

CATFISH (*Ameiurus albicus*) infested with *Leithyophthirus multifilis*.

REPORT ON A PARASITIC PROTOZOAN OBSERVED ON FISH IN THE AQUARIUM.

BY CHARLES WARDELL STILES, PH. D.,
Zoölogist, Bureau of Animal Industry.

INTRODUCTION.

During the early part of the Columbian Exposition season a lot of young catfish (*Ameiurus albidus*) were brought to the aquarium from the Potomac River and some time after were discovered to be seriously infested with a protozoan parasite. The general pressure of aquarium operations prevented any special study of the subject until a spread of the parasitic disease to other species adjacent, notably to young trout, compelled attention to it. Some preliminary studies made by Prof. S. A. Forbes, director of the aquarium, resulted in a determination of the parasite as a species of the genus *Ichthyophthirius* of Fouchet, and some practical experiments with solutions of common salt, copperas, carbolic acid, and other materials, showed that the parasite was easily destroyed in a free state by several of these substances. It seemed necessary, however, that solutions should be found capable of destroying the parasite while imbedded in the mucous layer of the skin without injury to the fish infested—an undertaking which required a large amount of continuous work, systematically planned with reference to both scientific and practical ends. Prof. Forbes consequently applied to Secretary Morton, of the U. S. Department of Agriculture, for the temporary transfer of the author of this report from the Agricultural Department to the U. S. Fish Commission, with a view to having such methodical experimental work carried forward until definite results were reached.

Arriving at the aquarium in July, I found that in some tanks scarcely a catfish or a trout was free from the parasites. The latter were perfectly visible to the naked eye, and were scattered over the entire external surface of the fish. They were numerous on the gills and in the mouth, and in the case of the catfish they were also found in the stomach, the latter specimens probably having been swallowed. The protozoa were not entirely superficial, but were imbedded in the epithelial layer of the skin, lying in small round cavities large enough to contain but one or two individuals, or in elongated galleries or pustules (especially in the case of the catfish) containing numerous specimens of the ciliate. In most cases the fish were covered with a thick slime, which extended also into the mouth and covered the gills more or less completely.

The temperature of the water was high, running some days up to 74° F. (23.3° C.), and this factor should be taken into consideration in connection with the high mortality of the infested fish.

Fish infested with this parasite usually preferred the upper half of the tank. They gasped very rapidly and seemed unable to eat. When they took a morsel of food into their mouths, it was retained but a moment and then expelled. Gradually they

would seek the surface of the water, become weakened and sluggish in their movements, and finally die.

The exact pathogenic action of the parasite on the fish I am unable at the present moment to explain, for although I examined a number of fish after death—many of which were considerably decayed before I could study them—I found no one gross lesion or set of lesions common to them all, other than the general injury to the epidermis and the enormous amount of slime present on the body and *over the gills*. I am convinced that the parasite is not a harmless object and that the mortality of the fish was not due solely to the high temperature of the water, for had this been the case fish in uninfested tanks would have been affected as well as those in the infested aquaria. Furthermore, even if one were inclined to claim that the temperature was the chief factor in the disease, he would be confronted by a fact which would be difficult to explain on that theory, *i. e.*, that the young catfish seemed to succumb more easily than the trout, although able to live in much warmer water. On the other hand, as the catfish were more seriously infested than the trout, the higher mortality of the former is more easily understood by assuming a pathogenic property of the protozoa in question.

Accordingly, while the specific pathogenic action still remains undetermined, I think there can be no reasonable doubt that this parasite contributed largely to the cause of the losses sustained by the aquarium.

The special problem before me was to find an inexpensive solution which would kill the parasites, but in which the fish could live. This in itself was not a difficult matter, for several solutions were soon made which were absolutely fatal to the protozoa when liberated from the fish, and in which the fish were able to live for several hours or even for several days. Upon applying these solutions to practical use, however, it was found that they had absolutely no effect upon the parasites as long as the latter remained in the epidermis or in the slime. Two methods of experimentation were then open: first, to find a solution which would cut or remove the layer external to the parasite and thus act as a carrier of the germicide; or, secondly, to study the life-history of the parasite in the hope of discovering some stage of its development during which it lived free in the water, then to kill this stage and thus prevent a reinfection of the fish.

While the details of these experiments* will be given below (cf. pp. 186-189), it may be stated here that the last-mentioned method was the only one which was found feasible. The three solutions which gave the best results were salt water, methylen blue, and eosine. To fully understand the action of these liquids, it will be necessary to give a description of the parasite and the details of its life-history.

*These investigations are to be looked upon as field experiments, rather than as minute researches into the exact effect which the various solutions mentioned below have upon the fish, as they were carried on in an extemporized laboratory, and with a view to meeting the epizootic at hand rather than placing on record details and minutiae as to the exact number of seconds that various species of fish or the parasites could live in different percentages of strength of various disinfectants. Owing to the fact that I was obliged to make the experiments upon the grounds of the Exposition, it was necessary for me to borrow my instruments from the different Government exhibits at the fair, and it is with pleasure that I acknowledge here my indebtedness to Dr. Wiley and Mr. Fairchild for placing various instruments at my disposal; Dr. Kinyoun, of the U. S. Marine Hospital Service; Dr. Lagard, of the U. S. Army Hospital; John Wyeth & Bro., chemists, Philadelphia, and Merck & Co., New York, kindly furnished me with various chemicals, thus saving much trouble and expense.

THE PARASITE. *Holophrya* (*Ichthyophthirius*) *multifiliis* (Fouquet).

SYNONYMY.

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| 1876. <i>Ichthyophthirius multifiliis</i> Fouquet. | 1887. <i>Holophrya</i> (<i>I.</i>) <i>multifiliis</i> (Fouquet) Bütschli. |
| 1884. <i>Chromatophagus parasiticus</i> Kerbert. | 1892. <i>Ichthyophthirius cryptostomus</i> Zacharias. |

HOSTS.

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| Schlammpeitzger (<i>Cobitis fossilis</i>) and others, Hilgendorf and Paulicki, 1869. | Lake trout (<i>S. namaycush</i> Walb.), new host, 1893. |
| Von Behr trout (<i>Salmo fario</i> L.), Fouquet, 1876. | White bass (<i>Roccus chrysops</i> Raf.), new host, 1893. |
| Tench (<i>Tinca vulgaris</i> var. <i>aurata</i>), Kerbert, 1884. | Pike perch (<i>Stizostedion vitreum</i> Mitchell), new host, 1893. |
| Bream (<i>Abramis brama</i> L.), Kerbert, 1884. | Spotted catfish (<i>Ictalurus punctatus</i> Raf.) new host, 1893. |
| White bream (<i>Blicca bjorkna</i> Sieb.), Kerbert, 1884. | Young Mississippi catfish (<i>Ameiurus nigricans</i> Le Sueur), new host, 1893. |
| Carp (<i>Cyprinus carpio</i> L., with varieties; <i>C. var. cyprinorum</i> and <i>C. nudus</i>), Kerbert, 1884. | Channel catfish (<i>Ameiurus albidus</i> Le Sueur), new host, 1893. |
| Goldfish (<i>Carassius vulgaris</i> Kröyer), Kerbert, 1884. | Yellow catfish (<i>Ameiurus natalis</i> Le Sueur), new host, 1893. |
| Golden ide (<i>Idus melanotus</i> var. <i>miniatus</i> Heck. & Kner), Kerbert, 1884. | Marbled catfish (<i>Ameiurus marmoratus</i> Holbrook), new host, 1893. |
| Atlantic salmon (<i>Salmo salar</i> L.), Kerbert, 1884. | Whitefish (<i>Coregonus clupeiforrmis</i> Mitchell), new host, 1893. |
| Von Behr trout (<i>Salmo fario</i> L.), Kerbert, 1884. | Large-mouthed buffalo (<i>Ictiobus urus</i> Agassiz), new host, 1893. |
| Brook trout (<i>Salvelinus fontinalis</i> Mitchell), Kerbert, 1884. | Small-mouthed buffalo (<i>Ictiobus bubalus</i> Raf.), new host, 1893. |
| Brook trout (<i>Salvelinus fontinalis</i> Mitchell), Forbes & Stiles, 1893. | Sheephead (<i>Archosargus probatocephalus</i> Walb.), new host, 1893. |
| Von Behr trout (<i>Salmo fario</i> L.), F. & S. 1893. | |
| Loch Leven trout (<i>Salmo terevensis</i> Walker), new host, 1893. | |
| Rainbow trout (<i>Salmo iridens</i> Gibbons), new host, 1893. | |

GEOGRAPHICAL DISTRIBUTION.

