ECTOSYMBIONTS OF SELECTED LARVAL ANURANS AND AQUATIC URODELES FROM KALAMAZOO AND BARRY COUNTIES, MICHIGAN

Thesis for the Degree of M. S.

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ECTOSYMBIONTS OF SELECTED LARVAL ANURANS AND AQUATIC URODELES FROM KALAMAZOO AND BARRY COUNTIES, MICHIGAN

By

Sigurd Nelson, Jr.

AN ABSTRACT OF A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

ECTOSYMBIONTS OF SELECTED LARVAL ANURANS AND AQUATIC URODELES FROM KALAMAZOO AND BARRY COUNTIES, MICHIGAN

by Sigurd Nelson, Jr.

Larval anurans and aquatic urodeles were collected from various habitats in Kalamazoo and Barry Counties, Michigan and examined for ectosymbiotic animals. An attempt was made to establish habitat preference and specificity of the various symbionts.

Amphibians were collected by means of a dip net or by trapping, isolated in containers and removed to the laboratory. Prior to microscopic examination, the amphibians were anesthetized with MS 222, which reduced undue activity without resulting in death to the animals. A record of the symbionts' attachment site, and relative abundance was made. Permanent slides, microphotographs and whole-mount preservations were prepared of ectosymbionts.

The most abundant ectosymbiont recorded was the peritrichous ciliate Trichodina sp. Trichodina sp. occurred on all larval anurans except Bufo sp. Necturus maculosus was the only urodele found with Trichodina sp. Other peritrichous ciliates were less frequent.

Sigurd Nelson, Jr.

Testacean sarcodines, not previously reported in the literature, were found on larval anurans at three collection stations.

Necturus maculosus served as the host for Trichodina sp., Sphyranura sp. (Monogenea), and clam glochidia.

Leeches were found on both aquatic urodeles and larval anurans.

No evidence was discovered indicating a specific ectosymbiont to be limited by habitat.

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Mrs. Bernadette Henderson has my sincere thanks for help in providing materials necessary for this study.

Illustrations of ectosymbionts were made by Richard Burbidge.

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ECTOSYMBIONTS OF SELECTED LARVAL ANURANS AND AQUATIC URODELES FROM KALAMAZOO AND BARRY COUNTIES, MICHIGAN

This study is based on the ectosymbiotic animals found on larval anurans and aquatic urodeles. Adult anurans and terrestrial urodeles are excluded. Lentic habitats were selected in Barry and Kalamazoo Counties located in S.W. Michigan in an attempt to relate habitat preference as well as host specificity of the symbionts. Ecological notes related to habitat selections are incorporated as well as some limnological data. The symbionts are presented qualitatively in a phylogenetic sequence with little attempt at a quantitative analysis.

Symbiosis involves the interaction of two or more species, one usually larger than the other. A symbiotic relationship may be subdivided into such categories as parasitism, commensalism and mutualism. Arbitrary limits must be used to distinguish the above forms of symbiosis as no distinct line separates them.

Parasitism is limited to those forms living on or within a host species. The host must be of a different species and larger than the parasite. In all instances, the parasite derives benefit at the host's expense. Rapid destruction of the host does not occur as in the

case of the predator-prey relationship. Gradual degradation of the host, in part or as a whole, is the rule.

Commensalism is limited to forms receiving benefit from a host species without causing harm, whereas mutalism involves the exchange of benefits between two or more symbionts.

Unless direct evidence is presented, either by observation of tissue damage or by literature citation, the symbiotic relationship is considered parasitic. Due to difficulty in ascertaining whether a host receives benefit, any mutualistic association may arbitrarily be considered as commensalistic.

An ectosymbiont on larval anurans and aquatic urodeles is any symbiont located on the external surface of such amphibians. Those forms frequenting body orifices, but generally found on the body surface are included. Subcutaneous forms are deleted.

A review of the literature indicated that a total of five phyla was represented as ectosymbionts of Amphibia. Included are Protozoa, Platyhelminthes, Annelida, Arthropoda and Mollusca.

Phylum: Protozoa
Classification after Kudo (1953)

Class: Mastigophora

Order: Polymastigina

Family: Tetramitidae

According to Wenrich (1924a), the ectozoic flagellate, Costia necatrix (Henneguy), occurred both on the skin and gills of Rana catesbeiana Shaw, R. clamitans Latreille and R. palustris Le Conte. The association did not appear to cause any serious pathological results although they did attach themselves to the skin and gills.

Class: Ciliata

Order: Holotricha

Family: Amphileptidae

Wenrich (1924b) described the protozoan Amphileptus branchiarum on the gills of Rana catesbeiana, R. clamitans and R. palustris. The former two frogs were collected in the vicinity of Philadelphia, while R. palustris was reared in the laboratory. A. branchiarum, according to Wenrich (1935), perhaps represents a transition between a predaceous status and a parasitic one. A. branchiarum has a free-swimming stage during which it roams about over the gills of the host tadpoles and if, while doing so, it meets an ectozoic Vorticella sp. or Trichodina sp. it may

indulge its predaceous tendencies by devouring one of them. At other times, and more commonly, it attaches itself to the gills by a thin membrane within which it gently swims in a rotating manner, pausing at appropriate times to engulf masses of gill cells to satisfy its food requirements.

Order: Peritricha

Family: Scyphidiidae

Scyphidia sp. was reported by Wenrich (1924a) on the gills of Rana clamitans and Hyla versicolor Le Conte at Woods Hole, Massachusetts. Wenrich also reported a species which closely resembles Glossatella tintinnabulum (Kent) on R. catesbeiana, R. clamitans and R. palustris tadpoles, which Kent ('80-82) found on the skin and gills of the larvae of the salamander Notophthalmus cristatus Laurenti.

