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Observations of the Enteric Protozoa of *Rana pipiens* during Larval Development and Metamorphosis[1]

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Introduction

The existence of protozoa in the intestine of frogs has been known since the observations of van Leeuvenhoek, 1683. Nearly two centuries elapsed before any considerable advance in the study of intestinal protozoa was made. The observations of Dobell (1909) initiated the modern phase in the investigation of the enteric protozoa of amphibia. In this work, on the fauna of frogs and toads, many of the species now recognized were described.

Numerous investigators since Dobell have added greatly to our knowledge of the protozoa of amphibia. Observations of a morphological and taxonomic character have predominated. Of these only a few of the more pertinent works can be cited. Alexeieff (1911) described *Trichomonas augusta* and distinguished it from *T. batrachorum*. More recently Samuels (1941), Honigberg (1951, 1953) and Buttrey (1954) have published a number of detailed morphological studies of trichomonad flagellates from amphibia. Bishop has made observations on *Trichomonas batrachorum* (1931), *Monocercomonoides* (1932) and *Chilomastix* (1935) from anurans. The small diplozoa of amphibia await critical investigation but Wenrich (1935) has provided figures of the species that occur in *Rana pipiens*. Hegner (1922) has redescribed *Giardia agilis*. Hegner (1923) and Wenrich (1924) have described *Euglenamorpha* from tadpoles of North American frogs. Becker (1925, 1928) has investigated *Mastigina* from tadpoles of *Rana*. Sanders (1931) has worked out the life-cycle of *Entamoeba ranarum*. Higgins (1929) studied variation in *Nyctotherus* and Wichterman (1937) described division and conjugation in this genus. Metcalf (1923 and 1940) has published monographs on opalinid ciliates. Walton (1949) has published a list of protozoa reported from *Rana pipiens*.

Few investigations of the enteric protozoa of amphibia have been carried out from an ecological or physiological point of view. Hegner (1923) made a study of the relations between the intes-

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tinal protozoa of frog and toad tadpoles and the food of the host. He found that certain protozoa, at one time were numerous but later disappeared completely, while other forms persisted through metamorphosis from tadpole to adult. Hegner concluded that faunal changes may result from changes in the diet of the host. Cairns (1953) carried out transfaunation experiments which demonstrated, in most cases, a lack of host-specificity of the enteric protozoa of amphibia.

It is the purpose of this paper to present data on the intestinal fauna of *Rana pipiens* of the Lake Okoboji region in Dickinson County, Iowa. Bradley (1941) has made a protozoological survey of frogs of this region. In the present study emphasis is placed upon the correlation of developmental stages of the host with the occurrence of parasite species.

Materials and Methods

Rana pipiens used in this study were secured from several ponds near the Iowa Lakeside Laboratory in Dickinson County in Northwestern Iowa. The locations of the ponds are shown on the map (Figure 1).

Animals were collected, usually three times per week from mid-June to the latter part of August in 1952 and 1953. Some additional animals were collected in 1954. After capture, animals of a particular collection were segregated on the basis of size; animals of approximately the same size were put together in containers where they remained until examined. Usually, hosts were examined on the same day they were collected.

Hosts were killed by evisceration. The alimentary tract was uncoiled and divided, at regular intervals, into a number of parts. Samples of the gut contents were taken from each part, placed in a drop of 0.6% saline on a slide and observed under the low power of the microscope to detect the presence of parasites. In the young frog the rectum appears, and subsequently it is within the fundus of this organ that the largest number of parasites occurs. The rest of the tract is practically devoid of animals with the occasional exception of a few flagellates, both endozoic and free living.

After examination the hosts were tagged and then staged by the method of Taylor and Kollros (1946). According to developmental characteristics these Taylor-Kollros stages were divided into four groups: (I) the limb bud stages, 1-5 (only stages 3-5 were collected); (II) the foot paddle stages, 6-10; (III) the premetamorphic stages, 11-17; (IV) the metamorphic stages 18-25. At least five animals (in some cases many more) were examined from each stage, 3-25.

