THE GLOCHIDIA (LARVAE) OF THE FRESHWATER MUSSEL *MARGARITIFERA HEMBELI* (UNIONACEA: MARGARITIFERIDAE) FISH HOST SPECIES, MORPHOLOGY, AND PERIOD OF FISH HOST INFECTION

by

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TABLE OF CONTENTS

ABSTRACT

Eight streams in the Evangeline Ranger District, Rapides Parish, Louisiana were sampled for fish by seining to determine the fish host species of Margaritifera hembeli glochidia. Glochidia occurred on the gill filaments and in gill arch epithelium of Notropis chrysocephalus, Notropis umbratilis, and Notemigonus crysoleucas. Infection occurred from March through August, 1986 and peaked April through July. Encysted glochidia were semi-oval and averaged 187.5, 191.7, and 105.9 µm respectively in length, width, and hinge length. Mounted sections revealed the glochidial adductor muscle, hinge ligament, stomach, foot, gill buds, mantle, and shell flanges. There was no evidence of glochidial teeth or a byssus.

TABLES AND APPENDICES

FIGURES

INTRODUCTION

Because their demibranchs possess non-elastic interlamellar junctions that allow for little expansion of the marsupia, the Margaritiferidae are considered a primitive group of freshwater mussels. Margaritifera hembeli was first described as Unio hembeli in 1838 by T.A. Conrad from a specimen of unknown locality sent to him from New Orleans. The species was renamed Margaritana hembeli and then again renamed Margaritifera hembeli, the currently accepted designation (Ortmann, 1912; Johnson, 1983).

Margaritifera hembeli was reported to occur in two disjunct populations, one in Alabama and one in Louisiana (Ortmann, 1912), but it was determined that the Alabama group is a different species (Johnson, 1983). There are approximately ten thousand M. hembeli scattered in beds that are limited to eleven streams of the Bayou Boeuf drainage. These streams are located in the Evangeline Ranger District of the Kisatchie National Forest, Rapides Parish, Louisiana. About 89% of the total known population occurs in four of the eleven streams (Louisiana Natural Heritage Program, 1985). Because of its limited distribution and population size, M. hembeli is a candidate for federal listing as an endangered species (Jim Stewart, USFWS, pers. comm., 1986).

The streams containing M. hembeli are typically clear shallow streams with sand and sand/gravel substrates with intermittant pockets of mud and clay. These streams are generally bounded by bottomland areas with undisturbed old growth trees of mixed hardwood-loblolly and beech-magnolia forest types. No M. hembeli have been found in streams bounded by developed or cultivated lands. Beds of M. hembeli are found in sand and sand/gravel substrates in water depths of about six to eighteen inches and may contain as many as two thousand individuals. The sand/gravel beds in which M. hembeli occur contain rocks coated with the black substance manganese oxide, and for the dark shelled mussels, these rocks provide excellent cover from potential predators (Louisiana Natural Heritage Program, 1985).

Like most freshwater mussels, M. hembeli reproduces by means of glochidia. These larvae are host specific parasites that infect the gills, fins, or body surfaces of fish. When the glochidia come in contact with the appropriate host species and tissues, they encyst and undergo a period of metamorphosis. During metamorphosis the glochidia develop organ systems of the adult and emerge as juvenile mussels that settle to the substrate where, if the habitat is suitable, they burrow in and continue to grow and develop.

Before successful encystment and metamorphosis of the glochidium can occur, the individual host must first be susceptible to glochidial infection (Karna and Millemann, 1978). Some fish species as a whole are resistant to such infections, either actively by immunological responses or passively by the fact that they do not possess certain physiological parameters required by the glochidia for successful metamorphosis. Within a susceptible fish species, many older individuals sometimes develop immunological responses as a result of past infections, limiting glochidial infections to the more numerous younger individuals. In addition to possessing certain physiological characteristics, host fish are generally of a species that spends much of its life over areas with substrate suitable for newly released juveniles. This factor is important in that it helps reduce an already extremely high mortality rate among the young (Young and Williams, 1984a).

There are a variety of mechanisms available to glochidia for attraction and attachment to potential hosts, but they all share some similar structures and behaviors. In general glochidial structure consists of two chitinoid valves lined with a single layer of cells that form the larval mantle, a single adductor muscle, and in many species, teeth on the shell's periphery opposite the hinge. A larval thread known as the byssus is present in some species, and along with the

teeth, it may aid in attachment to the host fish (Pennack, 1965). A characteristic behavior of glochidia is the "snapping" of their valves. The rate of this snapping increases significantly in the presence of certain stimuli. The basal snapping rate of M. margaritifera glochidia was shown to increase somewhat in the presence of host (Brown Trout) blood and greatly in the presence of host mucous, gill, and fin tissues (Young and Williams, 1984b). This behavior along with attachment structures and the immediate closure response upon mantle contact increases the glochidias' chances of successful attachment.