Family: Epistylidae

Epistylis sp., Rhabdostyla sp. and Opercularia sp., according to Wenrich (1924a), have been found only on the skin, the tail region in the vicinity of the anus being the most favored location on tadpoles of Rana catesbeiana, R. clamitans and R. palustris at Philadelphia.

Family: Vorticellidae

Vorticella sp. was reported by Wenrich (1924a)--(see family Epistylidae for host and locality data).

Family: Urceolariidae

Fulton (1923) reported Trichodina pediculus (Muller) from the gills of Necturus sp. and Notophthalmus sp. larvae, indicating that Trichodina sp. found on Hydra sp. are probably this same species. Wenrich (1924a) reported Trichodina sp. on the skin and gills of Rana catesbeiana, R. clamitans and R. palustris at Philadelphia indicating a commensalistic association. Diller (1928) reported Trichodina sp. from tadpoles of Bufo sp., R. clamitans, R. palustris, R. sylvatica Le Conte and R. pipiens in Pennsylvania.

Fulton (1923) reported one species of endozoic Trichodina sp. from the urinary bladder of amphibia. Wenrich (1924a) stated that the members of the family Urceolariidae, including the genus Trichodina sp. are all associated with other animals and for the most part lead commensalistic lives on the exterior of their hosts.

Miscellaneous Protozoa

Upon examination of mucus from the skin of tadpoles of Pelobates fuscus Laur., and Rana esculenta L. in Europe, Sassuchin (1928) found numerous ectosymbiotic

protozoans. Some of the tadpoles were found dead or were in the process of dying.

Phylum: Platyhelminthes
Classification after Yamaguti (1963)

Class: Trematoda

Order: Monogenea

Family: Sphyranuridae

Species of a single genus of monogenetic trematodes Sphyranura Wright, 1879, is ectosymbiotic on amphibians. The type species, S. osleri Wright was found on the skin and gills of Necturus maculosus in Canada. Price (1939) redescribed the original material and reported S. oligorchis Alvey from the skin of N. maculosus in Pennsylvania. Alvey (1936) described S. polyorchis without giving data on host, location and distribution.

Phylum: Annelida
Classification after Pennak (1953)

Class: Hirudinea

Mann (1962) stated that it is very difficult to draw a sharp distinction between parasites and predators among leeches. Not only do their habits vary during the life history of an individual, but effect on the host may vary according to size.

Order: Rhynchobdellida

Family: Glossiphoniidae

Autrum (1953) reported Placobdella sp. from Rana esculenta and Pelobates fusca in Europe. Barrow (1958) exposed Batrachobdella picta (Verrill) to Notophthalmus viridescens in an attempt to infect the newt with Trypanosoma diemyctyli Tobey. The transmission of the flagellate proved unsuccessful, but he concluded from the feeding experiments and from the presence of dead larvae in two ponds heavily infected with leeches that it appears that if a leech of any size feeds on the newt they may be killed by loss of blood. Mann (1962) stated that Theromyzon sp. and Oligobdella sp. attack Amphibia.

Order: Arhynchobdellida

Family: Hirudidae

Macrobdella decora (Say), according to Moore (1923), attacks anuran eggs, tadpoles and adults. Blair (1927) reported Hirudo medicinalis L. feeding occasionally on frogs and tadpoles.

Phylum: Arthropoda

Class: Eucrustacea
Classification after Pennak (1953)

Order: Eucopepoda

Family: Lernaeidae

Lernaea cyprinacea Linnaeus was reported by Okada (1927) on Notophthalmus pyrrhogaster Boie in Japan. He

indicated that the copepod was probably the same species as found by Watase on Rana catesbeiana near Tokyo. Stunkard and Cable (1931) described the copepod, Lernaea ranae, on tadpoles of R. clamitans in Ohio. Baldauf (1961) reported L. cyprinacea from tadpoles of R. catesbeiana kept in an aquarium for several months in Texas. Tidd (1962) introduced L. cyprinacea to tadpoles of R. pipiens and R. sylvatica and found a parasitic interaction. Tidd and Shields (1963) described the tissue damage by L. cyprinacea on tadpoles of R. pipiens.

Order: Branchiura

Family: Argulidae

Goin and Ogren (1956) reported Argulus americanus Wilson on the perennibranch salamander Pseudobranchus striatus axanthus Netting and Goin and the tadpole R. heckscheri Wright near Gainesville, Florida.

Class: Arachnida

Order: Acarina

The first record of Acarina as ectoparasites of Amphibia was reported by Ewing (1926). Ewing 1926 described Hannemania hylae (Ewing) which parasitized the tree toad Hyla arenicolor Cope in southern California. The mite frequently penetrated the skin and assumed the role of an endoparasite.

Subsequently many acari have been reported as ectoparasites of adult anurans, however the literature does not reveal acari as ectoparasites of larval anurans or aquatic urodeles.

Phylum: Mollusca

Class: Pelecypoda
Classification after Pennak (1953)

Glochidia of Simpsoniconcha ambiqua Say were reported by Howard (1951) to parasitize Necturus maculosus in Illinois. The glochidia were found to be deeply embedded in the gill tissue. Glochidia of Megalonaias gigantea (Barnes) were also found but failed to embed in the gill tissue.

Seshaiya (1941) reported relative degrees of encystment by glochidia of Lamellidens sp. after experimentally infecting the tadpoles of Rana sp.

FIELD METHODS AND MATERIALS

Larval anurans were collected by means of a dip net. Generally the tadpoles were not observed and success in capturing was attained by randomly dipping in the aquatic vegetation. Aquatic salamanders were usually observed before capture and collected by means of a dip

net or, at one collection station, baitless minnow traps. If the salamanders were not visible, then the procedure paralleled that of the tadpole collecting.

Animals, when collected, were isolated in containers at the collection site. This was done to prevent transfer of symbionts from one host to the other. Earlier collections were placed in distilled water while subsequent collections were placed in the water from the collection site.