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| Germany (Hamburg), Hilgendorf & Paulicki, 1869; (Plön), Zacharias, 1892. | United States of North America (Chicago), fish came originally from the Potomac River, Forbes & Stiles, 1893. |
| France (Paris), Fouquet, 1876. | |
| Holland (Amsterdam), Kerbert, 1884. | |

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HISTORICAL REVIEW.*

†Hilgendorf and Paulieki (1869) of Hamburg seem to have been the first to place on record observations concerning this form. They noticed that a number of different species of fresh-water fish in the aquarium of the zoological garden in Hamburg were troubled with a slimy excrecence, which was followed by a growth of fungus and finally by the death of the fish. In each excrecence was found one or more specimens of a large (0.5 mm.) protozoan. This parasite, which Hilgendorf and Paulieki thought might belong to Ehrenberg's genus *Pantotricha*, seemed to possess neither mouth nor characteristic form; it was covered with fine cilia arranged in longitudinal spiral lines and possessed a nucleus, contractile vacuole, vacuoles, and granules; it appeared to rotate always in the same direction. When the fish were placed in a special aquarium for study, it was noticed that the parasites left their host, fell to the bottom of the glass, encysted themselves, and multiplied by division. The small resulting oval bodies, Hilgendorf and Paulieki think, probably infest new fish. They believe that the function of the skin of the fish must be greatly disturbed by the presence of these parasites, and that the fungous growth was only secondarily concerned in the malady. Among the fish infested, the authors mention especially the "Schlammpeitzger" (*Cobitis fossilis* L.).

*As very little literature was at my disposal while on duty at the World's Fair, the greater part of this historical review has been written since returning to Washington. Through the kindness of Prof. Forbes, however, I had access to Bütschli (1887), a review of Kerbert's work (1885), and to Fouquet's article (1876), while still in Chicago.

† Bei dem allgemeinen Interesse, welches den niederen Organismen für die Entstehung verschiedener Krankheitsprocesse in neuerer Zeit beigelegt wird, dürfte nachfolgende Beobachtung, welche in dem Aquarium des Hamburger zoologischen Gartens angeführt wurde, einer Mittheilung werth erscheinen. In den sämtlichen Süßwasserbehältern wurde nämlich bereits seit langer Zeit an den verschiedensten Fischarten das Auftreten von schleimigen Excrencenzen beobachtet, die schliesslich zu Schimmelbildung und endlich zum Tode der befallenen Individuen führten. Die microscopische Untersuchung dieser Schimmelmassen zeigte nur die gewöhnlichen aufgequollenen Epithelzellen der Fischhaut. Aber schon bei der ersten Besichtigung trat eine Art von Infusionsthierchen, die sich durch verhältnissmässig enorme Grösse auszeichnete (bis 0.5 mm. im Durchmesser) in dem Gesichtsfelde auf, wurde indessen anfänglich für ein zufälliges Vorkommen angesehen. Die microscopische Besichtigung von besonders geeigneten Stellen des Fisches, z. B. den Bartfäden und Flossen, zeigte aber bald, dass jede einzelne Excrecenz im Innern einen scharf conturirten weissen Punct besass, der eine unverkennbare Aehnlichkeit mit dem macroscopischen Aussehen jener Thierchen verrieth, eine Vermuthung die sich bei Anwendung des Microscops völlig bestätigte.

Die betreffende Form, welche wohl zu der Ehrenberg'schen Gattung *Pantotrichum* gehören könnte, zeigt weder Mund, noch durch Grösse ausgezeichnete Wimperhaare oder Borsten, noch eine charakteristische Körpergestalt; sie ist überall mit feinen gleichmässig entwickelten, in schwach spiraligen, gedrängten Längslinien stehenden Wimpern besetzt, und lässt nur noch einen (bei grossen Exemplaren hufeisenförmigen) Kern, die contractile Blase, Vacuolen und Körnchen erkennen. Ein Bartfaden von einem Schlammpeitzger zeigt die Einbettung des Thieres unter dem (hier gelblichen) Epithel und über der Schicht der Pigmentzellen aufs deutlichste. Die Epithellage bildet, ohne sonstige Veränderungen zu bieten, einen ansehnlichen Hügel über dem Parasiten, welcher sich in einer fortwährenden, anscheinend stets nach derselben Seite gerichteter Rotation befindet. Wenn, wie es öfter beobachtet wurde, eine Anhäufung von den Schmarotzern an einer bestimmten Stelle stattfindet, so verbinden sich die epithelialen Decken der einzelnen Individuen zu zusammenhängenden, ziemlich ausgedehnten Massen, welche den ganzen Körper des Fisches, Augen, Nasenröhren, Flossen, etc., übersäen.

In ein besonderes Glassgefäss untergebracht, verloren die Fische bald einen Theil der Infusorien, welche sich auf dem Boden des Glases ansammelten, und an diesen abgesonderten Thieren konnte nun

Thus, as we shall see, the first observers, although they did not go into minute details in regard to the microscopic structure of the parasite, made very exact observations on its life-history, and all the work on this form which has been done since then confirms most of their observations, corrects some slight errors, and supplements their description by furnishing details which escaped their notice.

Fouquet (1876) found numerous nearly spherical parasites, 0·3 mm to 0·8 mm in diameter, on small trout in Prof. Balbiani's laboratory (Collège de France, Paris), which he identified as the form found by Hilgendorf and Paulicki. They rotated, according to his observations, from left to right as well as from right to left in a cyst formed at the expense of the epithelium; the cilia were arranged regularly in rows as already noticed by Hilgendorf and Paulicki. In the cortical layer of the ciliate were found trichocysts and numerous contractile vacuoles, 0·03 mm in diameter. The endoplasm contained granules which carmine would not color and which were insoluble in caustic potash and only slightly soluble in ether and alcohol.

The larger specimens contained irregular patches of blackish pigment. The mouth was on the anterior extremity, non-contractile, 0·04 mm in diameter and surrounded by cilia which are slightly longer than those of the body; an œsophagus was present. Fouquet does not believe that this orifice can be used as a mouth, but rather that it serves as a sucker, while the parasite absorbs its nourishment by endosmosis. The nucleus is described as horseshoe-shaped, 0·30 mm long by 0·09 mm broad; the nucleolus could not be discovered in the adult, but was found in the young. When the parasite has reached a certain stage, it leaves the fish, falls to the bottom of the aquarium, forms a cyst around itself and divides in the series 2, 4, 8, 16, etc., until about 1,000 small cells are formed. The division is completed in about 40 to 50 hours, the time varying according to the temperature. On the third or fourth day the young, which measure 0·046 mm by 0·028 mm, escape from the cyst and seek new hosts. The trichocysts are more visible in the young than in the adult. The nucleus is 0·015 mm in diameter and in part incases the nucleolus, but each has its own membrane. The nucleolus measures $3\cdot1 \mu$ in diameter. Fouquet's attempt to infect fish with the young parasites gave negative results. The epizootic began each year towards the end of May, and lasted two or three months. While Hilgendorf and Paulicki considered this parasite related to *Pantotricha* Ehrbrg., Fouquet considers it a representative of a new genus of *Heterotricha*, for which he proposes the name *Ichthyophthirius* (*I. multifiliis*).

eine weitgehende Theilung nach vorheriger Encystirung beobachtet werden, während die unmittelbar vom Fische herabgenommenen Individuen nie die Andeutung eines Theilungsprocesses erkennen liessen. Theilthierchen wurden in verschiedenen Beobachtungen 2, 4, 8, 16 bis zu etwa 100 und darüber gezählt. Wurde die gemeinschaftliche Hülle durch Druck zum Bersten gebracht, so drängten sich die kleinen Nachkommen des Mutterthieres hervor und schwammen in schneller Bewegung als anfänglich ovale, rotirende Gebilde lebhaft umher.

