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THE PARASITES OF THE CRAYFISH IN THE
CENTRAL MICHIGAN AREA

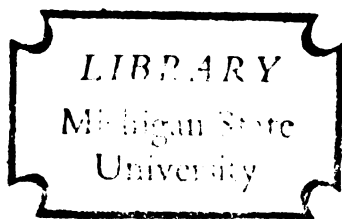
Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Donald Francis Blake

1959

THESIS



THE PARASITES OF THE CRAYFISH
IN THE CENTRAL MICHIGAN AREA

By

Donald Francis Blake

AN ABSTRACT

Submitted to the College of Arts and Sciences
Michigan State University of
Agriculture and Applied Science in partial fulfillment
of the requirements for the
degree of

MASTER OF SCIENCE

Department of Zoology

June, 1959

Approved by

T. Wayne Porter

AN ABSTRACT

The following three species of crayfish were taken during the survey, and they are listed in a decreasing order of abundance; Orconectes propinquus, O. rusticus and Cambaras bartoni robustus.

The Entocytherinae are ostracods with only one genus, Entocythere, and it is found closely associated with crayfish. Their teeth and leg structure seem well adapted for a parasitic way of life. There is little known information concerning the transfer of the ostracod from host to host. Marshall (1903) stated that this transfer took place during the time of copulation, but a lack of host specificity seems to indicate that this transfer occurs at times other than just during copulation. Also, the fact that the older crayfish are highly infected while the younger crayfish are rarely infected could be indicative of the fact that the transfer of the ostracod from host to host is a complicated one.

The taxonomic characters of the Branchiobdellidae are very good, and they can be readily identified. In a comparison of Augusta Creek with the Red Cedar River, the branchiobdellids appear to have an affinity for warm water.

There has been much discussion as to whether the Entocytherinae and the Branchiobdellidae are parasitic or free living organisms. Nevertheless, there seem to be certain morphological evidences which would warrant referring to them as parasites. True, they are not obligatory parasites in the same sense as are the Platyhelminthes and the Sporozoa,

yet they might still be classed as at least facultative parasites.

Internal parasites were found in crayfish collected from all of the collecting areas on the Red Cedar River and in one area on Augusta Creek. They were trematodes of the family Allocreadiidae and they can be easily identified by the six oral papillae. These trematodes use the crayfish along with several other crustaceans and aquatic insects as a second intermediate host.

In all of the forms discussed, there were no real indications of host specificity. Host specificity has been reported to occur in the Branchiobdellidae, but my observations suggest that the distribution of these forms is a case of geographic isolation caused by a physical barrier rather than host specificity.

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O U T L I N E

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And last, but in no wise the least, I would like to thank my beloved wife Vera, for her effervescent spring of encouragement and for typing this manuscript.

INTRODUCTION

While working with the decapod crustaceans in the invertebrate zoology class at Michigan State University, I became quite interested in the fauna which was found living in close association with the crayfish. It was this interest that provided the impetus for this survey.

From the time that parasitology became a distinct and definite branch of the sciences, much thought and writing has been given to developing a definition of a parasite. Although many attempts have been made to formulate an accurate definition of a parasite, none of them have been so completely anthropocentric as to be useless, according to Cameron (1956). Still others go so overboard in the other direction that they become either too comprehensive or insufficiently conclusive. Even today definitions of parasitism are given to suit particular needs or areas of study, e.g. physiological, ecological, morphological.

To most people, and even to many biologists, a parasite is merely a smaller animal living within or attached to the outside of a larger animal. Smith (1934) quotes Leuckhart as defining parasitism as the finding of food and lodgement by one organism in or on another. Cameron (1956) gives a definition which could be considered a little more conclusive. He defines a parasite to be an organism which in some stage of its life cycle requires some vital factor which it can obtain only from another living organism.

Rather than a mere condition, parasitism, as interpreted herein, represents a complete mode of life and more essentially, a mode of obtain-

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that this is crucial for ensuring transparency and accountability in the organization's operations.

2. The second part outlines the various methods and tools used to collect and analyze data. This includes both traditional manual methods and modern digital technologies, highlighting the benefits of each approach.

3. The third part focuses on the role of human resources in the data collection process. It discusses how training and support for staff can significantly improve the quality and reliability of the data collected.

4. The fourth part addresses the challenges and limitations of data collection. It identifies common pitfalls such as incomplete data, errors in recording, and difficulties in accessing certain types of information.

5. The fifth part provides recommendations for overcoming these challenges. It suggests implementing robust data management systems, conducting regular audits, and fostering a culture of data accuracy and integrity.

6. The sixth part discusses the importance of data security and privacy. It outlines the necessary measures to protect sensitive information from unauthorized access and ensure compliance with relevant regulations.

7. The seventh part explores the potential applications of the collected data. It highlights how the data can be used to inform decision-making, identify trends, and optimize organizational performance.

8. The eighth part concludes the document by summarizing the key findings and reiterating the importance of a systematic and reliable data collection process.

ing nutrition in which the holozoic type of nutrition is eliminated in part or entirely.

Parasitism, in its broadest sense, has become a very specialized way of life. The parasite, in its efforts to most efficiently pursue this very specialized mode of life, has adopted many morphological modifications. Many of the modifications have been of types which closely parallel those of free living sessile forms.

Most of the forms discussed in this paper are of the type that fall into the ranks of the more debatable, those which might not be considered parasites. Both the branchiobdellids and the entocytherids have been classed as commensals as many times as they have been classed as parasites. An examination of the tooth structure of both the branchiobdellids and the entocytherids might be an indication of their parasitic nature. The teeth of these two groups are fitted for rasping and tearing. Therefore the possibility exists that these teeth could be used to rasp off the ends of the gill filaments and allow the parasite to obtain haemolymph in this manner.

