Both parasites form attachment helices (0.52 × 0.04 μm), which may originate in the protozoan cell membrane and function for maintenance of parasite position on the host cell. There was no uptake of 14C, injected into host fish, via the attachment helices by the parasite that further substantiated the mechanical function for the spiral structure. Protozoan feeding on host tissue may be accomplished by use of necrotic gill tissue and mucus.

Trichophrya clarki (Heckmann 1970, 1971) was found on the gills of all adult cutthroat trout (Salmo clarki) examined from Yellowstone Lake, Yellowstone National Park, Wyoming, during the summers of 1968 and 1969. Trichophrya catostomii (Heckmann 1970, 1971) was present on the gills of 60% of the adult longnose suckers (Catostomus catostomus) examined from the same region.

Butschli (1889) reported Trichophrya in perch (Perca) and pike (Esox) from Europe and assigned the species name T. piscium. Davis (1937, 1942) was the first to report Trichophrya in the Northern Hemisphere. He assigned the name T. micropteri and T. ictaluri for the gill parasites of smallmouth black bass (Micropterus dolomieui) and channel catfish (Ictalurus punctatus), respectively. No name was given for Trichophrya in brook trout (Salvelinus fontinalis). He also was the first to suggest that it may have a pathogenic effect. Chen Chih-leu (1955) and Prost (1952) added to Chinese and European records by assigning T. sinensis to infested white and black Amur fishes and T. intermedia to infested salmon-fry (Salmo salar). Lom (1960) added to the host record for T. intermedia by including brown trout (Salmo trutta) and three other fishes in Czechoslovakia. Culbertson and Hull (1962) summarized all host records of Trichophrya and suggested T. piscium be used for all species found in fishes. This suggestion was followed by Sandeman and Pippy (1967), who reported on four salmon-ids of Newfoundland infested with Trichophrya. Hoffman (1967) stressed the need for further taxonomic study of trichophryan species and their symbiotic effects. Heckmann (1970), used the same criteria outlined by Culbertson and Hull (1962) and transmission electron microscopy of the protozoan, described two new species, one in cutthroat

Fig. 1. A gill macerate from an infested cutthroat trout from Yellowstone Lake showing numerous suctorian ciliates, Trichophrya clarki, next to gill filaments. (1000X)

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trout (*T. clarki*) and one in longnose suckers (*T. catostomi*).

The objective of this study was to examine the host-parasite relationship of *Trichophrya* on cutthroat trout and longnose suckers. Three methods were used for study of this problem: light microscopy, electron microscopy (transmission TEM and scanning SEM), and radioactive tracers. To date there has been no ultrastructural description of the host-parasite relationship or the interface between the ectoparasite and host cells. Meyer (1966) questioned the parasitic nature of *T. ictaluri* and stated the main effect may be mechanical interference with respiration. Davis (1967) reported heavy loss among fingerling and adult smallmouth bass, raised in hatcheries, due to *T. micropteri*. These were attached to the gills by a broad base, closely applied to the epithelium, causing hyperplasia and necrosis of host tissue.

**Materials and Methods**

**Light Microscopy**

Fishes ranging in size from 3.5 to 45.7 cm total length, were obtained from several sites in Yellowstone Lake and Yellowstone River. Intact gills, infested with *Trichophrya*, were scraped and the macerate was examined (Fig. 1). Infested gills were also fixed with 10% formalin and prepared by standard methods for histological examination (Davenport 1960). Sections were stained with the following: Harris’ hematoxylin and eosin, periodic acid Schiff (McManus 1956), mercuric bromphenol blue (Mazia et al. 1953), five-dye stain (Greenstein 1961), and Schiff’s reagent (Davenport 1960).

**Electron Microscopy**

Infested gill macerate and gill filaments were placed in small plastic vials containing 2.5% gluteraldehyde buffered with potassium phosphate (0.1M, pH 7.3). Post fixation was accomplished with 1% osmium tetroxide in the same buffer. Standard methods were used in preparing the tissue for sectioning (Daves 1971). The dehydrated material was embedded in Araldite epoxy plastic and sectioned, then poststained with uranyl acetate and Reynold’s lead citrate. In an attempt to determine possible differences in the cytochemical nature of magnified structures, the staining procedure was varied in the following manner: no poststain, uranyl acetate only, and lead citrate only. Fixed gill macerate and filaments were sent to Florida State University for examination by scanning electron microscopy.