Field notes were recorded concerning water temperature, depth of capture, distance of capture from shore and bottom type. Aquatic plants, at the capture site were identified. A record of the time, date and location--tier, range and section--was taken. Selected weather data were recorded.

LABORATORY METHODS AND MATERIALS

Specimens were removed from the collection-site medium and anesthetized with MS 222 Sandoz at a concentration of 1×10^{-3}, an anesthetic suitable for cold-blooded vertebrates. It was found that many of the symbionts were anesthesized making protozoan identification difficult. In addition, anesthesized symbionts were often freed from attachment to the host, consequently settling to the bottom of the container. This problem was partially

reduced by first observing the amphibians in distilled water, followed by anesthesia.

The entire external body surface of each amphibian was examined with a minimum of 27X. An examination of the media was also made. The symbionts' attachment sites were recorded.

The symbionts were identified by keys to the Protozoa: Jahn (1949), Kudo (1954) and Pennak (1953); Platyhelminthes: Yamaguti (1963); and Annelida: Pennak (1953). Amphibians were identified using Blair, Blair, Brodkorb, Cagle and Moore (1957), and Wright and Wright (1949). In addition, identifications were made by Dr. Gerald W. Esch (nematodes), Dr. M. M. Hensley (amphibians), Martin L. Kopenski (leeches and aquatic plants), Don L. McGregor (ostracodes) and Dr. T. Wayne Porter (other invertebrates). Symbionts were generally determined to genera. Anurans were identified to genera with the exception of Acris crepitans blanchardi Harper, and urodeles to species. The anurans represent the species of Bufo, Hyla and Rana found in Barry and Kalamazoo Counties. For a list of anurans representing the above genera see Appendix I.

Protozoan symbionts were preserved and stained with 10% nigrosin, and acidified methyl green (1% solution in 1% acetic acid). Platyhelminthes (Monogenea), nematodes,

arthropods and molluscs were preserved in 70% alcohol. Annelids (Hirudinea) were fixed in 5% formalin and stored in 70% alcohol. The Platyhelminthes were stained with paracarmine before mounting in balsam. Nematoda, Annelida, Arthropoda and Mollusca were stored in vials.

Due to difficulty in preparing protozoan slides, microphotographs were made of some protozoan ectosymbionts. A Spencer compound microscope adapted with a 35 mm. camera was used. Illumination was made with a Spencer Ortho-Illuminator on to which the microscope and camera were mounted. Photographs were made using Kodak Panatomic-X (FX 135-20) film at a shutter speed of one-tenth to one-twenty-fifth of a second with high intensity light. Photographs were made from magnification of 200X to 430X.

Total length of the amphibians was recorded along with snout-vent length of proteid salamanders. The amphibians were preserved in 5% formalin, placed in glass vials and jars, and deposited in the Michigan State University Museum. The 1965 collections of Necturus maculosus were examined, tagged and released at the capture site.

DESCRIPTION OF THE COLLECTION AREAS PRESENTED
WITH THE HOST-SYMBIONTS FOUND

Collection Station 1

Kalamazoo Co., T 1S, R 9W, S 28: Open alkaline system.

Amphibians were collected on two occasions at a depth of < 4 feet and within 12 feet of the shoreline among Chara sp. The substrate consisted of muck and marl. H_2S and methane were detected at the collection sites.

The first collection was made 31 October 1965 between 1500 and 1700 hours under 70% cloud cover. Water temperature was 9°C.

Amphibians and ectosymbionts: Rana sp.--12 tadpoles examined; ectosymbionts, Difflugia sp.--two tadpoles,

each with one specimen, and one tadpole with two specimens (found in MS 222 examining media); Trichodina sp.--few to abundant specimens on lateral body wall of ten tadpoles.

The second collection was made 14 November 1965 between 1500 and 1600 hours under a cloudless sky with bright sun. Water temperature was 7°C.

Amphibians and ectosymbionts: Rana sp.--eight tadpoles examined; ectosymbionts, Trichodina sp.--few specimens on the spiracle of one tadpole and abundant specimens on the entire body surface of a second tadpole.

Collection Station 2

Kalamazoo Co., T 1S, R 9W, S 21: Closed system with bog margin at south end.

Amphibians were collected on four occasions at a depth of < 3 feet and within 10 feet of the shoreline. Aquatic plants in the immediate vicinity included Sagittaria sp. (Arrowhead), Nuphar sp. (Yellow Water Lily), Nitella sp. (Characeae), and Utricularia sp. (Bladderwort). The substrate consisted of muck.

The first collection was made 20 August 1965 between 1800 and 2000 hours under a hazy sky. Water temperature was 24°C.

Amphibians and ectosymbionts: Notophthalmus viridescens--seven efts examined; ectosymbionts, Scyphidiidae--numerous specimens on the lateral body wall of one eft. N. viridescens--one adult examined; ectosymbionts, none. Acris crepitans--one tadpole examined; ectosymbionts, Vorticella sp.--few specimens attached to anal region. Rana sp.--seven tadpoles examined; ectosymbionts, Vorticella sp.--few specimens attached to two tadpoles; Trichodina sp.--few specimens on entire body surface of one tadpole.

The second collection was made 16 September 1965 between 1600 and 1800 hours under a hazy sky. Water temperature was 23°C.

Amphibians and ectosymbionts: Notophthalmus viridescens--seven efts examined; ectosymbionts, none. N. viridescens--two adults examined; ectosymbionts, none.

Rana sp.--fourteen tadpoles examined; ectosymbionts, Vorticella sp.--few specimens found on body and in MS 222 of 10 tadpoles; Trichodina sp.--few specimens on entire body surface of one tadpole; Placobdella sp.--two specimens, one each on body wall of both tadpoles.

The third collection was made 26 September 1965 between 1400 and 1600 hours under a cloudy sky. Water temperature was 13°C.