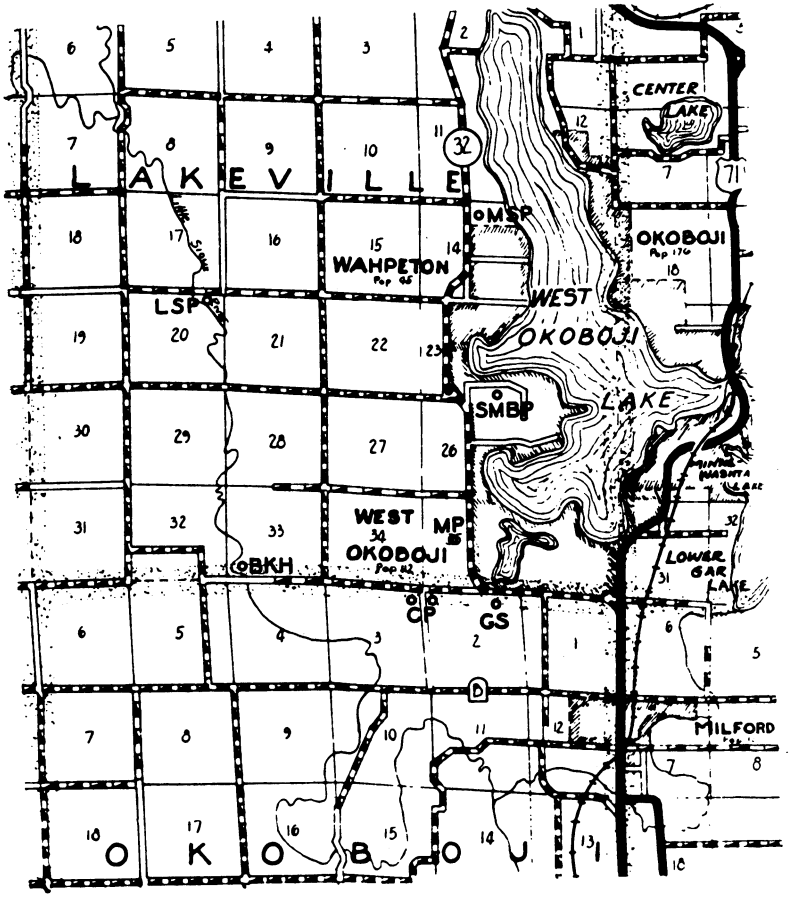

Figure 1. Map showing location of collecting areas (reproduced from highway map of Dickinson County, Iowa): S.M.B.P. = South Miller's Bay Pond; C.P. = Crossroads Ponds; G.S. = Gerlach's Slough; M.S.P. = Manhattan Slough Pond; B.K.H. = Big Kettle Hole; L.S.P. Little Sioux Pond; M.P. = Midway Pond.

Xenopus laevis tadpoles raised from eggs laid by adults of a stock maintained at the Zoology Laboratory of the State University of Iowa, were used to test the host-specificity of the protozoan fauna of *Rana pipiens*. Twelve larvae that had been reared together were paired for size and isolated. One member of each pair served as a control and the other individual was fed material from the alimentary tract of *Rana pipiens* tadpoles. Preliminary examination of material to be fed experimentally was made to insure that parasites were present in considerable quantity. Examinations were made on both the control and the infected *Xenopus* tadpoles one week after the experimental feeding. Slides were made

of the gut contents of infected animals and careful examinations were made to identify the organisms present.

More than 500 animals were examined in this investigation and observations were made on living material as well as fixed and stained slides. The preserved material consists of more than a thousand slides fixed in Schaudinn's or Hollande's fixatives or in fumes of osmic acid and stained with either Mayer's haemalum, Heidenhain's iron-haematoxylin or by the protein-silver method of Moskowitz (1950). Slides stained in iron-haematoxylin were differentiated with either 2% iron or 2% phosphotungstic acid. Best staining results were obtained with Heidenhain's iron-haematoxylin and with Mayer's haemalum.

The smaller endozoic protozoa can be reliably diagnosed only with stained material. The results to be reported are based upon the analysis of such material from 211 hosts. Diagnosis is based upon the morphological and taxonomic studies cited above. A host is considered to be infected when a single organism of unquestionable identity is found in material from that host. Few diagnoses depend upon single organisms. Organisms, when present, are usually found repeatedly.

Results

In the present study 15 species of enteric protozoa have been found. Of these *Mastigina hylae, Chilomastix* sp., *Trichomonas augusta, Opalina obtrigonoidea* and *Nyctotherus cordiformis* were found in hosts from each of the ponds from which collections were made. *Endolimax ranarum* and *Euglenamorpha* were found infrequently in only one pond and *Giardia agilis* was found in only two tadpoles from two ponds. The other protozoa, though not found in all ponds had a wide distribution.