Wahrscheinlich werden diese kleinen Theilsprösslinge wiederum einen Fisch aufsuchen, um an ihm durch reichliche Nahrung Wachstum und abermalige Theilungsfähigkeit zu erwerben, und um dann denselben, soeben dargestellten Kreislauf der Entwicklung zu wiederholen.

Die Fische selbst müssen natürlich, zumal durch massenhaftes Auftreten dieser Schmarotzerform bedeutend leiden, in dem sich die Oberhaut in Fetzen ablöst und die Function der Haut wesentliche Beeinträchtigung erfährt. Die Pilzbildung scheint erst auf dem abgestossenen Epithel als secundärer Process vor sich zu gehen, und darf somit wohl nicht als ein wesentliches Moment der Krankheit angesehen werden.

W. Saville Kent (1880-82, vol. I, p. 109) refers to *Ichthyophthirius multifiliis*, and creates (vol. II, p. 530) for it a new family, *Ichthyophthiriidae* S. K., with the diagnosis:—

Animalcules adherent, more or less ovate, ciliate throughout, oral cilia of larger size than those of the general cuticular surface, oral region adhesive, acetabuliform.

Saville Kent (vol. II, p. 531) seems to doubt the absence of a mouth because of the presence of black substance in the body and also upon other grounds. (Cited from Kerbert).

Kerbert (1884)* noticed a skin-disease on several of the fresh-water fish (see list of hosts, p. 175) of the Amsterdam Aquarium, caused by an infusorian parasite, which he thought was probably identical with the form described by Fouquet, but for which, on account of certain apparent differences in structure, he created a new genus and new species, *Chromatophagus parasiticus*, which he places in Saville Kent's family *Trachelocercidae*. This disease has been termed "spot disease" (Flecken-Krankheit), and should not, according to Kerbert, be confounded with the disease "Pocken-Krankheit or pox," observed by Wittmack in various cyprinoids, where, "on the surface of the skin, there appear bluish-gray spots of a slimy, fungus-like character, which spread more or less over the entire body, and extend to the eyes, fins, etc." Kerbert never discovered any infusoria in pox, although Wittmack thinks the cause of the disease may possibly be traced to them. Spot disease, according to Kerbert, also occurs on salt-water fishes, but the ciliated parasite he found upon *Mustelus vulgaris* Müll. & Henle, and *Acanthias vulgaris* Risso, in the pulp cavity of numerous placoid scales, is probably not the same as the form he found upon the fresh-water fish, although Kerbert simply states that the rapid decay of the fish prevented him "from making a thorough examination of this species of infusorians."

Kerbert then discusses the views of Hilgendorf and Paulicki, and Fouquet, at some length. He, agreeing with Saville Kent, looks upon the dark granules in the infusoria as the remains of cutaneous pigment of the fish, and hence as positive proof against the view that these parasites have no mouth. He mentions a paper by Livingston Stone, † in which a cutaneous parasite of *Salmo fario* is described and figured, which Kerbert thinks is probably a rotifer rather than an infusorian. According to Kerbert also, La Valette St. George refers to the articles of Hilgendorf and Paulicki (1869) and Stone, but makes no further statements. As Kerbert's paper appeared in full in the Fish Commission report, it will suffice here if the barest outline of his results is given. The parasites measured 0.615 mm by 0.408 mm; no trichocysts could be distinguished; mouth was lateral in position; pharynx was well developed; anus absent but the feces were seen to escape from various points of the body. Reproduction not by fission, but essentially the same as described by Fouquet, the division always taking place in the dark.

Bütschli (1887) considers that the parasites found by Hilgendorf and Paulicki, Fouquet, and Kerbert are identical. He does not consider that *Ichthyophthirius* should rank as a genus, but makes a subgenus of it in the genus *Holophrya* Ehrbg., 1831, (subfamily) ‡*Holophryina* Perty, 1852 (Fam.) emend., family §*Enchelina* (Ehrbg.)

* As Kerbert's original is not at my disposal, I quote from Kerbert 1885 and 1886.

† Domesticated trout: How to breed and grow them. Third ed.; Charlestown, N. H., 1877, p. 277. (Appendix I.)

‡ *Holophryina*, according to the rules of the International Zool. Congress.

§ *Enchelyida*, according to the rules of the International Zool. Congress.

Stein, 1860. Bütschli believes that the trichocysts of Fouquet are rather to be explained as a plasma structure,* and he seems to think that Fouquet's statement regarding the absence of a micronucleus in the adult is erroneous.

The following is Bütschli's definition of this family:

I. FAMILIE. ENCHELINA (Ehrbg.) Stein, 1860.

Gestalt meist mehr oder weniger monaxon; kurz bis recht lang gestreckt, jedoch häufig mit Neigung zur Bilateralität, seltener zur Asymmetrie. Der Mund liegt stets terminal oder ist doch nur sehr wenig nach hinten verschoben. Er ist meist rundlich, seltner etwas spaltartig in die Länge gezogen; gewöhnlich geschlossen, öffnet er sich nur während der Nahrungsaufnahme. Schlund, wenn deutlich entwickelt, eine gerade nach hinten ziehende kürzere oder längere, stets unbewimperte Röhre, die meist von einem mehr oder weniger deutlichen Stäbchenapparat umgeben ist. After in der Regel terminal. Die meist grobe Nahrung wird wohl immer durch Schlingen, nie durch Einstrudeln aufgenommen. Conjugation terminal.

I. UNTERFAMILIE. HOLOPHRYINA Perty, 1852 (Fam.) emend.

Bewimperung fast stets gleichmässig und allseitig, nur um die Mundöffnung zuweilen ein bis mehrere Kränze anscheinlicher Cilien. Selten ist das Wimperkleid auf die vordere Körperhälfte beschränkt. Tentakelartige Gebilde fehlen; ebenso eine panzerartige Umhüllung.

Holophrya. Ehrbg., 1831 und 1838; Dujardin (1841); Cohn (1853); Stein (1854, 1859, 1869); Cienk. (1855); Cl. u. L. (1858-61); Eberhard (1858); Quennerstedt (1865-69); Mereschkowsky (1877-78); Maupas (1883); Daday (1886); Stokes (1887, 1888).

Synon., *Leucophra*, p. p. O. F. M. (1786); *Euchelys*, p. p. Diesing. (1866).

Parasit. Infusor. Hilgendorf u. Paulicki = *Ichthyophthirius* Fouquet = *Chromatophagus* Kerbert. Taf. 56, Fig. 5-8 und 10.

Mittelgross bis ansehnlich (0.4). Gestalt theils regulär ellipsoidisch mit nahezu gleichen Polen, theils gestreckter bis cylindrisch; das Hinterende dann auch etwas zugespitzt. Biegsam, doch selten erheblich contractil. Mund terminal, selten ein wenig nach hinten verschoben (Lieberk.); spalt oder grubenförmig; manchmal von sphincterartigem Lippensaum umgeben, welcher auch etwas warzenförmig vorspringen kann. Zuweilen von dichter gestellten, etwas längeren, nach vorn gerichteten Cilien umgeben. Ein Schlund scheint theils zu fehlen, theils ist er deutlich und dann kurz spaltartig oder röhrig. Stäbchenapparat fehlen oder schwach entwickelt. After terminal. Contractile Vacuole gewöhnlich einfach, terminal; seltener daneben noch einige kleinere in einer oder mehreren Längsreihen über den Körper vertheilt (Lieberkühn) oder endlich sehr zahlreiche kleine (sog. *Ichthyophthirius*). Cilien zart. Ma. N. kuglig bis eiförmig, hufeisenförmig lang bandförmig und dann gewunden bis rosenkranzförmig; selten in zahlreiche kleine Bruchstücke zerfallen. Mi. N. unbekannt. Nahrung grob und fein. Encystirung in gallertiger oder zarter Hülle; Cysten kuglig; zuweilen in denselben energische Vermehrung zu sehr zahlreichen kleinen Sprösslingen (*Ichthyophthirius*). Sichere Arten ca. 5-6. Süßwasser und Meer. Auch parasitisch (*Ichthyophthirius*) im Epithel der gesammten Körperoberfläche von Süßwassertischen (Cyprinoiden, Salmoniden, Esox.)