Amphibians and ectosymbionts: Rana sp.--five tadpoles examined; ectosymbionts, Arcella sp.--one specimen on lateral body wall of one tadpole; Trichodina sp.--few to abundant specimens on three tadpoles.

The fourth collection was made 10 October 1965 between 1500 and 1600 hours under a cloudy sky with intermittent sunshine. Water temperature was 12°C.

Amphibians and ectosymbionts: Rana sp.--ten tadpoles examined; ectosymbionts, Difflugia sp.--two specimens on two tadpoles (found in MS 222); Scyphidiidae--few to numerous specimens on lateral body wall of six tadpoles; Vorticella sp.--five specimens on lateral body wall of one tadpole.

Collection Station 3

Kalamazoo Co., T 1S, R 9W, S8: Breeding pond adjacent to Wintergreen Lake.

Amphibians were collected at a depth of one foot and within 4 feet of the shoreline. Aquatic plants in the vicinity of the capture were Lemna minor (Lesser Duckweed), Scirpus sp. (Bulrush) and Typha sp. (Cattail). The substrate consisted of organic material in various stages of decomposition.

The collection was made 21 July 1965 between 1800 and 2000 hours under a partly cloudy sky. Water temperature was 25°C.

Amphibians and ectosymbionts: Rana sp.--seven tadpoles examined; ectosymbionts, Trichodina sp.--moderate number of specimens on entire body surface of two tadpoles and confined to oral and anal region of one tadpole.

Collection Station 4

Gull Lake, Kalamazoo Co., T 1S, R 9W, S 6 and 7.

During the spring of 1965 a pilot study of Necturus maculosus yielded the following ectosymbionts: Trichodina sp., Sphyranura sp. (Monogenea), and clam glochidia. During the spring of 1966 three collections were made. Amphibians were collected at a depth of < 4 feet and within 30 feet of the shoreline. The substrate consisted of sand and stones. Higher aquatic plants were not present at the collection site.

The first collection was made 4 April 1966 between 2100 and 2300 hours under an overcast sky. Water temperature was 4°C.

Amphibians and ectosymbionts: Necturus maculosus --two specimens examined; ectosymbionts, Trichodina sp.--abundant specimens on the gills of one salamander and few specimens on the gills of the second; Sphyranura sp.--one specimen on the gills of one salamander and less than 25 on the gills of the second salamander.

The second collection was made 20 April 1966 between 2200 and 2300 hours while raining. Water temperature was 11°C.

Amphibians and ectosymbionts: Necturus maculosus--five specimens examined; ectosymbionts, Trichodina sp.--abundant specimens on the gills of two salamanders while absent on three salamanders; Sphyranura sp.--few specimens on the gills of all five salamanders; Clam glochidia--one glochidia found encysted in the gill tissue of one salamander.

The third collection was made 18 May 1966 between 2100 and 2300 hours under a cloudy sky. Water temperature was 12°C.

Amphibians and ectosymbionts: Necturus maculosus--three specimens examined; ectosymbionts, Trichodina sp.--abundant specimens on the gills of two salamanders, and on the gills and general body surface of one salamander; Sphyranura sp.--five to 25 specimens on the gills of all

salamanders; Clam glochidia--one glochidia found partially encysted in the gill tissue of one salamander.

Collection Station 5

Kalamazoo Co., T 1S, R 9W, S 5: Excavated marl pits adjacent to Gull Lake Laboratories.

Amphibians were collected on three occasions at a depth of < 4 feet and within 10 feet of the shoreline. Aquatic plants consisted of Chara sp. and Potamogeton sp. (Pondweed). The substrate was marl.

The first collection was made 18 July 1965 between 1100 and 1200 hours under a partly cloudy sky. Water temperature was 23°C.

Amphibians and ectosymbionts: Ambystoma tigrinum--eight larvae examined; ectosymbionts, none. Notophthalmus

viridescens--three adults examined; ectosymbionts, none.

The second collection was made 15 July 1965 between 1300 and 1400 hours under a clear sky. Water temperature was 28°C.

Amphibians and ectosymbionts: Ambystoma tigrinum--two larvae examined; ectosymbionts, Cypridopsis vidua (Ostracoda)--one specimen about 1 mm. from left rear leg of one salamander.

The third collection was made 23 July 1965 at 900 hours under bright sunshine. Water temperature was 26°C.

Amphibians and ectosymbionts: Ambystoma tigrinum--four larvae examined; ectosymbionts, Placobdella sp.--one specimen on lateral body wall of one salamander.

Collection Station 6

Barry Co., T 1N, R 9W, S 27: Small ditch in the vicinity of Lawrence Lake.

Amphibians were collected on two occasions, at a depth of 1.5 feet. Higher aquatic plants were absent. The substrate was marl.

The first collection was made 17 August 1965 between 1000 and 1100 hours during a downpour. Water temperature was 23°C.

Amphibians and ectosymbionts: Rana sp.--18 tadpoles examined; ectosymbionts, Trichodina sp.--one specimen on one tadpole.

The second collection was made 11 September 1965 between 1400 and 1500 hours under a hazy sky. Water temperature was 20°C.

Amphibians and ectosymbionts: Rana sp.--three tadpoles examined; ectosymbionts, none.

Collection Station 7

Barry Co., T 3N, R 10W, S: Hall Lake--Alkaline System.

Amphibians were collected at a depth of 6 inches and within 5 feet of the shoreline. Aquatic plants in the immediate vicinity included Asclepias sp. (Milkweed), Carex sp. (Sedge Family), Spirodela polyrhiza (Big Duckweed), and Typha sp. (Cattail). The substrate consisted of decomposed organic material.

The collection was made on 12 August 1965 between 1100 and 1200 hours under a cloudless sky. Water temperature was 20°C.

Amphibians and ectosymbionts: Rana sp.--15 tadpoles examined; ectosymbionts, Trichodina sp.--specimens found on spiracle, anal region or entire body surface of 14 tadpoles.