Table 1 records the occurrence of 15 protozoan species in developmental stages 3-25, and indicates the percentage of host animals possessing specific parasites in each of the developmental stages of the host. The ciliates, *Opalina obtrigonoidea,* and *Nyctotherus cordiformis* are the only forms which were represented in every stage.

Seven protozoa have a high incidence in the digestive tract of *Rana pipiens*: *Trichomonas batrachorum, Trichomonas augusta, Hexamitus intestinalis, Octomastix* sp., *Entamoeba ranarum, Opalina obtrigonoidea,* and *Nyctotherus cordiformis* (see Table I). *Mastigina hylae* and *Chilomastix* sp. are present in a high percentage of tadpoles until the onset of metamorphosis. Other species appear to be of more sporadic occurrence.

Stages 1 and 2 of *Rana pipiens* were not collected because these stages were no longer available in the ponds at the beginning of the summer sessions at the Iowa Lakeside Laboratory. Observa-

Table I.

Percentage Distribution of Parasites in Developmental Stages of Host.

	Limb bud stages				Foot Paddle stages			
Host Stages	3	4	5	6	7	8	9	10
Number Examined	5	6	9	7	10	7	6	10
Euglenamorpha hegneri	0	16.6	11.1	14.3	0	0	0	0
Mastigina hylae	60	66.6	77.7	14.3	50	11.1	83	30
Monocercomonoides sp.	0	0	11.1	14.3	0	0	16.6	10
Chilomastix sp.	80	49.8	88.8	71.5	90	44.4	83	80
Trichomonas batrachorum	0	16.6	44.4	85.8	60	11.1	16.6	20
Trichomonas augusta	0	33.2	44.4	100	50	66.6	49.8	60
Hexamitus intestinalis	100	83	77.7	100	90	66.6	83	100
Urophagus sp.	20	16.6	33.3	42.9	10	22.2	33.2	20
Octomitus sp.	0	0	0	0	10	11.1	33.2	10
Octomastix sp.	80	83	33.3	85.8	60	44.4	66.4	80
Giardia agilis	20	0	0	0	0	0	0	0
Endolimax ranarum	0	0	11.1	14.3	10	0	33.2	10
Entamoeba ranarum	60	49.8	77.7	28.6	50	44.4	83	60
Opalina obtrigonoidea	100	83	88.8	57.2	80	55.5	100	90
Nyctotherus cordiformis	100	33.2	55.5	57.2	40	33.3	66.4	60

Table I.—Continued

Percentage Distribution of Parasites in Developmental Stages of Host.

	Premetamorphic stages							Metamorphic stages							
Host Stages	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Number Examined	8	13	10	10	5	6	12	10	15	6	5	9	5	5	32
Euglenamorpha hegneri	0	0	0	0	0	0	33.2	50	6.6	0	0	0	0	0	0
Mastigina hylae	75	30.8	50	60	60	49.8	58.1	50	19.8	33.2	20	0	0	0	0
Monocercomonoides sp.	25	0	10	10	20	33.2	16.6	10	6.6	0	0	0	0	0	6.2
Chilomastix sp.	75	100	90	80	80	100	74.7	80	72.6	66.4	20	0	0	0	0
Trichomonas batrachorum	37.5	30.8	40	10	0	49.8	33.2	60	13.2	16.6	20	11.1	20	20	31.2
Trichomonas augusta	62.5	38.5	60	50	60	66.4	41.5	80	39.6	16.6	40	0	80	20	53.0
Hexamitus intestinalis	75	92.4	90	80	100	100	83	80	79.2	86.4	40	0	20	0	15.6
Urophagus sp.	0	23.1	0	10	0	16.6	8.3	30	6.6	16.6	0	0	0	0	3.12
Octomitus sp.	12.5	0	20	10	0	0	0	0	13.2	0	20	0	0	0	0
Octomastix sp.	87.5	92.4	60	80	60	83	74.7	80	79.2	16.6	40	0	40	0	53.0
Giardia agilis	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0
Endolimax ranarum	0	7.7	0	0	0	16.6	0	0	0	0	0	0	0	0	0
Entamoeba ranarum	25	30.8	50	40	60	66.4	41.5	50	33	33.2	20	0	20	20	18.7
Opalina obtrigonoidea	100	84.7	80	100	100	100	83	90	92.4	83	100	100	100	100	68.6
Nyctotherus cordiformis	53.9	50	90	80	66.4	66.4	83	50	79.2	83	80	88.8	80	100	53.0

