Experimental and Natural Infection of Planktonic and Benthic Copepods by the Asian Tapeworm, *Bothriocephalus acheilognathi*

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ABSTRACT: The life cycles of pseudophyllidean tapeworms include a procercoid stage that inhabits the hemocoel of copepods. A variety of copepods from Belews Lake, North Carolina, were individually exposed to 5–10 coracidia of the Asian fish tapeworm, *Bothriocephalus acheilognathi*. The planktonic cyclopoid species *Diaicyclops thomasi*, *Mesocyclops edax*, and *Tropocyclops prasinus* proved susceptible to infection, whereas a sympatric calanoid copepod (*Skistodiaptomus pallidus*) could not be infected experimentally. In addition, lab experiments revealed that the benthic cyclopods *Eucyclops agilis* and *Paracyclops fimbriatus poppei* could also be infected. All cyclopoids appear to be susceptible to infection regardless of developmental stage. Natural infections during the period of fall recruitment of the parasite by the fish hosts were highest among the planktonic *T. prasinus*. Although no benthic copepods were found infected during summer months in the field, these organisms may be important in transmitting the cestode to the detritivorous fathead minnow (*Pimephales promelas*) during the spring and fall periods of recruitment.


*Bothriocephalus acheilognathi* Yamaguti, 1934 (*B. gowkongensis* Yeh, 1955), commonly referred to as the Asian fish tapeworm, has spread from Asia throughout Europe and parts of North America. This parasite is known to infect over 40 species of fish, mainly cyprinids (Riggs and Esch, 1987). In Belews Lake, North Carolina, the cestode is commonly found in mosquitofish (*Gambusia affinis*), red shiners (*Notropis lutrensis*), and fathead minnows (*Pimephales promelas*), and sporadically in green sunfish (*Lepomis cyanellus*).

Numerous intermediate hosts have been experimentally infected, but research has been confined to Asia and Europe. Species of *Bothriocercus* are generally non-specific for intermediate hosts (Liao and Shih, 1956; Jarroll, 1979). Thus, *B. claviceps*, *B. cuspidatus*, and *B. acheilognathi*, all parasites of freshwater fish, infect a variety of cyclopoid copepods. *Bothriocercus scorpii*, a common marine form, infects calanoid copepods. The only cestode in the genus for which unsuccessful copepod infection attempts have been reported is *B. rarus* (Jarroll, 1979), which is unusual in that it infects newts. Jarroll (1979) was unable to infect either *Eucyclops speratus* or *Paracyclops fimbriatus poppei* with coracidia of *B. rarus*.

Still fewer studies have attempted to determine which species serve as natural intermediate hosts. Jarroll (1979) found *Macrocyclops ater* to be the only copepod to be naturally infected with *B. rarus*, and prevalence was very low (0.82%). Liao and Shih (1956), however, stated that prevalence of *B. acheilognathi* in cyclopoid copepods can reach 7%.

Cyclopoid copepods and other microcrustaceans from Belews Lake were exposed under laboratory conditions to coracidia of *B. acheilognathi* in an effort to determine which species may serve as an intermediate host. Both planktonic and benthic copepods from Belews Lake were also examined for evidence of infection.

Materials and Methods

Planktonic copepods were obtained from Belews Lake by vertical and/or horizontal tows using a No. 20 (76-μm) Wisconsin net. Benthic copepods and ostracods were collected with a 15.1 × 15.1-cm Ekman grab. On return to the laboratory, organisms were isolated from the sediments using the method of Elmgrom (1959). Water was added to the sediments and the sample was agitated. After approximately 1 hr, the water above the settling sediments was filtered through bolting cloth fixed within a hoop. Animals trapped on the cloth hoop were transferred to a finger bowl. This process was repeated at least 3 times, or until no more copepods were recovered.

Gravid tapeworms were removed from red shiners

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Table 1. Experimental infections of individual copepods with Bothriocephalus acheilognathi.

<table>
<thead>
<tr>
<th>Potential host</th>
<th>No. exposed</th>
<th>No. infected</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diacyclops thomasi</td>
<td>28</td>
<td>12</td>
<td>42</td>
</tr>
<tr>
<td>Mesocyclops edax</td>
<td>54</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>Tropocyclops prasinus</td>
<td>5</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>Eucyclops agilis</td>
<td>45</td>
<td>13</td>
<td>29</td>
</tr>
<tr>
<td>Paracyclops fimbriatus poppei</td>
<td>13</td>
<td>6</td>
<td>46</td>
</tr>
</tbody>
</table>

and fathead minnows that were trapped or seined at Belews Lake. Worms were placed in water at room temperature to induce release of eggs. Coracidia hatched after a period of development (approximately 24 hr). Cyclopoid copepods were isolated in 2 ml of filtered lake water and were exposed to 5 or 10 coracidia each. Calanoid copepods, copepod nauplii, and ostracods were placed in 20 ml of filtered lake water or conditioned tap water and exposed in groups of 4–7 to 100–200 coracidia per group. All experiments were performed at room temperature (25°C). The cyclopoid copepods were identified according to Yeatman (1959), and the calanoids according to Wilson (1959). Ostracods were not identified.