After the completion of this survey and some study of the morphology of the animals found living in close association with the crayfish, it seems apparent that the two questionable forms, the Branchiobdellidae and the Entocytherinae are parasitic in nature. This is not to say that they subsist entirely on nutrients obtained from the crayfish, but rather feed intermittently on the body tissues of the host animal.

METHODS AND MATERIALS

This project was started by collecting crayfish from several areas in the vicinity of the Michigan State University campus and the W. K. Kellogg Biological Station. Several methods of capturing the crayfish were tried, and all but one proved useful. The most successful collecting technique was that of the electrical shocker as described by Haskell (1939). In this method a 110 volt AC generator powered by a gasoline engine was used. When the electrode was placed in the water where crayfish were present, the electrical charge caused the crayfish to approach the electrode. Usually the light colored ventral side was up which rendered them very easy to see, and they were collected by scooping them up with a Turtox Indestructible Dip Net and placed in a bucket of water. Another successful method was the use of the seining net. However, the disadvantage to both of the above mentioned methods was that they both required two men and it was not always possible to secure a partner. Most of the crayfish were hand collected with the dip net. Whenever a crayfish was seen on the substrate of a lake or stream, the net was placed behind it, the animal activated, and it would swim into the net. From 15 to 25 specimens could be collected in an hour or so. Trapping was attempted but did not prove very successful although others (Andrews, 1907) had found it to be very effective. Both single and double cone nets baited with fresh pork and beef were tried. These traps were placed in areas that were known to have large populations of crayfish, but only a very few specimens were taken. This may have been due to the fact that

these areas had a very abundant supply of natural food.

There were three species of crayfish taken during the survey; Cambaras bartoni robustus Girard, Orconectes rusticus, and O. propinquus Girard. Of the three O. propinquus was taken in the greatest abundance while C. bartoni robustus was taken in the smallest numbers. O. propinquus was found usually in small streams with clear, swift running water, and also in the larger streams and lakes. C. bartoni robustus was found in fast moving streams hiding among the rocks, and was usually in the same general areas as was O. propinquus. O. rusticus was found in the larger streams, and permanent ponds. It was also found in the same areas as was O. propinquus. O. rusticus and C. bartoni robustus were never taken together. Approximately 140 man hours were spent in the field collecting specimens. Four hundred specimens were taken of which 132 were examined for parasites.

The crayfish were brought into the laboratory and kept alive until they were ready to be examined for parasites. Several methods were investigated to determine how to kill the crayfish quickly and yet not disturb the parasites in or on the individual. Goodnight (1940) described a method of placing the crayfish in a solution of 85 parts of 85% alcohol, 10 parts of formalin, and 5 parts of glacial acetic acid (AFA), and after a time taking them out and collecting the Branchiobdellidae from the residue on the bottom of the container. Hoff (1942a) used a similar method for collecting Entocytherinae but used 95% alcohol instead of AFA, but unless each crayfish was placed in a separate container, all records of host specificity would be lost. In the early stages of this work it be-

came apparent that killing the crayfish in any type of liquid media was not satisfactory because many of the parasitic forms were washed off. Attempts to freeze the specimens were also tried and while this killed the animal rather quickly, the parasites were also killed or their metabolic rates were slowed down so much that the movement which was a valuable aid in finding them was markedly reduced. The method used most during this research was that of cutting through the cephalothorax immediately posterior to the eyes with a pair of heavy scissors so that the nerve connecting the brain to the posterior part of the nervous system was severed. The animal died quickly and the parasites were not disturbed. The dissected animal was placed in a Petri dish and the internal organs and external parts were examined for parasites. The organs were kept moist with physiological saline solution. When the examination was finished, the animal was removed from the Petri dish and the forms that had been washed off or had dropped off were removed from the dish.

All of the forms removed from the crayfish were preserved in vials of AFA. Smears were made of the contents of the gastric mill and the intestinal tract. The smears were stained with the Jenner - Giemsa method. The metazoan forms were stained with a solution of glacial acetic acid, paracarmine and alcohol. Then the specimens were destained, dehydrated in alcohol series, cleared in xylol, and mounted in permount. Several attempts were made to stain the parasites in borax carmine. This stain penetrated the tissues of the specimens very well, and stained them quite well. However, due to the small size of the specimens and the darkness of the stain, many of the specimens were lost when trying to decant the excess stain. This method of staining was not used owing to this difficulty.

*Key to the Parasites of the Crayfish of the
Central Michigan Area.

*(Modified from Goodnight - 1940)

-
1. Form found encysted in a capsule within the body cavity, metacercariae with six oral papillae.....Allocreadiidae (Trematoda)
 - Form not found encysted in a capsule.....2
 2. Body covered with a bi-valved shell.....Entocytherinae
 - Body not covered with bi-valved shell;3
 3. Body with appendages on trunk segments II-VIII (see fig. 2).....Pterodrilus distichus Moore
 - Body without appendages (see fig. 3).....4
 4. Accessory sperm duct present; head much wider than anterior body segments (see fig 1).....5
 - Accessory sperm duct absent; width of head approximately same as anterior body segments.....9
 5. Upper lip divided.....6
 - Upper lip entire except for small median emargination.....7
 6. Major annulations of body segments distinctly elevated over minor annulations; lower jaw with several median and lateral denticles.....Cambarincola chirocephala Ellis
 - Major annulations of body segments not distinctly elevated over minor annulations; lower jaw with several minute denticles on each side....Cambarincola philadelphia (Leidy)
 7. Major annulation of body segment VIII distinctly elevated over minor annulation (see fig. 1).....Cambarincola elevata Goodnight.
 - Major annulation of body segment VIII not distinctly elevated over minor annulation.....8
 3. Upper jaw with 5 teeth; middle tooth is very long and prominent (see fig. 4).....Cambarincola macrodonta Ellis