EXPLANATION OF TABLE

Table I shows the distribution of parasites in the various developmental stages of *Rana pipiens*. The occurrence of specific parasites is indicated by the percentage of hosts found to be infected in each developmental stage.

tions on material from limb bud stages 3, 4 and 5 indicate that the protozoan fauna characteristic of anurans normally is acquired early in larval development. All of the parasites listed with the exception of *Octomitus* were found in the limb bud stages 3 through 5. Only *Giardia,* of infrequent occurrence in the present study, was absent in developmental group II, the foot paddle stages 6 through 10; the remainder of the parasites with the exception of *Euglenamorpha,* were found in at least two of these foot paddle stages. *Giardia* was conspicuously absent in developmental group III, the premetamorphic stages 11 through 17; *Euglenamorpha* was present in only one of these stages; *Octomitus* and *Endolimax* were present in two stages; all additional parasites were well represented in the different stages of this group. In developmental group IV, the metamorphic stages 18 through 25, *Endolimax ranarum* was the only form not represented; however *Giardia* and *Euglenamorpha* were present in only one and two of these stages respectively (see Table I).

For most parasites, incidence records for hosts of stages 22 show a sharp drop in the percentage of hosts infected (Table I). In this stage even when incidence is high, as in the case of *Opalina* and *Nycototherus* the number of parasites present in a particular host may be small. The conditions found in stage 22 appear to carry over through stages 23 and 24. Only a few species of parasites are found in these stages and again the numbers of organisms present in a host is usually small. These stages (22, 23, 24) seem to be periods of "difficulty" experienced by the fauna: some disappear and do not reappear; some disappear, or become so reduced in numbers that their presence is not detected, and reappear as a result of either multiplication or reinfection; a few though reduced in number, persist throughout development of the host. *Euglenamorpha, Mastigina* and *Giardia* are three forms that disappear and do not reappear, i.e. they are not known to occur in adult *Rana pipiens. Chilomastix, Octomitus* and *Endolimax* seemingly disappear during metamorphosis. Since these genera are occasionally found in adult frogs, either infection is reacquired or a few organisms may survive in some hosts. The juvenile frogs of stage 25 exhibited faunas similar to those normally found in adult frogs.

The following six protozoa were successfully transfaunated to *Xenopus laevis* tadpoles and thus do not appear to be host specific for *Rana pipiens*: *Euglenamorpha, Trichomonas batrachorum, Trichomonas augusta, Hexamitus intestinalis, Octomastix* sp., and *Nyctotherus cordiformis.* The experimental infections of *Nyctotherus* in *Xenopus* larvae were light; the other experimental infections were comparable to those in *Rana* tadpoles. None of the above listed animals were present in the controls, however, *Protoopalina*

was present in both experimental and control *Xenopus* larvae. *Protoopalina* occurs in the parent *Xenopus* stock. Cysts from the parents apparently contaminated the eggs and were ingested by the larvae early in their development. *Opalina* from *Rana pipiens* failed to establish itself in *Xenopus*.

Opalina, the most common ciliate in *Rana pipiens,* varies considerably in size and form in the developing tadpoles as well as in the juvenile (recently metamorphosed) frog. The body form varies from rounded to ellipsoid, short tailed to long tailed, triangular tailed forms to triangular tailless forms, and short slender forms to elongate slender forms (Plate I). It was found that in developmental group I, stages 3-5, rounded, ellipsoid, short tailed and elongate tailed forms were present. Triangular forms were not seen in this developmental group, however they appear in stage 8. These tailless triangular forms are in addition to the other forms of opalinids found earlier in the development of the host. The opalinids found in stage 8 are definitely larger than those observed in the preceeding stages. The triangular tailed forms are seen for the first time in group III. Group III, the premetamorphic stages (11-17), and group IV, the metamorphic stages (18-25), possessed all of the different forms of opalinids found (Plate I). The elongate opalinids may be straight or slightly curved, plicate or twisted in the different hosts. These conditions are often striking in fresh material but seldom retained after fixation.