After successful attachment to the host, encystment begins. The glochidium is rapidly surrounded by a wall of host epithelial cells. Complete encystment for the glochidia of Lampsilis radiata on yellow perch requires only two to three hours after initial attachment (Tedla and Fernando, 1969). The period of glochidial encystment varies widely between species and depends on whether the species is a long term or short term breeder. Juveniles may appear as early as forty days after the initial infection, as in the case of L. radiata, or after as long as 293 days, as in the case of M. margaritifera (Tedla and Fernando, 1969; Young and Williams, 1984b). Juveniles are often released from the host over an extended period, possibly because release is due more to host tissue

fragility than glochidial activity (Tedla and Fernando, 1969).

The size and shape of glochidia vary between species and more so between genera. Though there are differences in larval morphology, it is often difficult to distinguish between the glochidia of two related mussel species as in the case of Villosa vanuxemi and Villosa nebulosa. The glochidias' valve shapes are nearly identical, having very similar breadth, length, and hinge length measurements (Zale and Neves, 1982). Mean length and breadth of glochidia may range from 382 and 383 μm respectively as in Anodonta cataracta to 60 and 80 μm as in M. margaritifera (Rand and Wiles, 1982; Young and Williams, 1984a). The valves may be oval, rounded, or triangular in shape (Pennak, 1965), but again, within a genus it may be difficult to distinguish between species based strictly on glochidia size and shape.

Prior to this study, all that was known about M. hembeli was the adult morphology, approximate population size, and distribution. The goals of this study were to determine the glochidias' fish hosts, the occurrence of glochidia from month to month, the streams in which glochidial infections occur, and the morphological characteristics of the larvae.

METHODS AND MATERIALS

Fish populations were sampled twice monthly from October, 1985 through September, 1986. The study area included eight streams that contain approximately 99.7% of the total M. hembeli population (Louisiana Natural Heritage Program, 1985). These streams are Loving Creek, Little Loving Creek, Bayou Clear, Brown Creek, Castor Creek, Mack Branch, and Long Branch of the Evangeline Ranger District, Rapides Parish, Louisiana. Each stream was sampled for fish by seining in both riffle and pool areas over and near existing beds of M. hembeli. Bottom and plankton samples were taken for glochidia downstream of M. hembeli beds as time allowed. Sampling at each site continued until a good sample size was obtained or until the maximum time allowable was invested in the sampling effort. Collected fish were immediately fixed in 10% buffered formalin and preserved for later examination in the laboratory. Specimens of M. hembeli were examined in the field for the presence of gravid females. A few individuals were preserved and taken back to the laboratory for further inspection. The field work for this study involved approximately 460 man hours over the course of 12 months.

All of the preserved fish specimens were keyed to species using Freshwater Fishes of Louisiana (Douglas, 1974) and The Fishes of Missouri (Pflieger,

1975). Each sample was kept separate, and a record was maintained of the number of each species caught. Dissecting scopes were used to inspect the fish for the presence of glochidia. Gills, fins, and body surfaces were examined for infection, and a record was kept of the site of the larval infection and the number of individuals per species infected. Because myxosporidian cysts appeared very similar to glochidia, gill filaments were routinely excised and more closely inspected to confirm the presence of larvae.

The gills of infected fish were excised to make whole mounts and sections of encysted glochidia. Whole mount specimens were washed in running water for one hour and treated successively with 15, 30, 50, and 70% ethyl alcohol for twenty to thirty minutes per treatment. After completion of partial dehydration, the specimens were stained in a one to one solution of Semichon's carmine stain and 70% ethyl alcohol. After one hour the stain was withdrawn and the specimens were washed with 70% alcohol. To partially destain the valves without destaining the glochidias' soft tissues, the specimens were bathed in 70% acid alcohol. The specimens were washed with three changes of 70% alcohol to remove the acid and treated with Li CO_3 to prevent further destaining. The dehydration process was completed by successively treating stained specimens with 80, 95, and two 100% ethyl alcohol solutions for

one hour each. The specimens were cleared in two changes of xylene for one hour each and then mounted using Lipshaw mounting media.

Mounted sections of glochidia were made from paraffin embedded gills. Preserved gills were washed in water for one hour and dehydrated successively with 30, 50, 70, 95, 95, 100, and 100% alcohol for one hour each. They were cleared in two changes of xylene for one hour each. The cleared specimens were treated with a series of paraffin/xylene solutions that were maintained at a temperature of about 63°C. These solutions included concentrations of 25, 50, 75, 100, and 100% paraffin for 30 minutes each to prepare the gills for embedding. The gills were embedded in paraffin and sectioned at a thickness of 13 um.

The gill sections were floated on warm water (approximately 50°C) to remove any irregularities in the paraffin and mounted on slides using egg albumin. After allowing the slides to dry completely, they were placed in two changes of xylene for 30 minutes each to remove the paraffin. The mounts were hydrated through successive five minute treatments in 100, 95, and 70% ethyl alcohol and water. They were placed in Harris' hematoxylin stain for approximately 20 minutes. After removing the excess stain with water and fixing the stain with $LiCO_3$ in water, the sections were placed in 70% alcohol for five minutes to prepare

them for counterstaining with eosin. After two to three minutes in eosin, the mounts were rinsed twice in 95% alcohol and dehydrated in two changes of 100% alcohol. The sections were cleared in two changes of xylene five minutes each and permanently mounted with Lipshaw mounting media.