Collection Station 8

Barry Co., T 3N, R 9W, S 30 and 31: Otis Lake--Closed system with bog margins at opposite ends.

Amphibians were collected on four occasions at a depth of <2 feet and within 20 feet of the shoreline. Aquatic plants in the immediate vicinity included Chara sp., Cladophora sp., Nymphaea sp. (Water Lily), Pontederia sp. (Pickerelweed) and Scirpus sp. (Bulrush). The substrate consisted of muck and peat.

The first collection was made 21 June 1965 between 1400 and 1500 hours under a cloudless sky. Water temperature was 33°C.

Amphibians and ectosymbionts: Rana sp.--two tadpoles examined; ectosymbionts, Epistylis sp.--small colony attached to anal region of one tadpole; Placobdella sp.--one specimen attached to base of fin on one tadpole. Hyla sp.--two tadpoles examined; ectosymbionts, Epistylis sp.--small colony attached to anal region of one tadpole.

The second collection was made 24 June 1965 between 1000 and 1100 hours under a partly cloudy sky. Water temperature was 27°C.

Amphibians and ectosymbionts: Rana sp.--one tadpole examined; ectosymbionts, Placobdella sp.--two specimens on lateral body wall. Bufo sp.--13 tadpoles examined; ectosymbionts, Arcella sp.--one specimen on one tadpole; Epistylis sp.--anal region of all tadpoles.

The third collection was made 26 July 1965 between 1300 and 1500 hours under a hazy sky. Water temperature was 33°C.

Amphibians and ectosymbionts: Rana sp.--one tadpole examined; ectosymbionts, Trichodina sp.--few specimens on general body surface; Pisicola sp. (Hirudinea)--one specimen attached to lateral body wall. Acris crepitans--eight tadpoles examined; ectosymbionts, Trichodina sp.--very few specimens found on three tadpoles.

The fourth collection was made 26 September 1965 between 1200 and 1300 hours under an overcast sky. Water temperature was 7°C.

Amphibians and ectosymbionts: Rana sp.--one tadpole examined; ectosymbionts, Trichodina sp.--few specimens within anal orifice.

The fifth collection was made 22 June 1966 between 1000 and 1100 hours under a cloudless sky. Water temperature was 35°C.

Amphibians and ectosymbionts: Rana sp.--four tadpoles examined; ectosymbionts, Glossatella sp. (Scyphiidae)--few specimens on lateral body wall of one tadpole; Epistylis sp.--abundant around anal region of two tadpoles; Vorticella sp.--eight specimens at left rear leg of one tadpole; Trichodina sp.--abundant on the general body surface of three tadpoles. Hyla sp.--two tadpoles examined; ectosymbionts, Trichodina sp.--moderate to abundant on both tadpoles.

Collection Station 9

Barry Co., T 3N, R 9W, S 32: Flooding for fowl and furbearers.

Amphibians were collected at a depth of 2-3 feet, and within 8 feet of the shoreline. Aquatic plants in the immediate vicinity included Alisma plantago-aquatica (water Plaintain), Sparganium sp. (Bur Reed) and Spirodela polyrhiza (Big Duckweed). The substrate consisted of sand and organic material.

The collection was made on 28 June 1965 between 900 and 1100 hours under bright sun. Water temperature was 23°C.

Amphibians and ectosymbionts: Rana sp.--seven tadpoles examined; ectosymbionts, Arcella sp.--one specimen on dorsal surface of one tadpole; Trichodina sp.--abundant and evenly distributed on body surface of one tadpole; Placobdella sp.--one specimen found on one tadpole. Hyla sp.--nine tadpoles examined; ectosymbionts, Trichodina sp.--abundant and evenly distributed on body surface of all tadpoles. Ambystoma tigrinum--one larval form; ectosymbionts, none.

TABULATION OF RESULTS

Table 1. Percentage of Amphibia with the following Ectosymbionts Related to the Collection Station

Collection Station	Amphibians Examined	No. Examined	Protozoa						Platyhelminthes	Annelida		Arthropoda	Mollusca
			Difflugia sp.	Arcella sp.	Scyphidiidae	Epistylis sp.	Vorticella sp.	Trichodina sp.	Sphyranura sp.	Pisicola sp.	Placobdella sp.	Cypridopsis vidua	Clam glochidia
1	Rana sp.	15			5			90					
2	N. viridescens	17			6								
	A. crepitans	1					100						
	Rana sp.	36	6	3	3			14					
3	Rana sp.	7						43					
4	N. maculosus	29						70	97				28
5	N. viridescens	3											
	A. tigrinum	14									7	7	
6	Rana sp.	18						6					
7	Rana sp.	15						93					
8	Bufo sp.	13		8		8							
	A. crepitans	8						37					
	Hyla sp.	4				25		50					
	Rana sp.	9			11	33	11	67		11	11		
9	A. tigrinum	1											
	Hyla sp.	9						100					
	Rana sp.	7		14				14					

Table 2. Percentage of Amphibia with the following Ectosymbionts

Amphibians Examined	No. Examined	Protozoa						Platyhelminthes	Annelida		Arthropoda	Mollusca
		Difflugia sp.	*Arcella* sp.	Scyphidiidae	*Epistylis* sp.	*Vorticella* sp.	*Trichodina* sp.	*Sphyranura* sp.	*Pisicola* sp.	*Placobdella* sp.	*Cypridopsis vidua*	Clam glochidia
N. viridescens	20			5								
Necturus maculosus	29						70	97				28
A. tigrinum	15									7	7	
Bufo sp.	13		8		8							
Acris crepitans	9					11	33					
Hyla sp.	13				8		85					
Rana sp.	112	4	2	7	3	12	47		1	4		

Table 3. Number of Amphibians Examined from each Collection Station

Amphibian	Total No.	Collection Station								
		1	2	3	4	5	6	7	8	9
Notophthalmus viridescens	20		17			3				
Necturus maculosus	29				29					
Ambystoma tigrinum	15					14				1
Bufo sp.	13								13	
Acris crepitans	9		1						8	
Hyla sp.	13								4	9
Rana sp.	112	20	36	7			18	15	9	7

DISCUSSION OF RESULTS

Protozoans were the most common ectosymbionts of amphibians examined. Ambystoma tigrinum was the only amphibian lacking protozoan ectosymbionts. The Ranidae had the most diversity of protozoan relationships with six taxa being represented from both sarcodine and ciliate Protozoa. The other anurans all possessed two genera of protozoans. No one protozoan ectosymbiotic genus was found on all the anuran genera examined. The urodeles, excluding A. tigrinum, each possessed one genus or family of protozoan.