- Upper jaw with 5 teeth; middle tooth is only slightly longer than the other 4 (see fig. 5).....Cambarincola vitrea Ellis
- 9. With 4 - 6 teeth in the upper jaw; middle tooth of upper jaw longest if teeth are odd in number, and middle pair longest if teeth are even in number (see fig. 6).....Xironodrillus formosus Ellis

THE ALLOCREADIIDAE

The Allocreadiidae of the order Digenea is a very interesting family of trematodes identified by their oral papillae. These papillae are projections of the oral sucker and there are six of them. These papillae are made up of strong muscle fibers that are continuous with the sucker wall. Four of the papillae arise from the dorsal wall of the sucker while the other two project laterally from the sucker near the ventral surface.

Little work has been done on the life cycles of the Allocreadiidae, therefore identification to genus of the immature forms is nearly impossible. As do many other digenetic trematodes, the Allocreadiidae have two intermediate hosts. The adult allocreadiids are found mostly in fish. The first intermediate host is usually a snail, and the second intermediate host varies widely among the Crustacea and the Insecta. Some of the crustacean intermediate hosts are the crayfish, scuds and fresh water shrimps. Certain aquatic insects are also known to serve as intermediate hosts. There are some species of the allocreadiids that use members of the Mollusca families Unionidae and Sphaeriidae as the secondary intermediate host.

Those metacercariae that parasitize the crayfish for the second intermediate host in this area were found encysted in the body tissues in the cephalothorax. They were found mostly in the digestive gland in the males and in the ovarian masses in the females. The females of crayfish had a greater number of cysts than did the males. These metacercariae were found encapsulated in a small spherical cyst. The outer covering of the

cyst was brown and tough. It appeared somewhat like sclerotized protein or some similar substance. Within this tough outer covering was a thin, clear membrane which encased the parasite. For the most part, the metacercariae were encysted singly, but there were several cases of two in a capsule, and two or three examples of three metacercariae in one capsule.

In most sites, the infection of the crayfish with these metacercariae was rather light, approximately 35% with one or two cysts in each of the infected animals. In contrast, the Red Cedar River presented a much different situation. Of those collected from this body of water almost every crayfish, approximately 97%, was infected. The males averaged five cysts while the females averaged eleven cysts.

In Augusta Creek, a very puzzling, but interesting situation was found. There were three areas in the creek from which crayfish were collected. Site 1 was under a bridge at 43rd Street and B Avenue (NW $\frac{1}{4}$ of the NW $\frac{1}{4}$ of Section 10, Range 9W, Tier 1S); site 2 was near the bridge at 43rd Street and C Avenue (SE $\frac{1}{4}$ of the SW $\frac{1}{4}$ of Section 10, Range 9W, Tier 1S); and site 3 was in the W. K. Kellogg Forest (NW $\frac{1}{4}$ of the SW $\frac{1}{4}$ of Section 22, Range 9W, Tier 1S) (see map in Appendix). The allocreadiids were not found in the crayfish taken from any area collected except at site 2 which is located about halfway between the other two locations. One possible explanation of this distribution could be the fact that the stream is artificially stocked with trout for fishing and stocking is done from the bridge located at site 2. Thus trematodes may be introduced from snails that were taken from the hatchery ponds along with fish.

HOST AND LOCALITY DATA

ALLOCREADIIDAE

Kalamazoo Co.: (O. propinquus) Augusta Creek at C Avenue and 43rd Street, 7 July - 18 August, 1958.

(O. rusticus) Augusta Creek at C Avenue and 43rd Street, 7 July - 18 August, 1958.

(Cambaras bartoni robustus) Augusta Creek at C Avenue and 43rd Street, 7 July - 18 August, 1958.

Ingham Co.: (O. propinquus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road, 1 October, 1958; MSU Campus, 1 October - 15 November, 1958.

(O. rusticus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road, 1 October - 15 November, 1958.

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THE ENTOCYTHERINAE

The Ostracoda are small bi-valved crustaceans which appear to the naked eye as a small seed, but with the aid of low magnification resemble minute clams. The small, rather interesting animals are difficult to identify since their shells are opaque and proper identification requires some microdissection. The exact number of species occurring in the United States is not known. Each time an investigator makes a new survey of some state or area, several new species are described.

The subfamily Entocytherinae has but one genus reported from the United States, and it is always associated with crayfish. According to Hoff (1942a), species of the closely related genus Sphaeromicola described from Europe are believed to live in a definite commensalistic relationship with some isopods and amphipods. He further states that the relationship between the American forms and the crayfish has not been definitely established. Marshall (1903) described the entocytherids as being definitely parasitic and he based his statement on the grounds that the homogenous food masses found in the intestine of the ostracod were blood. The same type of food masses, however, have been found in the intestines of the truly free living ostracods (Hoff, 1942a). Klie (1931) found Entocythere donaldsonensis living unassociated with crayfish. Furthermore, certain structural adaptations (mouthparts, teeth, long exopodite on second antenna, all legs morphologically similar) found in this species are not found in a true free-living form. A consideration of the mouthparts of the entocytherids would indicate that while they are