Whole mounts of freshly excised glochidia were measured for length, width, and hinge length by using an ocular micrometer. The host species and collection dates were recorded with the measurements in the event of any noticeable change in size over the period of encystment.

RESULTS

Approximately 3800 fish and thirty species were collected over a twelve month period from the study streams. With a few exceptions and some variation in abundance, the species collected were common to all eight of the streams, but predominant in all of the stream collections was the striped shiner, Notropis chrysocephalus.

Collected fish exhibited glochidial infections over a six month period from March through August, 1986 (Appendix A). Infected fish were collected in mid March from Castor Creek and Long Branch and from Little Loving Creek by late March. In the latter half of April, infections appeared in Loving Creek and Bayou Clear, and by early May they occurred in Brown Creek. Infected fish were not recovered from Little Bayou Clear or Mack Branch.

Of the thirty species of fish collected, three were infected with glochidia. The infected species were Notropis chrysocephalus (striped shiner), Notropis umbratilis (redfin shiner), and Notemigonus crysoleucas (golden shiner)(Figure 2). The most consistent glochidia infections occurred on N. chrysocephalus (Figure 3). Infections appeared by early March, peaked from April through June, began to decrease in frequency during July, and were no longer evident by early September. Notropis umbratilis were collected from all of the

streams at one time or another, but consistently, only from Bayou Clear, Brown Creek, and Castor Creek. Glochidial infection of the redfin shiner began occurring in late April - early May and ended by late August - early September (Figure 4). Only four N. crysoleucas were captured over the entire collection period, introducing a heavy sample bias for that species. Three of the four individuals were collected during the infection period and two of those three were parasitized by glochidia.

The first glochidial infections occurred when water temperatures were as low as 15.5°C. April through July, the months of peak infection, water temperatures ranged from 18.5°C to 23.5°C. Water temperatures gradually rose to approximately 24°C between July and the end of August and, during this period glochidial infections began to decrease. By the time water temperatures began to fall in September, glochidial infections no longer occurred. These observations only show the trend that occurred during this study (Figure 5). To show any correlation between temperature and glochidia release as has been done for some species would require several years of replicated research.

Margaritifera hembeli glochidia were encysted in the fishes' gill filaments and in the epithelium covering the gill arches and rakers (Figures 7 and 8). No glochidia were found on the fishes' fins or body

surfaces. Gill lamellae fused around the parasites to form cyst walls, creating a club-like appearance in the gill filaments when infection occurred near the filaments' tips.

The glochidia of M. hembeli have semi-oval valves (Figure 6) with mean lengths, widths, and hinge lengths of 187.5, 191.7, and 105.9 μm respectively (Table 1). There are no glochidial teeth on the shell's periphery opposite the hinge and, there is no evidence of a byssus. Two slight undulations in the shell's border are present, one on either side of and dorsal to the hinge. The significance of these structures is unknown though it is possible they may bear some relation to hinge teeth.

In sagittal view, the glochidia are oval (Figure 7). The adductor muscle is a prominent structure just ventral to the hinge. The glochidial mantle has two cell layers. The outer layer will form the mantle of the adult (Karna and Millemann, 1978). The foot of the glochidium is a large structure in the center of the section. It ranges from triangular to rounded in shape depending on where the section was made. The stomach is directly between the hinge and the foot. Gill buds are located to either side of the foot. Host tissue can be seen pinched between the two shell flanges, the ventral edges of the valves.

In transverse sections, the large adductor muscle is not centered in respect to the hinge (Figure 8). It is oval and occupies fully three fourths of the hinge's length. The glochidial foot is fairly large and much resembles the adult foot. The stomach is visible between the muscle and foot though it is not as distinct a structure. Many of the adult structures are visible in the developing larvae.

DISCUSSION AND CONCLUSIONS

No fish infected with glochidia were found in Mack Branch or Little Bayou Clear. Little Bayou Clear has a very small M. hembeli population of about 90 mussels. It is probable that the number of glochidia produced by this population resulted in a very low infection rate that was not detected by sampling. Mack Branch has approximately 380 M. hembeli, a population size comparable to Castor Creek (Louisiana Natural Heritage Program, 1985) where many infected fish were found. Most of the fish caught in Mack Branch were sunfish, small bass, cypress darters, and mosquito fish, all characteristic of slow moving or still water. Very few Notropis species were captured in this stream. The current in Mack Branch is slow compared to the other study streams and any newly released glochidia would settle to the bottom rapidly, reducing their already slim chances of attaching to the stream's scarce host species.