After 5 days live animals were fixed in 70% ethanol and stained in aceticarmine (Shostak et al., 1985). They were then examined under ordinary light microscopy at 400 x; or, alternatively, copepods were examined live under phase contrast. Examination of live copepods proved to be the better method for detection of procercoids. Copepods that died during the experiments were examined for infections but omitted from data analysis, unless otherwise indicated. No deaths of heavily infected copepods were observed.

In order to determine parasite prevalence in natural infections, zooplankton were sampled by towing the Wisconsin net for approximately 4 min during daylight hours in late October/early November 1986, a period of active parasite recruitment by the fish hosts. Night samples were also taken during the same period. Animals were fixed for 1 hr in 70% ethanol, stained in aceticarmine (Shostak et al., 1985), and examined microscopically for infection. Benthic copepods were captured during the summer of 1986 using the method described above, and fixed and stained as per the planktonic copepods, except that the duration of fixation and storage was not limited to 1 hr, but extended for a period of a few weeks.

Results

All cyclopoid copepods tested were susceptible to infection with coracidia of Bothriocephalus acheilognathi (Table 1). The planktonic copepods included Diacyclops thomasi (S. A. Forbes, 1892), Mesocyclops edax (S. A. Forbes, 1891), and Tropocyclops prasinus (Fischer, 1860). The numbers of T. prasinus were limited because the animals were very difficult to maintain in the laboratory, and most died early in the experiments. The benthic copepods Eucyclops agilis (Koch, 1838), and Paracyclops fimbriatus poppei (Rehberg, 1880), also proved susceptible to infection.

In the group exposures, 1 of 7 ostracods developed an infection, and 3 of 4 copepod nauplii were found to harbor procercoids (Table 2). The nauplii were early larval stages of the benthic copepod E. agilis. No calanoid copepods (Skistodiaptomus pallidus (Herrick), 1879) became infected on exposure to coracidia.

The copepod infection data were further analyzed to determine which developmental stages were susceptible to infection by B. acheilognathi. All stages examined proved vulnerable to infection (Table 3); development of the parasite seemed to be hindered by the presence of egg masses in females. For example, 3- and 5-day-old worms remained small in the fifth-stage copepodite female M. edax with developing eggs, as compared to procercoids in other copepod species.

The majority of the copepods captured during the night-time surface tows in the autumn were M. edax (97%), a strong vertical migrator. Few of these were infected (0.6%) (Table 4). The majority of the copepods taken during daylight hours were T. prasinus (92%). A much higher proportion of these were parasitized (7.1%) (Table 4). All benthic copepods sampled during the summer proved negative for infections (Table 4).

Discussion

All cyclopoid copepod species tested in the present study could be experimentally infected with B. acheilognathi. Thus, this tapeworm is not restricted to certain species of intermediate host in North America, an observation which confirms those of others in Europe and Asia who have successfully infected numerous cyclopoid species (Liao and Shih, 1956; Shcherban et al., 1963 in United States Department of Agricul-
Table 3. Copepod stages experimentally exposed.*

<table>
<thead>
<tr>
<th>Potential host</th>
<th>Nauplii</th>
<th>Copepodites</th>
<th>Adults</th>
<th>Female + eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C1</td>
<td>C2</td>
<td>C3</td>
</tr>
<tr>
<td>Diacyclops</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mesocyclops</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tropocyclops</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eucyclops</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Paracyclops</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* - = not exposed; + = exposed, became infected; † = exposed, became infected, and died.


The successful infection of benthic copepods has important implications for the population dynamics of the parasite in the definitive host. Mosquitofish and red shiners are planktivorous (Riggs, 1986), and most likely acquire the parasite by ingesting infected planktonic copepods (D. thomasi, M. edax, and T. prasinus). The fathead minnows are benthic detritivores (Riggs, 1986). The eggs of B. a cheilognathi sink and stick to the substratum (pers. obs.). When coracidia hatch from eggs on the substratum, benthic copepods, such as E. agilis and P. f. poppei, are thus exposed to infection. These species in turn transmit the parasite to the fathead minnows feeding on the bottom.