- 1990: 1.1 million people in the world were infected with HIV
- 1995: 2.5 million people in the world were infected with HIV
- 2000: 4.5 million people in the world were infected with HIV
- 2005: 6.5 million people in the world were infected with HIV
- 2010: 8.5 million people in the world were infected with HIV
- 2015: 10.5 million people in the world were infected with HIV
- 2020: 12.5 million people in the world were infected with HIV
- 2025: 14.5 million people in the world were infected with HIV
- 2030: 16.5 million people in the world were infected with HIV
- 2035: 18.5 million people in the world were infected with HIV
- 2040: 20.5 million people in the world were infected with HIV
- 2045: 22.5 million people in the world were infected with HIV
- 2050: 24.5 million people in the world were infected with HIV
- 2055: 26.5 million people in the world were infected with HIV
- 2060: 28.5 million people in the world were infected with HIV
- 2065: 30.5 million people in the world were infected with HIV
- 2070: 32.5 million people in the world were infected with HIV
- 2075: 34.5 million people in the world were infected with HIV
- 2080: 36.5 million people in the world were infected with HIV
- 2085: 38.5 million people in the world were infected with HIV
- 2090: 40.5 million people in the world were infected with HIV
- 2095: 42.5 million people in the world were infected with HIV
- 2100: 44.5 million people in the world were infected with HIV

adapted for straining out particulate organic matter from the water, they can also be readily used for rasping off epidermal tissue as well as for the ingestion of mucus from the gills of the host crayfish. Hoff (1942a) states that while he is inclined to believe that these ostracods are for the most part commensals, there exists the possibility that they are facultative parasites.

Another problem existing in the relationship between the ostracod and the crayfish and one which needs more research is the method of transfer of parasite from host to host. Marshall (1903) stated that the transfer occurred during the copulation of the crayfish or when an ostracod leaves a host for some reason to find another host. Hoff (1942a) supported this idea by stating that the dark sheltered places in which the crayfish hide are well suited for the transfer of an ostracod from one crayfish to another while the crayfish are copulating. Nevertheless, the lack of host specificity is some indication that the transfer of parasite from host to host occurs at times other than during the period of copulation. If the transfer occurred during copulation only, then there would be a greater display of host specificity. Also, there are some indications which tend to show that the transfer of the ostracod from one crayfish to another crayfish is complicated. Older crayfish which molted very infrequently were highly infected, the younger crayfish which molted repeatedly were rarely infected.

Since they are found only on crayfish, the distribution of the Entocytherinae is solely dependent upon the distribution of the crayfish.

HOST AND LOCALITY DATA

Entocytherinae

Salmon Creek Co.: (O. propinquus) Augusta Creek at B Avenue and 43rd Street, 7 July - 13 August, 1958; C Avenue and 43rd Street, 7 July - 13 August, 1958; Kellogg Forest, 7 July - 13 August, 1958.

Gull Lake, 15 July - 7 August, 1958.

(O. rusticus) Augusta Creek at B Avenue and 43rd Street, 7 July - 13 August, 1958; C Avenue and 43rd Street, 7 July - 13 August, 1958; Kellogg Forest, 7 July - 13 August, 1958.

(O. bartoni robustus) Augusta Creek at B Avenue and 43rd Street, 7 July - 13 August, 1958; C Avenue and 43rd Street, 7 July - 13 August 1958; Kellogg Forest, 7 July - 13 August, 1958.

Ingham Co.: (O. propinquus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road 1 October - 15 November, 1958; MSU Campus 1 October - 15 November, 1958.

(O. rusticus) Red Cedar River at Zimmer Road 1 October - 15 November, 1958; Dobie Road 1 October - 15 November 1958; MSU Campus 1 October - 15 November, 1958.

THE BRANCHIOBDELLIDAE

The Branchiobdellidae are unique in structure and habits and they are closely allied to the Hirudinea. They are so closely allied to the leeches that they were considered to be a family in this class until their definite oligochaete characteristics were established about 1912. According to Pennak (1953), the Branchiobdellidae form a morphological link connecting the typical Oligochaeta and the Hirudinea.

The Branchiobdellidae have body lengths that range from 1 mm. to 12 mm. The body consists of a head, trunk, and a highly muscular caudal sucker. The head is cylindrically shaped, and it may or may not be lobed. The trunk is posterior to the head and it contains eleven true segments. This region has many shapes in the different species and in some cases even genera can be identified on the basis of the shape of the trunk alone. The presence of sulci on the segments make the appearance of eleven true segments hard to define. Many of the segments are divided with a transverse sulcus and this gives the appearance of two rather than one segment. At times the last three segments are indistinct and hard to define, and are somewhat fused into the base of the caudal sucker. Some forms have dorsal appendages found on the segments of the trunk. These appendages usually consist of round fleshy protruberances having ridges of transverse muscle fibers attached to them.

The excretory system of the Branchiobdellidae consists of two pairs of nephridia, the anterior pair being located in segment III and the posterior pair located in segment VIII. •