Notropis umbratilis and Notropis chrysocephalus were both infected by glochidia, but N. chrysocephalus yielded infections two months before any were found on N. umbratilis. Notropis umbratilis had higher percentages of infection during peak months than N. chrysocephalus (Figures 3 and 4), but the overall percentages (Total number of infected fish/Total number of collected fish during infective months) were 13.4%

for N. chrysocephalus and 14.9% for N. umbratilis, a difference of only 1.5%. From general observations, N. umbratilis yielded six to eight glochidia per infected fish while N. chrysocephalus regularly yielded twice that number. A greater percentage of N. umbratilis carried glochidia, but a comparable percentage of the much more abundant N. chrysocephalus carried a larger number of glochidia per fish.

Notropis chrysocephalus were also more widely distributed through the streams than N. umbratilis. They were as frequently found in riffle areas as in pools while N. umbratilis were generally restricted to pools and deeper slower moving water. Notropis chrysocephalus appear to spend more time in waters with suitable substrate for M. hembeli than do N. umbratilis, giving the newly released juveniles a greater chance for survival. Because of their abundance, habits, and tendency to carry more glochidia, N. chrysocephalus appear to be the primary hosts for M. hembeli glochidia. Notropis umbratilis is a host species, but its habits and tendency to carry few glochidia make it a less efficient vector. Notemigonus crysoleucas is susceptible to infection by glochidia, but its status as a host cannot yet be determined.

No gravid female M. hembeli were observed throughout the study period. Since inspections were made every two weeks, it is possible that the gravid

period was missed. It is also possible that the percentage of gravid females at any one time was low making them difficult to find or that this researcher did not inspect a large enough proportion of the population. However, some specimens were collected mid-June that possessed remnant accessory material in their demibranchs as described by Smith (1979, 1976) in M. margaritifera, indicating that the gravid state had indeed occurred.

There are three other mussel species in the Bayou Boeuf drainage. They are Villosa lienosa, Ligumia subrostrata, and Fusconaia flava (Louisiana Natural Heritage Program, 1985). Without larvae taken directly from M. hembeli to verify species, the literature had to be consulted for information on the other species' glochidia. Villosa species are approximately 100 µm wider and 40 µm longer than the herein described glochidia and are more egg shaped than semi-oval (Zale and Neves, 1982a). They parasitize centrarchids and the banded sculpin (Zale and Neves, 1982b). Ligumia subrostrata glochidia parasitize Lepomis species rather than shiners (Stern, 1978). There is no literature known to this researcher on the glochidia of Fusconaia flava. It is possible that some of the glochidia observed were from F. flava, but not likely. There are very few F. flava in the study streams and large numbers of M. hembeli near the

sampling sites. The valves of the observed glochidia are very similar in shape, size, and morphology to other Margaritifera species (Smith, 1976; Karna and Williams, 1979). This researcher is confident that these glochidia are M. hembeli.

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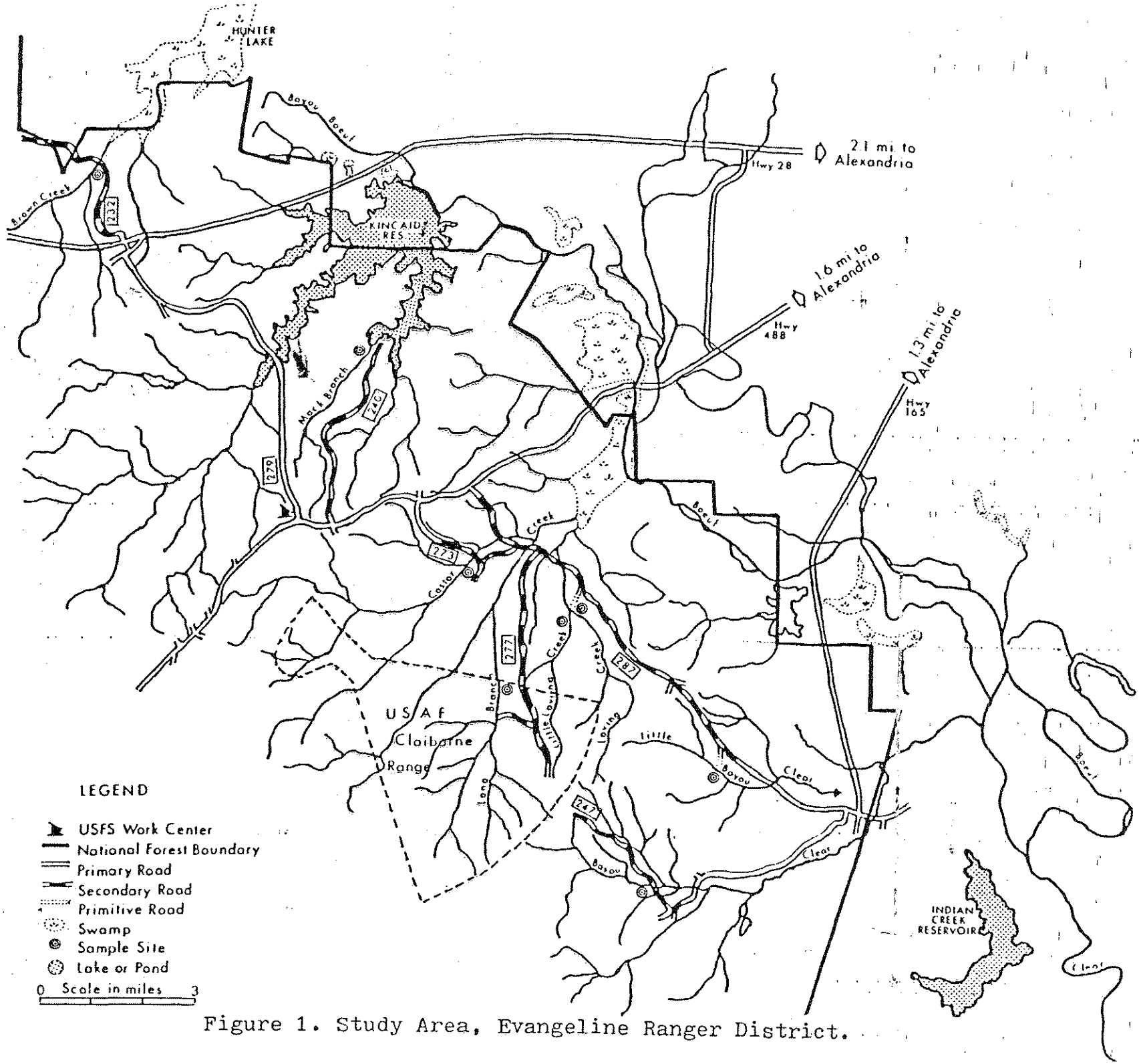

