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# PREVALENCE AND RELATIONSHIPS OF TWO ALLOCREADIID METACERCARIAE IN THE BURROWING MAYFLY, *HEXAGENIA LIMBATA* (SERVILLE IN GUERIN), OF BLUESTEM LAKE, LANCASTER COUNTY, NEBRASKA

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All naiads of the burrowing mayfly, *Hexagenia limbata,* examined from Bluestem Lake, Lancaster County, Nebraska, were infected by trematode metacercariae. The naiads hosted two species of trematode, *Megalogonia ictaluri* Surber, 1928, and *Crepidostomum cooperi* Hopkins, 1931. Three thousand sixty-three cysts from 24 mayflies were inspected to confirm their identity and to determine their distribution on the naiads.

† † †

## INTRODUCTION

*Crepidostomum cooperi* Hopkins, 1931, and *Megalogonia ictaluri* Surber, 1928 (Trematoda: Allocreadiidae) are parasites of freshwater fishes. *Crepidostomum cooperi* has been reported from at least 20 genera of fishes (Hazen and Esch, 1982), while *M. ictaluri* has been reported from four genera (Amato, 1979). *Hexagenia limbata* (Serville in Guerin), the burrowing mayfly, serves as an intermediate host to both species. Metacercariae of *C. cooperi* are also found in the amphipod, *Hyalella azteca* (Saussure) (Hazen and Esch, 1982). Previous studies (Amato, 1979; Hazen and Esch, 1982; and others) have examined the host-parasite relationships of these allocreadiids in the burrowing mayfly.

Janine Caira (unpublished, The Manter Laboratory) discovered metacercariae of both *M. ictaluri* and *C. cooperi* on *H. limbata* naiads from Bluestem Lake. The metacercariae of these species are practically identical in appearance, and it was not apparent that both species were present until numerous larvae in such cysts were mechanically excysted. Previous data collected from Bluestem Lake that assumed all cysts contained *M. ictaluri* may, therefore, be slightly inaccurate. The present study was undertaken to determine whether different conclusions could be drawn when both species are known to be present and to compare results from a lake with both species to those obtained by others from sites where only *C. cooperi* occurs.

Although Hazen and Esch (1982) reported "no apparent difference in the distribution of metacercariae [of *C. cooperi*] in subimagoes of the two sexes" of *H. limbata,* a critical study of possible differences in recruitment by male and female mayflies has not been previously made. The relationship between mayfly sex and the number of *M. ictaluri* and *C. cooperi* in naiads at Bluestem Lake was studied.

The conclusion reached by Amato (1979) that pre-adult *M. ictaluri* in Bluestem Lake demonstrate "no indication of site preference between abdominal segments and gills" was reconsidered in view of the knowledge that both *M. ictaluri* and *C. cooperi* occur there.

Finally, Hazen and Esch (1982) reported that the "fourth, fifth, and sixth abdominal segments [of *H. limbata*] carry nearly 70% of all metacercariae [of *C. cooperi*] in the host." Amato (1979) did not study distribution of *M. ictaluri* within mayflies. The present study demonstrates site preferences within the mayflies for both species.

## MATERIALS AND METHODS

Twenty-four naiads of *Hexagenia limbata* were collected between September 1983 and January 1984 from the northwestern end of Bluestem Lake, southwestern Lancaster County, Nebraska. During the autumn months, mud was sifted on-site with a 710 $\mu$m or 1 mm testing sieve to collect the naiads. During the winter months, an ice auger was used to penetrate the ice, and an Ekman dredge was used to collect mud which was sifted upon arrival in the laboratory.

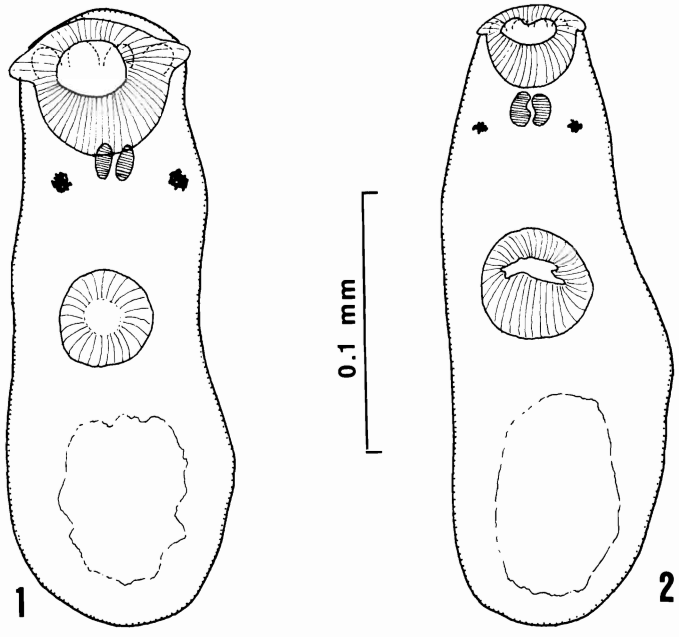
Naiads were kept alive in a large culture dish with a mud substrate and a 1:1 mixture of lakewater and tapwater. An aerator was installed, and a sheet of glass covered the bowl to reduce evaporation. Under these conditions, naiads survived several months at room temperature.