**Tracer Study**

A tracer experiment was conducted with four infested cutthroat trout of approximately equal size from Yellowstone River. Each fish was anesthetized with MS 222 and injected intracardially with 5 microcuries of *¹⁴*C-D-Glucose (U). Previous to the injection blood samples were qualitatively checked for
glucose, using chromatography. The fish were sacrificed 1, 2, 4, and 8 hours after injection. The gills were removed and washed in physiological saline and fixed in 10% formalin. Samples of 25 *Trichophrya* and 0.4 grams of gill filament were analyzed from each fish using liquid scintillation counting (Arnoff 1960, Chase and Rabinowitz, 1962). The suctorian and gill filaments were placed in liquid scintillation vials containing 0.5 and 1.0 ml, respectively, of hydroxide of Hyamine (Rohm and Haas) for 12 hours to disrupt the cell membranes. Scintillation fluid was added and each sample was counted for a period of 20 minutes. The trichophyrians were fixed with 10% formalin and washed three times with changes of formalin.

The wet film method for autoradiography (Pelc 1947, MacDonald et al. 1948) was used to corroborate the data from liquid scintillation counting. Gills from the injected fish were prepared histologically (Davenport 1960) and sectioned at 20 μm. Duplicate thin sections from each fish were spread on glass slides that were immersed in water along with unexposed film (Fuji plate film; ET2F-9327). A strip of film was then removed from the plate and placed over the tissue sections. The tissue-film preparation was subsequently removed from the water, air dried, and stored in light-tight film boxes. The preparation was developed and stained with Mayer’s hematoxylin after 2, 4, 6, and 8 weeks’ exposure (Shigematsu 1969a, 1969b). It was then observed with a compound microscope.

**Results**

**Light Microscopy**

**Histology.**—The suctorian parasites on cutthroat trout are usually concentrated on the lamellar tips of the gill filament where they are closely attached to the epithelial cells (Figs. 2 and 3). Cutthroat trout samples, with the largest number of trichophyrians, had 7.1% of the gill surface covered by the parasite. In one 36 cm trout, there was an average of 31 suctorians per gill filament totaling about 42,000 organisms. The macro- and micronucleus of *T. clarki* were Feulgen positive (Schiff’s reagent) and the mercuric bromphenol stain showed an intense blue area between the parasite and the epithelial host cell. Periodic acid Schiff staining revealed similar particles in both protozoans and the adjacent surface of the gill epithelium (Fig. 4).

**Histopathology.**—Sections of gills from cutthroat trout infested with *T. clarki* had no apparent cytological damage (Figs. 2 and 3), whereas longnose suckers inhabited by *T. catus* were definitely affected by the parasite (Figs. 5 and 6). There was definite damage to the gill lamellae, characterized by hyperplasia and hemorrhaging of the adja-

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*Fig. 4. This is a section of cutthroat trout gill tissue stained with periodic acid Schiff to emphasize mucopolysaccharides. Note the dark staining granules in the host (H) epithelial cells and similar granules in the cytoplasm of the suctorian ciliate (P). The protozoan could be using mucus as a source of food (400X)*
This is a section of longnose sucker gill tissue. The suctorian ciliates are causing pathological damage to the host (arrow and box) tissue that includes hemorrhaging and hyperplasia of the gill lamellae. Necrosis (N) also occurs in the infested tissue with subsequent clubbing of filaments. (400X)

Transmission Electron Microscopy

The ultrastructural characteristics of *T. clarki* and the gill epithelial cells have been described (Heckmann 1970).

Sections of the interface between the host epithelial cell and parasite were prepared. A helical structure in the interface attaches the parasite to the gill epithelium (Fig. 7). This structure (referred to as attachment helix) has the following measurements: length, 0.52 μm (range 0.20 to 0.82 μm) and width 0.04 μm (range 0.03 to 0.06 μm). The filament making up the helix appeared circular in transverse section (Fig. 8) and revealed an electron transparent center bound by an opaque ring. Lom, 1970, described a similar structure for suctorian infested fish concurrent with this initial description (Heckmann 1970). The attachment helix is found only on the side of the protozoan next to the host cell (Fig. 7). It appears to originate as a cleft in the outer wall of the protozoan. The protozoan membrane, in the cleft, moves into the space between the host cell and parasite and expands into a long filament (Figs. 9 and 10). The filament then contracts to form the helix. Cytochemical evidence from ultrathin sections along with histochemical analyses of specimens suggest that the origin of the helix is the outer wall of the protozoan. The helix was osmophilic when unstained thin sections of oxmium-fixed protozoa were viewed. It was mercuric bromphenol blue positive. Lipo-protein material, found in biological membranes, have an affinity for osmium whereas protein stains blue with bromphenol blue.