Nyctotherus, in developing *Rana pipiens* also exhibits morphological variability. Some of the animals are wide at the posterior end making an egg shaped body, others have at the posterior end a projecting tip which may be thought of as a tail. Some of these forms are twice as long as broad while others are more rounded to ellipsoid in shape. Tailed *Nyctotherus* appear first in stage 20 and are found in all of the remaining five stages.

Some of the variability of Nyctotherus may be correlated with the phenomena of fission and conjugation, (Wichterman 1937). Numerous stages of binary fission, conjugation and macronuclear re-organization have been observed. The author has not been able to find cysts.

Discussion

The results presented above clearly demonstrate that the protozoan fauna of *Rana pipiens* is acquired early by the developing host (see Table I). Young tadpoles typically harbor a large and mixed assemblage of protozoa. The fauna of tadpoles, however, is apparently influenced markedly by metamorphosis: a time of change in the morphology and physiology of the host. It is at this time that endozoic protozoa are drastically reduced in numbers. Some species are eliminated completely (i.e. they are not found in the juvenile or adult frog). Examples of these forms are *Euglena-*

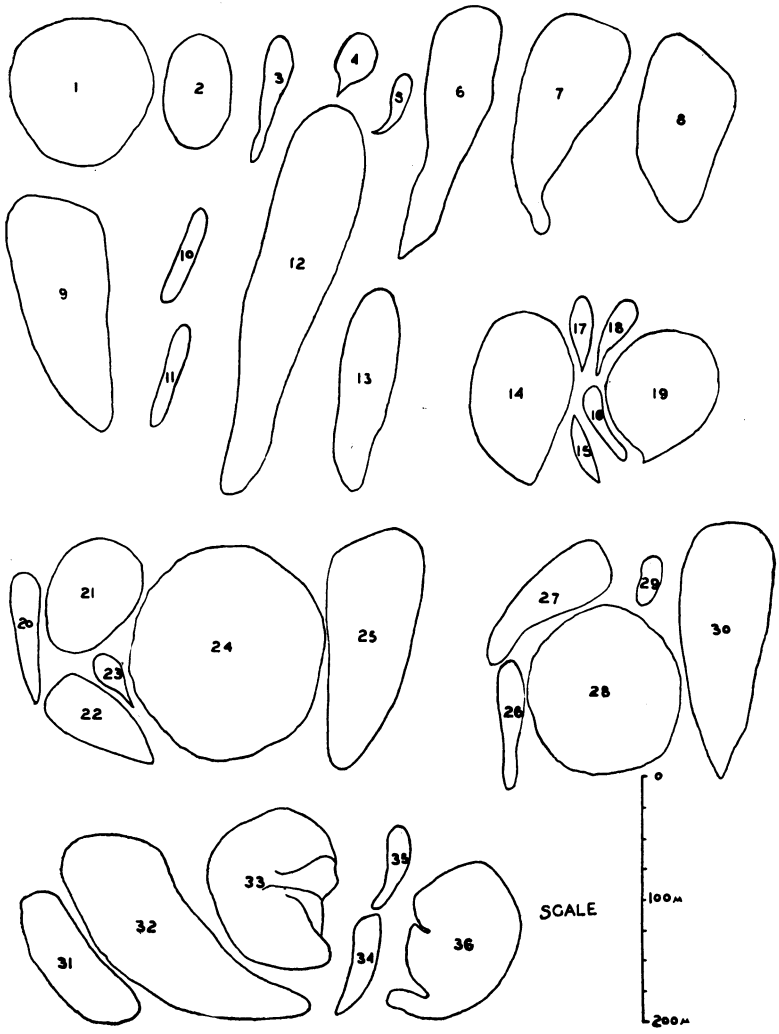

Plate I. Outline drawings of opalinids from *Rana pipiens,* drawn, from prepared slides, with aid of camera lucida.

Figure 1-13 show the variations in size and form of the opalinids found in *Rana pipiens* during development: (1) rounded; (2) ellipsoid; (3) elongate, tailed; (4, 5) short, tailed; (6) elongate; (7) triangular, tailed; 8, 9) triangular, tailless; (10 11) small, elongate, slender; (12, 13) large, elongate.

Figure 14-19 are typical forms found in tadpoles of group I, developmental stages 3 through 5.

Figure 20-25 are typical forms found in tadpoles of group II, developmental stages 6 through 11.

Figure 26-30 are typical forms found in tadpoles of group III, developmental stages 12 through 17.