Each naiad was measured and its sex was determined. Two characters were available to determine sex: the very obvious genitalia of males and the difference in size and shape of the eye. The eye of males is larger than that of females, and the posterior margin is angled whereas in females it is rounded. The use of the eye character is necessary only with very small (4 or 5 mm) individuals (Hunt, 1952).

Record sheets bearing dorsal and ventral diagrams of the naiad were prepared for each mayfly. In addition to noting the length and sex of the naiad, the location of each metacercarial cyst was recorded on the diagram. The cyst was isolated as to segment or gill of the host, as well as to whether it occurred on a dorsal or ventral surface. Body cysts were examined segment by segment the first day of dissection. Each gill was taken from the naiad and placed in a separate, labeled well of a glass concavity plate. If the entire naiad could not be examined in one session, cysts were refrigerated *in situ* in tapwater.

Each of the 3,063 metacercariae was excysted using two pairs of very fine forceps, one as a brace for the minute cyst, the other to clasp and break the cyst in one quick motion. Because it is difficult to identify living metacercariae under a dissecting microscope, a temporary wet-mount with a vaseline-edged coverslip was made for each excysted metacercaria for study at higher magnification.

The two species of metacercariae (Figs. 1 and 2) were distinguished on the basis of three structural characters. First, and most apparently, *C. cooperi* has six large oral papillae of approximately equal size. Two project laterally from the oral sucker, and four occur on the dorsal margin of the sucker. *Megalogonia ictaluri* also has six oral papillae, but all are relatively small and only the lateral papillae are conspicuous. In fact, the four dorsal papillae were not mentioned in the original description of the species by Surber (1928). Secondly,




FIGURES 1 and 2. Camera lucida drawings of excysted metacercariae. 1. *Crepidostomum cooperi*. 2. *Megalogonia ictaluri*.

*M. ictaluri* tapers toward the anterior end of the body while *C. cooperi* does not. Thirdly, the ventral sucker of *M. ictaluri* is larger than the oral sucker whereas the oral sucker of *C. cooperi* is usually conspicuously larger than the ventral sucker.

Although the data were not recorded as such, it was observed that while the majority of cysts occurred on the surface of the mayfly naiad, some occurred in the deeper tissues. These cysts were drawn on the ventral surface of the naiad on the data sheets.

Prevalence and intensity are as defined by Margolis et al. (1982), i.e., prevalence = number of infected individuals ÷ number of individuals sampled, and mean intensity = total number of parasites ÷ number of infected individuals. These values were calculated to compare infections of naiads by the two species. The prevalence and intensity of each species were also determined for male and female mayflies separately as well as for three arbitrarily assigned size-classes of mayfly. For these calculations, the smallest mayflies were designated as those less than or equal to 11 mm in length, the intermediate mayflies were those from 12 to 19 mm, and the largest mayflies were those 20 mm or longer. Finally, each gill and each segment of the mayfly naiad were examined separately and the prevalence and intensity of each species of parasite on these body parts were calculated for comparative purposes.

A Chi-square test was made to compare infections between males and females of both species, as well as to compare

infections between the body and gills of both species. An F-test and Duncan's Multiple Range Test were used to compare infection intensities between segments for both species. The statistical tests were performed as described by Steel and Torrie (1980).

## RESULTS

Table I shows the prevalence and mean intensity for *M. ictaluri* and *C. cooperi* overall, in males and females, and in the three size-classes. The data show these values vary between the two species. Figures 3 and 4, respectively, show the prevalence and intensity for *M. ictaluri* and *C. cooperi* on each segment and each gill of the mayfly naiad, and again, there are differences in these values between species.