Figure 1. Study Area, Evangeline Ranger District.

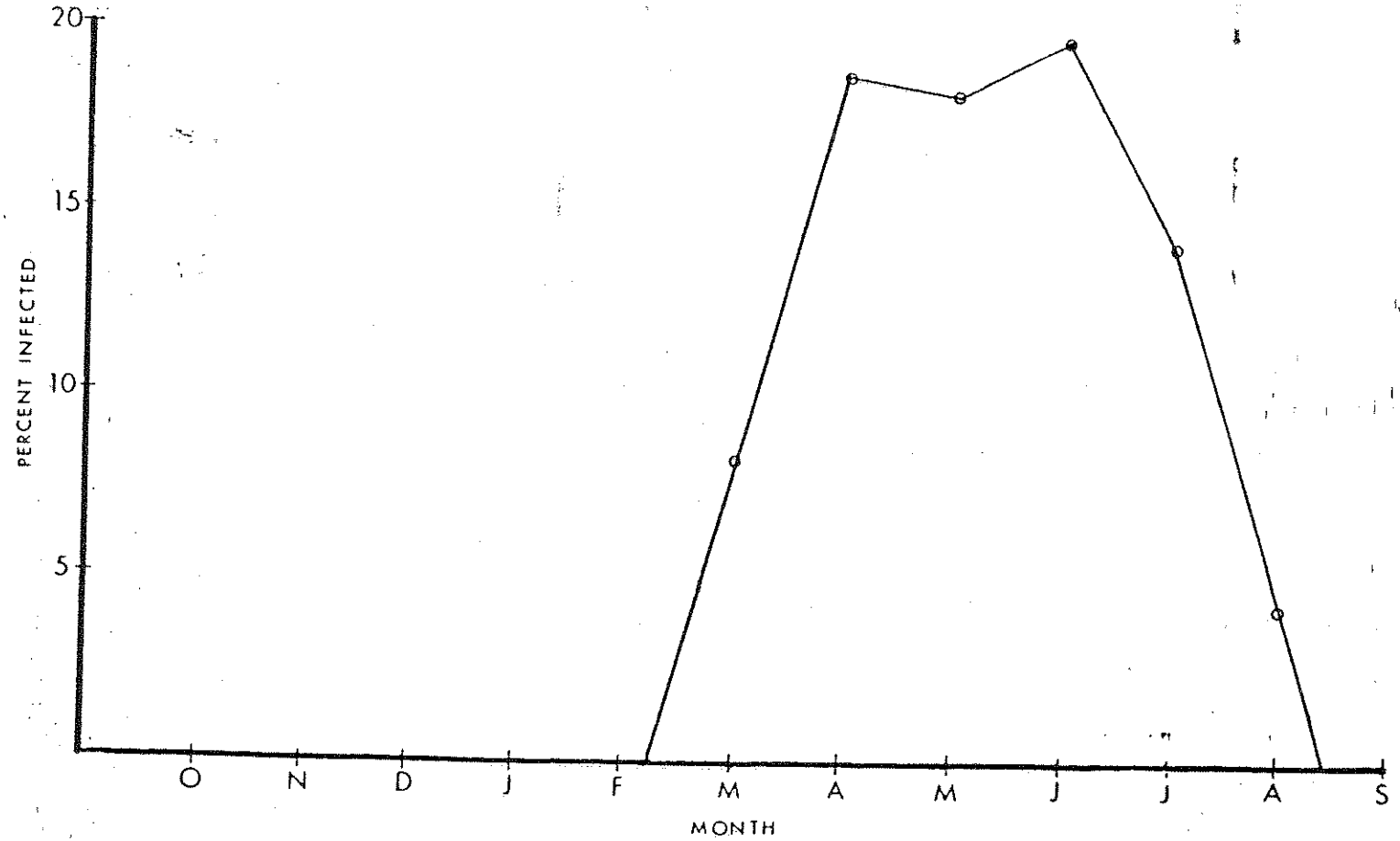

Figure 3. Overall percentages of N. chrysocephalus infected with glochidia each month in the streams with infected fish.