Appendix 2

1

1. The first step in the process of identifying a research problem is to select a topic of interest.
2. The second step is to conduct a literature review to determine what has already been studied.
3. The third step is to identify a specific research question or hypothesis.
4. The fourth step is to design a study to test the hypothesis.
5. The fifth step is to collect data and analyze the results.
6. The sixth step is to write a report of the findings.
7. The seventh step is to present the findings at a conference or in a journal.
8. The eighth step is to discuss the implications of the findings.
9. The ninth step is to conclude the study.
10. The tenth step is to publish the findings.
11. The eleventh step is to disseminate the findings.
12. The twelfth step is to evaluate the study.
13. The thirteenth step is to reflect on the experience.
14. The fourteenth step is to share the experience.
15. The fifteenth step is to continue the research.
16. The sixteenth step is to seek funding.
17. The seventeenth step is to recruit participants.
18. The eighteenth step is to obtain ethical approval.
19. The nineteenth step is to develop a protocol.
20. The twentieth step is to pilot the study.
21. The twenty-first step is to collect data.
22. The twenty-second step is to analyze data.
23. The twenty-third step is to interpret results.
24. The twenty-fourth step is to write a report.
25. The twenty-fifth step is to present findings.
26. The twenty-sixth step is to discuss implications.
27. The twenty-seventh step is to conclude the study.
28. The twenty-eighth step is to publish findings.
29. The twenty-ninth step is to disseminate findings.
30. The thirtieth step is to evaluate the study.
31. The thirty-first step is to reflect on the experience.
32. The thirty-second step is to share the experience.
33. The thirty-third step is to continue the research.
34. The thirty-fourth step is to seek funding.
35. The thirty-fifth step is to recruit participants.
36. The thirty-sixth step is to obtain ethical approval.
37. The thirty-seventh step is to develop a protocol.
38. The thirty-eighth step is to pilot the study.
39. The thirty-ninth step is to collect data.
40. The fortieth step is to analyze data.
41. The forty-first step is to interpret results.
42. The forty-second step is to write a report.
43. The forty-third step is to present findings.
44. The forty-fourth step is to discuss implications.
45. The forty-fifth step is to conclude the study.
46. The forty-sixth step is to publish findings.
47. The forty-seventh step is to disseminate findings.
48. The forty-eighth step is to evaluate the study.
49. The forty-ninth step is to reflect on the experience.
50. The fiftieth step is to share the experience.
51. The fifty-first step is to continue the research.
52. The fifty-second step is to seek funding.
53. The fifty-third step is to recruit participants.
54. The fifty-fourth step is to obtain ethical approval.
55. The fifty-fifth step is to develop a protocol.
56. The fifty-sixth step is to pilot the study.
57. The fifty-seventh step is to collect data.
58. The fifty-eighth step is to analyze data.
59. The fifty-ninth step is to interpret results.
60. The sixtieth step is to write a report.
61. The sixty-first step is to present findings.
62. The sixty-second step is to discuss implications.
63. The sixty-third step is to conclude the study.
64. The sixty-fourth step is to publish findings.
65. The sixty-fifth step is to disseminate findings.
66. The sixty-sixth step is to evaluate the study.
67. The sixty-seventh step is to reflect on the experience.
68. The sixty-eighth step is to share the experience.
69. The sixty-ninth step is to continue the research.
70. The seventieth step is to seek funding.
71. The seventy-first step is to recruit participants.
72. The seventy-second step is to obtain ethical approval.
73. The seventy-third step is to develop a protocol.
74. The seventy-fourth step is to pilot the study.
75. The seventy-fifth step is to collect data.
76. The seventy-sixth step is to analyze data.
77. The seventy-seventh step is to interpret results.
78. The seventy-eighth step is to write a report.
79. The seventy-ninth step is to present findings.
80. The eightieth step is to discuss implications.
81. The eighty-first step is to conclude the study.
82. The eighty-second step is to publish findings.
83. The eighty-third step is to disseminate findings.
84. The eighty-fourth step is to evaluate the study.
85. The eighty-fifth step is to reflect on the experience.
86. The eighty-sixth step is to share the experience.
87. The eighty-seventh step is to continue the research.
88. The eighty-eighth step is to seek funding.
89. The eighty-ninth step is to recruit participants.
90. The ninetieth step is to obtain ethical approval.
91. The ninety-first step is to develop a protocol.
92. The ninety-second step is to pilot the study.
93. The ninety-third step is to collect data.
94. The ninety-fourth step is to analyze data.
95. The ninety-fifth step is to interpret results.
96. The ninety-sixth step is to write a report.
97. The ninety-seventh step is to present findings.
98. The ninety-eighth step is to discuss implications.
99. The ninety-ninth step is to conclude the study.
100. The hundredth step is to publish findings.

In the reproductive system, some forms have one pair of testes and some have two pairs. Those with the single pair have them located in segment V and those with the two pairs have them located in segment V and VI. The sperm duct usually opens on the ventral midline of segment V and if there is a male genital pore present, it is located on the ventral midline of segment VI. The female organs consist of a pair of ovaries located in segment VII. There are two ventrally located female genital pores.

There are several good taxonomic characters in the Branchiobdellidae that are used in the preparation of the key. They are the shape of the trunk, the presence or absence of a male genital pore, dorsal appendages, position of the caudal sucker, the number of testes, and the shape and arrangement of the jaw teeth.

The distribution of the Branchiobdellidae is wholly dependent on the distribution of crayfish and they have been reported from all regions where crayfish have been found. There were certain factors noted which indicate that there might be a possible correlation between the water temperature and the number of branchiobdellids present. In Augusta Creek, a trout stream in Kalamazoo County, these forms were somewhat sparse. This stream runs crystal clear, and during the time that crayfish were collected, the stream had an average temperature of 55 degrees Fahrenheit. Likewise Gull Lake, also in Kalamazoo County, is clear but with a somewhat higher average temperature, 59 degrees F. to 61 degrees F. The collecting was done during the months of July and August. The number of branchiobdellids collected from the crayfish taken in these two bodies of water were very small in comparison to the number taken in other

areas, and they were usually found under the branchiostegites. The branchiobdellids taken from the external surface were usually found on the ventral side of the cephalothorax between the appendages.

In the Red Cedar River in Ingham Co. large numbers of branchiobdellids were found on the outside of the body as well as under the branchiostegites. Several crayfish were almost completely covered with the branchiobdellids. The crayfish taken from this river were caught during the months of October and November and the average temperature of the stream was 68 degrees F. to 70 degrees F.

A review of the literature indicates that some study has been given to the possibilities of host specificity. Except for the fact that those found in the United States on the western crayfish Astacus have not been found on the eastern forms Cambaras, Procambaras, and Orconectes and visa versa, there has been no evidence of host specificity. The possibility exists that this one case of host specificity develops as the result of geographical isolation caused by a physical barrier which prevents the crayfish from expanding the range. Accordingly, the parasites would also be limited in their distribution.