TABLE I. Prevalence and mean intensity of infection, between sexes and between size-classes, for *Megalogonia ictaluri* and *Crepidostomum cooperi.*

| | *Megalogonia ictaluri* | *Crepidostomum cooperi* |
|---|---|---|
| Prevalence overall | 100% | 63% |
| Mean intensity | 126.5 | 1.8 |
| Prevalence in females | 100% | 73% |
| Prevalence in males | 100% | 54% |
| Mean intensity in females | 143.2 | 1.8 |
| Mean intensity in males | 112.4 | 1.9 |
| Prevalence ⩽11 mm | 100% | 33% |
| Prevalence 12–19 mm | 100% | 69% |
| Prevalence ⩾20 mm | 100% | 60% |
| Mean intensity ⩽11 mm | 22 | 1.0 |
| Mean intensity 12–19 mm | 114 | 1.9 |
| Mean intensity ⩾20 mm | 229.2 | 1.7 |

Table II compares the intensity of *M. ictaluri* and *C. cooperi* in male and female naiads. Female mayflies have significantly more *M. ictaluri* cysts than do males, but no difference between sexes in the number of cysts of *C. cooperi* could be demonstrated.

Table III gives results of Chi-square tests of differences in intensity between the abdomen and gills for *M. ictaluri* and *C. cooperi.* Gills had significantly more cysts of *M. ictaluri* than did the abdomen, but the abdomen had more cysts of *C. cooperi* than the gills did.

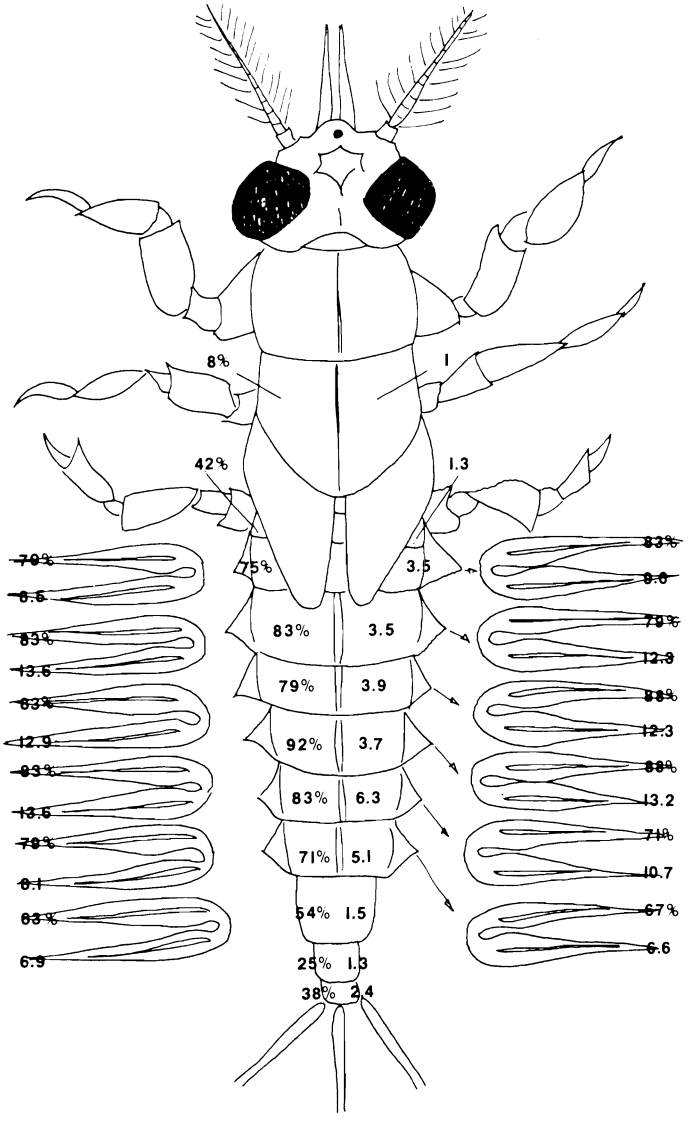



FIGURE 3. *Hexagenia limbata* showing prevalences (%) and mean intensities for thorax, each gill, and each abdominal segment infected with *Megalogonia ictaluri.*

Tables IV and V compare the intensity of infection between body segments (segments in this case include abdominal segments plus the attached gills). No significant difference occurred between the numbers of cysts of *C. cooperi* on any of the abdominal segments of the mayfly naiad. At least one significant difference did appear, however, between the numbers of cysts of *M. ictaluri* among abdominal means.