Protozoan ectosymbionts were taken from all but Collection Station 5, where urodeles were taken exclusively. Protozoans were not found on A. tigrinum from Collection Station 9, but were found on anurans taken from this same collection station. Protozoans were on urodeles from Collection Station 4 (Gull Lake) and Collection Station 2 where anurans were taken with protozoan ectosymbionts.

Trichodina sp. was the most common protozoan found (Appendix IV, Plate 2). This peritrichous ciliate was on amphibians taken at all collection stations, except 5. Necturus maculosus was the only urodele with ectosymbiotic Trichodina sp. Only at Collection Station 2

was there a greater percentage of protozoan found than Trichodina sp., if Trichodina sp. occurred on an anuran genus at a particular collection station. Only three collection stations yielded other peritrichous ciliates. Eighty-four percent of the Hyla sp. examined possessed Trichodina sp., followed by 70% of the Necturus sp., 46.6% of the Rana sp., and 33.3% of the Acris crepitans. N. viridescens, A. tigrinum and Bufo sp. lacked Trichodina sp. No other protozoan exceeded 11.6% infestation for Amphibia.

The percentage (5.5) of Trichodina sp. given for Rana sp. at Collection Station 6 is extremely misleading. One specimen of Trichodina sp. was found on one tadpole. Trichodina sp., when present, generally exceeded 10 or more per individuals infested. Collection Station 6 was a closed marl ditch, and the Trichodina sp. present might have been introduced via adult anurans frequenting the habitat.

The aloricate peritrichs lacking a stalk have been reported only as members of the family Scyphidiidae. At times, Scyphidia sp. (Appendix IV, Plate 1) and Glossatella sp. could readily be identified, while in other instances generic identification was not possible. Scyphidiidae were found on N. viridescens, Collection Station 2, and were the only ectosymbiotic protozoans

which occurred on urodeles other than Trichodina sp. Scyphidiidae were found on Rana sp. at this same collection station.

An occurrence of testacean sarcodines Arcella sp. and Difflugia sp. had not previously been reported in the literature. According to Jepps (1956) testaceans are very widespread, and often abundant, in all habitats where protozoans occur, except in salt water, and that no parasitic forms are known. Arcella sp. were found on Rana sp. exclusively. Difflugia sp. have not been observed on tadpoles but were always found in the MS 222 examining media. Heinis (1928) found one Difflugia constricta alive after washing the feet of a freshly killed Perdix cinerea and stated that it was probable that bird transportation was of greater significance than usually supposed. The writer felt that larval anurans may transport testaceans in much the same manner.

No evidence has been observed in the present study which would indicate that the protozoans found were ectoparasitic. However, in the absence of histological studies, definite symbiotic levels could not be determined.

Ninety-six percent of all Necturus maculosus were parasitized by the monogenetic trematode Sphyranura sp. (Appendix IV, Plate 3). Price (1939) separated the

Sphyranura sp. on the basis of the number of testes, S. osleri 12-16, S. oligorchis 5-7 and S. polyorchis 20-23. Price further stated that should a re-examination of the hooks of S. polyorchis reveal lateral prominences, S. polyorchis is possibly a synonym of S. osleri.

An examination of specimens of Sphyranura sp. from Gull Lake, revealed that the testes varied from three to nine in number, thus not falling within the precepts of the three previously described species. It is the writer's opinion that perhaps S. osleri and S. oligorchis may represent the same species. Together with the doubt Price stated, this would place the three Sphyranura sp. into a single species, S. osleri.

Leeches were found on Ambystoma tigrinum and Rana sp. at three collection stations. Ratio of body size of the leech to body size of the amphibians indicated a parasitic rather than a predator-prey relationship. No amphibians were found with Macrobdella decora attached, as reported by Moore (1923). One tadpole of Rana was collected with a large triangulated lesion on the ventral surface which appeared to be the result of a dislodged leech. It would appear that if adult M. decora attacked amphibians, such interactions would generally result in death to the amphibians and go unobserved during this study.

A single arthropod Cypridopsis vidua (Ostracoda) was found about 1 mm. from the left rear leg of one Ambystoma tigrinum. This ostracode was a free-living form and may have been caught in the surface tension when transferring the salamander from distilled water to MS 222.

Twenty-seven percent of all Necturus sp. were parasitized by clam glochidia which were partially embedded within the gill tissue. Without culturing glochidia to maturity, generic identification is impossible.

No evidence was found which would indicate that an ectosymbiont was endemic to any particular habitat. Epistylis sp. and Vorticella sp. were found only on amphibians from bog areas, but both ciliates have been found in most aquatic habitats. Scyphidiidae were found in at least two diverse habitats. The multi-cellular ectosymbionts of N. maculosus may have shown habitat preference, but without collecting the salamander from other collection stations such habitat preferences could not be determined.

Water temperature did not appear to be critical during this study. Trichodina sp. ectosymbionts were present on amphibians collected in habitats ranging from 4-35^{o}C. Scyphidiidae were found on amphibians in areas ranging from 12-35^{o}C. Multicellular ectosymbionts were not influenced by water temperature.