Die Gattung *Holophrya* in dem hier angenommenen Umfang liesse sich in einige Sectionen oder Untergattungen zerlegen, welche aber durch Uebergänge wohl zu innig zusammenhängen, um als besondere Genera betrachtet zu werden.

Section I. umfasst die Formen mit einfacher terminaler Vacuole und verschiedenartig gestaltetem, einfachem Ma. N. (Typus *Holoph. discolor*).

Section II. diejenigen, bei welchen der Mund nicht mehr ganz terminal, sondern etwas nach hinten gerückt ist (eine unedirte von Lieberkühn beobachtete Form).

Section III. diejenigen, bei welchen zahlreiche kleine Vacuolen über die gesammte Körperoberfläche vertheilt sind (alleiniger Typus der sog. *Ichthyophthirius multifiliis* Fouquet = *Chromatophagus* Kerbert) und

* "Die helle äussere Zone des Körpers (Corticalplasma wahrscheinlich) zeigt eine sehr deutliche radiäre Streifung, welche Fouquet auf Trichocysten bezieht (ich möchte sie für eine Plasmastruktur halten). Dass allein diese kleinen Sprösslinge einen Mikronucleus besitzen, wie Fouquet versichert, rührt jedenfalls nur von der Schwierigkeit her, welche die Beobachtung dieses Gebildes bei den Erwachsenen bereitet."

Section IV. die langcylindrische sog. *Holophrya oblonga* Maupas mit fein zertheiltem Nucleus, die wahrscheinlich identisch mit der sog. *H. marina* Daday's (Makronucleus rosenkranzförmig) ist und welcher auch wohl der *Prorodon marinus* (Clap. u. L. 301, sowie Quennerstedt 408b und Möbius 1888) nahe steht. Letztgenannte Formen besitzen jedoch auch wohl Beziehungen zu *Chaenia* Quennerst.

Weissmann (1891) makes a passing reference to Fouquet's species as "an example of the phyletic origin of germs among the lower flagellata and gregarines."

Zacharias (1892a and 1892b) found an *Ichthyophthirius* on *Leuciscus rutilus* and *Alburnus* sp. kept in a large aquarium in the biological station in Plön. The parasites formed pustules in the skin of their hosts, and were situated especially on the head and along the lateral lines; in some cases the fish were practically covered with them. The ciliates measured 0.65 to 0.80 mm long by 0.50 to 0.55 mm broad; the dorsal surface is described as convex, the ventral surface as flat, and the entire body is covered with short (5 μ) cilia; the protozoan possesses a large horse-shoe shaped nucleus, which is situated in the pointed anterior half of the body. By direct light the animal appears white; by transmitted light, grayish yellow. The anterior end is somewhat lighter in color than the middle and posterior portion, due to the uneven distribution of granules and crystals in the endoplasm. The endoplasm has a vacuolated structure and contains in the plasma between the vacuoles numerous round and angular refringent bodies. Besides these are found collections of dark granules (= pigment of other authors) probably to be looked upon as metabolic products. The thickest portion of the nucleus is at the bend and measures 0.05 mm. Like Fouquet, Zacharias believes that although the nucleolus is present in the young it is absent in the adult.

Zacharias states that no mouth is present, but on the ventral surface he found a depression 0.035 mm deep, 0.015 mm in diameter, which he interprets as a sucker by means of which the young fasten themselves to their host. He found no portions of food in the endoplasm, and experiments to feed powdered carmine were negative, from which Zacharias assumes that the parasites feed in some way upon the body fluids of their hosts. Contractile vacuoles were absent. As Fouquet's species presented a terminal anterior "mouth," and as this organ in Zacharias's species is situated ventrally in the anterior third, Zacharias looks upon it as a new species, for which he proposes the name *I. cryptostomus*.

Zacharias believes that the parasite which he observed is identical with the form found by Hilgendorf and Paulicki, basing this opinion on the statements of Hilgendorf and Paulicki that in their form the "mouth" was absent, while Zacharias doubts whether it could have been easily overlooked had it been terminal, as in the case of *I. multifiliis*. Furthermore, Hilgendorf and Paulicki state that the encysted parasite segmented into 100 to 150 young, this agreeing with the form examined by Zacharias, while Fouquet's species segmented into nearly 1,000.

Regarding the reproduction, Zacharias states that the ciliates which have left the fish lose their cilia and form a cyst around themselves, which he looks upon as the thrown-off cuticle, the content dividing until the young cells measure 0.075 mm in diameter. The macronucleus of the young measures 0.025 mm, the micronucleus 0.004 mm, and the cilia of the young are about twice as long as the cilia of the adult. As the young globular animals become oval or ovoid the globular nucleus elongates and becomes crescentic. While former authors had not found any stages of reproduction while the parasites were still on the fish, Zacharias states that he frequently

found "encysted" individuals on young *Leuciscus rutilus*, but does not say whether these were in process of segmentation.

According to this author the injury to the fish is due to the fact that the epithelium is injured by the ciliate, and thus places are exposed where fungi (especially *Saprolegnia ferax*) gain a hold. The latter then retard the function of the skin to such an extent that the fish soon succumb.

OBSERVATIONS AT THE WORLD'S FAIR.

The parasite (fig. 1) found at the World's Fair is unquestionably an *Ichthyophthirius*; whether *Ichthyophthirius* should, however, be given generic rank, as Fouquet and Zacharias have concluded, or whether it should be considered as a subgenus of the genus *Holophrya*, as Bütschli contends, is perhaps still an open question. Personally I lean decidedly to the view of Fouquet and Zacharias, that *Ichthyophthirius* is entitled to generic rank, but prefer at present nevertheless to follow rather than oppose an authority like Bütschli, unless supported by stronger evidence against his view than I have at present. Accordingly, I have accepted *Ichthyophthirius* as subgenus. The question of the specific relations between the form I have studied and the animals studied by Fouquet and others will be discussed further on.

In many respects the observations made agree perfectly with observations of other authors, as given previously; some of my observations may, however, be interesting and worthy of record, as they tend to complete the descriptions reviewed above as well as to explain some differences of opinion expressed by various authors.

The size of the animals now under consideration agrees approximately with the figures given by Fouquet and others, *i. e.*, up to 0.7 mm or 0.8 mm in diameter. These figures are, however, *estimated*, as no means for exact measurement were at my disposal. The body is round to elongate oval in shape when at rest, but changes its form almost as much as an amoeba. This change is naturally not due to pseudopodia, but to its twisting, turning, and contracting. Deep grooves may extend across its outer surface, or the body may be perfectly smooth superficially; it may be flat on one surface and convex on the other, or it may be nearly spherical; in fact, it may assume almost any form when in motion. When at rest the body becomes globular or oval, and this should perhaps be looked upon as the normal form, as it agrees with the form of the young. Even when not progressing in the water, but remaining apparently in one place, the animal is in constant motion, revolving not only to the right and left, but in every other direction, that is, dorso-ventrally, ventro-dorsally, diagonally, etc. This may perhaps explain the statements of different authors in regard to the position of the mouth, for, as we saw above, Hilgendorf and Paulicki state that the mouth is absent (in other words, they observed none); Fouquet found the mouth (sucker) terminal and anterior; Kerbert placed it on the left side, and Zacharias on the ventral surface in the anterior third.

According to my observations, when the animal has assumed an elongated form, one end being more pointed than the other, the mouth (fig. 1) is *frequently* situated terminally at the pointed extremity, while the nucleus is nearer the other extremity. If we observe the animal for some time, however, we notice that any given point of the surface of the animal may come to lie at the pointed portion; that is, any portion

of the animal may become pointed, and hence appear as if it were the anterior extremity, and as fast as the pointed portion shifts the mouth will also apparently change its position, at one moment being apparently at the anterior end, at the *apparent* posterior end, on the right or left side, or on the dorsal (fig. 2) or ventral surface. In all cases, however, I find the mouth at the pole opposite to the nucleus. On account of this constant apparent shifting of the mouth in position, I am led to doubt the validity of its apparent position as a specific character, and hence to call into question Zacharias's species, *I. cryptostomus*, distinguished from *I. multifiliis*, as we saw above, by the *antero-ventral* instead of *antero-terminal* position of the mouth. If we now compare figs. 1 and 2 of Zacharias's species with the statements just made, we see that our statements agree in so far that the mouth and nucleus are opposite each other, for he figures the mouth (fig. 2) as ventral, the nucleus (fig. 1) apparently as dorsal of it, *i. e.*, he has drawn a dorsal view, according to his interpretation of the topography, and in this view the nucleus is anterior. I have seen this same relation dozens of times in the form observed at Chicago.