Because all of the developmental stages of cyclopoid copepods that were examined could be experimentally infected with B. a cheilognathi (Table 3), it appears that the potential of a copepod becoming infected is not dependent on the host’s developmental state. Inasmuch as molting of infected copepods was observed, parasitism also does not seem to affect development of the copepods, a situation that is consistent with that described by Dupont and Gabrion (1987), who worked with B. claviceps in copepods. However, we have no data regarding the impact of parasitism on the rate of copepod development or the effect of copepod age on procercoid development, except the observation that parasite development seemed to be impeded by the presence of eggs in the body cavity of the fifth copepodite female M. edax. Michajlow (1953) was able to infect fourth and fifth copepodites and adults of Cyclops strenuus with Triaenophorus lucii, but noted development was retarded in the juvenile forms. Together with Halvorsen’s (1966) observations that the development of Diphyllobothrium latum is retarded in juvenile C. strenuus, these various observations suggest that although infection by cestodes can occur in various developmental stages of the copepod intermediate hosts, the subsequent development of the parasite may be influenced by host maturity (Humes, 1950; Guttowa, 1956; Watson and Lawler, 1965; Kuperman and Kireev, 1976).

It is possible that adult male copepods are poor hosts for B. a cheilognathi inasmuch as males of E. agilis and P. f. poppei became infected but died soon afterward (Table 3). Michajlow (1938) and Guttowa (1956) also found that males and females of the calanoid D. gracilis exhibited differing abilities to serve as hosts for the cestode T. lucii, although Humes (1950) and Michajlow (1938) found no sex-specific differences in susceptibility of a variety of diaptomids to Dibothriocephalus latus.

There are few estimates of the extent of infection in copepods by procercoids in nature. Jarroll

Table 4. Natural copepod infections recorded from Belew’s Lake.

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>Species</th>
<th>N</th>
<th>No. infected</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Night (fall)</td>
<td>Mesocyclops</td>
<td>704</td>
<td>4</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Tropocyclops</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Diacyclops</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Eucyclops</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Day (fall)</td>
<td>Tropocyclops</td>
<td>85</td>
<td>6</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>Mesocyclops</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Paracyclops</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Benthic (summer)</td>
<td>Eucyclops</td>
<td>46</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Paracyclops</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mesocyclops</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
(1979) found *Macrocylops ater* to be the only copepod naturally infected with *Bothriocephalus rarus*, and prevalence was exceedingly low (0.82%). Liao and Shih (1956) stated that prevalence of *B. acheilognathi* may reach 7%. Prevalence of 1% or 2% were recorded for *Triaenophorus* spp. in *D. thomasi* at a number of different localities over a number of years (Watson and Lawler, 1965). Because infected *D. thomasi* were more abundant in inshore, shallow waters as compared with offshore sites (Watson and Lawler, 1965), and because prevalence of *Triaenophorus* spp. in *D. thomasi* has been shown to vary from 43% in early June to 0 in July (Miller, 1952), such seasonal and spatial heterogeneity complicates the determination of prevalence in the field.

*Tropocyclops prasinus* was the dominant planktonic copepod during the autumn recruitment of *B. acheilognathi* by the fish hosts in Belews Lake and harbored the parasite at a prevalence of 7.1%, which is comparable to that observed by Liao and Shih (1956) in China. Because *M. edax* is not an important component of the zooplankton community during the autumn and is parasitized at a very low level (0.6%), it probably is not important in the transmission of *B. acheilognathi* in the fall. Furthermore, strong vertical migrants such as *M. edax* (Williamson and Magnien, 1982) experience reduced vulnerability to fish predation (Zaret and Suffern, 1976; Zaret, 1980; Stich and Lampert, 1981; Gliwicz, 1986), which in turn reduces transmission rates of parasites utilizing migrants as intermediate hosts.

Despite the fact that no benthic copepods harbored natural infections, these zooplankters may still be important in transmission of the cestode to detritivores such as fathead minnows. Sample sizes are low and the benthic animals were collected in the summer when no recruitment occurs (Riggs and Esch, 1987); thus, it is not surprising that none was parasitized in the present study. On the other hand, benthic copepods are susceptible to infection by coracidia in the laboratory, and may indeed acquire the parasite during the spring and fall periods of transmission. It is unlikely that organisms other than cyclopoid copepods serve as intermediate hosts for *B. chei-
lognathi* in nature since only 1 ostracod and no *S. pallidus* became infected after extensive exposure to coracidia in the group experiments (Table 2).

The ubiquitous distribution of this parasite and its continued spread to new areas (Heckman et al., 1987; Riggs and Esch, 1987) are in part attributable to the lack of specificity not only for its definitive host, but for its intermediate host as well. *Bothriocephalus acheilognathi* infects over 40 species of fish belonging to a variety of families (Riggs and Esch, 1987). Because the cestode is found primarily in cyprinids, and because all cyclopoid copepods that have been tested may serve as intermediate hosts, this cestode is likely to expand its present range in North America and probably other parts of the world as a result of the cosmopolitan distribution of both cyclo-
poid copepods and cyprinid fishes.

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