Table VI shows that no significant difference occurred in the mean number of cysts of *M. ictaluri* on abdominal segments 5, 4, 3, 6, and 2. No significant difference was found in the mean number of cysts of *M. ictaluri* on abdominal segments 6, 2, and 7, and no significant difference was found in

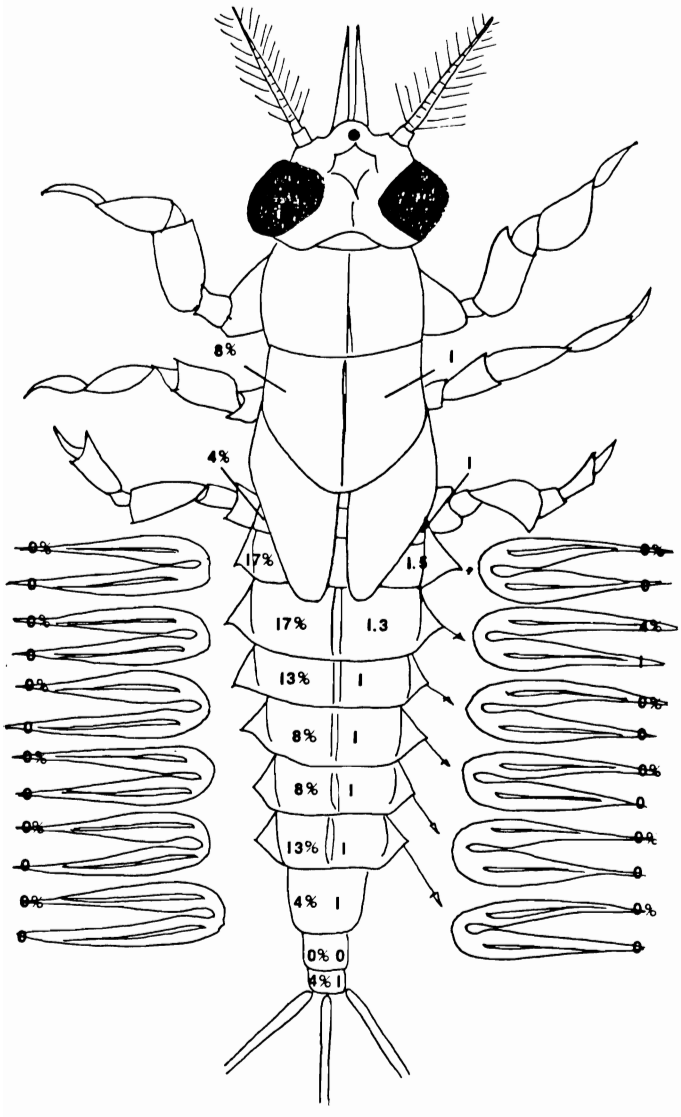



FIGURE 4. *Hexagenia limbata* showing prevalences (%) and mean intensities for thorax, each gill, and each abdominal segment infected with *Crepidostomum cooperi.*

TABLE II. Data for Chi-square test of differences in intensity between naiad sexes for *Megalogonia ictaluri* and *Crepidostomum cooperi.*

| | *Megalogonia ictaluri* | *Crepidostomum cooperi* |
|---|---|---|
| Observed total cysts–female | 1,575 | 14.0 |
| Observed total cysts–male | 1,461 | 13.0 |
| Expected total cysts–each sex | 1,518 | 13.5 |
| Critical $\chi^2$ value, p = 0.05 | 3.84 | 3.84 |
| Calculated $\chi^2$ | 4.2806 | 0.037 |

TABLE III. Data for Chi-square test to compare intensity between the abdomen and the gills for *Megalogonia ictaluri* and *Crepidostomum cooperi.*

| | *Megalogonia ictaluri* | *Crepidostomum cooperi* |
|---|---|---|
| Observed total on body | 567 | 26.0 |
| Observed total on gills | 2,469 | 1.0 |
| Expected total on either | 1,518 | 13.5 |
| Critical $\chi^2$ value, p = 0.05 | 3.84 | 3.84 |
| Calculated $\chi^2$ | 1,191.56 | 23.148 |

TABLE IV. Data for F-test of intensity differences between the abdominal segments for *Crepidostomum cooperi.*

| | df | Sum of squares | Mean square | $F_{calculated}$ |
|---|---|---|---|---|
| Treatments | t-1 = 9 | 1.604 | 0.178 | 1.3295* |
| Error | t(r-1) = 230 | 30.792 | 0.13378 | |
| Total | rt-1 = 239 | 32.396 | | |