Kerbert's figures, 1 and 3, support the statements here made, that the mouth and nucleus are at opposite poles and that the mouth apparently shifts* in position.

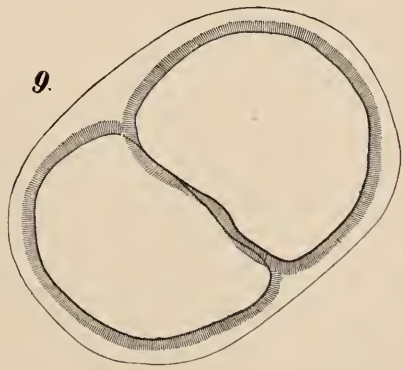
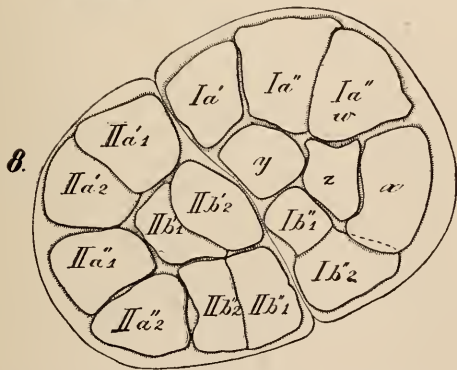
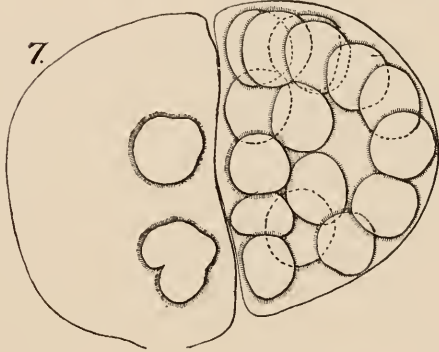
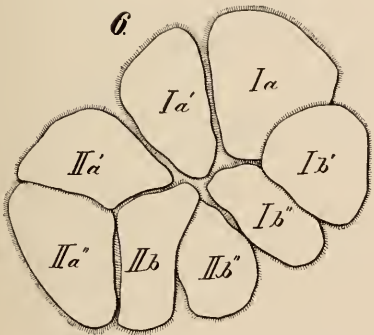
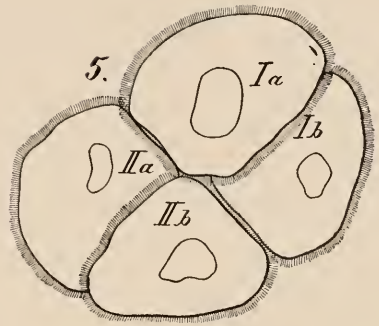
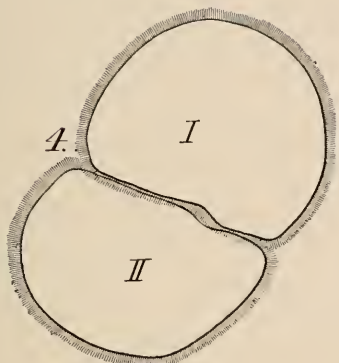
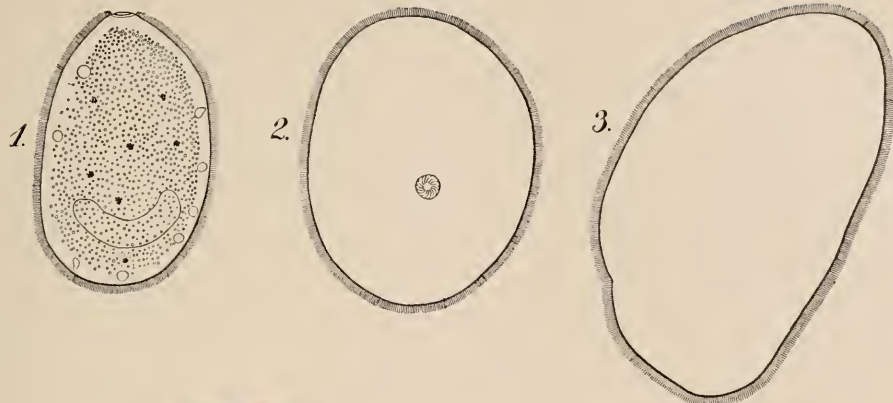
Agreeing with Fouquet and others, I find the mouth round and noncontractile; surrounding the aperture is an area which is thickened slightly and devoid of cilia (others have found the cilia longer in this position than over the rest of the body), but the inner margin of the oral aperture, as well as the œsophagus, is provided with them. A short ciliated œsophagus is present. As stated previously, Fouquet and Zacharias believe that this oral aperture acts as a sucker rather than as a mouth. While not denying the possibility of its acting as a sucker, although I have seen no proof for the view, I see no reason for denying that food can enter the body through this opening. The oral and œsophageal cilia certainly would be of more use in forwarding food than in sucking fast to an object, yet at the same time I have not seen any food enter through this aperture, nor, as I intimated above, have I seen it act as a sucker. No anus was discovered, neither could I confirm Kerbert's statement that the feces were expelled from various points on the surface; I was also unable to discover any trichocysts.

The entire body, with the exception of a narrow zone immediately surrounding the oral aperture, is covered with short cilia arranged regularly in rows.

The protoplasm of the body is indistinctly divided into an endoplasm and an ectoplasm. In the ectoplasm are found numerous globules, such as have been described by former authors, and numerous contractile vacuoles. The latter are arranged, apparently in no regular order, throughout the entire ectoplasm, but each vacuole seems to be a more or less permanent structure, *i. e.*, as soon as a vacuole has emptied itself to the exterior through a minute but perfectly visible canal it appears again at the same point. Following one vacuole under the microscope for some time I found that it contracted on an average every 7 seconds.

The endoplasm contains very numerous globules and a crescentic nucleus, both of which have been sufficiently described by former authors.

*This apparent change in the position of the mouth is, of course, nothing more nor less than a general change in orientation of the entire body.



1. *Ichthyophthirius multifiliis* showing ectoplasma, endoplasma, granules, pigment, contractile vacuoles, nucleus, mouth, and cilia.
 2. The same; the animal has changed its orientation so that the mouth is turned towards the observer.
 3-8. Semidiagrammatic figures of the division. The cyst appears in the 16-cell stage (beginning of the 16-cell stage).
 3. One-cell stage, seen at 1 o'clock p. m.
 4. Two-cell stage, at 1:45 p. m.
 5. Four-cell stage, 3:15 p. m.
 6. Eight-cell stage, 4 p. m.
 7. Sixteen-cell stage, 5 p. m.; the membrane is present.
 8. Thirty-four-cell stage, formed at 7:30; membrane broke at 8:30.
 9. One of the ciliates encysted in its 2-cell stage.

Reproduction.—Former authors have described the multiplication of these parasites after leaving the host as taking place only in an encysted stage, but, as will be seen below, I have found several variations in this multiplication which may serve as connecting links between a simple division, as seen in other protozoa, and the reproduction as described by the authors mentioned above. In the case of one specimen, for instance, which I liberated from the fish and then placed in a hanging drop, the movements, which were at first very rapid and in all directions, gradually became slower and the animal revolved in but one plane. At the end of about an hour, without forming any cyst around itself, it divided and each half swam off independently of the other, resuming the rapid motion. This observation demonstrates beyond a doubt that *Ichthyophthirius* is capable of reproduction by division, without first becoming encysted, which is, I believe, a matter of considerable importance from a practical standpoint, for former authors have observed—and I have above confirmed their statements in this regard—that it is not unusual to find several specimens of the parasite in a single epithelial pustule or gallery, and this may occur even when infection is comparatively light. There are only two possible explanations for such an occurrence. Either a multiple infection has taken place at the same point, or a single infection has taken place and the parasite has grown and multiplied *in situ*. The probabilities appear to me to favor the latter explanation, especially in view of the above observation upon a specimen artificially liberated from the host.