HOST AND LOCALITY DATA

Cambarincola chirocephala

Kalamazoo Co.: (O. propinquus) Augusta Creek at B Avenue and 43rd

Street, 7 July - 13 August, 1958; C Avenue and 43rd Street, 7 July - 18 August, 1958; Kellogg Forest, 7 July - 18 August, 1958.

(O. rusticus) Augusta Creek at B Avenue and 43rd Street, 7 July - 18 August 1958; C Avenue and 43rd Street, 7 July - 18 August, 1958; Kellogg Forest, 7 July - 18 August, 1958.

(Cambaras bartoni robustus) Augusta Creek at B Avenue and 43rd Street 7 July - 18 August, 1958; C Avenue and 43rd Street, 7 July - 18 August, 1958.

Ingham Co.: (O. propinquus) Red Cedar River at Zimmer Road, 1 October - 15 November 1958; Dobie Road, 1 October - 15 November, 1958.

(O. rusticus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road, 1 October - 15 November, 1958.

Cambarincola philadelphica

Ingham Co.: (O. propinquus) Red Cedar River at Zimmer Road, 1 October - 15 November 1958; Dobie Road, 1 October - 15 November, 1958.

Cambarincola elevata

Kalamazoo Co.: (O. propinquus) Augusta Creek at B Avenue and 43rd

Street, 7 July - 13 August 1958; C Avenue and 43rd Street, 7 July - 13 August 1958; Kellogg Forest, 7 July - 18 August, 1958.

Gull Lake, 15 July - 7 August, 1958.

(O. rusticus) Augusta Creek at B Avenue and 43rd Street, 7 July - 18 August 1958; C Avenue and 43rd Street, 7 July - 18 August 1958; Kellogg Forest, 7 July - 18 August, 1958.

(Cambaras bartoni robustus) Augusta Creek at B Avenue and 43rd Street, 7 July - 18 August, 1958; C Avenue and 43rd Street, 7 July - 18 August, 1958; Kellogg Forest, 7 July - 18 August, 1958.

Ingham Co.: (O. propinquus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road, 1 October - 15 November, 1958; MSU Campus, 1 October - 15 November, 1958.

(O. rusticus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road 1 October - 15 November, 1958; MSU Campus, 1 October - 15 November, 1958.

Cambarincola macrodonta

Kalamazoo Co.: (O. propinquus) Augusta Creek at B Avenue and 43rd Street, 7 July - 18 August, 1958; C Avenue and 43rd Street, 7 July - 18 August, 1958; Kellogg Forest, 7 July - 18 August, 1958. Gull Lake, 15 July - 7 August, 1958.

(O. rusticus) Augusta Creek at B Avenue and 43rd Street, 7 July - 18 August, 1958; C Avenue and 43rd Street, 7 July - 18 August, 1958; Kellogg Forest, 7 July - 18 August, 1958.

(Cambaras bartoni robustus) Augusta Creek at B Avenue and 43rd Street, 7 July - 18 August, 1958; C Avenue and 43rd Street, 7 July - 18 August, 1958; Kellogg Forest, 7 July - 18 August, 1958.

Ingham Co.: (O. propinquus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road, 1 October - 15 November, 1958; MSU Campus, 1 October - 15 November, 1958.

(O. rusticus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road, 1 October - 15 November, 1958; MSU Campus, 1 October - 15 November, 1958.

Carbarincola vitrea

Kalamazoo Co.: (O. propinquus) Augusta Creek at B Avenue and 43rd Street, 7 July - 18 August, 1958; C Avenue and 43rd Street, 7 July - 18 August, 1958.

(Carbaras bartoni robustus) Augusta Creek at B Avenue and 43rd Street, 7 July - 18 August, 1958; C Avenue and 43rd Street, 7 July - 18 August, 1958.

Ingham Co.: (O. propinquus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road, 1 October - 15 November, 1958; MSU Campus, 1 October - 15 November, 1958.

(O. rusticus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road, 1 October - 15 November, 1958.

Xironocrilus formosus

Kalamazoo Co.: (O. propinquus) Augusta Creek at C Avenue and 43rd Street, 7 July - 18 August, 1958; Kellogg Forest, 7 July - 18 August, 1958.

(O. rusticus) Augusta Creek at C Avenue and 43rd Street, 7 July - 18 August, 1958; Kellogg Forest, 7 July - 18 August, 1958.

(Carbaras bartoni robustus) Augusta Creek at C Avenue and 43rd Street, 7 July - 18 August, 1958.

Ingham Co.: (O. propinquus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road, 1 October - 15 November, 1958.

(O. rusticus) Red Cedar River at Zimmer Road, 1 October - 15 November, 1958; Dobie Road, 1 October - 15 November, 1958.

Pterodrilus distichus

Ingham Co.: (O. propinquus) Red Cedar River at Dobie Road, 1 October - 15 November, 1958.

S U M M A R Y

1. While the true nature of the relationships between the crayfish and the Allocreadiidae and the Entocytherinae is in doubt there appears to be some indications of their parasitic nature.

2. The metacercariae of the Allocreadiidae parasitize the crayfish in great numbers in some bodies of water.

3. There were two genera and three species of crayfish collected during this survey.

4. There were three genera and seven species of Branchiobdellidae collected.

5. There were numerous specimens of the ostracod genus Entocythere spp. and the trematode family Allocreadiidae collected.