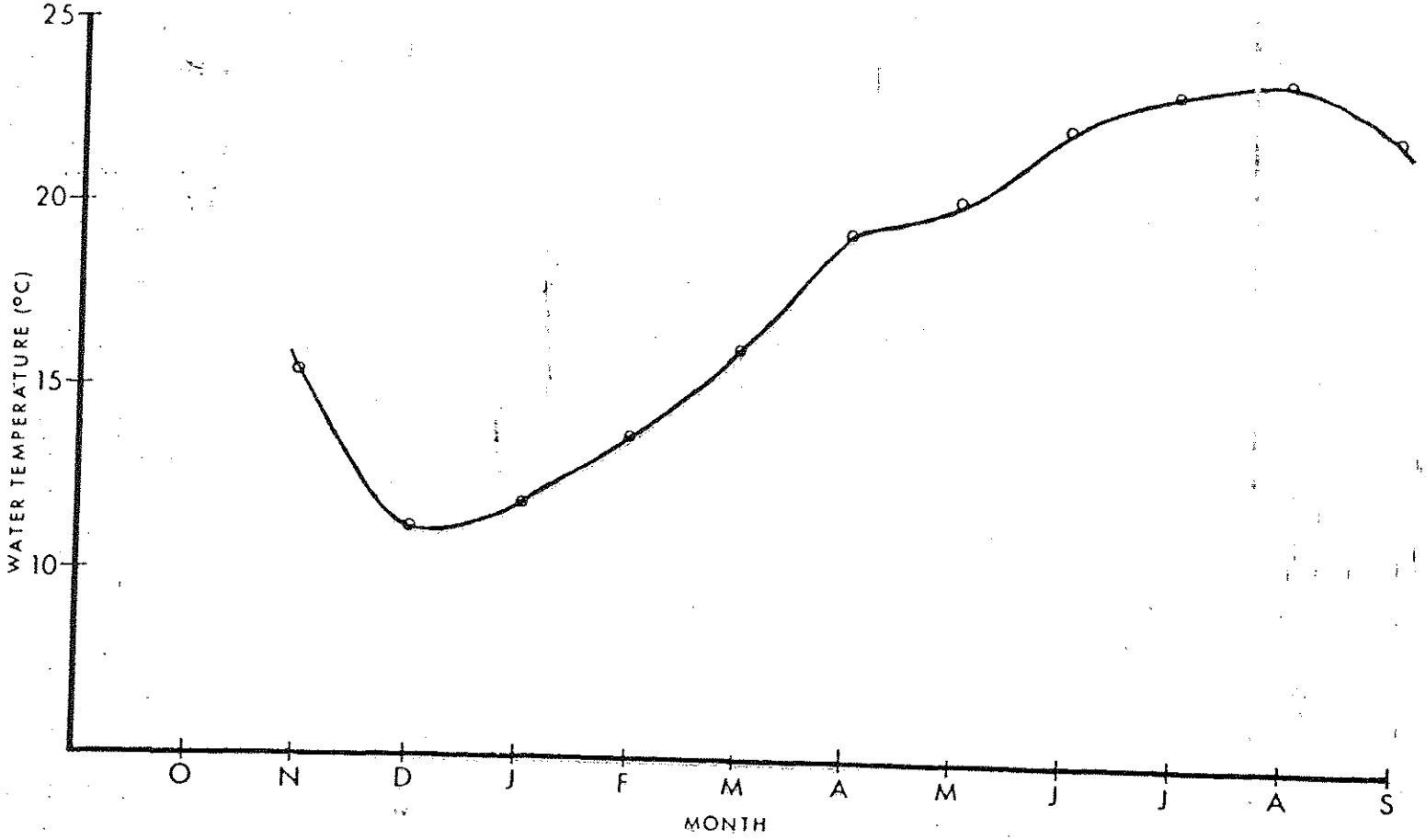

Figure 5. Average temperature trend of the study streams.

