

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

DAVID J. MARCOGLIESE¹ AND GERALD W. ESCH²

Department of Biology, Wake Forest University, Winston-Salem, North Carolina 27109

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 *Diacyclops 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.

KEY WORDS: cyclopoid copepods, benthic copepods, *Bothriocephalus acheilognathi*, *Diacyclops thomasi*, *Mesocyclops edax*, *Tropocyclops prasinus*, *Eucyclops agilis*, *Paracyclops fimbriatus poppei*, experimental infection.

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 *Bothriocephalus* are generally non-specific for intermediate hosts (Liao and Shih, 1956; Jarrecka, 1964; Korting, 1975; Jarroll, 1979; Dupont and Gabrion, 1987). Thus, *B. claviceps*, *B. cuspidatus*, and *B. acheilognathi*, all parasites of freshwater fish, infect a variety of cyclopoid copepods. *Bothriocephalus 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 *Macrocyclus 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- μ m) Wisconsin net. Benthic copepods and ostracods were collected with a 15.1 \times 15.1-cm Ekman grab. On return to the laboratory, organisms were isolated from the sediments using the method of Elgmork (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

¹ Present address: Department of Fisheries and Oceans, Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Halifax, Nova Scotia, Canada B2Y 4A2.

² To whom reprint requests should be sent.

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

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

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 or artificial pond water and exposed to 5 or 10 coracidia each. Calanoid copepods, copepod nauplii, and ostracods were placed in 20 ml of filtered lake 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 acetocarmine (Shostak et al., 1985). They were then examined under ordinary light microscopy at 400×; or, alternatively, copepods were examined live under phase contrast. Examination of live copepods proved to be the better method for detection of proceroids. 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 acetocarmine (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

Table 2. Experimental infections of groups of organisms with *Bothriocephalus acheilognathi*.

Potential host	No. exposed	No. infected	%
<i>Skistodiaptomus pallidus</i>	8	0	0
Ostracods	7	1	14.3
<i>Eucyclops agilis</i> nauplii	4	3	75

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 proceroids (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 proceroids 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.*

Potential host	Developmental stage								
	Nauplii	Copepodites					Adults		Female + eggs
		C1	C2	C3	C4	C5	Male	Female	
<i>Diacyclops</i>	-	-	+	+	+	+	-	+	-
<i>Mesocyclops</i>	-	-	-	+	+	+	-	-	-
<i>Tropocyclops</i>	-	-	-	-	-	+	-	+	-
<i>Eucyclops</i>	+	-	-	-	+	+	†	-	+
<i>Paracyclops</i>	-	-	-	-	+	+	†	-	-

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

ture, 1972; Körting, 1975; Esinenko-Marits et al., 1968 in United States Department of Agriculture, 1976).

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. acheilognathi* 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. acheilognathi* (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 proceroid 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. acheilognathi* 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 proceroids in nature. Jarroll

Table 4. Natural copepod infections recorded from Belews Lake.

Sampling period	Species	N	No. infected	Prevalence (%)
Night (fall)	<i>Mesocyclops</i>	704	4	0.6
	<i>Tropocyclops</i>	8	0	0
	<i>Diacyclops</i>	7	0	0
	<i>Eucyclops</i>	7	0	0
Day (fall)	<i>Tropocyclops</i>	85	6	7.1
	<i>Mesocyclops</i>	5	0	0
	<i>Paracyclops</i>	2	0	0
Benthic (summer)	<i>Eucyclops</i>	46	0	0
	<i>Paracyclops</i>	12	0	0
	<i>Mesocyclops</i>	9	0	0

(1979) found *Macrocyclus 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 migrators 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. acheilognathi* 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 cyclopoid copepods and cyprinid fishes.

Acknowledgment

Publication is supported by a grant from the Wake Forest University Research and Publication Fund.