Figure 31-36 are typical forms found in tadpoles of group IV, developmental stages 18 through 25.

morpha, Mastigina and *Giardia.* Other forms are either eliminated and later reestablished after metamorphosis or they are reduced in numbers to the point that their presence is not detected in metamorphosing tadpoles i.e. they are not found in stages 22 through 24, but known to occur in the adult. Among such forms are *Monocercomonoides, Chilomastix, Urophagus, Octomitus* and *Endolimax.*

Several genera, (*Chilomastix, Endolimax, Opalina*), warrant some additional comment. Bishop (1935 made a study of *Chilomastix* from *Bufo vulgaris* and considered the possibility that both *C. caulleryi* and *C. aulastomi* occur in amphibia. She attempted to separate the two species on the basis of differences in the division process. These differences have not been observed in the present study, however, populations of *Chilomastix* encountered in this survey have shown morphological variation sufficient to suggest that more than one species may occur in *Rana pipiens.* The solution of this problem will require detailed morphological study probably under controlled environmental conditions.

Organisms comparable to *Endolimax ranarum* (Epstein and Ilovaiski, 1914) Wenyon, have been found. The identification of these small amoebae as *Endolimax* follows a practice common among protozoologists, but in the absence of cytological analysis of division such an identification should be considered as tentative.

On the basis of Metcalf's work (1923), the opalinids of *R. pipiens* are identified as *Opalina obtrigonoidea.* Metcalf (1923, 1926, 1940) found that opalinids of tadpoles differ from those of the adult. He is of the opinion that in *Rana clamitans* and *R. catesbeiana* a chain of larval stages occurs. To certain of the opalinids of tadpoles Metcalf has applied the name *Opalina larvarum. Opalina* of the *larvarum* type have been reported *from* tadpoles of *R. pipiens* (Metcalf, 1940). The author of the present study has found in the opalinids of larval *Rana pipiens* morphological variations that may well be developmental stages. However it is not clear what relationship these various forms bear to the opalinids found in adult frogs. The author recognizes several alternative explanations It is possible that cysts picked up by the tadpoles release rounded individuals that under-go a series of changes in form which might account for the variations in shape of the opalinids found in the different stages of the developing host. An alternative presumes that the tadpoles pick up cysts which release either rounded or elongated forms depending upon the type of cyst ingested. This possibility suggests that more than one species of opalinid may occur in tadpoles. If an assumption of a specific diversity is made one may speculate further that the rounded and

elongate forms may not be related to the triangular forms. The resolution of this problem will probably require an experimental approach.

The results of the transfaunation of organisms from *Rana pipiens* to *Xenopus laevis* are in agreement with Cairns' demonstration (1953) of a lack of host-specificity for many of the enteric protozoa of amphibia and support the assertion of Wenrich (1953) that species of parasites will presumably establish themselves within hosts in which they find favorable conditions for survival.

Summary

I. *Rana pipiens* tadpoles and frogs were collected from ponds located in the vicinity of the Iowa Lakeside Laboratory in Dickinson County, Iowa. These hosts were examined for enteric protozoa and the occurrence of parasites was correlated with the development stages of the hosts.

II. Protozoa most commonly found in *Rana pipiens* tadpoles were *Opalina obtrigonoidea* and *Nyctotherus cordiformis.* Common organisms (i.e. those found in almost every developmental stage) were *Mastigina hylae, Chilomastix* sp., *Trichomonas batrachorum, Trichomonas augusta, Hexamitus intestinalis, Octomastix* sp. and *Entamoeba ranarum.* Less common species were *Euglenamorpha hegneri, Monocercomonoides* sp., *Urophagus* and *Octomitus* sp., species rarely encountered were *Giardia, agilis* and *Endolimax ranarum.*

III. Organisms which most frequently survived metamorphosis were *Trichomonas batrachorum, T. augusta, Hexamitus, Octomastix, Entamoeba, Opalina* and *Nyctotherus.* Protozoa not found to survive metamorphosis were *Euglenamorpha, Mastigina, Chilomastix* sp., *Octomitus, Giardia,* and *Endolimax.*

IV. *Euglenamorpha, Trichomonas batrachorum, T. augusta, Hexamitus, Octomastix* and *Nyctotherus* are not host specific for *Rana pipiens.* The preceeding protozoa were successfully transfaunated to *Xenopus laevis* tadpoles.