Fig. 3 represents a specimen which had escaped from a trout and come to rest at the bottom of a glass jar at about 1 o'clock p. m. The animal was revolving very slowly in different directions, so that the mouth was turned toward the observer at one instant, in the opposite direction, or to the right or left the next instant.

At 1:45 it had divided (fig. 4) into 2 cells, I and II. Each cell revolved independently of the other, but they remained close together, although no surrounding cyst membrane could be discovered.

At 3:12 the cells had divided again (fig. 5), I giving rise to Ia and Ib, II giving rise to IIa and IIb. No cyst was visible; cilia were present and the 4 cells were in constant motion.

At 4 p. m. (fig. 6) Ia had divided into Ia' and Ia''; Ib into Ib' and Ib''; IIa into IIa' and IIa''; IIb into IIb' and IIb''. By no test, either by direct or oblique light—coloring methods could of course not be used—could I distinguish any cyst-membrane around this 8-cell stage. Every cell continued to revolve slowly in its own area.

At 5 p. m. the 16-cell stage (fig. 7) was reached. IIa' had given rise to IIa'₁ and IIa'₂; IIa'' to IIa''₁ and IIa''₂; IIb' to IIb'₁ and IIb'₂; IIb'' to IIb''₁ and IIb''₂; Ia' to Ia'₁ and Ia'₂; Ib' to Ib'₁ and Ib'₂.

All of these divisions were observed directly and continuously. There can, therefore, be no doubt as to the origin of the various cells. The origin of the cells *w*, *x*, *y*, and *z*, however, can be given with less certainty, although it is almost beyond question that *w* arose from Ia'' and *x* from Ib' and it is probable that *y* arose from Ia'', and *z* from Ib'.

During the formation of this 16-cell stage a distinct surrounding membrane gradually came into view, not, however, a common membrane inclosing all 16 cells in one cavity, but a separate cyst for each group of 8 cells which had resulted from the two cells I and II (of fig. 7) respectively, while between the two groups the membranes

appeared to unite in a common wall. Whether they really united, however, or whether the distance between them was so slight as not to be recognized (cf. fig. 8), I am of course not able to say, as any attempts to demonstrate the true condition would have destroyed the specimen for further observation.

At 5:30 the cells had changed position slightly, but no further division had taken place.

By 7:30 the 32-cell stage was reached, but it was impossible to follow the origin of the various cells, as they changed position so rapidly.

At 8:30 the membrane surrounding the small cells resulting from cell II (fig. 9) broke, and all the young ciliates except three escaped.

By 10 o'clock the cells of cyst I had undergone another division. I was able to count 27 with certainty, and in all probability this represented a 32-cell stage of this cyst (64-cell stage of the original animal), as several cases of doubtful division were not included in the number 27. One of the cells remaining in cyst II had divided, but the other two remained intact.

At 11 p. m. another division had taken place in cyst I; the resulting cells were about the size of the cells of the 64-cell stage, and this theoretically evidently represented a 64-cell stage of cyst I (128-cell stage of the original animal), although the young animals now revolved and changed place so rapidly that it was utterly impossible to count them. No change was noticed in cyst II, or in the cells which had escaped from cyst II and were swimming around in the preparation.

At 12 m. no change had taken place, and the preparation remained the same until 1:30 a. m., when it was placed aside for the night.

At 11 a. m. nothing could be seen of cyst II; cyst I had broken, and its contents, for the most part, had escaped, while the cells remaining in the cyst were dead.

A number of other cases of division were observed, which were essentially the same as the one just described. In many cases, however, it was impossible to follow the origin of the various cells beyond the 8-cell stage, and considerable variation was noticed in the time of the appearance of the surrounding membrane. The following are some of the more important observations:

Many of the ciliates become encysted while still on their hosts, and there undergo a division. I have found cysts of the 1, 2, and 4 cell stage on the host, but never any beyond this stage. [*The hosts had been dead for several hours.*] Many of the parasites encyst themselves shortly after leaving their hosts *before* any division takes place.

The first division may take place before any encystment, one cell swimming off and apparently undergoing no further division (for the time being, at any rate), while the other cell remains quietly in its place, encysts itself and divides into numerous small ciliates; or the first division may take place before any membrane appears, the two cells may swim around independently of each other; each may then become encysted and proceed to divide.

So far as my observations go, the surrounding membrane may appear in or at the end of the 1, 2, 4, or 8 cell stage. That is to say, I have observed these stages when, so far as I could discover, there was no membrane present, but when the membrane would gradually appear in the next succeeding (2, 4, 8, 16 cell) stage. I repeat, for emphasis, that I did not attempt to prove the presence of a membrane by resorting to any staining methods.

Zacharias has stated that the animal loses its cilia before encystment and has expressed his belief that the cyst-membrane is the thrown-off cuticle. From the above description it will be seen that my results do not agree with his. Admitting the possibility that the forms we have studied represent two different species, in which case we might perhaps expect to find some differences in the mode of reproduction, etc., I will repeat here that in the form Forbes and I have seen the cilia are not thrown off either before or during encystment; that the cyst-membrane in this case is not a discarded cuticle is shown conclusively by the fact that it appears at different stages of division. The origin of the cyst-membrane is not clear to me. The only explanation of its formation which occurs to me is that it is formed by an excretion of the cells which through the constant rotation of the latter is gradually moved centrifugally, condensing at a short distance from the organism.

I was unable to directly observe the division of any specimen beyond the 128-cell stage, as described above, but the division certainly goes beyond that stage, for not only have former authors described and figured it, but I have also found cysts containing more than 128 cells. Furthermore, the youngest stages found on the fish are smaller than the cells observed in the 128-cell stage.

In one case a cyst contained 4 cells at 3 p. m., 8 at 4:10, 16 at 4:25. At 11 a. m. on the following day it corresponded to the stage given by Fouquet as the final stage of division, and must have contained from 500 to 800, or possibly 1,000, minute cells, the latter corresponding to the description of the young given by Fouquet, except that I could not distinguish the trichocysts.

These young were also found swimming around on the bottom of a glass in which several trout had been confined, and were found in great numbers on the fish, so there can be no doubt that this is the stage which serves to spread the infection from fish to fish. It will be noticed that this stage was reached in less than 24 hours in the specimen referred to.

From the life-history as just given, in confirmation of the results obtained by former authors, it is evident that, although the parasites may, in all probability, multiply by division while on the fish, yet they eventually leave their host, swim around in the water for a time, become encysted, and run through successive divisions in the geometrical series 2, 4, 8, 16, etc., and reinfect fish as a minute body, invisible to the naked eye and about 30μ (estimated) long. As all experiments to kill the parasites while on the fish were unsatisfactory, it is evident that we still have a mode of prevention open to us in attacking the protozoan while it is free in the water. The problem of treatment now resolves itself into finding an inexpensive solution in which the fish can live until all the parasites have left the body, but the same solution must be fatal to the protozoa. The duration of life of the individual protozoan is a factor of considerable importance in this connection. It will be naturally expected that this will be subject to some variation and that no definite estimate can be made of it. Nor is it possible to directly observe how long one of the protozoa is able to live on the fish. In one of my experiments, however, a catfish was completely cleared of its parasites in about a week's time, while a trout was cleared in ten days' time, and these figures may form a basis for the estimation of the length of time it is necessary to subject the fish to treatment.

EXPERIMENTS.

A saturated salt solution kills the protozoa instantly, while the young trout, upon being transferred to it from fresh water, are killed in 10 to 40 seconds.

By placing salt at the bottom of a deep (about 4 feet) aquarium and allowing a constant supply of fresh water to run in with as little commotion as possible, we obtain a strong salt solution at the bottom, with a layer of fresh water at the top, while the water at different depths will be of different degrees of salinity. As the salt gradually dissolves, the fish seek the upper half of the aquarium as their abode, but frequently dive down into the briny water, sometimes even wallowing in the salt for an instant. The fish will live almost indefinitely in an aquarium arranged as just described, as has been demonstrated at the aquarium at the World's Fair, and elsewhere. As the parasites leave the fish they will, however, be killed upon reaching the salty water.