6. Little if any host specificity was noted in any of the parasitic forms.

7. Maps of the collecting areas, as well as an illustrated key to the forms identified are presented.

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A P P E N D I X

LIST OF APPENDIXES

- Appendix 1. Illustrations for the key of the Branchiobdellidae.
- Appendix 2. Composite list of the crayfish and the parasites collected.
- Appendix 3. Composite chart showing the distribution of the crayfish and the parasites.
- Appendix 4. Map of the collecting areas on Augusta Creek.
- Appendix 5. Map of the collecting areas on the Red Cedar River.

A P P E N D I X 1

- Fig. 1. Lateral view of Cadherincola elevata Goodnight.
Fig. 2. Lateral view of Pterodrilus distichus Moore.
Fig. 3. Lateral view of Cadherincola macrodonta Ellis.
Fig. 4. Upper jaw of C. macrodonta Ellis.
Fig. 5. Upper jaw of C. vitrea Ellis.
Fig. 6. Upper jaw of Microdrilus formosus Ellis.

- a. Spermatheca
b. Accessory sperm duct
c. Genital pore
d. Testes

A P P E N D I X 2

The following species of crayfish were taken and specimens obtained from them.

Phylum-Arthropoda
Class - Crustacea
Order - Decapoda
Carthagen bartoni robustus Girard
Orconectes rusticus
Orconectes propinquus (Girard)

The following specimens were taken from the above named crayfishes with no evidences of host specificity.

Phylum - Annelida
Class - Oligochaeta
Order - Opisthopora
Family - Branchiobdellidae
Subfamily - Cambarincolinae
Cambarincola chirocephala Ellis
Cambarincola philadelphia (Leidy)
Cambarincola elevata Goodnight
Cambarincola macrodonata Ellis
Cambarincola vitreus Ellis
Xironodrilus formosus Ellis
Pterodrilus distichus Moore

Phylum - Arthropoda
Class - Ostracoda
Order - Podocopa
Family - Cytheridae
Subfamily - Entocytherinae
Entocythere spp.

Phylum - Platyhelminthes
Class - Trematoda
Order - Digenea
Family - Allocreadiidae

A P P E N D I X 3

DISTRIBUTION OF THE CRAYFISH AND THE PARASITES

Station Parasite	AUGUSTA CREEK			RED CEDAR RIVER			GULL LAKE
	1	2	3	4	5	6	
<u>Cambarincola</u> <u>chirocephala</u>	0 x @	0 x @	0 x	0 x	0 x		7
<u>Cambarincola</u> <u>philadelphica</u>				x	x	x	
<u>Cambarincola</u> <u>elevata</u>	0 x @	0 x @	0 x @	0 x	0 x	0 x	x
<u>Cambarincola</u> <u>macrodonata</u>	0 x @	0 x @	0 x @	0 x	0 x	0 x	x
<u>Cambarincola</u> <u>vitrea</u>	x @	x @		0 x	0 x	x	
<u>Xironodrilus</u> <u>formosus</u>	0 x	0 x @	0 x	0 x	0 x		
<u>Pterodrilus</u> <u>distichus</u>					x		
<u>Entocytherinae</u>	0 x @	0 x @	0 x @	0 x	0 x	0 x	x
<u>Branchiobdellidae</u>		0 x @		0 x	0 x	x	