Bibliography

Alexeieff, A., 1911, Sur la spécification dans le genre *Trichomonas* Donné. Compt. rend soc. biol. 71:539-541.

Becker, E. R., 1925. Morphology of *Mastigina hylae* Frenzel from the intestines of the tadpole. J. Parasitol. 11:213-216.

————, 1928. Streaming and polarity in *Mastigina hylae* Frenzel. Biol. Bull. 54:109-116.

Bishop, Ann, 1931. The morphology and method of division of *Trichomonas.* Parasitology. 23:129-156.

————, 1932. A note upon *Retortamonas rotunda* n. sp., an intestinal flagellate in *Bufo vulgaris.* Parasitology. 24:233-237.

————, 1935. Observations upon *Chilomastix* from *Bufo* vulgaris with notes on *Chilomastix aulastomi.* Parasitology. 24:507-519.

Bradley, William, 1941. A protozoological survey of frogs of the Okoboji Region. Thesis—State University of Iowa Library.

Buttrey, B. W., 1954. Morphological variations in *Tritrichomonas augusta* Alexeieff) from amphibia. J. Morphol. 94:125-164.
Cairns, John J., 1953. Transfaunation studies on the host-specificity of the enteric protozoa of amphibia and various other vertebrates. Proc. Acad. Nat. Sci. Phila. 105:45-69.
Dobell, C. C., 1909. Researches on the intestinal protozoa of frogs and toads. Quart. J. Microscop. Sci. 53:201-277.
Epstein, H. J. and Ilovaiski, S. A., 1914. Researches on parasitic amoebae. (In Russian). J. Soc. Amis Sci. Nat., Anthrop., Ethnogr. (Sect. Zool.) Moscow. n.s. 2:1-55 (cited from Wenyon, 1926).
Hegner, R. W., 1922. The systematic relationship of *Giardia lamblia* Stiles, 1915, from man and *Giardia agilis* Kunstler. Am. J. Hyg. 2:435-441.
————, 1923. Observations and experiments on Euglenoidina in the digestive tract of frog and toad tadpoles. Biol. Bull. 45:162-180.
Higgins Helen T., 1929. Variations in *Nyctotherus* (Protozoa Ciliata) found in frog and toad tadpoles and adults. Trans. Am. Microscop. Soc. 68:141-157.
Honigberg, B. M., 1951. Structure and Morphogenesis of *Trichomonas prowazeki* Alexeieff and *Trichomonas brumpti* Alexeieff. Univ. Calif. Publs. Zool. 55:337-394.
————, 1953. Structure, taxonomic status, and host list of *Tritrichomonas batrachorum* (Perty). J. Parasitol. 39:191-208.
Metcalf, M. M., 1923. The Opalinid Ciliate Infusorians. Smithsonian Institution, U. S. Natl. Museum, Bull. 120.
————, 1926. Larval stages in a protozoan. Proc. Natl. Acad. Sci. U. S. 12:734-737.
————, 1940. Further studies on the opalinid ciliate infusorians and their hosts. Proc. U. S. Natl. Museum. 87:465-634.
Moskowitz, N., 1950. The use of protein silver for staining Protozoa. Stain Tech. 25:17-20.
Samuels, R., 1941. The morphology and division of *Trichomonas augusta* Alexeieff. Trans. Am. Microscop. Soc. 60:421-440.
Sanders, Elizabeth P., 1931. Life cycle of *Entamoeba ranarum* Grassi (1879). Arch. Protistenk. 74:365-371.
Taylor, A. C. and Kollros, J. J., 1946. Stages in the normal development of *Rana pipiens* larvae. Anat. Rec. 94:7-18.
Walton, A. C., 1949. Parasites of the Ranidae (Amphibia) XIV Trans. Illinois State Acad. Sci. 42:161-164.
Wenrich, D. H., 1924. Studies on *Euglenamorpha hegneri* n.g., n. sp., a euglenoid flagellate found in tadpoles. Biol. Bull. 47:149-174.
Wenrich, D. H., 1935. Host parasite relations between parasitic protozoa and their hosts. Proc. Am. Phil. Soc. 75:605-650.
Wenyon, C. M., 1926. Protozoology. Bailliere Tindall and Cox, London.
Wichterman, R., 1937. Division and conjugation in *Nyctotherus cordiformis* (Ehr.) Stein (Protozoa, Ciliata) with special reference to the nuclear phenomena. J. Morphol. 60:563-611.

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