With the exception of Amphileptus branchiarum, reported by Wenrich (1924b), little explanation can be made concerning the absence of previously reported ectosymbionts during this study. A. branchiarum was commonly found swimming around the gills or embedded in the gill tissue of tadpoles. No tadpoles were examined during this study with gills exposed. The mastigophoran Costia necatrix and the peritrichous ciliates Rhabdostyla sp. and Opercularia sp. reported by Wenrich (1924a) were not found. Both ciliates are members of the family Epistylidae. Trichodina sp. reported by Fulton (1923) on N. viridescens and by Diller (1928) on Bufo sp. were not found on these anurans during this study. Perhaps too few Notophthalmus sp. and Bufo sp. were examined by the writer.

The results are presented with no allowance being made for the periodic ecdyses which are characteristic of the Amphibia. Ectosymbionts attached by means of haptors, or embedded in the skins, are not influenced by this shedding. However, those ectosymbionts that were sessible or swimming freely on the surface of the amphibian would be dislodged and would have to re-establish their relationship. Generally, those amphibians examined immediately after capture had a greater number of ectosymbionts, while amphibians examined after some delay had fewer

ectosymbionts. Also, if an amphibian remained in the MS 222 examining media too long, ecdysis occurred.

Endoparasites found during this study are reported in Appendix II. Ectosymbiotic mycophyta are reported in Appendix III.

CONCLUSIONS

1. The peritrichous ciliate Trichodina sp. was ectosymbiotic on larval anurans at all collection stations where anurans were taken. Trichodina sp. occurred on all larval anurans examined with the exception of Bufo sp. The only urodele found with Trichodina sp. was Necturus maculosus.

2. Other peritrichous ciliates were less common and occurred on amphibians at three collection stations. Both anurans and urodeles had ectosymbiotic Scyphidiidae.

3. Testacean sarcodines, not previously described in the literature, were found on larval anurans at three collection stations.

4. Necturus maculosus served as the host for three phyla of ectosymbionts: Protozoa, Platyhelminthes, and Mollusca (Glochidia). Other urodeles were largely free of ectosymbionts.

5. Leeches were found on both aquatic urodeles and larval anurans.

6. Ectosymbionts do not seem to be limited by habitat.

LITERATURE CITED

LITERATURE CITED

Alvey, C. H. 1936. The morphology and development of monogenetic trematode Sphyranura oligorchis (Alvey, 1933) and description of S. polyorchis n sp. Parasitol., 28: 229-253.

Autrum, H. J. 1932. Ein neuer Fundort von Haementeria costata (Fr. Muller) in Deutschland und Bemerkungen über den möglichen Wirt des Egels. Zool. Anzeiger, 98 (1/2): 45-46.

Baldauf, R. J. 1961. Another case of parasitic copepods on amphibians. Jour. of Parasitol., 47 (2): 195.

Barrow, J. H., Jr. 1958. The biology of Trypanosoma diemyctyli Tobey. Jour. of Prot., 5: 161-170.

Blair, W. F., A. P. Blair, P. Brodkorb, F. R. Cagle and G. A. Moore. 1957. Vertebrates of the United States. New York: McGraw-Hill Book Co., 819 p.

Blair, W. N. 1927. Notes on Hirudo medicinalis, the medicinal leech, as a British species. Proc. Zool. Soc. Lond., 999-1002.

Chandler, A. C., and C. P. Read. 1961. Introduction to parasitology. New York: John Wiley and Sons, 822p.

Diller, W. F. 1928. Binary fission and endoximis in the Trichodina from tadpoles. Jour. Morphol. Physiol., 46: 521.

Ewing, H. E. 1926. The life history and biology of the tree-toed chigger, Trombicula hylae Ewing. Ann. Ent. Soc. Amer., 19(3): 261-267.

Fassett, N. C. 1957. A manual of aquatic plants. The University of Wisconsin Press, Madison, 405 p.

Fulton, J. F., Jr. 1923. Trichodina pediculus and a new closely related species. Proc. Boston Soc. Nat. Hist., 37: 1.

Goin, C. J. and L. H. Ogren. 1956. Parasitic copepods (Argulidae) on amphibians. Jour. of Parasitol., 42: 72.

Heinis, F. R. 1928. Betrag zur Moosfauna des Krakatau. Verh. Naturf. Ges. Basel, 39: 57-65.

Howard, A. D. 1951. A river mussel parasitic on a salamander. Nat. Hist. Miscellanea, No. 77.

Jahn, T. 1949. How to know the protozoa. Dubuque, Iowa: Wm. C. Brown Co., 234 p.

Jepps. M. W. 1956. The protozoa, sarcodina. Edinburgh: Oliver and Boyd.

Kudo, R. 1954. Protozoology. Springfield: Charles C. Thomas, 966 p.

Mann, K. H. 1962. Leeches (Hirudinen) their structure, physiology, ecology and embryology. New York: Pergamon Press.

Moore, J. 1923. The control of blood-sucking leeches with an account of the leeches of palisades interstate park. Roosevelt Wild Life Bulletin, Vol. 2, No. 1, 9-53.

Okada, Y. K. 1927. Copepode parasites des amphibians. Annotationes Zoologicae Japonenses. 11: 185-187, French trans.

Pennak, R. W. 1953. Fresh-water invertebrates of the United States. New York: The Ronald Press Co., 769 p.

Price, E. W. 1939. North American monogenetic trematodes. Vol. IV. The family Polystomatidae. 6:80-92.

Sassuchin, D. 1928. Zur Froge uber die ecto- und endoparasitischen Protozoen der Froschkaulqueppen. (Ecto- and endoparasitic protozoa of tadpoles.) Arch. Protistenk. 64 (1/2):71-92.