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| <p>1. Saturated salt solution. 22° C.</p> <p>2. Saturated salt solution. 22° C.</p> <p>3. Saturated salt solution, 50 cc.; lake water, 500 cc. 22° C.</p> <p>4. Saturated salt solution, 50 cc.; lake water, 1,000 cc. 22° C.</p> <p>5. 9:45 a. m. Lake water, 500 cc.; saturated salt solution, 1 cc. 22.8° C.</p> <p>9:46 a. m. Saturated salt solution added, 1 cc.</p> <p>9:47 a. m. Saturated salt solution added, 1 cc.</p> <p>9:48 a. m. Saturated salt solution added, 2 cc.</p> <p>9:53 a. m. Saturated salt solution added, 5 cc.</p> <p>9:59 a. m. Saturated salt solution added, 5 cc.</p> <p>10:02 a. m. Saturated salt solution added, 5 cc.</p> <p>10:03 a. m. Saturated salt solution added, 5 cc.</p> <p>10:05 a. m. Saturated salt solution added, 5 cc.</p> <p>10:06 a. m. Saturated salt solution added, 5 cc.</p> <p>10:07 a. m. Saturated salt solution added, 15 cc.</p> <p style="text-align: right;">Total saturated salt solution, 50 cc.</p> <p>6. Lake water, 1,000 cc.; saturated salt solution, 150 cc. 24° C.</p> <p>7. Lake water, 3,000 cc.; layer of ordinary salt on bottom of jar. 23.5° C.</p> | <p>Young trout (4 months old) overcome in 30 seconds; removed to fresh water, but did not recover. Parasites on fish for the most part alive. Those which fell from the trout during its rapid movements and struggles were killed instantly.</p> <p>Young trout (4 months old) affected instantly; killed in 10 seconds. Parasites on fish still alive.</p> <p>Young trout affected immediately; overcome in 10 seconds, but revived in fresh water. Parasites on fish still alive.</p> <p>Two young trout affected immediately; overcome in 10 seconds; revived in fresh water; placed again in solution No. 4; remained unaffected for 12 minutes, when they began to weaken; transferred to fresh water they revived again. Parasites on fish still alive; protozoa in water dead.</p> <p>9:45. Young trout placed in solution No. 5.</p> <p>Trout begins to weaken at 10:18, is gradually overcome, but revives in fresh water. Returned to same solution it is overcome in 40 seconds, but revives again in fresh water. Parasites on the fish are all alive, but those which have dropped into the water die instantly.</p> <p>(a) One trout overcome in 4 minutes.</p> <p>(b) One trout overcome in 5 minutes.</p> <p>(c) One trout overcome in 12 minutes.</p> <p>Parasites on fish alive.</p> <p>(a) One trout overcome in 15 seconds.</p> <p>(b) Five trout remain alive 4 hours and are then taken out.</p> <p>During experiment the fish frequently showed signs of weakening and were then removed temporarily to fresh water and returned to the solution immediately upon recovery. Parasites on fish still alive; those which fell into the water were killed.</p> |
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In the above experiments, except Nos. 1 and 2, fresh air was continually forced through the water.

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| 8. Large aquaria, 4 feet deep; about 25 pounds of salt placed in each aquarium. Fresh lake water was constantly running in at the surface, but no attempt was made to force air through the water. Experiment performed by the attendants of the aquarium. | Tanks contained badly infested trout and catfish. Most of them stood the test well, and after two weeks the infection was greatly lessened. Some of the fish, however, still had parasites. |
| 9. Methylene blue, 1 per cent aq. sol., 1 cc.; lake water, 200 cc. | (a) Trout alive at end of 2 minutes. Parasite also alive.
(b) Trout died in 18 minutes. |
| 10. Methylene blue, 1 per cent aq. sol., 10 cc.; lake water, 200 cc. | Trout alive; parasites unaffected at end of 5 minutes. |
| 11. Methylene blue, 1 per cent aq. sol., 20 cc.; lake water, 2,000 cc. | Trout unaffected at end of an hour. Parasites on fish still alive; those which escaped from the fish die in the solution. |
| 12. Methylene blue, 1 per cent aq. sol., 15 cc.; lake water, 500 cc. | Parasites, upon being freed from the fish and placed in this solution, died in 12 minutes. Trout were unaffected at the end of an hour. |

For experiments Nos. 9 to 12 no air-pressure was available; temperature varied from 22° C. to 24° C.

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| 13. Lake water, 10 L.; methylen blue, 1 per cent aq. sol., 30 cc.; constant aëration. 22° to 24° C. | Friday, 5 p. m. Five small trout badly infested, three of the fish very weak.
Saturday, 9 a. m. One trout dead.
Sunday, 9 a. m. One trout dead.
Monday, 9 a. m. One trout dead.
Wednesday, 9 a. m. Two catfish added. |
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Many of the parasites had fallen from the fish, and considerable scum had left them. This scum, together with the parasites, was colored blue by the methylen blue, and the solution was much weakened; 30 cc. 1 per cent methylen blue was added.

Friday, 9 a. m. One catfish dead; the trout had lost most of their parasites. Monday, 12 m. Trout entirely free from parasites; a few parasites left on the catfish.

From this experiment it is evident that methylen blue is a substance which may be used to kill the parasites. It is, however, fatal to the fish if used in too strong solution, or if the fish are very weak. Although this experiment does not justify the unqualified use of methylen blue, it is very suggestive as pointing out a line for future research and experiment.

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| 14. Eosin, 1 per cent aq. sol., 5 cc.; lake water, 500 cc. | Both fish (trout) and parasites alive after 10 minutes. |
| 15. Eosin, 1 per cent aq. sol., 15 cc.; lake water, 500 cc. | Trout unaffected after two hours; parasites on fish still alive; those which fell from the fish died. |
| 16. Lake water, 10 L. Eosin 1 per cent aq. sol., 60 cc. 23° C., constant aëration. | Friday, 5 p. m., 2 trout.
Monday, 9 a. m., 2 catfish.
Wednesday, 9 a. m., all alive. |
| 17. Eosin solutions used in experiments 14 to 16 kill the free protozoa in 15 to 60 minutes. | The parasites have lessened in number; accident to jar ends experiment. |

As the fish live very well in these solutions, eosin can be looked upon as another possible remedy for parasitical diseases of fish.

At the same time that the above 17 experiments were made, numerous other experiments were also carried on. Although the details of some of the other experiments will be given below, as of possible use to anyone who takes up this subject in the future, I am inclined to look upon the experiments thus far given as the most suggestive for future work. It was my intention to experiment with these three substances—salt, methylen blue, and eosin—on a much larger scale, but at this point a combination of circumstances prevented my carrying out the matter further.

In most of the experiments given below, it will be noticed that the solutions had little or no effect upon the parasites, or that the fish were affected in so short a time that it was impracticable to experiment further with the solutions named. Constant aëration was employed.