Literature Cited

- Dupont, F., and C. Gabrion. 1987. The concept of specificity in the proceroid-copepod system: *Bothriocephalus claviceps* (Cestoda) a parasite of the eel (*Anguilla anguilla*). *Parasitology Research* 73:151-158.
- Elgmork, K. 1959. Seasonal occurrence of *Cyclops strenuus strenuus* in relation to environment in small water bodies in southern Norway. *Folia Limnologica Scandinavica* 11:1-196.
- Gliwicz, M. Z. 1986. Predation and the evolution of vertical migration in zooplankton. *Nature* 320:746-748.
- Guttowa, A. 1956. Proof of experimental definition of the main first intermediate host of broad fish tapeworm—*Diphyllobothrium latum* (L.)—in the area of Poland. *Acta Parasitologica Polonica* 4: 781-802. (In Polish with English summary.)
- Halvorsen, O. 1966. Studies of the helminth fauna of Norway. VIII. An experimental investigation of copepods as first intermediate hosts for *Diphyllobothrium norvegicum* Vik (Cestoda). *Nytt Magasin for Zoologi* 13:83-117.
- Heckman, R. A., P. D. Greger, and J. E. Deacon. 1987. New host records for the Asian fish tapeworm, *Bothriocephalus acheilognathi*, in endangered fish species from the Virgin River, Utah, Nevada, and Arizona. *Journal of Parasitology* 73:226-227.
- Humes, A. G. 1950. Experimental copepod hosts of the broad tapeworm of man, *Dibothriocephalus latus* (L.). *Journal of Parasitology* 36:541-547.
- Jarecka, L. 1964. Cycle évolutif à un seul hôte intermédiaire chez *Bothriocephalus claviceps* (1). (Goeze, 1782), cestode de *Anguilla anguilla* (L.). *Annales de Parasitologie* 39:149-156.
- Jarroll, E. L., Jr. 1979. Population biology of *Bothriocephalus rarus* Thomas (1937) in the red-spotted newt, *Notophthalmus viridescens* Raf. *Parasitology* 79:183-193.
- Körting, W. 1975. Larval development of *Bothriocephalus* sp. (Cestoda: Pseudophyllidea) from carp

- (*Cyprinus carpio* L.) in Germany. *Journal of Fish Biology* 7:727-733.
- Kuperman, B. I., and V. K. Kireev.** 1976. The effect of procercooids of *Triaenophorus nodulosus* on the biology of their first intermediate hosts, *Cyclops strenuus*. *Parazitologija* 10:434-438. (In Russian with English summary.)
- Liao, H., and L. Shih.** 1956. Contribution to the biology and control of *Bothriocephalus gowkongensis* Yeh, a tapeworm parasitic in the young grass carp (*Ctenopharyngodon idellus* C. and V.). *Acta Hydrobiologica Sinica* 2:129-185. (In Chinese with English summary.)
- Michajlow, W.** 1938. Über das Bedürfnis einer Vereinheitlichung der Forschungsmethoden, die sich auf die Copepoden als Zwischenwirte der Cestoden beziehen. *Zoologica Polonica* 3:15-22.
- . 1953. The intraspecies relationships in the procercooid populations of *Triaenophorus lucii* (Mull.). *Acta Parasitologica Polonica* 1:1-28. (In Polish with English summary.)
- Miller, R. B.** 1952. A review of the *Triaenophorus* problem in Canadian lakes. *Bulletin of the Fisheries Research Board of Canada*, No. 95. 42 pp.
- Riggs, M.** 1986. Community dynamics of the Asian fish tapeworm, *Bothriocephalus acheilognathi*, in a North Carolina cooling reservoir. Ph.D. Dissertation, Wake Forest University, Winston-Salem. 340 pp.
- , and **G. W. Esch.** 1987. The suprapopulation dynamics of *Bothriocephalus acheilognathi* in a North Carolina cooling reservoir: abundance, dispersion, and prevalence. *Journal of Parasitology* 73:877-892.
- Shostak, A. W., R. B. Rosen, and T. A. Dick.** 1985. The use of growth curves to assess the crowding effect on procercooids of the tapeworm *Triaenophorus crassus* in the copepod host *Cyclops bicuspidatus thomasi*. *Canadian Journal of Zoology* 63:2343-2351.
- Stich, H. B., and W. Lampert.** 1981. Predator evasion as an explanation of diurnal vertical migration by zooplankton. *Nature* 293:396-398.
- United States Department of Agriculture.** 1972. Index-catalogue of medical and veterinary zoology. Suppl. 18, part 3. U.S.D.A. Agriculture Research Service, Beltsville, Maryland. Page 273.
- . 1976. Index-catalogue of medical and veterinary zoology. Suppl. 20, part 3. U.S.D.A. Agriculture Research Service, Beltsville, Maryland. Page 197.
- Watson, N. H. F., and G. H. Lawler.** 1965. Natural infections of cyclopoid copepods with procercooids of *Triaenophorus* spp. *Journal of the Fisheries Research Board of Canada* 22:1335-1343.
- Williamson, C. E., and R. E. Magnien.** 1982. Diel vertical migration in *Mesocyclops edax*: implications for predation rate estimates. *Journal of Plankton Research* 4:329-339.
- Wilson, M. S.** 1959. Free-living Copepoda: Calanoida. Pages 738-794 in W. T. Edmondson, ed. *Fresh-water Biology*, 2nd ed. John Wiley & Sons, Inc., New York.
- Yeatman, H. C.** 1959. Free-living Copepoda: Cyclopoida. Pages 795-815 in W. T. Edmondson, ed. *Fresh-water Biology*, 2nd ed. John Wiley & Sons, Inc., New York.
- Zaret, T. M.** 1980. Predation and freshwater communities. Yale University Press, New Haven. 187 pp.
- , and **J. S. Suffern.** 1976. Vertical migration in zooplankton as a predator avoidance mechanism. *Limnology and Oceanography* 21:804-813.