Seshaiya, R. V. 1941. Tadpoles as hosts for the glochidia of fresh-water mussels. Current Science, Bangalore, Oct. 12, 1941, 535-536.

Stunkard, H. W. and R. M. Cable. 1931. Notes on a species of Lernaea parasitic in the larvae of Rana clamitans. Jour. of Parasit., 18: 92-97.

Tidd, W. M. 1962. Experimental infestations of frog tadpoles by Lernaea cyprinacea. Jour. of Parasit., 48: 870.

Tidd, W. M. and R. J. Shields. 1963. Tissue damage inflicted by Lernaea cyprinacea Linnaeus, a copepod parasitic on tadpoles. Jour. of Parasit., 49: 693-696.

Wenrich, D. H. 1924a. Protozoa on the skin and gills of tadpoles. Trans. Am. Microsc. Soc., 43: 200-202.

Wenrich, D. H. 1924b. A new protozoan parasite, Amphileptus branchiarum, n. sp. on the gills of tadpoles. Trans. of the Amer. Microsc. Soc., 43: 191-199.

Wenrich, D. H. 1935. Host-parasite relations between parasitic protozoa and their hosts. Proc. Am. Phil. Soc., 75: 605-50.

Wright, A. H. and A. A. Wright. 1949. Handbook of frogs and toads of the United States and Canada, 3d ed., Ithaca, New York: Comstock Publishing Associates, Inc.

Yamaguti, S. 1963. Systema helminthum. Vol. IV monogenea and aspidocotylea. Interscience, New York, 699 p.

APPENDICES

APPENDIX I

SPECIES LIST OF ANURANS RECORDED FOR KALAMAZOO AND BARRY COUNTIES, MICHIGAN

Bufonidae:

Bufo americanus Holbrook

B. fowleri Hinckley

Hylidae:

Acris crepitans blanchardi Harper

Pseudacris nigrita Schwartz

Hyla versicolor LeConte

H. crucifer Wied

Ranidae:

Rana clamitans Yarrow

R. sylvatica Le Conte

R. pipiens Schreber

R. palustris Le Conte

R. catesbeiana Shaw

APPENDIX II

ENDOPARASITES FOUND DURING THIS STUDY

Endoparasites found in the examining media following defecation by amphibians.

Endoparasite	Host	Collection Station
Opalinidae (Ciliate)	*Rana* sp.	1 and 6
Nyctotherus sp. (Ciliate)	*Rana* sp.	1 and 6
Cestoda	*N. maculosus*	4
Nematoda	*Rana* sp.	1 and 2

Subcutaneous nematodes identified as Filariodea were found on 17 *Necturus maculosus* at Collection Station 4.

APPENDIX III

ECTOSYMBIOTIC MYCOPHYTA

Ectosymbiotic mycophyta of the family Saprolegniaceae were found on the skin and gills of Necturus maculosus from Gull Lake. Two of the 29 N. maculosus examined were parasitized by the Saprolegniaceae.

APPENDIX IV

ILLUSTRATIONS OF COMMON ECTOSYMBIONTS FOUND

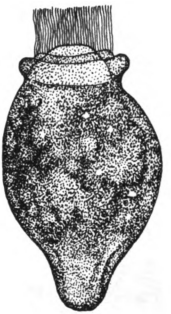

PLATE 1. Scyphidia sp.

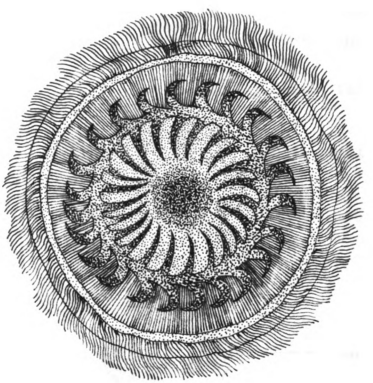

PLATE 2. Trichodina sp.

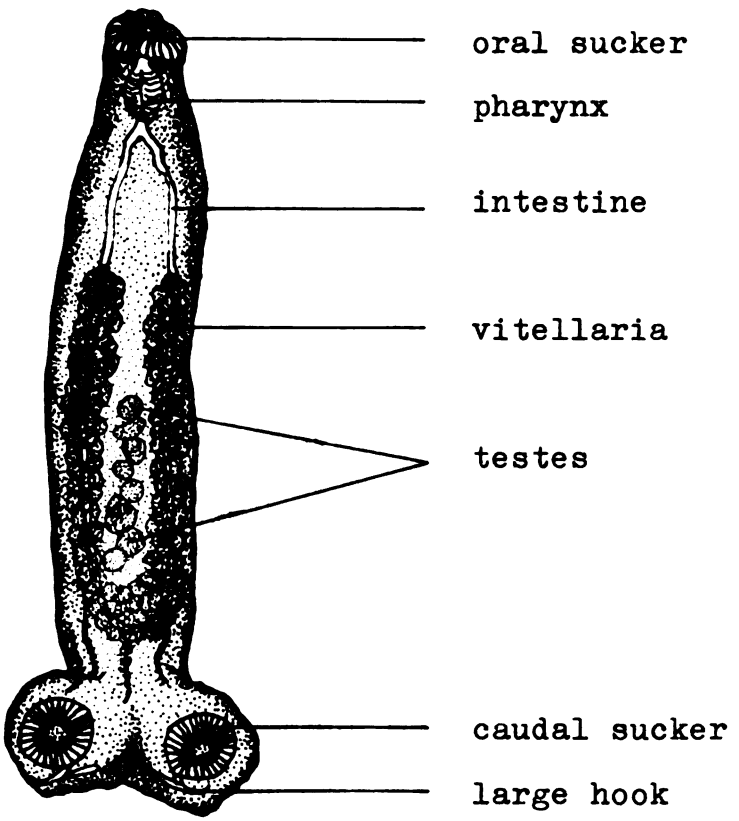

PLATE 3. Sphyranura sp.