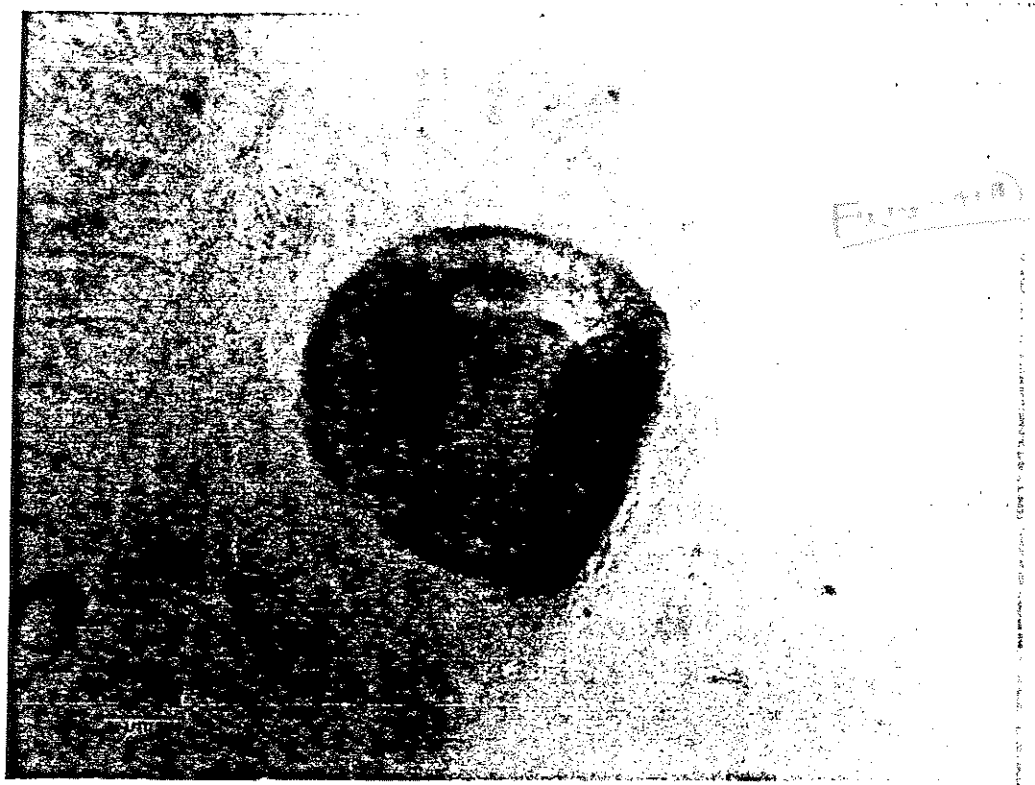

Figure 6. Whole Mount of Margaritifera hembeli Glochidia Encysted in Fish Host Gill Filament.