A series of electron micrographs shows organelle changes to the epithelial cells of cutthroat trout due to *T. clarki*. In gill epithelial cells not infested with *T. clarki*, there are numerous mitochondria with well-defined...
cristae (Fig. 11). Infested cells show, in comparison to normal cells, swollen mitochondria that have fewer cristae and lack the outer enveloping membrane (Fig. 12). Thus damage is detected as the number of host cell mitochondria decrease and disappear. Similar damage was observed for *T. catostomi* in longnose suckers.

Scanning Electron Microscopy

Scanning electron microscopy shows *T. clarki* to be saucer-shaped, with the convex
surface attached to the gill epithelium. There are fine filaments between the suctorian and the host cells that are probably aggregations of attachment helices (Fig. 13).

Tracer Study

Liquid scintillation counts and autoradiography indicate there is no uptake of $^{14}$C by $T. clarki$ from the fish host during eight hours following injection. The isotope was still present in the gill epithelium after two hours and was presumably available to parasites using sustenance directly from the host. Silver grains in the autoradiographic film developed in all four gill samples after four weeks exposure. Samples from fish sacrificed at one- and two-hour intervals after $^{14}$C injection had
exposed silver grains only over the lumen of blood vessels and capillaries of the gills. The four-hour sample demonstrated radioactivity in these same regions and also in the epithelial cells of the gill filament. Distribution of radioactivity in the eight-hour sample was similar to that in the four-hour sample but of less intensity (approximately 50% fewer grains visible).

Discussion

Host-Parasite Relationship

There is no uptake of $^1$C by T. clarki, but TEM disclosed definite changes in the mitochondria of host epithelial cells. The mitochondria decrease in number and apparently disappear, which is probably due to the masking effect the parasite has on respiratory activity (Davis 1942, Meyers 1966). Strobel (1965) observed a reciprocal response by mitochondria for hydrating spores of Puccinia striiformis that was also related to respiration. The pathological signs in longnose suckers infested with T. catostomi are more extensive. Infested gill tissue shows areas of hyperplasia and necrosis, which is visible with light microscopy, in addition to the previously described fine structure.

The exclusive use of free-living protozoa as food for fish suctoria has been questioned (Davis 1942). The tentacles of other suctoria are used in obtaining food, immobilizing prey, and transporting cytoplasm to the central body (Hull 1961a, 1961b). Phialocysts (Batisse 1967a, 1967b) or haptocysts (Bardele and Grell 1967) at the tip of the tentacles are used to hold and impale prey. Rudzinska (1954, 1965, 1966) described Tokophrya infusionum feeding on live ciliates using its tentacles as described. No other protozoa were observed in the fish gills or on the trichophyran tentacles in infested fishes (T. intermedia) of Czechoslovakia (Lom 1960). Prost (1953) suggested that T. intermedia feed on host necrotic tissue. In this study no free-living protozoa were found impaled on the tentacles of the two suctorian species, and it was observed that T. catostomi may feed on the necrotic gill tissue of longnose suckers. The mucus layer on the surface of gill epithelium may also be a source of food. Periodic acid Schiff preparations show particles (complex polysaccharides) of similar stain intensity both in T. clarki and on the surface of host epithelium.

The attachment helix is an organelle that functions in holding the parasite next to the epithelial host cell. This structure may hold the parasite in position when water flows across the gill surface. Other structures in the interface of the parasite and host have been
Fig. 10. This figure shows a sequence in the development of the attachment helix. The cell membrane strands lengthen out (a) in the cell interface (b) and start to form a spiral (c, d), with the helix being the final product (e) (10,500X). The protozoan is on the left-hand side of the figure, and the epithelial cell of a fish gill is on the right-hand side.

described. Uspenskaja (1966) found small cytoplasmic extensions ("rootlets") from *Myxidium* into the urinary bladder epithelium of *Esox lucius*. He considered these to function primarily as absorption organelles rather than for attachment. Scholtynseck and Hammond (1966a, 1966b) noted ribbonlike extensions (15 mm by 2 µm) from *Eimeria* macrogametocytes into host cells and postulated that their function was ingestion of nutrients.
Fig. 11. This is a fish gill epithelial cell containing characteristic mitochondria (m). (10,500x)

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Fig. 12. When the Trichophryan attaches to the gill filament, the immediate epithelial cells show mitochondrial (m) and organelle degeneration, probably due to the masking effect of the protozoan. (10,500X)
Fig. 13. This is a scanning electron microscopy of a cutthroat trout gill. The Trichophrya is attached to the gill lamellae (box) and appears to be saucer-shaped. (1000X)

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