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| <p>18. Peroxide of hydrogen (H₂O₂), 1 cc.; lake water, 400 cc.
 19. H₂O₂, 2 cc. Sat. salt sol., 3 cc.; lake water, 1,000 cc. 34°C.
 20. H₂O₂, 1 cc. Sat. salt sol., 1 cc.; lake water, 500 cc. 24.8° C., 4:11 p. m.
 4:42 H₂O₂ added, 3 cc.
 4:45 Sat. salt sol., added, 3 cc.
 4:54 Sat. salt sol., 2 cc.
 5:00 H₂O₂, 1 cc.
 5:02 H₂O₂, 1 cc.
 5:15 Sat. salt sol., 1 cc.
 21. * Ker. emul., 100 cc.; lake water, 400 cc.
 22. Kerosene emulsion, 666 cc.; lake water, 1000 cc.
 23. Kerosene emulsion, 100 cc.; methylen blue, 4 cc.; lake water, 900 cc. 23° C.
 24. Ker. emul., 50 cc.; lake water, 500 cc. 23° C.
 11:39. Methylen blue, 1 cc.
 25. Kerosene emulsion, 30 cc.; lake water, 500 cc.
 26. Kerosene emulsion, 500 cc.</p> | <p>Small trout overcome in 17 minutes; parasites were unaffected.
 Small trout lived 1 hour and then gradually weakened; parasites were unaffected.
 4:11. Small trout.
 4:48. Small trout.
 5:16. Both fish slightly overcome but revived in water. Parasites on fish alive. Considerable scum had fallen from the fish and those protozoa which fell from the trout were killed.
 Small trout overcome in 3 minutes, recovered in water. Parasites unaffected.
 Small trout overcome in 22 min., revived in water.
 About half the parasites and considerable scum fell from fish, but the protozoa were unaffected.
 Small trout overcome in 2 minutes, revived in water. Parasites unaffected.
 10:36. Small trout.
 11:51. Some of the scum had fallen from trout.
 12:10. Trout slightly overcome; revive in water.
 12:20. Trout replaced in the solution.
 4:00. Still alive. Parasites on fish and in water were unaffected.
 Fish and parasites were unaffected after 3 hours; some of the scum was removed.
 (a) Trout overcome in 2 minutes.
 (b) Trout alive and well after 1 hour. Parasites unaffected. Some scum had fallen.</p> |
| <p>Kerosene cleans the fish to some extent, but has little or no effect upon the protozoa. The individual fish are affected very differently by the emulsion.</p> | |
| <p>27. Creosote, 1 drop; lake water, 200 cc.
 28. Creosote, 1:300 1 cc; lake water, 300 cc
 29. Vinegar 1 cc.; lake water, 200 cc.
 12:35. Vinegar added, 1 cc.
 30. Vinegar, 15 drops; lake water, 105 cc.
 31. Salycilic acid, $\frac{1}{2}$ gram; lake water, 200 cc.
 32. Permanganate of potassium, 1 minute crystal; lake water, 700 cc.
 33. Chlorinated soda sol., 2 cc; sat. salt sol., 5 cc.; lake water, 1,000 cc.
 34. Same as No. 33.
 35. Tannic acid, 1 cc.; lake water, 500 cc.
 36. Chl. soda sol., 2 cc.; salt sat. sol., 5 cc.; lake water, 500 cc.
 37. Same as No. 36.
 38. Seiler's tablets, 2; lake water, 2,000 cc.
 3:45. Seiler's tablets, 2.
 39. Ferrous sulphate (copperas), small crystal; lake water, 2,000 cc.</p> | <p>Trout overcome in 2 minutes but recovered in water. Parasites unaffected.
 Fish dies in 8 min., parasites alive at end of 3 hrs.
 12:30. Small trout.
 2:00 p. m. Trout died, parasites alive.
 Trout affected immediately; free parasites die in 2 minutes.
 Trout dies in 4 minutes; parasites unaffected.
 Trout lives 2 minutes; parasites unaffected.
 Small trout died in 3 minutes; parasites unaffected.
 Same result.
 Trout unaffected after 1 and 1½ hours; parasites unaffected.
 Trout overcome in 3 minutes; parasites alive.
 Trout overcome in 2 minutes; parasites alive.
 12:55. Small trout.
 4:23. Fish partially overcome; parasites on fish alive, those in the solution dead.
 11:43. Catfish.
 3 p. m. Fish dead; parasites in solution dead, those on the fish still alive.</p> |

* Two parts kerosene to 1 part milk.

40. Cupric sulph. sol. (blue vitriol), 15 drops; lake water, 2,000 cc. 24-30.	Fish dies in 7 minutes; parasites unaffected.
41. Cupric sulphate, 1 cc.; lake water, 200 cc. 3:36. Cup. sul., 1 cc. 3:38. Cup. sul., 1 cc.	3:34. Small trout. 3:42. Trout overcome; revived in water. 3:56. Returned to solution. 4:10. Dead; parasites unaffected.
42. 3:06. Potassium ferrocyanide, 1 per cent, 3 cc.; lake water, 700 cc. 3:12. Pot. fer., 3 cc. 3:15. Pot. fer., 9 cc. 3:20. Pot. fer., 30 cc.	Trout lived 3 hours. Parasites unaffected.
43. 10:24. Pepsin, 1 teaspoonful; lake water, 1,000 cc. 10:35. Sat. salt sol., 5 cc. 10:50. Sat. salt sol., 5 cc. 2:30. Salt sol., 5 cc.	Trout died in 3 minutes. Parasites still alive. 10:55. New trout. Trout overcome at 3:00. Slime leaves fish to some extent. Parasites unaffected.

This experiment was tried with the hope of removing the slime from the fish by means of the pepsin, and thus reaching the parasites with the salt. The experiment was repeated a number of times, but the results were extremely variable.

44. Ichthyol, 7 drops; lake water, 500 cc. 23.5° C.	3:55. Catfish. 4:32. Partially overcome and removed to fresh water; parasites alive.
45. Ichthyol, 3 drops; lake water, 3,000 cc. 35° C.	9:55. Weak catfish. 10:30. Fish died. 11:07. Second catfish. 11:29. Parasites in water alive.
11:30. Ichthyol, 6 drops. 11:45. Ichthyol, 6 drops.	
12:01. Salt sol., 15 cc.	12:00. Parasites in water alive.
3:16. Ichthyol, 9 drops; salt sol., 15 cc.	3:15. Parasites on fish alive; those in solution dead.
46. Methyl violet, 2 cc.; kerosene emulsion, 20 cc.; lake water, 2 L., 23° C.	4:45. Second fish partially overcome; died in fresh water; parasites on fish still alive. (a) 11:23. Trout. 11:44. Overcome; revived in water. 11:50. Returned to solution. 2:20. Dead; parasites on fish and many of those in the solution are still alive. (b) 2:25. Trout. 3:45. Fish dies; parasites unaffected.

It need hardly be stated that most of the above experiments were repeated two or three times. When the result was similar and unsatisfactory on two or three different fish, the experiment was discontinued.

SUMMARY.

The following may serve as a brief summary of the foregoing studies upon this parasitic disease:

(1) Two factors in particular, a protozoan parasite and the high temperature of the water, caused considerable loss among the fresh-water fish in the aquaria of the U. S. Fish Commission at the World's Fair in Jackson Park, Chicago. Although some of the fish of the aquarium were infested with fungi, the latter were not present upon many of the fish which I examined.

(2) This ciliated protozoan, belonging to the genus *Holophrya* (*Ichthyophthirius*), was imported by young catfish (*Ameiurus albidus*), and after a time spread to other fish.

(3) The species observed is evidently identical with Fouquet's *Ichthyophthirius multifiliis*.

(4) From a study of its morphology and reproduction, it agrees almost equally well with Zacharias's *I. cryptostomus*, and as the characters upon which his species is founded appear to me to be open to question, I am led to doubt, although not to positively deny, the validity of his species.

(5) *I. multifiliis*, as observed at the World's Fair, may multiply by simple division, or by division into numerous small ciliates after becoming encysted. The two modes of reproduction are, however, not sharply separated, as numerous gradations may be observed in this species. This reproduction is not confined to the night time, as Kerbert supposed.

(6) The encystment may take place either on the fish or after the parasite leaves the fish.

(7) The cyst-membrane may appear before division, or at any period up to and including the 16-cell stage.

(8) It is impracticable to try to kill the parasites while they are on the fish, for liquids endurable by the fish will not penetrate the slime in which the parasites lie.

(9) Experiments to digest this slime with pepsin or to wash it off with kerosene emulsion did not meet with sufficient success to warrant recommendation at present.

(10) The most practical method of destroying the parasite is to attack it during its free stage prior or subsequent to encystment, or during its encysted stage.

(11) The encysted stage lasts, according to my observations, about a day; according to Kerbert and others, from $2\frac{1}{2}$ to 4 days.

(12) The young cells resulting from the division during encystment swim around in the water and then attack new hosts.

(13) Placing salt in the bottom of the aquarium and allowing a constant supply of fresh water seems at present to be the most feasible method of treatment.

(14) Very weak solutions of methylen blue and eosin give good results, but have the disadvantage of coloring the water. Fish can live in these solutions for a number of days, but the parasites which leave the fish to reproduce are killed in a few minutes. If these solutions are used, aëration must be resorted to.