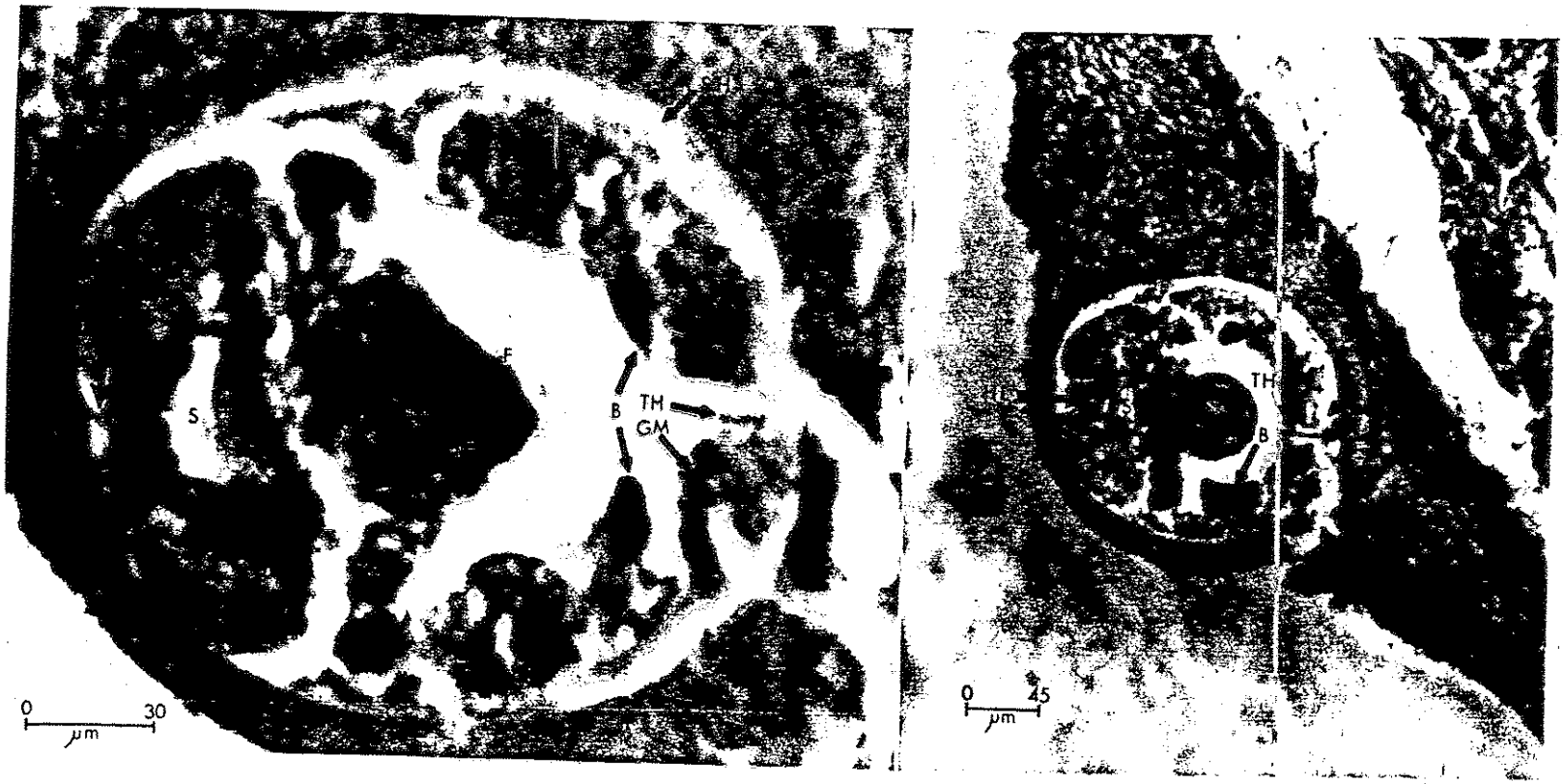

Figure 7. Sagittal Sections of Margaritifera hembeli Glochidia. F, glochidial foot; S, stomach; HL, hinge ligament; M, adductor muscle; B, gill buds; TH, host tissue; AE, gill arch epithelium; GM, glochidial mantle; SF, shell flange; SH, glochidial shell.

Figure 8. Transverse Section of Margaritifera hembeli Glochidia. F, glochidial foot; M, adductor muscle; S, stomach; DM, definitive mantle; GF, gill filament of host; GL, gill lamellae.

Table 1. Dimensions (μm) of the glochidia of *M. hembeli*

	Mean	SD	Range
Length	187.5	9.7	165.0-215.0
Width	191.7	16.4	150.0-225.0
Hinge Length	105.9	8.2	85.0-115.0

APPENDIX A

Stream	Species	No. Infected/No. Collected Per Infective Month					
		March	April	May	June	July	August
Loving Creek	N. chrysocephalus	0/19	3/8	2/22	1/14	2/16	1/14
	N. umbratilis	0/0	0/0	0/0	0/0	0/0	0/0
Little Loving Creek	N. chrysocephalus	1/18	0/8	4/29	8/20	1/13	0/21
	N. umbratilis	0/0	0/12	0/0	0/0	0/0	0/2
Bayou Clear	N. chrysocephalus	0/13	5/16	4/13	1/7	4/14	0/6
	N. umbratilis	0/11	1/12	4/18	5/13	3/3	1/7
Brown Creek	N. chrysocephalus	0/38	0/46	2/30	7/51	2/55	0/68
	N. umbratilis	0/7	0/8	1/11	2/17	1/5	1/12
Castor Creek	N. chrysocephalus	3/9	9/16	9/19	4/6	7/16	5/20
	N. umbratilis	0/0	0/4	1/5	0/5	1/3	1/1
Long Branch	N. chrysocephalus	6/25	2/7	1/7	2/18	3/22	0/15
	N. umbratilis	0/1	0/0	0/0	0/0	0/0	0/0
TOTALS	N. chrysocephalus	10/122	19/101	22/120	23/116	19/136	6/143
	N. umbratilis	0/19	1/44	6/34	7/35	5/11	3/22

APPENDIX B

Stream	Species	Percent Infected Each Month With Glochidia					
		March	April	May	June	July	August
Loving Creek	N. chrysocephalus	00	37.5	9.1	7.1	12.5	7.7
	N. umbratilis	-*	-	-	-	-	-
Little Loving Creek	N. chrysocephalus	5.6	00	13.8	40.0	7.7	00
	N. umbratilis	00	00	-	-	-	00
Bayou Clear	N. chrysocephalus	00	31.3	30.8	14.3	28.6	00
	N. umbratilis	00	8.3	22.2	38.5	100**	14.3
Brown Creek	N. chrysocephalus	00	00	6.7	13.7	3.6	00
	N. umbratilis	00	00	9.1	11.8	20.0	4.5
Castor Creek	N. chrysocephalus	33.3	56.2	47.4	66.7	43.8	25.0
	N. umbratilis	-	00	20.0	00	33.3	100**
Long Branch	N. chrysocephalus	24.0	28.6	14.3	11.1	13.6	00
	N. umbratilis	00	-	-	00	-	-

* No specimens of the species collected.
**Sample size of only 1-3 specimens.

APPENDIX C

Host Species	Date Collected	Glochidial Dimensions (μm) Length	Width	Hinge Length
N. chrysocephalus	Apr. 13	185	180	105
		190	195	110
		165	150	110
		190	185	-*
N. chrysocephalus	May 11	185	190	-
		190	210	115
		185	185	105
		185	190	105
		195	190	110
		175	175	110
N. chrysocephalus	June 12	180	180	110
		190	200	110
		185	185	115
N. umbratilis	June 27	190	205	90
		190	200	110
		190	195	105
		190	210	100
		215	225	85