x = O. propinquus 0 = O. rusticus @ = Cambarus bartoni robustus

A P P E N D I X 4

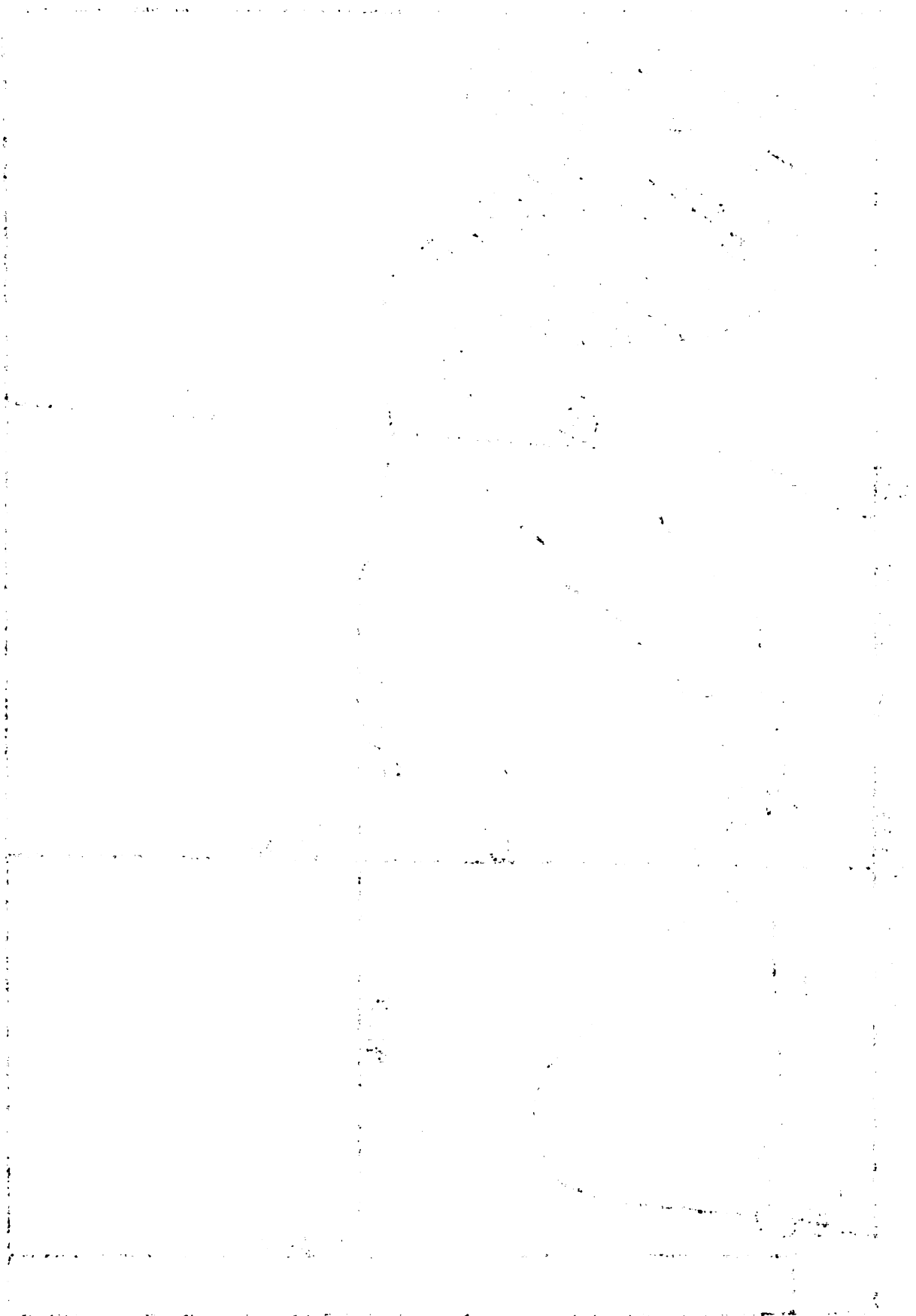
A P P E N D I X 4

Map of Augusta Creek Collecting Areas

Site 1. B Avenue and 43rd Street

Site 2. C Avenue and 43rd Street

Site 3. Bellogg Forest



A P P E N D I X 5

A P P E N D I X 5

Map of the Red Cedar River Collecting Areas

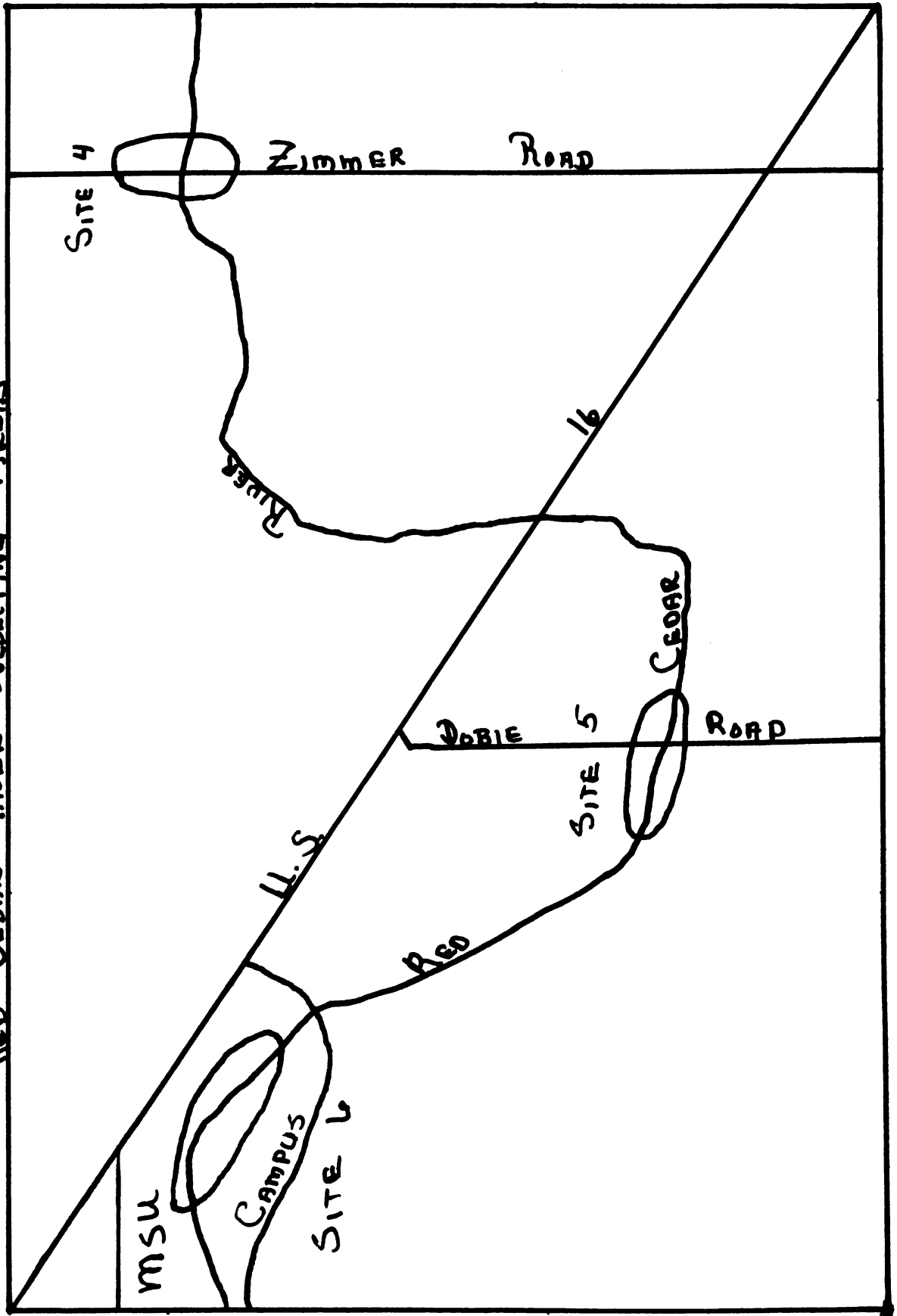
Site 4. Zimmer Road

Site 5. Dobie Road

Site 6. Michigan State University Campus

(Behind the main library and at
the dam)

RED CEDAR RIVER COLLECTING AREAS



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