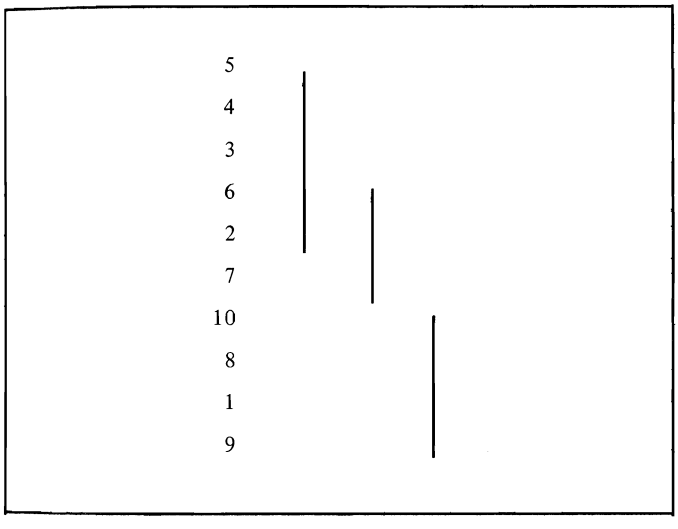
*Critical F-value, p = 0.05 is 1.88.

TABLE V. Data for F-test of intensity differences between the abdominal segments for *Megalogonia ictaluri.*

| | df | Sum of squares | Mean square | $F_{calculated}$ |
|---|---|---|---|---|
| Treatments | t-1=9 | 26,385.016 | 2,931.668 | 13.83* |
| Error | t(r-1)=230 | 48,754.167 | 211.9746 | |
| Total | rt-1=239 | 75,139.183 | | |

*Critical F-value, p=0.05 is 1.88.

TABLE VI. Results of Duncan's Multiple Range test for equality of cysts means for abdominal segments of *Megalogonia ictaluri.*




the mean number of cysts of *M. ictaluri* on abdominal segments 10, 8, 1, and 9. For this table, segments were numbered one to ten from anterior to posterior of the mayfly. Segment numbers connected with the same line are not significantly different.

## DISCUSSION AND CONCLUSIONS

Mayfly naiads were more often, and much more heavily, infected with *Megalogonia ictaluri* than with *Crepidostomum cooperi.* All of the mayflies examined were infected with *M. ictaluri* while 63% were infected with *C. cooperi,* and the intensities, respectively, were 126.5 as opposed to 1.8. The range in number of *M. ictaluri* found on one host was 1 to 444, whereas *C. cooperi* infections ranged from 1 to 5. Infections of adult *C. cooperi* in fishes in Bluestem Lake have been found to range from 1 to 109 (Caira, personal communication). This suggests that *C. cooperi* has another second intermediate host in this lake, or that the fish there consume relatively large numbers of mayfly naiads. In either case, it seems this allocreadiid is successful in making the transition from intermediate to definitive host.

In examining the intensities of arbitrarily assigned size-classes, it appeared that the number of *M. ictaluri* cysts per naiad increased as the naiad increased in size. This was consistent with what Amato (1979) found, and when the mayfly naiad is considered a resource provider for the parasite, this follows as more resources could conceivably support more parasites. Hazen and Esch (1982) found "a positive relationship between total body length and the prevalence and density [of *C. cooperi*]" infections also, but this did not appear to be the case for *C. cooperi* in Bluestem Lake where *C. cooperi* occurred in low numbers (Table I).

It did appear that there was a significant difference in the intensity of infection between male and female mayflies for *M. ictaluri,* although not for *C. cooperi.* Possibly there exists a significant size difference between the sexes that would account for this intensity difference, or possibly females exhibit a behavior different from males that attracts more *M. ictaluri* or makes the female naiad more accessible as an intermediate host.

It also appeared that there was a significant difference in the intensity of infection between the gills and abdomen proper for both species, especially in the case of *M. ictaluri.* The perpetual activity or structure of the gill may make it a preferable site for *M. ictaluri,* or the gills may be more frequently inhabited by this species simply because a cercaria of *M. ictaluri* could penetrate a host more easily there than it could through the exoskeleton of the body. In any case, the gills did not present such an advantage to *C. cooperi* as it was found significantly more often on the abdomen proper.

It appeared that there was no significant difference in infection intensities between abdominal segments on the naiads for *C. cooperi,* but that there was a significant difference for *M. ictaluri.* For the latter, segments 5, 4, 3, 6, and 2 were the most heavily infected, and there was no significant difference in intensity among these five segments. They were, however, significantly different from segments 6, 2, and 7, which, in turn, were not significantly different among themselves. Segments 10, 8, 1, and 9 were the least heavily infected and did not differ significantly in infection intensity among themselves, although they did differ from other segments. The most obvious reason for this difference is that segments 10, 8, 1, and 9 do not include gills.

Thus, the most suitable habitat for *M. ictaluri* metacercariae appears to be segments 5, 4, 3, 6, and 2 of large, female mayfly naiads. On mayfly naiads, *C. cooperi* is most often found on the abdomen proper of either a female or male. In either case, the thorax, for the most part, seems poorly suited.

## ACKNOWLEDGMENTS

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